Radu Popa

Between Necessity and Probability: Searching for the Definition and Origin of Life

Springer

Between Necessity and Probability:
Searching for the Definition and Origin of Life

Springer
Berlin
Heidelberg
New York
Hong Kong
London
Milan
Paris
Tokyo

Physics and Astronomy ONLINE LIBRARY
springeronline.com

Advances in Astrobiology and Biogeophysics

springeronline.com

This series aims to report new developments in research and teaching in the interdisciplinary fields of astrobiology and biogeophysics. This encompasses all aspects of research into the origins of life – from the creation of matter to the emergence of complex life forms – and the study of both structure and evolution of planetary ecosystems under a given set of astro- and geophysical parameters. The methods considered can be of theoretical, computational, experimental and observational nature. Preference will be given to proposals where the manuscript puts particular emphasis on the overall readability in view of the broad spectrum of scientific backgrounds involved in astrobiology and biogeophysics.
The type of material considered for publication includes:

- Topical monographs
- Lectures on a new field, or presenting a new angle on a classical field
- Suitably edited research reports
- Compilations of selected papers from meetings that are devoted to specific topics

The timeliness of a manuscript is more important than its form which may be unfinished or tentative. Publication in this new series is thus intended as a service to the international scientific community in that the publisher, Springer-Verlag, offers global promotion and distribution of documents which otherwise have a restricted readership. Once published and copyrighted, they can be documented in the scientific literature.

Radu Popa

Between Necessity and Probability: Searching for the Definition and Origin of Life

With 58 Figures

Springer

Dr. Radu Popa
University of Southern California
Department of Marine Biology
3651 Trousdale Parkway, AHF 107
90089-0371 Los Angeles, CA, USA

Cataloging-in-Publication Data applied for

Bibliographic information published by Die Deutsche Bibliothek Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at <http://dnb.ddb.de>

ISSN 1610-8957

ISBN 3-540-20490-3 Springer-Verlag Berlin Heidelberg New York

Springer-Verlag is a part of Springer Science+Business Media
springeronline.com

Printed in Germany

Cover design: Erich Kirchner, Heidelberg

Printed on acid-free paper 54/3141/ts - 5 4 3 2 1 0

Everything we hear is an opinion not the fact.
Everything we see is a perspective not the truth.

Marcus Aurelius

Preface

The most complicated machines are made from words.
Jacques Lacan

Where does life come from? This is a question that has fascinated mankind since the beginning of time. As soon as somebody or 'something' (either life form or machine) becomes aware of itself, it also asks questions about its world and about its own origins. It is no mystery that the oldest myths and legends of human culture are so often centered on genesis motifs. Yet despite tremendous progress in science during the last century, we are still far from understanding the origin of life. Because we do not know exactly what life is (or maybe because we cannot agree upon this issue), a wide variety of theories and pseudo-theories have been proposed about the origin of life. The palette of visions concerning the origin of life has reached out in many directions: life being generated by incomprehensible-to-us mystical forces, spontaneous generation (Buchner 1855), creationism (Bernard 1878b), random genesis, panspermia (Arrhenius cited in Servos 1996), qualitative upgrades of quantitative accumulations (Oparin 1924), gradualist (smooth) 'upgrades' from lifeless matter into life, life as an inevitable natural consequence (Klabunovsky 2002), life as an emergent property of matter (Turian 1999), life as an extraterrestrial manipulation or a phenomenon from another physical dimension. Because human knowledge is a shared type of knowledge, our understanding of the world is collective. Therefore, most theories about early life are not fully independent of each other and show significant overlap.

The scientific field studying the physical meaning of life on Earth and in the Universe, its origin and its fundamental properties has been given various names such as prebiology (Rossler 1983), exobiology (Ponnamperuma 1972) or originology (Kompanichenko 1996). Another possible name that might better suggest the physical ambiguity of its study subject is 'parabiology'. Because this book is a quest and a challenge for the understanding of life anywhere and, whatever its physical substance, I prefer to use the term exobiology. Astrobiology, bioastronomy and artificial life are connected disciplines which, although interested in the same major issues, have more pragmatic purposes such as searching for life elsewhere, the quest for non-terrestrial types of life and the creation of artificial types of life.

Among all sciences, exobiology holds an unmatched record. It has the highest ratio between the number of hypotheses and the number of relevant findings. The reasons are quite simple. Earth is an old planet, almost 4.6 Gyr (giga years). Therefore the Earth we observe today is very different from the Earth in its beginnings. Without a time machine that would allow one to probe the early Earth, we will never be sure whether our models are an accurate representation of the environment in which life originated. Moreover, modern life is not simple but appears as an intricate web of large and complex molecules that seem very unlikely to have appeared spontaneously and that cannot exist independently of each other. For a biochemist or molecular biologist, this 'Gordian' knot has no apparent beginning but only endless loops and interdependencies. Therefore, modern life appears as a large collection of interlocked chicken-and-egg paradoxes. Consequently, life cannot be understood through either purely deductive logic or through purely experimental approaches. Facing such an enormous challenge, a society predisposed to philosophical, contemplative and intuition-based approaches is as helpless as a super-technological, pragmatic and deductive society. An integrated and realistic attitude seems the only sound approach toward scientific satisfaction.

Being aware that the vision presented here might contradict certain postulates that other theories about the origin of life consider as fundamental, I support the postulate that life emerged very early in the universe, that life is probably present in other forms in other parts of the universe as well, that life was 'pushed' into existence by understandable and foreseeable forces and that an intelligent mind is capable of understanding life as a general concept. Certainly, nothing is forever settled in science and no theory must be taken for granted, irrespective of how much experimental evidence we humans may have for it, and how precise that evidence may be. This is just a reminder of the relativity of our 'scientific truths', a warning that "in science one can proclaim a theory about reality as being the latest but never as the last theory." Consequently, although as an author I might be tempted to envision my opinion as legitimate, the interpretation of the origin, properties, meaning and definition of life presented here can offer no more than what current scientific knowledge would allow. Aware of this unavoidable caveat, I have tried throughout this study to be as open as possible to alternative interpretations. If not deliberate, it might sound ironical that quotes given at the beginning of some chapters are actually contradictory to what the chapters have to say.

Probably every scientific generation before us believed that the end of their quest was in sight. Yet to this day they have all failed to resolve their questions. Over and over, research has demonstrated that the intrinsic complexity of the living state is too overwhelming to resolve. Because later generations will scrutinize life and its origin with more experience and better insight than us, and with an appropriate respect for the magnificence of this subject, the purpose of any sound theory about life cannot be to clarify the

problem, but to point questions in the right direction, to identify a plausible and not an ultimate answer and to navigate on a likeliest path through the fog of often conflicting experimental observations and alternative hypotheses. In exobiology one can never expect ultimate answers, only illumination.

Acknowledgements

This book is dedicated to my family for their support. I would like to acknowledge all my professors, mentors and friends who cultivated in me the passion for this fascinating discipline. Special thanks to Cezar Radu, Arthur Marx and Kenneth Nealson for their inspiring guidance.

University of Southern California *Radu Popa*
Los Angeles
September 2003

Contents

1 Introduction

> One can separate the logical form of an organism from its material basis of construction, ... its capacity to live and reproduce is a property of the form, not the matter.
>
> Emmeche 1992

According to Ernst Haeckel, "any detailed hypothesis whatever concerning the origin of life must, as yet, be considered worthless, because up till now we have no satisfactory information concerning the extremely peculiar conditions which prevailed on the surface of the earth at the time when the first organisms developed" (Haeckel 1866). Erwin Schrödinger (1943) also expressed pessimism regarding the chances of understanding life. In the same vein: "Life is like consciousness. If you think you can explain what it is, you got it all wrong" (Shaw 2002). Niels Bohr interpreted life as a fundamental property of matter analogous to certain quantum properties, to be taken as given, and thus allowing little if any logical scrutiny (Bohr 1933):

> 'The existence of life must be considered as an elementary fact that cannot be explained, but must be taken as a starting point in biology, in a similar way as the quantum of action, which appears as an irrational element from the point of view of classical mechanical physics, taken together with the existence of elementary particles, forms the foundation of atomic physics.'

The modern day belief is that the origin of life may be unclear but that it is not an impossible problem (Scott 1986, Morowitz 1992, Bedau 1998, Lahav 1999, Brooks 2001, Buiatti and Buiatti 2001). Overwhelmed by life's incredible complexity, some scientists prefer to consider definitions of life as uninteresting, or even doubt the scientific need for them, or the chances of ever finding, a satisfactory definition for life (Gilat 2002). But if we cannot describe life on Earth, how will we be able to understand life as a general concept. That represents biological and nonbiological life, terrestrial and extraterrestrial life, material and cybernetic life.

R. Popa, *Between Necessity and Probability: Searching for the Definition and Origin of Life*, Adv. Astrobiol. Biogeophys., pp. 1–14 (2004)
http://www.springerlink.com/

Four major questions are generally formulated in exploratory astrobiology, the origin of life and artificial life:

- Is life a unique thing, or is it a unique collection of common features?
- Is the origin of life mere probability, or is it the result of some special circumstances?
- Is life a physical necessity?
- Is it possible to formulate a representation of life on Earth in a way that will address life elsewhere?

Understanding life is not just a theoretical exercise; it is a quest directly connected with its origin and has many practical applications (Bedau 1998, Kuhn 2002, Russell 2002, Lacey et al. 2002). Future explorers of the outer space (either man or machine), challenged with facing life forms unlike anything on Earth (Friedman 2002, Nealson 2002), have to be 'armed' with accurate guidelines capable of discriminating the essence of life from its composition and from its physical appearance. Many things in nature might seem related to life, whereas they are simple natural phenomena or complex processes, and conversely, many things that may seem lifeless, might be alive or even dangerous (to us). The creation of artificial life (making fully fledged autonomous systems) cannot advance without understanding its general properties and the circumstances leading to life and supporting it (Langton 1989, Ronald et al. 1999, Ruiz-Mirazo et al. 1999, Standish 1999). The assessment of successful early life simulations involves identifying specific and interconnected objectives derived from the essential attributes of life (Ronald et al. 1999, Korzeniewski 2001) or from the logic behind its self-organization (Boden 1996). Finally, anticipating the overall consequences of life on another planet will become an important part of mission planning for all extraterrestrial exploration (Brack 2000, Reichhardt 2001) and the terraforming of other planets.

Several approaches are commonly used to represent life: mechanistic–reductionist, dialectic–materialism, holism, and vitalism (Pályi et al. 2002). The mechanistic–reductionist approaches interpret all life-related phenomena through physicochemical processes but explain little about the origin of life. The dialectic–materialism views describe the origin of life as a set of qualitative changes (jumps) driven by quantitative (gradual) accumulations. Holistic views interpret life as a collective property, while vitalist theories attribute life to a hidden (vital) force. Although these approaches might sound different from each other, it is not always easy to tell where one ends and the other begins. An extensive collection of definitions of life has been formulated using such approaches (Maynard Smith and Szathmáry 1995, Muller 1935, Gánti 1975, Lahav 1999, Pályi et al. 2002, Szathmáry 2002, Appendix B). Some definitions of life are subjective and depend upon an individual standpoint and religious subconscience (Hennet 2002), ranging from pure materialism to pure spiritualism (Apte 2002; Appendix B). A committed materialist defines life in terms of matter and energy, denying the need for a spirit, while a pure spiritualist would only consider the reverse. Although some address major

requirements of life, many definitions do not identify the essentials of life, while others focus heavily on features that are particular to the terrestrial type of life. A comprehensive interpretation of life must address issues and identify properties that are independent of its physical nature (Dix 1983, Kauffman 2001), otherwise the model becomes flawed with exceptions and liable to unanswerable criticism. A correct interpretation of life must address the essential properties of life, must be capable of relating them to the early history of life, and must help reveal the forces driving its emergence and evolution.

Holistic and Mechanistic Definitions of Life

Two scientific approaches dominate modern theoretical debate about how life should be interpreted: holistic (as opposed to reductionist) and mechanistic (as opposed to vitalist) (Tamponnet and Savage 1994, DeLoof and Broeck 1995, Buiatti and Buiatti 2001, Nevo 2001, Rosslenbroich 2001).

Holistic interpretations of life are function- and purpose-related descriptions. The classic example of holism is "nothing is alive in a cell except the whole of it" (Olomucki 1993). In holism, life is viewed as a collective property. It was suggested that an appropriate approach to obtaining a holistic definition of life is to address questions such as:

- How are different forms of life at different levels of the vital hierarchy related to each other (Bedau 1998)?
- Is there a gigantic hiatus or a phenomenological continuum between life and non-life (Bedau 1998)?
- What is more important for life: the form, the shape or the composition (Bedau 1998)?
- Are life and matter intrinsically related (Von Liebig 1868)?
- Are life and mind intrinsically connected (Bedau 1998)?

Holistic approaches show considerable limitations in experimental practicality because they often neglect the particular properties of living entities.

Reductionist interpretations of life try to demystify the explanation of some very complex activities otherwise thought by some to have a non-material explanation (Arrhenius 2002). One of the paradigms of modern molecular evolution is that minimal life consists only of molecules and their mutual interactions (Luisi 2002). Despite the popularity of reductionism, many scholars agree upon the existence of some emergent properties of matter, i.e., properties that appear only at certain levels of complexity. The structure at each level of organization is made of the components of a lower level, but the lower levels cannot explain some of the qualities of a higher level. Thus, structures may be considered the subject of a reductionist approach while some properties cannot (Luisi 2002).

The mechanistic interpretations of life are pragmatic approaches. They describe living forms as complex machines, whose parts function in a thermodynamic direction that is somehow fortunate for the survival of the overall system (Prigogine 1980, Schrödinger 1944). This need for thermodynamic justification feeds most mechanistic interpretations of life. Most mechanistic descriptions of life tend to explain it as a probabilistic paradigm. When this philosophy is taken to its extreme (i.e., interpreting the origin of life strictly as a collection of chance-like events), it fails to identify any meaning for life in general, to understand life as a concept or even to consider the need to look for attractors that might have 'pushed' life into physical existence. The purely mechanistic interpretations are closer to 'not seeing the forest for the trees' than any of the other approaches.

Vitalism is an ancient belief that living entities exist due to a mysterious force called the 'vital force', 'perfecting principle', 'entelechy', or 'mneme'. Although modern science generally dismisses this stand, vitalism has never been scientifically demonstrated as erroneous. However, vitalism has fallen behind in the ranks of approaches to describe the origin of life because of its non-scientific belief in a transcendental principle. On the other hand, because modern science is still unable to provide an articulate model for the origins of life, vitalistic ideas still surface now and then. After all, vitalists can always claim that they said nothing more than 'the force(s) behind life is (are) unknown', and on this issue they are perfectly right. Vitalists would be wrong only if they claimed that the situation would remain so forever. Finding a physical attractor for life would actually provide some reconciliation between modern science and the old-fashioned vitalism.

Generalist vs. Minimalist Definitions of Life

Although the need to represent life in a simultaneously broad and detailed manner seems obvious (Dix 1983), some descriptions of life use a conceptual generalization to an excessive degree. These descriptions either use an obscure language with disguised meanings and thus become eclectic to any non-specialist, or else they render themselves useless by providing insufficient information to discriminate life from non-life. They sometimes even go beyond what life actually is (Hotchkiss 1956, De Loof 1993, Baltscheffsky 1997, Korzeniewski 2001, Hennet 2002, Appendix B). Notable examples are:

- "Life is a historical process of anagenetic organizational relays" (Valenzuela 2002).
- "Any system that creates, maintains and/or modifies dissymmetry is alive" (Krumbein 2002).

The minimalist approach considers that life can and must be defined on the least amount of information (necessary and sufficient) to distinguish it from inanimate matter (Korzeniewski 2001). This approach was defended as an attempt to avoid dogmatic assumptions and arbitrary requirements about

life (Hennet 2002). However, the products of this approach are not by design meant to explain how and why life emerged. Although sometimes praised as being intellectually challenging, minimalist approaches are often irrelevant or unable to convey true illumination. They do not make life clearer to whoever or whatever (observer or instrument) has already experienced life, nor are they insightful to whoever (or whatever) has never seen life before.

It has been claimed that a minimalist description of life would also implicitly address the problem of its essence (Korzeniewski 2001). According to such an interpretation, a minimal and sufficient dictionary-type description of an object or a phenomenon (such as growing crystals, fire, clocks, hurricanes, wars or religious cults) must implicitly explain the forces and circumstances behind their emergence. A theoretical attempt has been made to describe life through the definition of a minimum unit of life (Szathmáry 2002). Apart from using the word 'minimalist', this interpretation reaches far beyond the dogma of a minimalist type of definition. It is rather an attempt to find a generalist interpretation of a minimal unit. The objection here is that the individual level of existence might not be enough to fully explain the meaning of life in general (at the supra-individual level). Life as an overall phenomenon has a considerable bearing on the understanding of its essence. Explaining life requires both individual and collective attributes. Generalist approaches to life are nevertheless valuable tools because of their built-in tendency to ignore the composition of individual life forms and address general concepts. They are the closest to a non-Earth-centric interpretation of life.

Cybernetic Definitions of Life

Cybernetic definitions of life are a product of the belief that it is possible to derive a definition of life from computer simulations, thus describing life entirely as a cybernetic 'thing' (Emmeche 1992, Milosavljevic 1995, Clark and Kok 1998, Bedau et al. 2000, DeBeer and Kourie 2000, Korzeniewski 2001). Yet caution must be exercised when using them. Statements such as "a living individual is as a network of inferior negative feedbacks (regulatory mechanisms) subordinated to (being at the service of) a superior positive feedback (potential of expansion)" (Korzeniewski 2001) are so unconstrained that many otherwise lifeless 'things' can fit the profile and be considered alive. Examples are not only plasmids and viruses but also the development of cloud systems, liquids at boiling point, fire, fluid vortices, magmatic extrusions, or uplifts associated with plate tectonics. Many of these 'things' display some form of intrinsic expansive tendency and use some form of regulatory feedback to adjust themselves to the tolerance limits of their available space and resources, thus apparently 'avoiding' functional collapse. If such things are alive, why not so consider other things such as the capital market, art, religion, or other phenomena related to the field of psychosociology such as the dispersion of news, lies, and gossip? They all

display regulatory mechanisms at lower levels supporting the need for an expansion.

Cellularist vs. Genetic Definitions of Life

Cellularist and genetic definitions are descriptions based solely on experimental knowledge of life on Earth. Most classical interpretations of early life were either cellular or genetic (Luisi 2002). A 'cellularist' believes that the formation of a cell (a semi-permeable physical enclosure or a compartment) represents the relevant turning point toward life and that all other properties of life are consequent. In contrast, a 'geneticist' considers replication and variability as being the true starting points for life. In the last few years it has become more and more obvious that these two visions are not actually antagonistic to each other, but rather entangled, complementary and temporally connected. They are different facetes of the same thing. Although cellularist and genetic definitions of life are no longer much favoured, they are often used for teaching purposes.

Parametric Definitions of Life

Parametric (or criterion-based) definitions of life try to identify a list of the most relevant features of life (Appendix B). The most extreme (and most mistaken) parametric approach tries to identify one single feature of life as explaining everything including its origin. So far this attempt has proven fruitless and it is likely to remain so. One single step toward life is extremely unlikely. It is no different from viewing life as an extraterrestrial experiment, a miracle, alchemistry or magic. The philosophy of most criterion-based definitions is to gather the smallest bundle of features of life, features that in each author's mind are the most striking, the easiest to perceive (or to measure) and the most comprehensive. The most popular parameters used in parametric definitions are replication, metabolism and evolution (Eigen et al. 1981, Gánti 1974, Békés 1975, Jibu et al. 1997).

Despite their analytic appeal, parametric interpretations of life fail to discriminate between properties of life that are primeval or causal and properties of life that are derived (i.e., consequential and therefore subsequent). Some features from the physicochemical world such as electrical charge, the notion of spin from quantum mechanics, the electromagnetic force, mass and gravitation are considered more fundamental than others such as temperature, energy, viscosity, the strength of a chemical bond or the shape of a crystal. The latter are generally considered derived because they can be explained as consequences. Analogously, life also displays some features that are fundamental (either causal or phase transitions) and others that are derived (deterministic or emergent). This separation is not trivial, especially when early life is discussed. Although features such as energy balance, preservation of molecu-

lar and cellular architecture, metabolism, replication, reproduction, complexity, Darwinian evolution, homeostasy, motion, genetic blueprints, response to stimuli or intelligence are excellent discriminators for life (Oparin 1924, Schrödinger 1943, Gatlin 1972, Gánti 1974, Eigen and Winkler-Oswatisch 1981, cited by Dyson et al. 1997, Horowitz 1986, Fontana 1992, Joyce 1994, Dyson 1997, Hazen 2001), they are not primordial. These features are deterministic consequences of other preexisting circumstances, outcomes of the way living nature functions and are often not a confirmation that life is present.

Numerous lifeless physical realities display some life-analogous properties. Computer programs are capable of replication, fire is capable of growth, oceans have boundaries, the economy displays a means to achieve homeostasy, and the arts have an evolutionary history. Simply assembling such features indiscriminately, without regard for their type, meaning and interdependencies would be superficial. An endless plethora of overlapping combinations of parametric definitions of life can be (and have been) formulated (Appendix B). Although never completely wrong and usually not mutually inconsistent, they have no special merits in themselves and often tolerate each other. All these definitions are snapshots of the same multidimensional 'thing' we call life, viewed from different angles. The biggest caveat of the definitions of life that use exclusively derived properties is their inability to relate to the early history of life. This is because, instead of viewing life as a qualitative accretion punctuated by stepwise probabilistic novelties, a thing in harmony with its surrounding universe, parametric definitions contemplate life as a mere collection of unusual attributes, a spatiotemporal coincidence of remarkable properties. They tend to ignore the conditions and the factors that lead to these properties. A potential trap for a purely parametric description of life using exclusively derived properties is that of failing to recognize prelife forms as relevant. This applies especially when elaborate features such as hierarchical networking, oxidative phosphorylation, semi-conservative replication, or Darwinian evolution are used as criteria to formulate a definition for life.

Material-Related Definitions of Life

Although "living organization is ... characterized by a deep interrelation between form and materiality" (Patee 1977, Emmeche 1992, Moreno et al. 1994), the universal properties of life are probably independent of its material nature (Maturana and Varela 1980, Langton 1989, Emmeche 1992, Morán et al. 1997). Despite the fact that life on Earth should only be used as one possible example of life, many descriptions of life are Earth-centric (Farmer 2002). The extension of this bias is also known as the 'weak anthropic principle' or the 'anthropic cosmological principle' (Barrow and Tipler 1986). The anthropic mentality implies that some very restrictive conditions are required

for the existence of life, such as the particular properties of water at terrestrial temperatures (Jibu et al. 1997), the properties of the carbon atom (Benn 2001, Altstein 2002), or the critical events of phase transitions (Glassman and Hochberg 1998).

A common downside consequence of this vision is to impose Earth-related physical and chemical limits on where life may exist in the Universe and even to establish theoretical habitable zones based on material composition (Gonzalez et al. 2001, Lineweaver 2001, Kasting 2002). In a few cases particular types of molecule such as nucleic acids and proteins are used to describe life (Kunin 2000). As far as we know, life on Earth might be the only type of life in the entire Universe using proteins and nucleic acids. Not only do protein/DNA-centric visions put any other possible form of life out of scale, but they interfere with a fundamental goal in exobiology, which is to understand life as a general concept. On the basis of an Earth-centric 'policy', an extraterrestrial flying to Earth, its body made of tar, its blood liquid ammonia and breathing chlorine gas, should be treated no more than as a bag of chemicals, irrespective of how complex its technology, how efficient its metabolism, or how elaborate its mathematics and its music. The 'Earth-centrist commission' would declare the 'extraterrestrial bag' to be 'a non-living thing' because it did not have ribosomes or DNA and it was too much unlike us. They would then draw some fluid from it with a syringe, make a chemical analysis and put the 'thing' in a freezer. How does this differ from the Amazonian indians killing explorers simply because they were unlike anything they had ever seen before in the jungle?

What is Needed to Explain Life?

"Definitions are usually like a fisherman's net: too small to encompass Leviathan, but with a mesh too large to hold many of the denizens of the deep" (Lauterbur 2002). Contemplative visions such as "the mystery of life isn't a problem to solve, but a reality to experience" (Herbert 1965), approach life as something that cannot be understood, considering the subject too magnificent for our tiny brains. This mentality denies the spirit of the scientific method, portraying scientists as lost beings, doomed to wander forever in seas of incertitude and jungles of paradox. Most scientists believe that even though we may not yet be up to the task, life and its origins remain decipherable natural phenomena. Although life appears very complex, it may be simple in its basic principles. The main objective in exobiology is to find a way to approach the Gordian knot of life? Without cause–effect events regulated by the laws of chemistry and physics, there can be no science of the origin of life (DeDuve 1991). It appears obvious that one should start from simple physical principles and follow their causality until lifelike properties are achieved.

The most common approach used by modern science to explain the origin of life is molecular Darwinism. Chemical simulations may be able to tell us

something about how life can accrete, but early life is so far from us in the past that we will never know its exact chemistry. When details are not available, what is left are the basic principles. Whatever pushed life into existence was not its material nature but the determinism of its very essence. Thus some properties of life in the Universe may be presented independently of its material basis. An appropriate theory of the origin of life should identify a general sequence of events (Dyson 1997), rather than trying to solve the abiotic genesis of its individual pieces, e.g., its particular molecules (Luisi 1993). Since the assemblage of life was stepwise and nonrandom, its description must also be hierarchical (von Bertalanffy 1968, Orstan 1990, Hazen 2001). In a hierarchical scenario, events of a different causal rank are presented in a connected web.

Both criterion-related descriptions and purpose-related descriptions of life must be used to outline the essence of life (Korzeniewski 2001) and to understand its universal properties. The criterion-based component of such an interpretation must focus more on fundamental features, covering both the emergence and the historical survival of life, addressing the causality of its derived properties, and allowing a numerical discrimination of the living state from lifeless physical realities. The purpose-related component of such an interpretation must shed light on the meaning of life (its purpose-like nature), and it must also address the question of whether some physical force(s) pushed or assisted life into existence, and eventually lead to the identification of the general and the minimal conditions required for the emergence of life. A correct interpretation of life should be minimal yet elaborate enough to discriminate life from non-life. It must address both modern life and early life, and must be scientifically sound and unambiguous, yet comprehensible to a person with a non-scientific training. A definition of life cannot be so esoteric as to become hard to convey.

In the present work, the aim has been to seek an interpretation of life which achieves the following:

- To describe life independently of any material, size or time frame considerations (Maturana and Varela 1980). This approach is based on the common assumption from cybernetics, artificial intelligence, computer and systems sciences and artificial life that "the material or energetic aspects of an organization do not affect its logical essence" (Bergareche and Ruiz-Mirazo 1999).
- To define the minimal conditions required for life to emerge, considering the constraints which formal operational requirements put on the material components (Thompson 1942, Vogel 1988).
- To use criteria that are comprehensive (universal) enough and at the same time simple enough to apply to everything that may be alive.
- To address both material life and cybernetic life and ask whether life can be reduced to an intrinsically computational algorithm (Emmeche 1992).

- To allow the identification of non-Earth-centric biosignatures that are measurable.
- To provide a set of tools that optimizes the possibility of finding life whenever present and minimizes the possibility of false positives.
- To generate a construct that can be related to the early history of life on Earth.
- To address the emergence of life as both a causal (deterministic) and a fortuitous (chance-like, probabilistic) 'thing'.

Outline of a Historical Reconstruction of Life

The origin of life is often separated into three main stages: geophysical, chemical, and biological. Geophysical and early geochemical evolution deal with the formation and evolution of our planet, the formation of minerals, rocks, water, oceans, dry land and atmosphere before life emerged. The geochemical evolution of Earth is a common starting point and framework for most origin-of-life theories. It has some relevance for astrobiology but it is almost useless in the field of artificial life. Chemical evolution is the best-explored segment of the history of early life. In the footsteps of Darwin and Oparin, most scientists believe that small organic molecules formed first through abiotic processes, that monomers subsequently emerged and were followed by polymers. Other features were added later, such as cellularization, molecular mechanisms and metabolism. The vast majority of early life simulation experiments (also called biomimetic studies) aim to clarify a number of precise chemical questions (Miller 1953, 1955, 1987, 1992, Arrhenius et al. 1973, Miller and Orgel 1974, Oró and Stephen-Sherwood 1976, Henderson-Sellers and Schwartz 1980, Brack 1998, Knauth 1993, 1998, Orgel 1995, Ponnamperuma et al. 1982, DeDuve 1988, 1995, Lahav 1999). Although life on Earth cannot be understood without investigating its particular chemistry, most scholars now agree that the existence of proteins, lipids and nucleic acids is not an absolute requirement for the living state in general.

The least explored, yet by far the most interesting part of the origin of life is the transition between lifelessness and life. Only a limited number of biomimetic experiments try to make the transition between chemistry and biology. The elaborate intricacies of life make it obvious that life cannot just pop out from randomness in a single swift step. Numerous physical and chemical steps were required for life to emerge. This study focuses on rules and patterns followed throughout the early accretion of life, on the various stages and types of primitive life, and on the 'lifeward' developments that were subsequent to abiotic geochemical evolution. Considering the transition: geophysical evolution $\longrightarrow$ chemical evolution $\longrightarrow$ biological evolution, this study is positioned somewhere between chemical evolution and biological evolution.

Although relevant for understanding the composition and functioning of life on Earth, prebiotic geophysical and chemical evolution on Earth goes

beyond the scope of the present work because what is questioned here is how many of the properties of life can be understood independently of its material nature. No chemical details are discussed, and nor do I address the questions as to where and how the first radicals, amino acids, pentoses, amphipates or cofactors were formed. I am concerned instead with the overall consequences of their existence and to some extent the general circumstances surrounding their formation. All the changes that followed once a Darwinian-type evolution had emerged and all the events that are subsequent to the establishment of a planetary homeostasy fall beyond the scope of this study and they are in any case beautifully covered in several classical studies.

Dominant Models

There are three dominant models concerning the origin of life:

- Phase separated systems (PSSs) or vesicles formed first, then enzymes were added, followed by genes in a third stage (Oparin 1924, 1961, 1968, Haldane 1929).
- Template synthesis (replication) was the first meaningful event, followed by metabolism and then by cells (Bernal 1967, Eigen et al. 1981).
- Abiotic activity (proto-metabolism) emerged first, followed by cellularization and then molecular evolution in third place (Wächtershäuser 1988, Clegg and Wheatley 1991). The preeminence of metabolism over genetic mechanisms was also hypothesized by Robert Hazen (Hazen 2001).

It is posited here that during the early evolution of life major features such as cellularization, metabolism or genetic mechanisms could not have been successive as they coevolved. None of them was primordial because none of them was dispensable. The hierarchical history of life (von Bertalanffy 1968, Orstan 1990, Hazen 2001) is not a succession of its major topics but a presentation of their concerted upgrades. The history of early life was a stepwise (punctual) accretion of achievements which can now be represented artificially through some major issues such as energy, boundary, metabolism, order, information and handedness. Thus one should not ask what major issue came first (energy or information or metabolism) but rather what type of mechanism they were represented by in different evolutionary episodes. One should also explore the attractors (forces) that lead to the upgrade of one evolutionary state into another. When applying this knowledge to artificial life, a mathematical theory of these transitions is also required. Yet the focus of the present study has been purposefully narrowed down to the logic rather than the mathematics behind the transition toward life, in order to construct a theoretical basis for the deconvolution of the tree of early life. For in the absence of purpose, logic and meaning, mathematical models are indistinguishable from combinatorial games or from infinite kaleidoscopes.

A reconstruction of early life brings out some guidelines:

- The structure, the function and the origin of life are inseparable (Bernal 1965).
- A description of life must be more than a picture of the present. It must also reflect its early history, ranking its achievements, and it must be purpose-oriented.
- Despite some historical step-like phase transitions, the separation between life and non-life was not very sharp. Many 'things' in nature exist at the boundary between non-life and life. Thus the non-life/life distinction is somewhat loose, especially at the transition zone (the origin of life) and a variety of interpretations of where lifelessness ends and life begins may be tolerated.
- Although it was sometimes argued otherwise (Woodger 1929), 'life' and 'living entities' are not synonymous things, but display certain independent features (Langton 1989, Emmeche 1992, Bedau 1998, Altmann 2002, Altstein 2002, Guimarães 2002, Pályi et al. 2002, Nair 2002, Valenzuela 2002). This distinction is essential to understanding both (Anbar 2002). A description of early life evolution must discriminate between 'units of life' and 'units of evolution' (Szathmáry 2002, Appendix C).
- Although early life forms were simpler than their modern successors (MaCallum 1908, Oparin 1957), their overall functional principles and their evolutionary tendencies were similar.
- The type of life on Earth (referred to as the 'terrestrial type of life') is only one possible type of life amongst many others equally possible.
- Although living entities in the Universe can be very 'different' from each other with respect to their material substrates, constructive details, size, density and metabolic rates, they must all share some common properties (the universal features of life).
- In contrast to a commonly held belief, the overall evolution of the biochemical network was not dominated by "a convenient import of clue components at critical moments out of a bountiful palette of external abiotically-made molecules." Although the terrestrial type of life did start with a handful of very common organics of abiotic origin, the build-up of the internal molecular network proceeded through the development of a selective import and novel biosynthetic achievements, and self-directed itself toward a peculiar chemistry that was purposefully uncommon to the outer world. In order to draft their own destiny (achieving some level of self-maintenance and gaining some functional independence), living units must beget distinction from outside chemistry. They must purposefully display a compositional personality. Thus trying to identify abiotic conditions leading to complex molecules of life such as nucleic acids, chlorophyll or proteins may be a mistaken line of research, as these molecules may have developed completely within life itself.

- Because modern life is hierarchical and emerged hierarchically (von Bertalanffy 1968, Morán et al. 1999, Hazen 2001), it is preferable to center the description of early life on how various states transited into various other states, rather than to list all possible disjoint steady states.
- The origin of life was both probability and necessity. A gradual accretion of life (Baltscheffsky and Jurka 1984), using stepwise (fundamental) phase transitions and their consequences (derived achievements) allows one to interpret its origins as both probabilistic (chance-like) and deterministic (causal).
- The overall properties of life did not evolve independently of each other. Instead they have coevolved. Rather than being a smooth transition, the accretion of life was a stepwise (punctual) addition of achievements belonging to all major features of life.
- The derived properties of life are consequential (deterministic), i.e., direct outcomes of defined circumstances. The fundamental phase transitions toward life are chance-like (probabilistic) events.
- The 'technological achievements' of life (either probabilistic or deterministic) were not added randomly but in a meaningful sequence that was progressive with respect to its overall efficiency.
- The driving forces behind all historical upgrades toward life (i.e., the agents that pushed life into physical existence) are somehow related to a unique purpose-like tendency. This 'purpose-like aspect of life' must have applied from the earliest, the smallest and the simplest individual (the protobiont), to the latest, the largest and the most complex supra-individual level (the biosphere). It must also exist in abiotic nature. It is posited here that the major driving force behind the early evolution of life is energy-related and not some form of molecular selfishness. Molecular selfishness is a mere byproduct, a consequence of the need for the propagation of the structural and functional consistency of living units, and it is not a major drive toward life.
- In order to avoid cyclic arguments resulting from the use of biological terms in the definition of life, one has to define life in purely physicochemical terminology (Lahav and Nir 2002).

The major issues needed to understand life as discussed here are: energy, boundary, metabolism, specificity and information. Handedness is interpreted as a particular case of specificity that is meaningful for a type of life based on polymers. Most of the achievements of life did not end their evolution after their emergence. Yet for graphical simplicity many of them will be illustrated as pop-out boxes in most figures.

A Large World for Lonely Life

Life on Earth displays a remarkable constructive unity. All living organisms use the same genetic code. Proteins only contain twenty amino acids and they

are the dominant catalysts, while phospholipids are present in all membranes. This unity is hard to explain if the concept of life is truly independent of its material composition. Could life have emerged only once? Do life forms need to exchange genetic information, which eventually led to structural unification? Is it possible that any given habitat may host no more than one form of life? Are our experimental approaches making us miss other forms of life here on Earth?

These two characteristics of terrestrial life (solitude and constructive unity) complicate the understanding of what life is in a more general context and thus limit our ability to locate extraterrestrial life forms, because a false mentality may persist wherein life can only be constructed this way. The absence of other types of life to be used as examples impede the establishment of the general principles of life, and this often leads to difficulties in interpreting the meaning and the origin of some 'things' present on Earth such as viruses, prions, desert varnish, manganese nodules or nannobes. Such ambiguities 'at home' are warning signs for the potential pitfalls in identifying extraterrestrial (ET) life.

The properties of life most often emphasized in the literature are derived either from classical biology (Lahav 1999) or from computer simulations (Milosavljevic 1995, Ruiz-Mirazo et al. 1999). In the simplest case, a group of such properties can be additively assembled with or without a causal relationship among them. Even if ET forms of life may be chemical and polymeric, it is unnecessary (and potentially misleading) to expect them to be based on the same materials as the terrestrial type of life, i.e., the same 20 amino acids contained in proteins, the same ATP, the same four nitrogenous bases contained in DNA, RNA, and the same phospholipid membranes. While it seems unlikely that ET life forms would share identical mechanisms such as chemiosmotic energy transduction, DNA replication or ribosome-based translation, all forms of life (biological and nonbiological, terrestrial and extraterrestrial, material and cybernetic) must share some common properties and obey some common rules (Conrad 1997, Hoyle and Wickramasinghe 1999, Santoli 2000, Kauffman 2001). Thus, the parameters depicting life must be selected in a way allowing the extension of the quest for life in the Universe beyond the reductionist search for a few particular molecules, for the chirality in amino acids, for DNA, and in general for any other terrestrial-life-related or Earth-centric fingerprints (Conrad and Nealson 2001, Farmer 2002). These universal parameters should also provide tools to understand the early evolution of life and monitor progress in artificial life simulation experiments (Langton 1989). Biological life displays many measurable properties such as respiration, C-assimilation, motion, handedness, chemical disequilibrium, heterogeneity, homeostasy, Darwinian evolution, isotope fractionation, or biomineralization (Campbell 1993) which, although good indicators of life on Earth, are derived features. In order to understand life in general, to clarify its origin, to focus on the very moments that made a difference in its early history and comprehend life independently of its material substrate, its form, its size, its shape, its kinetic activity and its evolutionary level, non-derived parameters of life are also needed that have a broad yet well-defined physical meaning.

2 The Early History of Bioenergy

> Basic biological organization is brought about by a complex web of energy flows.
>
> Bergareche and Ruiz-Mirazo 1999

Energy is most commonly defined as the potential to do work. The maintenance of the living state requires a constant flow of energy through the system. The concept of energy is not easily implemented in computational models of life and is therefore often ignored in artificial life models. Some models even "regard as irrelevant the energetic problematic (dissipation, irreversibility, couplings, energy currencies), in the physical realization of a biological system" (Ruiz-Mirazo et al. 1998). Examples of such models are Rosen's (M,R)-system, Varela's autopoietic models, Kauffman's autocatalytic set, and Fontana's algorithmic chemistry (see Appendix A). However, many origin-of-life theories maintain the primordial importance of energy for early life. Although everyone accepts that energetic constraints are important when describing material-based living systems, a problem arises when we have to consider whether or not they affect the very logic of the organization (Morán et al. 1999). It is argued here that energy considerations are not only primordial, but intimately related to the essence of life as well.

2.1 Energy-Related Phase Transitions Toward Life

Throughout the accretion of life, a variety of energy-related achievements have been added. Some of these features are probabilistic phase transitions while others are derived (emergent) properties. Three of the most important phase transitions in the early history of bioenergy are catalysis, reflexive activity and energy transduction.

R. Popa, *Between Necessity and Probability: Searching for the Definition and Origin of Life*, Adv. Astrobiol. Biogeophys., pp. 15–37 (2004)
http://www.springerlink.com/

2.1.1 Catalysis

A catalyst is defined as a component accelerating a reaction without changing its direction or its equilibrium and without being consumed in the process (see Fig. 2.1). Living forms can only exist if an external energy source supports them, and yet they cannot expect the environment to do the work for them. They must therefore be able to 'arrest' part of the external energy flow. In fact they have to redirect the external energy flow through themselves. Catalysis is one of the easiest ways to achieve this and has three basic purposes in living forms: it speeds up sluggish reactions, it competes with external processes, and it directs reactions along desirable paths.

From an energy balance point of view, a life form resembles a small village fed by a mill on a river shore. The relationship between the river and the village is not reciprocal. The river flow exists whether or not the village uses its force. However, the village cannot exist without the river. Because the water level in the river varies, it is better for the village to use the river wherever and whenever it flows. The villagers may stockpile some energy reserves to make it through periods of drought, but the river itself is too large to store. Because energy is always something external to life, life can exist in a 'closed system' but not in an 'isolated system' (see Appendix C). Life cannot exist in a 'thermally dead universe'. The wheel of the mill only

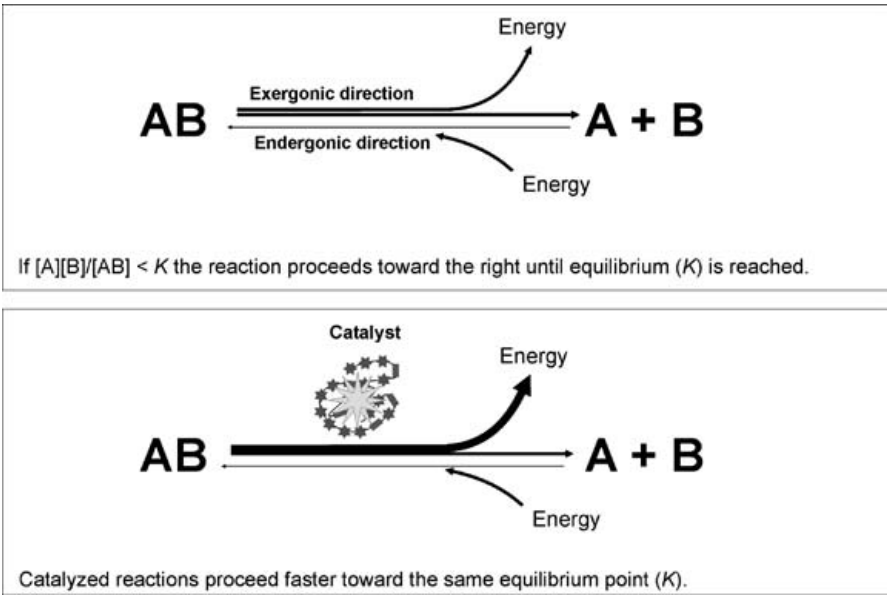


Fig. 2.1. Catalysts speed up downhill reactions. [A], [B] and [AB] are the concentrations of compounds A, B and AB, respectively. K is the value of [A][B]/[AB] at equilibrium

works if installed in the path of the flow and not on a lakeshore. The faster the flow, the more energy can be extracted from it. Therefore, whenever the water is flowing too slowly, the flow must be increased and directed toward the mill (such as building a dam). This ability to direct the water flow toward the mill wheel is analogous to catalysis.

Natural History of Life's Catalysts

Each modern cell uses hundreds of enzymes. Most enzymes are polypeptides, while some are nucleic acids. Enzymes are grouped into six classes (oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases). Although some enzymes do not use a cofactor, most of them do. Enzymes of the terrestrial type of life use more than 90 cofactors. Although catalysis is a primordial requirement for life (possibly its most fundamental prerequisite), the addition of catalytic skills was not a singular event. The concept of catalysis is primordial, yet large catalytic diversity has to be subsequent to the origin of life. Various types of catalysts and catalytic skills were mobilized throughout the evolution of life. Old catalysts were also continually upgraded during the entire history of life for better performance. Because the origin of life happened so long ago, (3.6–3.8 Gyr), it is impossible to predict the precise sequence of the earliest catalysis-related innovations. Some basic assumptions can still be made, and some evolutionary patterns and priorities identified, based primarily on the relative complexity of the added catalysts. (Fig. 2.2)

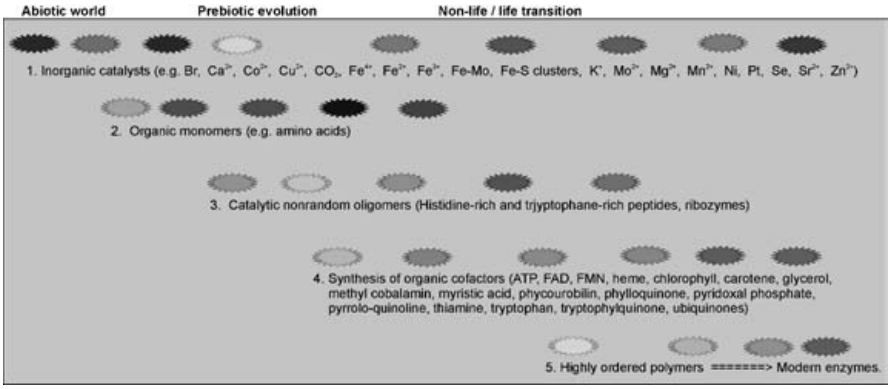


Fig. 2.2. Various catalytic innovations were added throughout the early history of life. The earliest catalysts (1) were probably simple inorganic chemicals that were available in the environment. As a proteic part was invented, these primordial catalysts became cofactors. Organic monomers (2) and nonrandom oligomers (3) were then used as catalysts. Later in evolution, as biochemistry became more sophisticated, the synthesis of organic cofactors (4) became possible and so too did the control over highly ordered enzymes (5)

Catalysis is not a life-restricted innovation. It is also present in nature. More than 90 enzymatic cofactors are now known.[1] Although most of these cofactors are organic constructs, in many cases inorganic chemicals are used. In general, the simpler a chemical is, the easier it is to find in the environment. Atoms are virtually indestructible, while simple molecules are generally more stable than larger molecular constructs.

Many inorganic cofactors used by life are common in abiotic nature, where they could be easily accessed by early life forms and used as 'tools' in catalytic processes. The simplicity of the inorganic cofactors compared to organic cofactors (see Fig. 2.3) may indicate that they were the first to be used by life forms (see sequence 1 in Fig. 2.2). Inorganic chemicals are not the only simple natural catalysts. Simple monomers such as amino acids could also have been used for their catalytic capabilities (Bar-Nun et al. 1994). Although simple catalysts are thermodynamically more reliable, their catalytic performances are somewhat limited while their substrate specificity is rather low. Early life forms exclusively using such catalysts could not have reached satisfactory efficiency while competing with external processes. One must assume that during the early stages of life (the beginning of sequences 1 and 2 in Fig. 2.2), catalysis was used solely as a means to speed up sluggish external reactions and to provide direction to internal processes, whilst it would have done little to outcompete the environment.

As soon as competition emerged (either with external processes or with other energy-dissipative entities), an increased catalytic performance was required for which simple catalysts (inorganics or simple organics) were no longer appropriate. A new brand of more efficient catalysts was needed. Terrestrial life's approach to solving this problem was to use polymers, a strategy still used by modern life (Fig. 2.2). Polymers make good chemical 'handles' capable of specifically binding many types of cofactors and substrates. Sometimes polymers can act as catalysts by themselves, on the basis of the radicals their monomers carry and a preserved architecture. Large molecular chains from modern enzymes improve reaction kinetics and substrate specificity. It would seem logical to assume that the formation of nonrandom oligomers capable of catalysis was the next pattern sequence (sequence 3 in Fig. 2.2) added to the history of catalysis. The question as to whether nonrandom oligomers can display catalysis has also been addressed experimentally and answered positively. Cysteine-rich peptides have a propensity to form metal–sulfur clusters, thus acting as electron transfer mediators (oxidoreductases). Moreover, Ni-binding oligomers are used in hydrogenation reactions while histidine-rich peptides formed in hydrophobic conditions are active in the hydrolysis of *p*-nitro-phenyl-esters (Luisi 2002).

The next important trend in the history of catalysis was the use of internally synthesized cofactors such as ATP, FAD, FMN, heme, chlorophyll, carotene, glycerol, methyl cobalamin, myristic acid, phycourobilin, phylloqui-

[1] http://wwwbmcd.nist.gov:8080/bmcd/bmcd.html

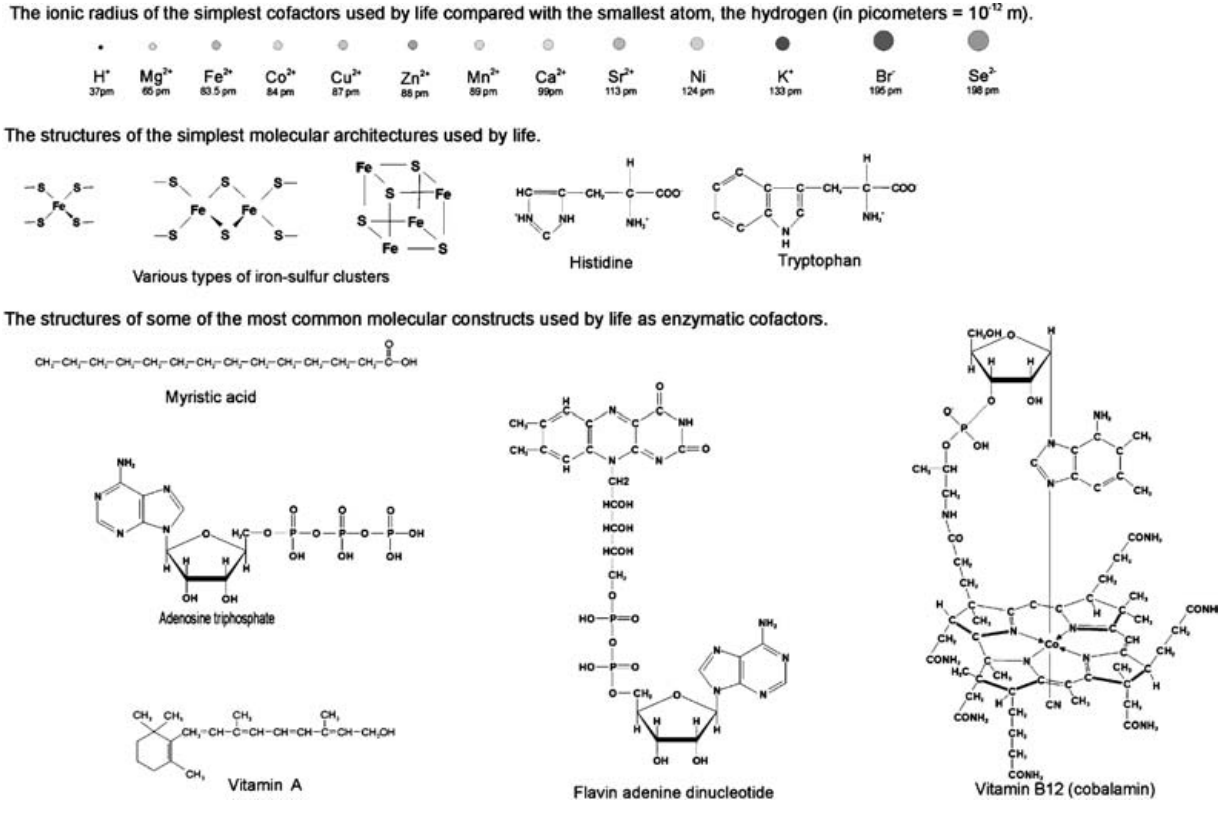


Fig. 2.3. Cofactors used by life display a wide range of sizes and complexity

none, pyridoxal phosphate, pyrrolo-quinoline, thiamine, tryptophan, tryptophylquinone, ubiquinones (sequence 4 in Fig. 2.2). Sequence 3 (synthesis of nonrandom monomers) was situated ahead of sequence 4 (synthesis of organic cofactors), simply because catalyzing condensation reactions in a chemically biased environment appears simpler than synthesizing organic molecules *de novo*. The last trend in the history of catalysis (sequence 5) was the improvement of control over the synthesis of highly ordered enzymes (see Fig. 2.2). Although these trends may have started at different moments in the early history of life, most of their evolution was consequential. Irrespective of the exact sequence of events, the overall trend in the evolution of catalysis was directed toward obtaining maximum functional diversity and the highest catalytic performances based on the least chemical complexity.

2.1.2 Reflexive Activity

Not every possible type of catalysis can be regarded as a lucky circumstance favouring life. In most cases catalysis is deleterious because in its presence the environmental thermodynamic equilibrium is reached faster while the energy supply may become discontinuous. In the analogy between life and the village with its water mill, no benefit is obtained if the wheel rotates, but the water flow is wasted and no work (either flour or electricity) is produced. Living entities cannot strategically afford to be wasteful because it is the least favorable condition that controls their existence. Even if a continual and abundant energy resource had ever existed, the intensity of catalyzed activity would have gradually decreased, because molecular catalysts would themselves have been exposed to degradation (see Fig. 2.4). A continual replacement and/or restoration would have been required to counteract the effect of this degradation. It would have been essential for their existence to balance the internal energy of the energy-dissipative units capable of performing catalytic activities. This is very likely to have been one of their earliest priorities.

This requirement is not only primordial, but it remained a condition for life from the beginnings to its present stage. Only systems able to function in a way that restores (extends or protects) at least part of their physical identity have any chance of becoming alive. A steady physical 'personality' (or identity) of a system is generally referred to as its quasi-state Θ. One system may display a multitude of quasi-states with different energy levels, entropy levels and complexity levels (Rosen 1991). Short-lived transition stages are not generally considered as quasi-states. A quasi-state is not necessary stable by itself? A quasi-state is defined by the possibility of being maintained once reached. Systems maintaining quasi-states must function in such a way that at least a fraction of the energy derived from their own activity may beneficially affect their stability. This second fundamental condition for a system to set off along the 'becoming alive' path is what is referred as reflexive activity.

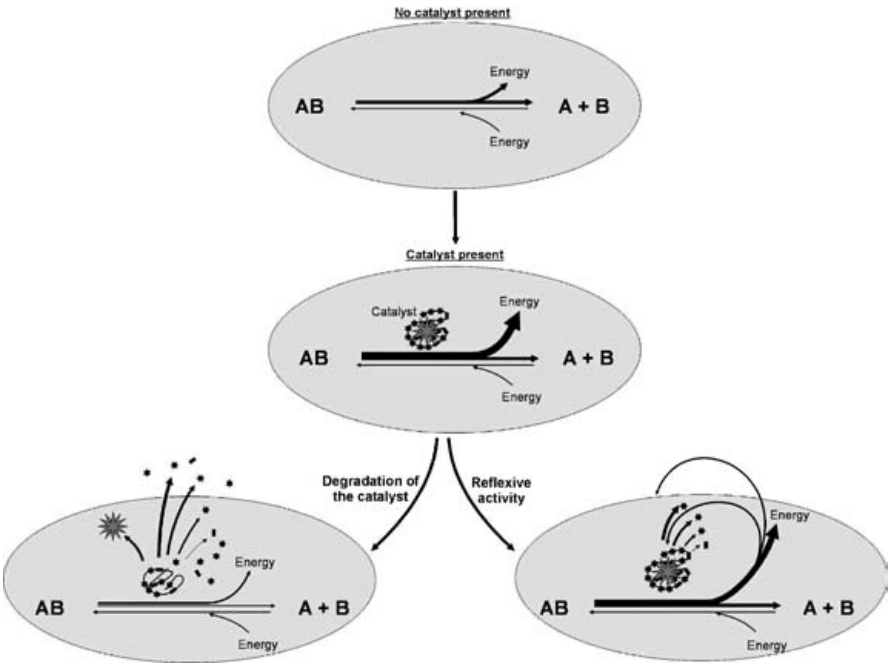


Fig. 2.4. The addition of reflexive activity increases the temporal existence of a catalyst

Reflexive activity is present whenever part of the energy derived from a process is used either to lower the degradation rate of the entity that is performing that process, or to increase the life expectancy of the performer, or to restore the integrity of a catalyst, or to reinitiate the process (see Fig. 2.4). If two systems A and B have the same catalytic performances ($\text{cat}_A = \text{cat}_B$) and are exposed to similar degradation conditions ($\Delta S_A = \Delta S_B$) but have different reflexive abilities ($\text{Ref}_A < \text{Ref}_B$), the temporal existence of B is longer than that of A. If the quasi-state is the quintessence of the physical existence (permanence) of a system, reflexive activity is the condition for the existence of a quasi-state. Reflexive activity is one of the most valuable concepts in identifying life and how much it stretches across space. If one system X performs an activity that does not affect itself, while another system Y uses the product of X and performs work toward the quasi-states of both X and Y, then Y alone or X plus Y may be alive, but the system X by itself cannot be alive.

A variety of concepts and processes imply some level of reflexive activity such as self-replication, constructive assimilation (Rizzotti 2002), autocatalysis (Farmer et al. 1986, Kauffman 2001, Lippmann 2002), self-repair (Ortega and Tyrrell 1999), self-renovation (Kompanichenko 2002), self-maintenance, self-sustenance (Baltcheffsky 2002) and constructive assimilation

(Rizzotti 2002). Reflexive activity is analogous to a very large parasol with many holes in it. This parasol may shadow many objects on the ground but none of them is fully shadowed by it. None of the shadowed objects (i.e., the concepts listed above) stretches over space (i.e., has a symbolic meaning) as wide as the parasol, while the parasol itself only explains one aspect of each of them. Thus none of these terms is identical in meaning with reflexive activity. The closest to the meaning of reflexive activity is 'the system doing work on itself'. Although one can say that during reflexive activity the activity of the system feeds back into the system, reflexive activity includes feedback regulation but feedback regulation is not enough to explain reflexive activity (see the entry for feedback regulation in Appendix C for more details).

Reflexive activity indicates the capacity of a system to invest energy and/or materials in itself by using energy derived from its own activity. Feedback regulation means regulating the intensity of the activity through its products. The reflexive activity concept means that energy and/or matter is directed backward, while feedback regulation means information is being directed backward. In reflexive activity, the state (the stability) of the system is the major objective (the target), while regulation of the activity is consequential. In feedback regulation, regulation of the activity is the objective (the target), while the state (the stability) of the system is consequential. The energy invested in the system during feedback regulation is seldom relevant, and is represented by the products lost during signalling.

Whenever a system with reflexive activity is catalytically active (it dissipates energy), its ΔS is less than would be expected in an inactive state. Therefore, if the material basis of the system is to be ignored (which is the case in computer-based artificial life), or if it is unknown (which is the case in extraterrestrial exploration), the existence of reflexive activity can be identified as a positive correlation between the state of the system or its performance at time t and the energy dissipation at t_{-1}. Quantitatively, reflexive activity represents how much of the energy needed for maintaining the system's quasi-state is derived from the energy that flows through the system. If the living system is compared to the village benefitting from a water mill, reflexive activity represents how much of the village's maintenance expenses is produced by using the water mill. Unless work is invested in the system by an external 'agent', in order for life to be possible, the energy flux passing through the system E_{fd} has to be larger than the energy flux θ_{E} needed to maintain the system in a given state. In practice Θ_{E} can be estimated as the difference between the expected degradation rate in the absence of any activity ($\Delta S_{\mathrm{inactive}}$) and the observed degradation rate of the active system ($\Delta S_{\mathrm{active}}$), $\Theta_E = (\Delta S_{\mathrm{inactive}}) - (\Delta S_{\mathrm{active}})$, while E_{fd} can be estimated as the difference between the system-independent energy flux of the environment in which the system is embedded ($\mathrm{Act}_{\mathrm{control}}$) and the energy flux in the presence of the active system ($\mathrm{Act}_{\mathrm{sample}}$), $E_{\mathrm{fd}} = (\mathrm{Act}_{\mathrm{control}} - \mathrm{Act}_{\mathrm{sample}})$. One may speculate that Θ_{E} represents "the extra energy price for being alive".

If reflexive activity is described as a self-renovation process ($E+$) (Kompanichenko 2002) and the result of the energetic effect of the destruction process is ($E-$), a living organism must display a positive balance ($E+ > E-$) for a sustainable development.

Although the primordial need for reflexive activity seems obvious, it is difficult to pinpoint the earliest reflexive activity mechanism used during the emergence of the terrestrial type of life, because the exact environmental conditions on Earth 4 Gyr ago are difficult to ascertain. A variety of mechanisms have been hypothesized that display some level of 'reflexiveness'. These mechanisms are of various types: macromolecular build-up (Lahav and Chang 1976), protective thermodynamic seclusion (Deamer 1998), auto-catalysis (Farmer et al. 1986, Orgel 1992, Kauffman 1986, Lippmann 2002) or self-repair (Ortega and Tyrell 1999). The ability to resist hydrolysis is considered by Martino Rizzotti as the most meaningful metabolic property of living cells (Rizzotti 2002). Examples of pre-life processes that could have been coupled with reflexive activities are the use of thioesters to drive condensation (Micheel et al. 1947, Weber and Orgel 1979, Weber 1981, 1984, 1987, DeDuve 1995), the formation of energy-rich pyrophosphate during the hydration of apatite-related minerals (Handschuh et al. 1973, Hermes-Lima 1990, Weber 1982), thermal condensation on mineral surfaces (Fox 1964, Fox and Harada 1964, Hulshof and Ponnamperuma 1976, Lahav and Chang 1976, Lahav et al. 1978, Coyne 1985, Keller et al. 1991, Holm et al. 1993), the bio-mineralization of iron sulfides (Koch 1985, Koch and Schmidt 1991, Wächtershäuser 1988, 1990, 1993, 1998, Russell et al. 1994, Russell 1996), surface metabolism based on the reduction of the activation energy in a bidimensional world (Wächtershäuser 1988), and self-production (and repair) of the membrane-related components (Badahur and Ranganayaki 1970, Schmidli et al. 1991, Bachman et al. 1992, Luisi 1993).

Whatever the primordial reflexive activity was, it is probably no longer present or conspicuous. It was probably wiped out by evolution, replaced or changed into something else, or it is now hindered by more elaborate and more efficient mechanisms. Examples of modern reflexive-activity-related processes are energy transduction, substrate-level phosphorylation, carbon fixation and biosynthetic pathways. The addition of reflexive activity to systems capable of catalysis resulted in a remarkable increase in their thermodynamic resilience, especially when coupled with catalysis which produced a significant increase in the energy flow (see Fig. 2.5).

Although reflexive activity is a condition for the existence of life, its presence alone is not proof of life. Many lifeless 'things' in the abiotic nature display reflexive activity. Autocatalysis, which is the type of reflexive activity most often claimed as primeval, is common in growing crystals, fire, positive feedbacks and iron rusting (Lippmann 2002).

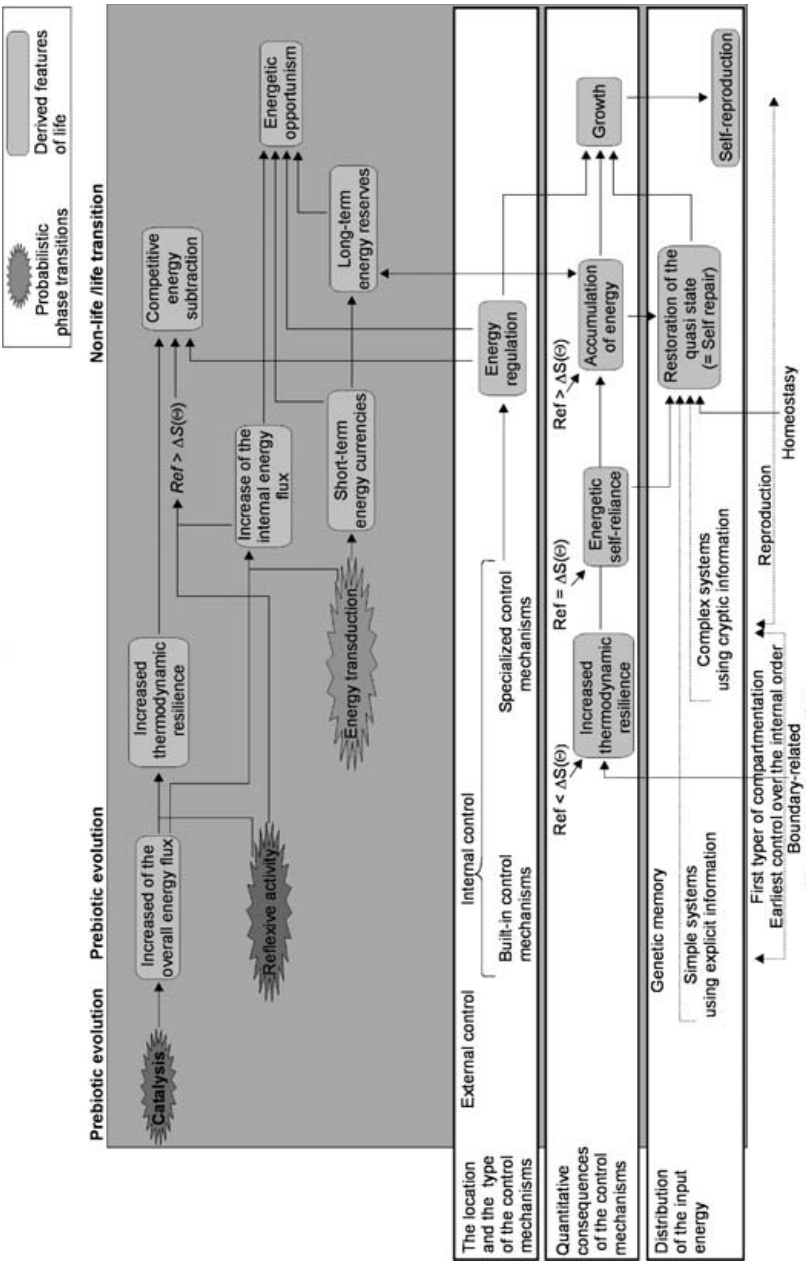


Fig. 2.5. Network of energy-related causalities during the accretion of early life

2.1.3 Energy Transduction

In the analogy between life and the village using a water mill to earn its living, the water flow is not used directly but it is first transformed. The mill wheel converts the energy of the water flow into mechanical energy that can be used either to grind seeds or to make electricity. Analogously, in living cells external energy is never used as is, and direct coupling with the external energy flow seldom occurs (Skulachev 1992). The process of transforming one energy form into another is known as energy transduction. During transduction, some form of external energy is first transformed into an intermediate form called energy currency that is compatible with the requirements of the internal mechanisms.

In modern human society the most common energy currency is electricity. In the living world, it is adenosine triphosphate (ATP) and membrane gradients (Δ) (see Fig. 2.6). Other energy currencies are acetyl-CoA, acetyl phosphate (Acetyl-Pi), pyrophosphate (PP or PPi), guanosine triphosphate (GTP), cytosine triphosphate (CTP), uracil triphosphate (UTP), and adenylyl sulfate (APS). Analogies have often been made between the energy currencies used by life and currencies used by the financial system (Skulachev 1992, Morán et al. 1999). A living cell always has some currency in cash (ATP) and some in cheques (Δ_{H^+} or Δ_{Na^+}) (Skulachev 1992). It does not matter how income is received, in cash or cheques, as long as they are interconvertible (Skulachev 1992). Energy reserve materials such as sugars, lipids or polyphosphate granules can also be considered as energy currencies but with a considerably longer life. Eventually all currencies are used to feed the internal network with energy (see Fig. 2.7).

There are many advantages to using energy transduction. Energy currencies provide some degree of functional independence from the outside world, thus allowing the existence of an internal steady state resilient to environmen-

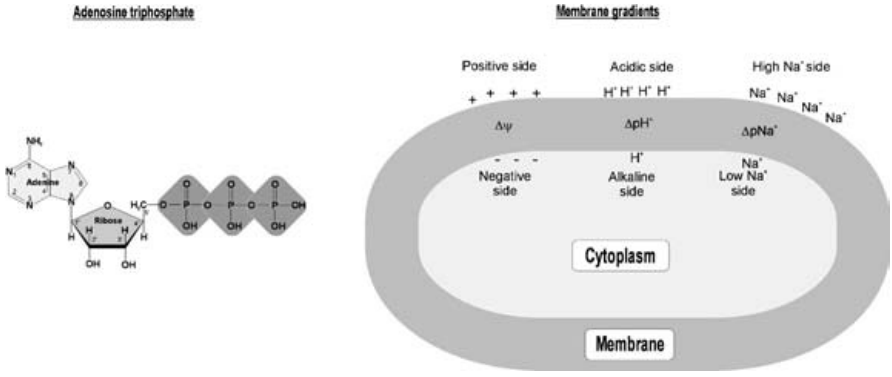


Fig. 2.6. The most common energy currencies used by modern life are ATP and membrane gradients

tal changes. Energy currencies such as ATP can only be used to store limited amounts of energy. Other components such as fats and sugars are specialized in storing larger amounts of energy. These energy-storing 'specialists' have high energy content, longer half-life than ATP, and lesser reactivity. Energy currencies such as ATP (or electricity) are not common in the abiotic world. This makes them less popular to external users (abiotic processes) and they are accordingly hard to 'steal'. Energy currencies are easier to handle than external resources, i.e., they are familiar to the way the internal mechanisms function. Energy currencies are easier to control because external processes can intervene less in their regulation. Energy currencies are a great way to manipulate and synchronize the overall intensity of internal activity. And finally, energy currencies are easy to direct along a desired route, because special pathways can be constructed for their delivery.

It seems apparent that life requires at least one energy currency to gain its functional independence from external energy fluctuations. An interesting question about life is: how many types of energy currency are required to maintain the living state? In simple models of life, a single energy currency (such as pyrophosphate) is believed to suffice. Yet according to Skulachev's laws of bioenergetics (see Appendix C), two energy currencies are required to maintain the viability of a biological system: one related to membrane processes and one to internal reactions (Skulachev 1992). One example of such a pair is present in photosynthetic organisms, where solar light is used

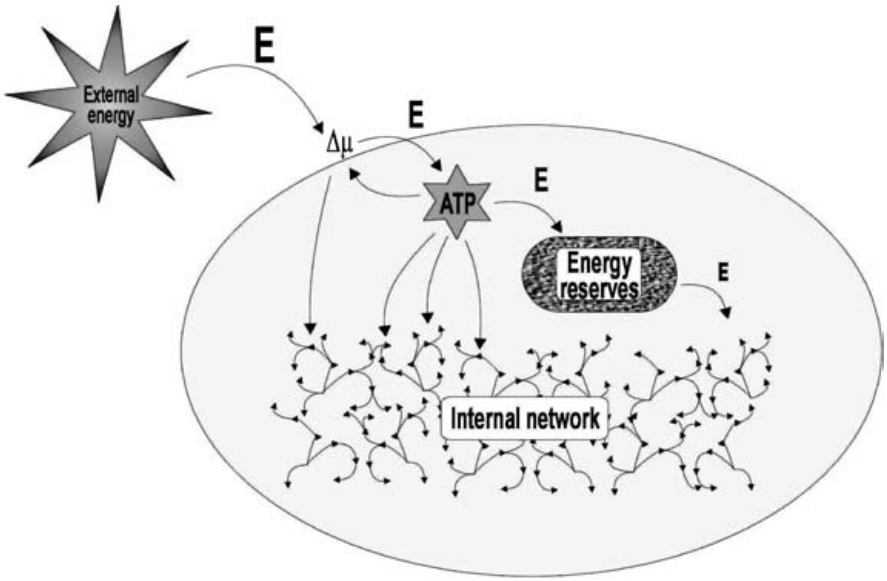


Fig. 2.7. Living forms seldom use external energy directly, but rather transform it into intermediary energy currencies in a process called energy transduction

to produce an electro-osmotic force, which is in turn used to produce ATP (Mitchell 1966). At least one of the energy currencies must activate internal reactions fast enough to ensure that catalysis-derived kinetic advantages would not be lost. However, energy must also be stored in order to allow the system to withstand energy shortages. A connection exists between how steady the external energy resource is and how many energy currencies there are (see Fig. 2.8). ATP degrades in seconds, membrane gradients can hold for minutes, pyrophosphate is stable for many hours, and lipids may resist oxidation for months. If disruptions in the energy supply are longer than the backup energy allows, new longer-lasting energy storage units must be added for the system to remain stable. The value of an energy currency (see Table 2.1) as a means to help the quasi-state of a living system elude energy shortages can be calculated from its concentration, its relative energy load and its half-life.

No satisfactory theory exists so far concerning the early history of energy transduction. Some of the mechanisms used by modern life to transduce energy are membrane-related, while some occur in solution. The most common membrane-related energy transduction mechanism used by modern life is chemiosmotic phosphorylation (see Fig. 2.9). The most important solution-related energy transduction mechanisms are collectively referred to as substrate-level phosphorylation. The multi-step process called glycolysis is the most common type of substrate-level phosphorylation (see Fig. 2.10). Other types of substrate-level phosphorylation occurring in fewer steps are coupled with oxidation reactions (e.g., sulfite oxidation through APS), fermentation reactions (e.g., from pyruvic acid to acetic acid), or disproportionation reactions (e.g., from acetate into methane and carbon dioxide). Although some mechanisms of substrate-level phosphorylation are less complex than the mechanism of oxidative phosphorylation, no modern day energy transduction mechanisms are simple enough to have been primordial. More-

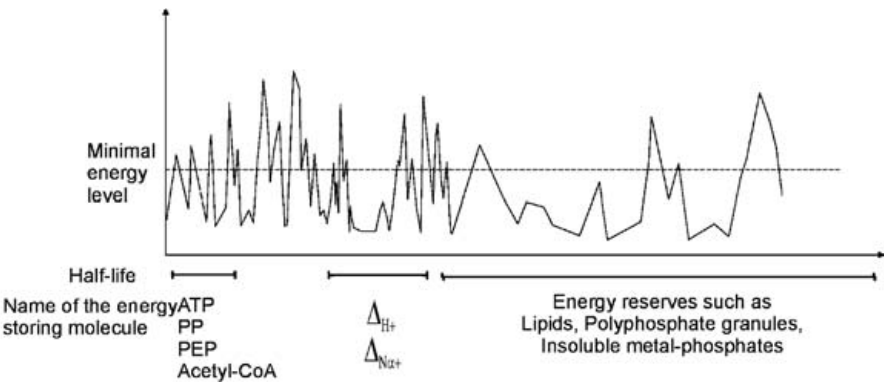


Fig. 2.8. The number of energy currencies used by living organisms and their half-life is dictated by the frequency and duration of disruptions in the energy supply

Table 2.1. The energy content of some of the most important energy currencies used by modern life forms

Name of energy currency	Energy load ($DG^{\circ\prime}$) at pH 7, 20°C, 1 M and 1 atm [$kJ\,mol^{-1}$]
Acetyl adenylate	55.59
Acetyl imidazol	55.59
Phosphoenolpyruvate	54.34
Acetoacetyl ScoA	43.89
Acetyl phosphate	43.89
S-adenozyl methionine	41.80
Phosphocreatine	37.62
AA esters	35.11
Acetyl thioesters	32.19
Uridine diphosphate glucose	31.77
$ATP \Longrightarrow AMP + PP_i$	31.77
$ATP \Longrightarrow ADP + P_i$	30.93
Phosphodiesters	25.08
Aldozo-1-phosphates	20.9
Glutamine	14.21
Glycosoids	12.54
Phosphomonoesters	12.54

over, the energy currency they form (ATP) is not a simple or even a stable molecule. ATP was probably not the energy currency of the earliest forms of life, it was invented later.

According to current theories, the most relevant energy resources for early life were solar light, temperature fluctuations, redox chemistry and hydration/dehydration processes. The earliest energy transduction mechanisms involved condensations, group transfer and maybe chemiosmotic processes, while the earliest energy currencies were membrane gradients, pyrophosphate or thioesters. At the present time there is an infatuation in the literature with imidazolide derivatives and adenylate derivatives as possible early energy currencies. Yet these components seem much too complex and unstable to be primordial. Certainly, this does not exclude the possibility of them being used later during biochemical evolution. The use of two types of energy currencies by the living world, one water-related and one membrane-related, is probably due to the fact that cells themselves are made of two functional phases (hydrophilic cytoplasm and hydrophobic membranes).

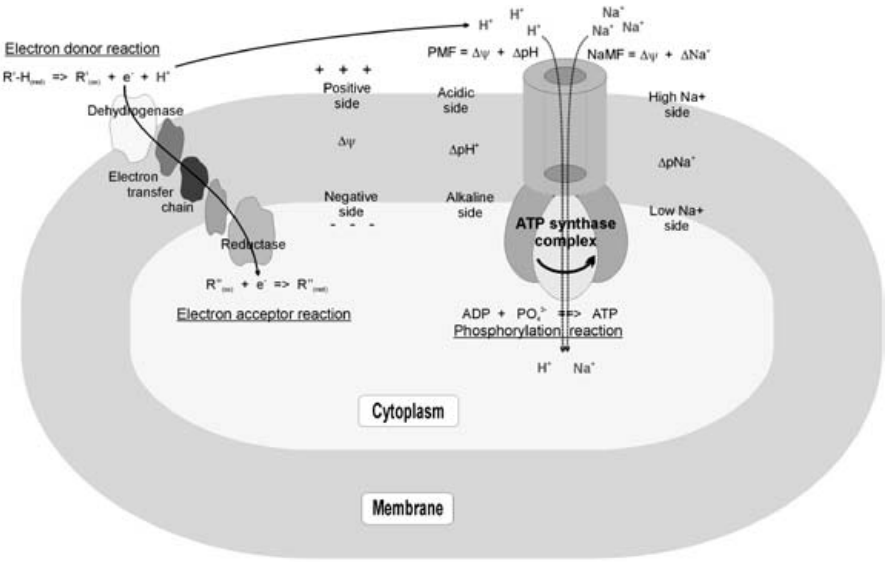


Fig. 2.9. Chemiosmotic phosphorylation is a complex mechanism used by modern life forms to obtain energy (i.e., ATP). $\Delta\Psi$ is the electric gradient, ΔpH^+ is the proton gradient, ΔpNa^- is the sodium gradient, PMF is the proton motive force, and NaMF is the sodium motive force

Three major energy-balance-related features of life have been presented here: catalysis, reflexive activity and energy transduction. It seems obvious that these features were much simpler in the beginning, and that they have evolved in time into the complex and diverse forms we observe today in modern life. Rather than being extensions without beginning of some ubiquitous features from the abiotic nature, and rather than evolving smoothly, these three energy-balance-related features of life were added as phase transitions. They were added to (or amassed by) pre-life forms as discrete events (sudden upgrades), and they were followed by gradual improvement (evolution). It is legitimate to ask which feature (catalysis, reflexive activity or energy transduction) came first? Can they be prioritized in order of their fundamental significance for the maintenance of energy-dissipative systems?

Considering the complexity involved and the fine-tuning required to render energy transduction useful, it is assumed that the earliest forms of catalysis and the earliest forms of reflexive activities preceded the earliest form of energy transduction (see Fig. 2.5). Energy transduction is also considered to be subsequent because catalysis is rather common in the geochemical world and because some life-independent types of reflexive activity involving very simple mechanisms may exist. The transduction of external energy into water-soluble currencies was probably invented after the encasement in

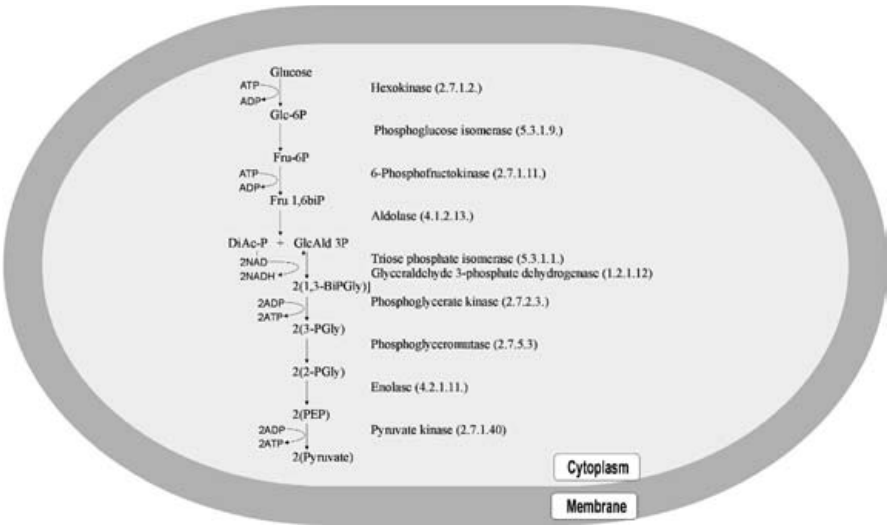


Fig. 2.10. Substrate-level phosphorylation. Glycolysis is one of the major types of substrate-level phosphorylation. This pathway is used to make energy currencies (e.g., ATP) in solution. Glc glucose, Glc 6P glucose 6-phosphate, Fru 6P fructose 6-phosphate, Fru 1,6-biP fructose 1,6-biphosphate, DiAc-P dihydroxyacetone phosphate, GlcAl 3P glyceraldehyde 3-phosphate, 1,3-BlPGly 1,3-biphosphoglycerate, 3-PGly 3-phosphoglycerate, 2-PGly 2-phosphoglycerate, PEP phosphoenolpyruvate, ADP adenosine diphosphate, ATP adenosine triphosphate

a secluded compartment yet must have been subsequent to the earliest mechanisms controlling internal order (see Fig. 2.5).

This ranking of the achievements of life serves two major purposes. Firstly, it will eventually allow the alignment of energy balance history with the history of the early boundaries and with the evolution of order. Secondly, it can be used as a tool to rank priorities in the construction of artificial life. Although their efficiency is very important, the number of types of catalysis, reflexive activity and energy currency is not relevant for the existence of the living state in general. These numbers are defined by local composition and by the diversity and fluctuation of energy sources. In the particular case of the carbon-based terrestrial type of life, a large number of catalytic activities and catalysts were added during its history, but in other types of life these numbers may be very different (smaller or larger). Artificial life is the discipline that will eventually allow such prediction.

Catalysis, reflexive activity and energy transduction have been described as fundamental phase transitions throughout the history of energy balance (see Fig. 2.5). Once these features emerged, some important consequences followed which were also very important for life. One example is the increase in thermodynamic resilience (temporal pervasiveness) of the system. This is

mainly due to the system doing work on itself (reflexive activity), while the system outcompetes the external energy flux (catalysis). Other examples are the increase in the internal energy flux which is mainly due to catalysis and the use of an energy currency produced via energy transduction (see Fig. 2.5).

2.2 Energy Control Mechanisms

The properties of life derived from energy balance features can be better understood by discussing the location and type of the energy control mechanisms, their quantitative consequences, and the way the resulting energy is distributed (see Fig. 2.5). The regulator of the energy flux in dissipative systems is either external or internal. External regulation is usually based on the particulars of the energy resource (amplitude fluctuation, frequency fluctuation, and periodicity). Regulation of the energy flux from within the system may occur through either built-in or specialized mechanisms.

In most abiotic energy-dissipative systems, energy flux regulation is a built-in property of the system, either an outcome of the way the system functions or a consequence of its architecture. When a fuse burns, the amplitude of the energy dissipation and the change in kinetics are independent of how long the fuse is (i.e., how large the energy reserves are). Such processes often become exponential (e.g., forest fires, burning piles of paper, positive feedback mechanisms), 'behaving' as though indifferent to the duration of their existence. If one takes the same amount of energy (e.g., the same amount of paper) and shapes it in such a way that the energy dissipation process cannot increase exponentially (e.g., a very long thin strip of paper), the half-life of the burning process increases. The dissipation kinetics in this case is limited by the available resource and by the time required to reinitialize the process. The same type of built-in regulation occurs in a fuse or a candle flame. These systems are constructed in such a way that, as long as conditions do not change significantly and as long as energy remains available, once the process (i.e., the burning) is initiated, these systems are prevented from shutting down or from increasing their kinetics too fast or too much. Using this strategy, the pace of the energy flow may be slowed just enough to allow the energy resource to recover, so that the energy dissipating unit would be 'insensitive' to resource fluctuations. Built-in energy regulators are common in the abiotic world, but specialized mechanisms are seldom used outside life. Many artificial systems have been imagined and constructed using specialized internal mechanisms to regulate their energy flux.

Only fragmentary information exists in the literature on the early evolution of energy control. Living things use both built-in mechanisms and specialized mechanisms to regulate their energy flux (see Fig. 2.5), and they seldom couple their internal activities with the external energy flow. The regulation of energy flux in life forms is influenced by external mechanisms

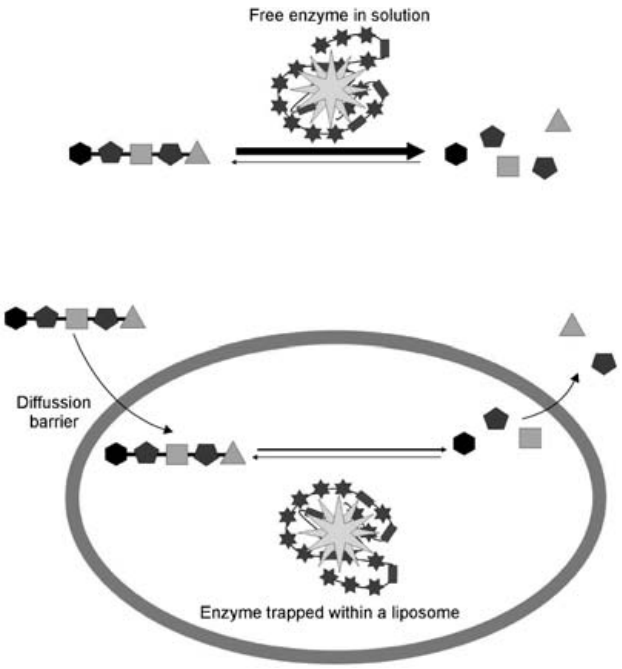


Fig. 2.11. In systems made of catalysts entrapped within a boundary, the catalytic activity can be regulated by the diffusion barrier

but never fully controlled by them. One can state with confidence that systems lacking energy flux regulation are lifeless (see Fig. 2.5). Although it may appear straightforward (thus implying simplicity and primitiveness), the dependence of the energy flux on an external controller goes against the functional safety of a living system.

One can hypothesize that the earliest energy-regulation-related breakthroughs toward life were represented by built-in mechanisms (see Fig. 2.5). The stability of such systems was maintained by the equilibrium between environmental availability and the limitations which the make-up of the system put on their energy dissipation kinetics. For structures made of organic molecules, existence within a narrow temperature range is an important requirement for optimal functioning. Consequently, a protocell made of organic molecules cannot 'abuse itself' by burning more energy than is physically safe (i.e., producing more heat than can be safely dissipated). Most experimental models of early life forms using built-in regulation comprise some form of enzyme-containing vesicle (see Fig. 2.11). When an enzyme is mixed in a watery phase with the substrate, the activity is largely controlled by the enzyme and substrate concentrations. Yet if the enzyme is confined within a liposome, the diffusion rate through the lipid barrier controls most of the activity.

To many it may appear that life in its most fundamental form does not require a specialized mechanism to regulate its energy flux, as long as it contains a built-in regulator. This is an important element of the logic behind artificial life. Yet all life forms we know of display specialized mechanisms as well. Is the use of a specialized mechanism a primordial requirement, or is it just a late upgrade? A built-in control is only good as long as the environmental conditions do not fluctuate beyond a reasonable range and as long as there is an unrestricted ecological niche. Indeed, for a living entity present in a mild and endless world, there is no fundamental need for any elaborate internal regulation of the energy flux. The external control and some built-in limitations should be enough. But the environmental reality is not a smooth ride. As soon as space becomes filled to capacity with living entities and energy becomes limited or discontinuous, energy, space and material limitations are created and the environment ceases to be an Eden. An energy limitation of any type creates the need to 'fill some form of internal batteries' as fast as possible (i.e., create energy reserves) whenever external energy is available, and never to exceed an upper threshold for the energy intake kinetics. This feature of life is defined as energetic opportunism (see Fig. 2.5). Energetic opportunism requires potential for high energy flux (catalysis), energy flux regulation, and the ability to make energy reserves. Because of the fine tuning required for the production and use of energy, life forms added one or more specialized mechanisms to the built-in controllers. It appears that in the absence of energy restrictions (e.g. competition for energy resources), the functional logic of life does not require specialized control of the energy flux.

2.3 Quantitative Consequences of Energy Control Mechanisms

Living systems are self-reliant. An increase in the efficiency of reflexive activity beyond ΔS is required for the full restoration of the quasi-state (Θ) of the system. In systems that do not display reflexive activity, the energy flux does not support their stability and hence the entropy of the system increases continually unless negentropy is supplied from the outside. Only after a system has become associated with reflexive activity, is it possible for its energy dissipation activity to have consequences on its temporal resilience. The quantitative consequences of reflexive activity are thus tightly linked with the very essence of life. In pre-life forms, reflexive activity may have resulted in the recovery of only a subliminal part of the energy lost by the system through changes in its quasi-state (Ref $< S_\Theta$) (see Fig. 2.5). The term S_Θ represents changes in entropy associated with departure from the quasi-state and can be estimated from how much energy would be needed to bring the system back to its initial state. Although early dissipative systems

with Ref $< S_\Theta$ could not 'survive' alone, they still displayed a longer existence (increased thermodynamic resilience) than competitors lacking reflexive activity, and they opened the way to further improvements (see Fig. 2.5).

On the other hand, although this technological novelty gave these systems a selective edge, from a life-history perspective such systems still remained dead ends. They owed their genesis to a continual abiotic and external source of materials. For such systems, reproduction could not have represented an advantage because the material and energetic investment in the offspring would have severely impaired the survivability of the 'parental entity', while all the energy the 'parent' could afford to concede to the offspring would be at best subliminal. Therefore, it is upheld here that the achievement of Ref $\geq S_\Theta$ preceded reproduction in the early history of life (see Fig. 2.5).

The only process analogous to reproduction that could have existed before the Ref $\geq S_\Theta$ episode was replication in which matter and energy were provided from the exterior while the system predominantly contributed with information. Environmentally supported replication may be such an example. Several models have been proposed using hydration/dehydration cycles and/or heating/cooling cycles to obtain the replication of early nucleic acids. Genetic memory, which is the ability to remember alterations as soon as they occur, was an essential requirement of life, probably implemented very soon after the initiation of reflexive activity, or even simultaneously with it (see Fig. 2.5). The Ref $\geq S_\Theta$ state is a crucial criterion in assessing the performances of early life experiments and artificial life simulations. A state of thermodynamic equilibrium could only have been achieved if enough energy was available and if the energy flux density of the transduction process was larger than the value of S_Θ for the system. This represents one of the minimal conditions for the existence of life.

2.4 Distribution of Energy Input

Importing more energy than is lost through the degradation of the quasi-state (S_Θ) does not necessarily make the system more stable and nor is it enough for growth. If the achievement of true self-repair is sought (Bergareche and Ruiz-Mirazo 1999, Ortega and Tyrrell 1999, Ruiz-Mirazo et al. 1999) the energy recovered from the exterior must also be managed. The distribution of the input must match the characteristics of the output. In the absence of distribution-related regulation, increases in catalytic efficiency would merely result in a nonspecific accumulation of energy. Another undesirable consequence would be the accumulation of waste energy faster than it can actually dissipate, leading to a self-destructive 'short-circuit of the metabolism'.

Most abiotic systems are simple and thus only require straightforward management. In early stages of prebiotic evolution, the use of simple explicit information may have been enough to maintain the system (see Fig. 2.5). This explicit type of information is either embedded in the nature of the system by

virtue of its structure and its way of functioning, or it is a part of the particular environment in which the system exists. Explicit information is obvious as given and does not require secondary processing such as translation to implement. The most common example of regulation through explicit information is the use of negative feedbacks. A wide variety of energy-dissipative phenomena such as waterspouts, tornadoes, hurricanes and crystal growth owe the maintenance of their structural, functional and developmental patterns to explicit mechanisms. All these phenomena are energy-dissipative "things" displaying reflexive activity and an explicit built-in control. Yet these systems display no secondary processing of the input energy and their physical 'longevity' is merely the result of their constructive processes (their make-up).

Because the diversity of explicit information can only go so far, only a limited level of complexity may be achieved using it. However, for simple systems, explicit information remains a robust and reliable way to achieve operational steadiness. In complicated systems though, explicit information is not enough to control the system, because the number of virtual directions toward which the system can deviate (i.e., the number of ways to fix it) increases exponentially with complexity. In technical devices, straightforward control systems become incapable of bringing the system back to equilibrium once an 'unfamiliar' state is reached. In very complex devices such as airplanes, more than one computer is required for their functioning, yet in extreme situations, it may not be enough. One case was even cited when the pilots of an airplane struggled against airplane's computer to avoid disaster. In order for the system to achieve self-repair, i.e., maintain its quasi-state, the energy recovered must be prioritized (directed specifically toward those subsystems displaying the highest degradation), irrespective of any virtual direction toward which the system might drift. Therefore, as prebiotic systems become more complex, an upgrade is required, based on a more comprehensive form of information (see Chap. 6). The use of cryptic information allows a system to store a large amount of information and thus to control its recovery from many virtual directions and even to dictate the path toward S_{Θ}.

Besides self-repair, the addition of energy regulation (management) to the energy-related phase transitions of life also assisted other achievements of life such as Ref $> \Delta S_{\Theta}$, competitive energy subtraction, growth and reproduction (see Fig. 2.5), and later contributed to the stability of supra-individual systems. The achievement of Ref $> \Delta S_{\Theta}$ is not a given feature of life but the result of a gradual improvement in the efficiency of reflexive activity and catalysis (see Fig. 2.5). Because all systems tend to move continually downhill from the point of view of ΔS, the quantitative achievement Ref $> \Delta S_{\Theta}$ had to be supported by a rich, easily manipulated source of energy, a large sink, and a continual pressure (drive) toward functional upgrade. The fourth law of thermodynamics (see Appendix C) was proposed to support the evolution of a system toward increased order. The implication is that a system can be 'pushed to become alive' only if it is built in such a way that the efficiency of

the energy flux is somehow coupled with its self-repair abilities. The reflexive activity has to address catalysis directly so that each upgrade significantly improves energy dissipation.

Competitive energy subtraction is the ability to extract energy from the environment by outrunning external competitors. The achievement of competitive energy subtraction requires the presence of catalysis, a reflexive activity larger than the energy associated with the internal degradation Ref $> \Delta S_{\Theta}$, and the regulation of the internal energy (see Fig. 2.5). The need for competitive energy subtraction by life means that the evolution of reflexive activity had to be linked with changes in catalytic performance. Because catalysis pushes competitive energy subtraction up while reflexive activity lowers it, the reflexive activity cannot be increased without appropriate changes in the catalytic efficiency. The achievement of competitive energy subtraction was not a sharp and definitive step. It was obtained through a continual improvement during the entire evolution of life as living systems competed first with lifeless phenomena and later among themselves. A measurable consequence of the existence of competitive energy subtraction is the increase in the rate of overall entropization (the system plus its surroundings), whenever life forms are present.

The existence of reflexive activity without a regulation mechanism is a double-edged sword. Reflexive activity is required for further developments, but without exercised control the system may lose its efficiency because reflexive activity acts contrarily to the competitiveness of energy subtraction and against energy dissipation. If the energy recovered is not directed properly while the intensity of the energy flow is controlled, an accumulation of energy results, without the quasi-state being preserved. This avalanche-like effect makes the system invest more energy in its action with each step, disregarding management of the energy reserves. If a system is to become truly energetically opportunistic, it is required to display all these features: reflexive activity, energy transduction, energy reserves and regulation of the energy distribution (see Fig. 2.5). Energetic opportunism is one of the most common strategies used by life to withstand discontinuous shortfalls in the energy supply.

Self-repair (restoration of the quasi-state) and growth, are consequences derived from energy regulation and accumulation of energy, but also from the existence of metabolic homeostasy. Self-reproduction is an upgrade derived from self-repair and growth, one of the most conspicuous and most often cited properties of life. As no growth can be imagined without energy being regulated, self-reproduction is itself an energy-dependent property of life. No single parameter presented so far may be solely responsible for the origin of life. Moreover, because life does not hold exclusive rights over some energy-related features (see the properties of fire, engines, fluid vortices, self-catalytic processes such as crystal growth, rolling stones, avalanches, rolling snow balls), these features are only minimal requirements for life, rather than

Table 2.2. The main energy-related features in the early history of life

Minimal requirements	Fundamental features	Major derived properties
Power density > 0	Catalysis	Competitive energy subtraction Ref $\geq \Delta S_{\Theta}$
Power density $> S_{\Theta}$	Reflexive activity	Energetic opportunism Energy currencies
Natural diversity	Energy transduction	Energy reserves Energy regulation Self-repair Growth Self-reproduction

exclusive life-discriminating properties. Living entities have often been compared with such energy-dissipative entities and also with high order (low entropy) equilibrium structures (such as crystals) (Prigogine 1980, Morán 1999). The main difference between physical dissipative structures and living structures is that the latter, in addition to their external constraints, display self-maintenance, or what have also been called functional constraints (Csanyi 1989).

Some of the minimal energy-related conditions required for life to emerge are:

- A large energy source, a large energy sink and a slow downhill abiotic process.
- The power density, which represents the available energy per unit volume, has to be significantly larger than zero (Sertorio and Tinetti 2001) to ensure that the reflexive activity does not become a drawback.
- The power density of the transducible energy resource has to be larger than the energy required to maintain the state of the system.
- A natural diversity (variability, plasticity) satisfactory for the emergence of the major energy-related phase transitions, catalysis, reflexive activity and energy transduction.
- A direct coupling between self-maintenance and the kinetics of energy dissipation.

3 The Origin of Cell Boundaries and Metabolism

> The living systems have special properties which arise primarily not from the substances of the system, but from their special organizational manner.
>
> Gánti 1975

The wide gap between the properties of non-living phenomena and the simplest living forms cannot be explained without some intermediate stages (Ruiz-Mirazo et al. 1999). Although some achievements of life suggest that a continuum might have existed between non-life and life (Browning 1869, Hazen 2001), many features of life appear to have emerged in a stepwise way. The addition of a boundary capable of separating the interior from the exterior is one of those achievements of life showing a pronounced stepwise (phase transition) character. Many authors believe that the formation of phase-separated systems (PSSs) was necessarily one of the earliest if not the absolute precondition for the origin of the living state (Oparin 1938, 1968, Oró and Lazcano 1990, Lyubarev and Kurganov 1995, Turian 1999). This seclusion from the external environment was given a variety of names such as compartmentalization, cellularization, territorial separation, segregation or encapsulation (Oparin 1924, 1968, Haldane 1929, Fox 1964, Edwards and Peng 1998, Edwards et al. 1998, Deamer 1998, Arrhenius 2002, Guimarães 2002). A variety of names was also used to describe the earliest PSSs, such as coacervates (Oparin 1921, 1968), bioids (Decker 1973), proteinoid microspheres (Fox and Dose 1977), aggregates (Kaplan 1978), marigranules, marisomes (Yanagawa and Egami 1980), liposomes (Deamer 1986, Schmidli et al. 1991), 'jee-wanu' meaning 'life particles' in Sanskrit (Badahur and Ranganayaki 1970), probotryoids (Russell et al. 1994), microvesicles, microspheres (Turian 1999) or droplets (Dyson 1997).

Separation from the exterior and spatial organization of living systems is believed to be primordial for some very important reasons. As a consequence of the second law of thermodynamics, an increase in order within

R. Popa, *Between Necessity and Probability: Searching for the Definition and Origin of Life*, Adv. Astrobiol. Biogeophys., pp. 39–62 (2004)
http://www.springerlink.com/

a system (i.e., a decrease in its entropy) has to be associated with an increase in the overall entropy (i.e., the entropy of the system plus the entropy of its surroundings). Without a directed channeling of order across space, random exchanges of matter and energy would 'smooth out' any heterogeneous distribution of entropy. Therefore, all systems requiring a defined level of negentropy to function (such as living forms) must be able to 'protect' their internal order and complexity from being dissipated. The simplest way to achieve such protection is by spatial confinement, limiting the exchange of entropy (Markó 2002).

Separation from the environment is also needed for the emergence of a metabolism and the installment of an evolutionary process (Morán et al. 1999, Altstein 2002, Pályi et al. 2002). In open fluids, substrates and products diffuse freely and this results in a gradual loss of individuality (Guimarães 2002). No living form has its internal chemistry fully merged within the chemistry of its environment. Although an active exchange of matter and energy between life and its environment has to be preserved, the interior also has to remain different. If the separation is not complete, the spatial heterogeneity associated with a boundary also results in selective diffusion and consequently in molecular selection (Pitsch et al. 1995, Zubay 1998). A physical separation leading to an overall spatial heterogeneity of the cellular metabolism is also axiomatic in autopoiesis (Varela et al. 1974). Despite these many advantages, one should not exaggerate the importance of a physical boundary in describing life, because not all space-separated entities are alive, while parts of prebiotic evolution may have proceeded without a boundary in open fluids or even on surfaces (Wächtershäuser 1988, Clegg and Wheatley 1991).

Pioneers of this discipline such as Oparin attributed an exaggerated role to boundaries in the origin of life. It was envisioned at one time that, once a boundary was added to a chemical system, most other life-related events would necessarily follow. Yet experiments and computer modeling showed that the addition of a boundary is insufficient to trigger life. Certain advanced features of life such as replication or encryption may even have been initiated outside the early protocells (Kuhn and Waser 1981). A popular mentality in current textbooks is to envisage early life forms as membrane systems protecting some energy-dissipative mechanism. Although both these features (boundary and energy dissipation) are stringent requirements for life, they do not necessarily lead to functional autonomy. In life forms, energy dissipation and boundaries are not added to each other but connected to each other. Autonomy implies an active participation of the system in the construction and modification of its state, which must include its own boundary. Only those systems capable of adjusting themselves (including their boundaries) to external perturbations can be called autonomous (Ruiz-Mirazo et al. 1999). It must be therefore emphasized that the ability of a system to do work on itself (reflexive activity) was at least as primordial for life as the existence of a boundary.

Many scientists try to create virtual life (mathematically-based computer simulations) in the hope of understanding the properties of real life. Yet a fundamental difference exists between the real world and a numerical representation of it. Mathematical operators are not real 'things' but merely theoretical concepts, symbolic representations of physical reality. Therefore, mathematical operators are virtually indestructible. In contrast, physical realities such as living entities, enzymes, metabolic chains, molecular mechanisms and ecosystems are continually subjected to the deleterious effect of time and are thus perishable. Unlike computer simulations, living entities in the real world degrade over time and deteriorate via exchanges of matter and energy with the outside world. For various reasons, most current mathematical models of life and most computer simulations of life avoid representing these material and energetic exchanges. For example, they are irrelevant for the stability of virtual life models, they are not trivial to model, some computational limitations still exist, the dynamics of the exchanges is hard to monitor, and thus our knowledge of their consequences remains incomplete. Moreover, many properties of artificial life are not derived from the way they function, but rather they are features deliberately built into the program for the purpose of fitting or verifying empirical observations.

This inability to integrate consequential features remains the biggest weakness in the field of artificial life and may also be something required to obtain artificial intelligence. Many artificial life models have difficulty explaining the origin and hierarchical accretion of derived properties of life, thus leaving a hiatus in our understanding of the early evolution of life on Earth, which in turn leaves this field open to endless speculation. Therefore, most experimentalists legitimately regard computer-based simulations of life as teaching instruments rather than as true research tools. Better communication among computer modelers and experimentalists is required in order to upgrade the scientific significance of artificial life models.

The implementation of true life determinism in artificial life systems is hampered by the fact that we have only fragmentary knowledge of the causal connection between a boundary and the ability of a system to self-maintain. Ignoring the consequences of spatial requirements is one of the major caveats of the theoretical models of life (Ruiz-Mirazo et al. 1999). Certain artificial life models that are either based on autocatalytic reactions leading to autocatalytic networks (Farmer et al. 1986), or based on algorithmic chemistries leading to self-maintaining function algebras (Fontana 1992), consider energy-related issues as being important, but do not approach the consequences of spatial constraints (Gánti 1975, Ruiz-Mirazo et al. 1999). Only a couple of artificial life models consider the requirements and consequences of compartmentation. Examples are Varela's autopoietic model (Varela et al. 1974, McMullin 1997, McMullin and Varela 1997), Rosen's extension of the (M,R)-system (Rosen 1981), compartmentation applied to Eigen's hypercycle model (Eigen and Schuster 1979) and the compartmentation of autocatalytic nets (Nuño et al. 1995).

3.1 The Make-up of Life's Boundaries

The boundaries of life-related entities are made of a variety of materials. The capsules of viruses are mosaics of interlocked proteins. Membranes from the

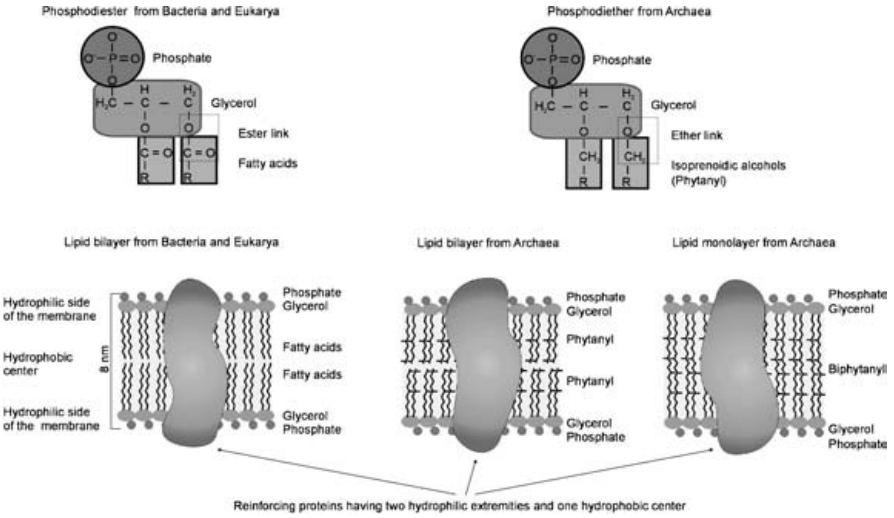


Fig. 3.1. Basic structure of membranes made from phospholipids in the living world

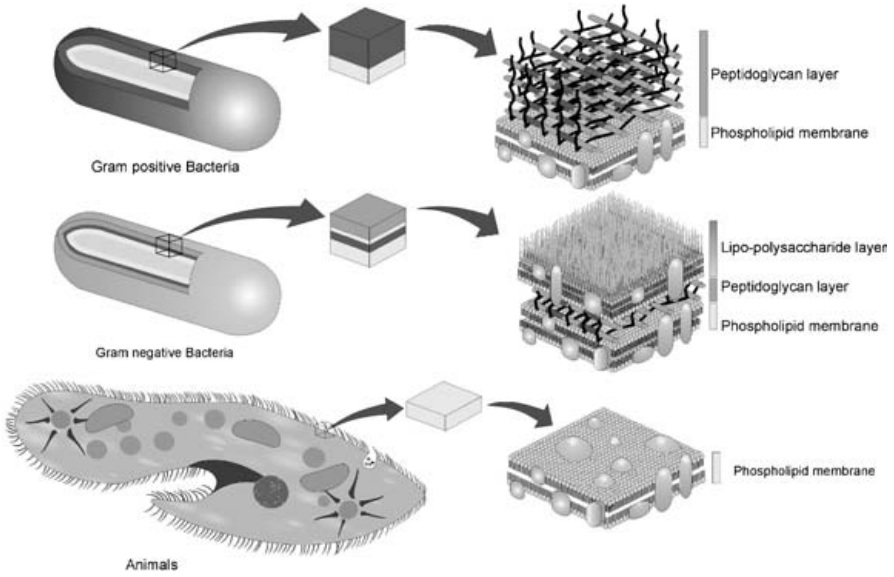


Fig. 3.2. Diversity of cell walls in the living world. Animal cells have only membranes and no cell walls

Archaea group of microorganisms are layers of lipid ethers reinforced with proteins. Lipid esters plus proteins make membranes in bacteria and Eukaryotes (see Fig. 3.1). A cell wall is also added in most Prokaryotes, resulting in very complex envelopes (see Fig. 3.2). A capsule made of insoluble polysaccharides (see Fig. 3.3) and an S-layer made of proteins arranged in a surface mosaic is also frequently present in many Prokaryotes (see Fig. 3.4) (Langworthy et al. 1974, Beveridge 1981, Morii and Koga 1994, Brown et al. 1998).

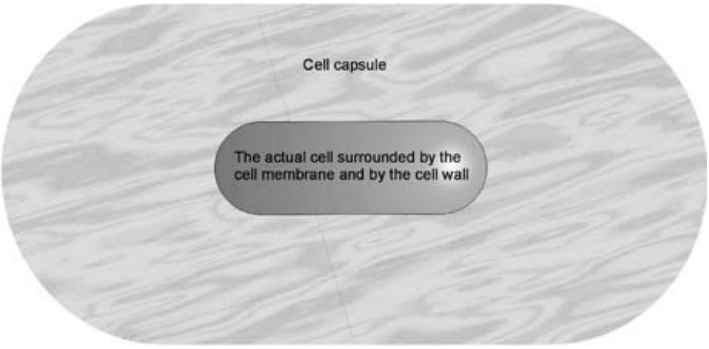


Fig. 3.3. Capsules (slime layers or glycocalix) are made of polysaccharides and lipoproteins and can be very large in some Prokaryotes

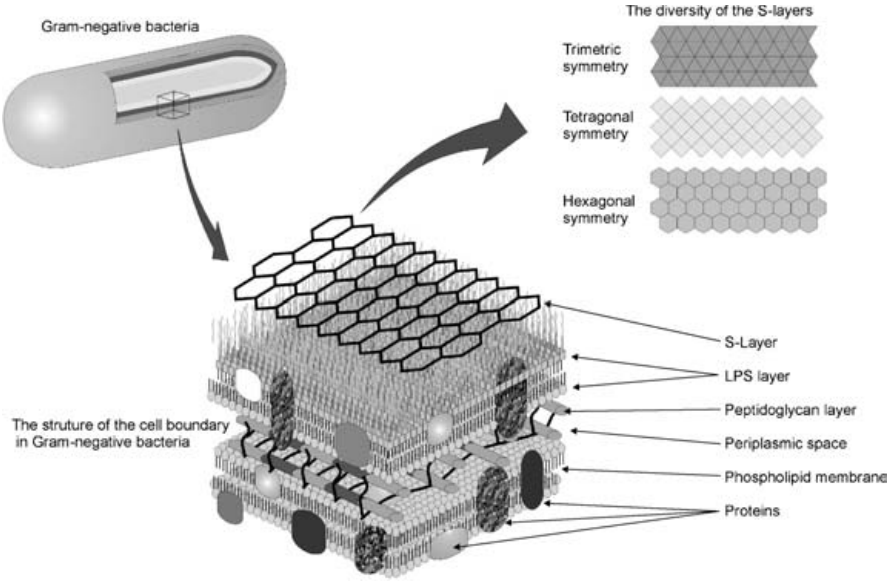


Fig. 3.4. Paracrystalline surface layers (S-layers) are surface mosaics made of proteins or glycoproteins and exist in every group of bacteria and almost all the Archaea. S-layers show various symmetries (trimetric, tetragonal and hexagonal)

The ubiquitous presence of a boundary in all material forms of life irrespective of its physical nature suggests that all boundaries share some universal life-supporting properties. Virtual life forms (cybernetic constructs) do not have a boundary. One may speculate that the physical limits they occupy in the computer's hardware can be used as a representation of a non-specialized boundary. However, these physical limits are irrelevant in the functioning of artificial life, because they have no particular structure, no particular properties and no consequences upon the stability of these life-related simulations.

3.2 The Early Evolution of Cellular Boundaries

Observing the complexity of a bacterial boundary (see Fig. 3.4) reveals that cellular layers in modern organisms are much too elaborate to have emerged in a single step. The way the boundaries of the earliest preliving entities accreted remains unclear, because we do not know which components of modern boundaries were invented first. Were early life forms surrounded by phospholipids, polypeptides or polysaccharides? The phylogeny of modern organisms offers little information about the early boundaries of life, because the boundaries of the organisms closest to the root of the tree of life are themselves very complex. Some modern organisms show simple envelopes, but they are phylogenetic branches that only require a limited structural complexity, or they are the result of a subsequent simplification or of a parasitic lifestyle. A popular approach to this problem is to apply the principle of simplicity and try to determine which of the structures from modern life forms are easier to form, easier to assemble and result in satisfactory outcomes (boundary-wise). Yet if the primitive life forms were surrounded by a now extinct type of material or if the composition of the boundaries changed significantly over the last 3.6 billion years of evolution (which is very likely), an approach based purely on simplicity would never provide a satisfactory answer.

Three types of chemical components have been used in models of primeval boundaries:

- amphipatic molecules (Deamer 1986),
- inorganic chemicals (Russell and Hall 1997),
- polypeptides (Fox and Dose 1977).

Other early forms of spatial confinement could have been represented by pores within rocks with their entrances blocked by natural colloids (Turian 1999), high viscosity fluids, or fluids trapped within capillary spaces. The earliest living entities might also have been microscopic grains surrounded by a thin layer of fluid and a membrane. It is also possible that the earliest boundaries were self-assembled polypeptides or fullerene-like mosaics that later developed into S-layers.

Most scientists believe that the earliest boundary in the history of life was a sandwich-like layer that self-assembled from amphipatic molecules (Calvin

1969, Nicolis and Prigogine 1977, Tanford 1980, Liebl et al. 1984, Wirth et al. 1997, Deamer 1998), or a spheroid induced by osmotic variations (Fox and Dose 1977, Fox and Matsuno 1983). Amphipatic molecules are chemical structures made of one hydrophilic (water attractive) part and one hydrophobic (water repellent or lipophilic) part (see Fig. 3.5). Soaps and detergents are common examples of amphipates. When mixed with water, amphipatic chemicals have a natural tendency to assemble into micelles and vesicles with the hydrophilic part toward the water and the hydrophobic part away from the water (see Fig. 3.6). Whenever exposed to hydrophobic fluids, such as organic solvents and oils, amphipatic molecules orient themselves with their hydrophobic part toward the fluid and their hydrophilic part away from the solvent (see Fig. 3.7).

If the lipids from modern membranes are indeed their oldest parts, and if primeval life forms were water-based, the early membranes may have accreted in the following hierarchical sequence (see Fig. 3.8):

Step 1. A pre-living state existed first with simple inorganic chemicals formed abiotically in an aqueous solution. In the absence of a boundary, all chemical processes occurred freely in the solution and only limited spatial heterogeneity was possible.

Step 2. Simple organic chemical precursors such as aliphatic alcohols, hydrocarbons, carboxylic acids, isoprenoids and sugars formed abiotically. Although certain features common to life forms could have been present

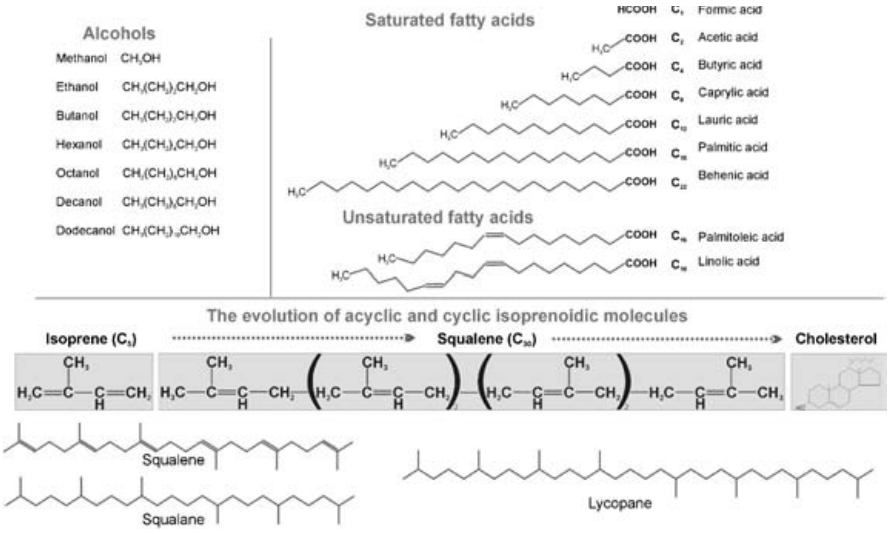


Fig. 3.5. Amphipatic molecules have one hydrophilic end and long hydrophobic tails. Many large hydrophobic molecules of life (such as squalane, cholesterol and lycopane) are derivates of isoprene

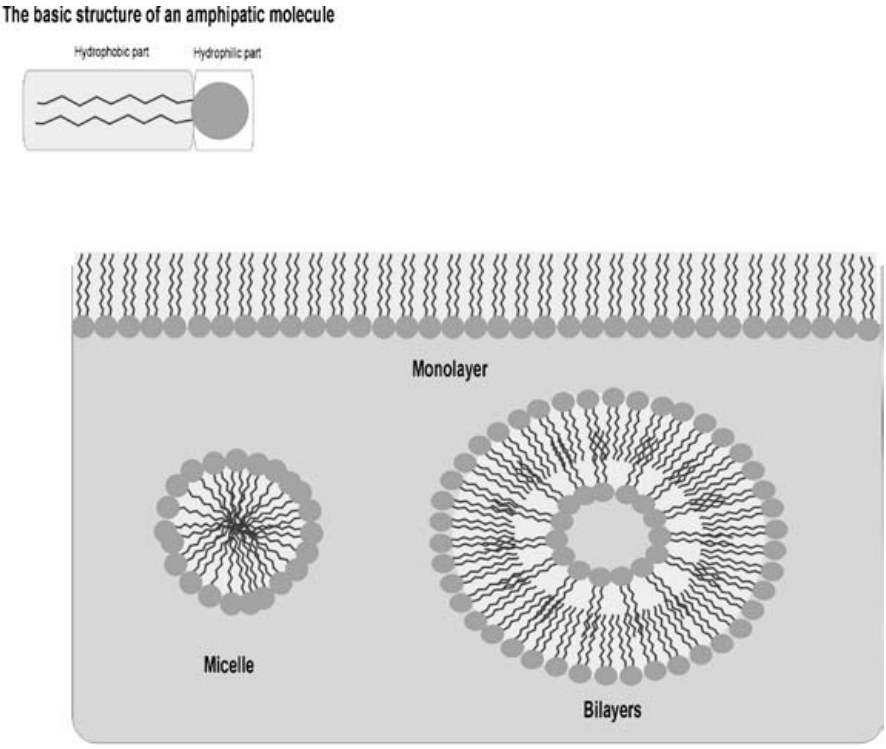


Fig. 3.6. When exposed to water, amphipatic molecules (e.g., soaps, detergents, phospholipids, phosphodiesters, aliphatic alcohols, carboxylic acids) self-assemble into monolayers, micelles bilayers, and vesicles with the hydrophilic (water attractive) part oriented toward the water and the hydrophobic (water repellent) part oriented away from the water. Adapted from (Edwards and Pen 1998)

(e.g., catalysis, energy dissipation, reflexive activity, chiral disruption and conformation-based conveyance of information), no living entity could actually have existed at this stage.

Step 3. The abiotic condensation of precursors produced large molecules and amphipatic molecular structures (see Fig. 3.9). It is generally believed that the reactions responsible for this step were of a condensation type.

Step 4. The concentration of amphipatic molecules in a watery fluid resulted in the formation of micellar structures and vesicles (see Fig. 3.8). The cohesiveness of this type of structure is not trivial to model and depends on the equilibrium between attractive and repulsive forces acting within heterogeneous fluid phases. The size of the early vesicles was rather limited and factors such as temperature had significant influence over their physical stability. At this stage in early evolution, the primordial vesicles were no more than little pockets suspended in water with some random chemicals trapped inside.

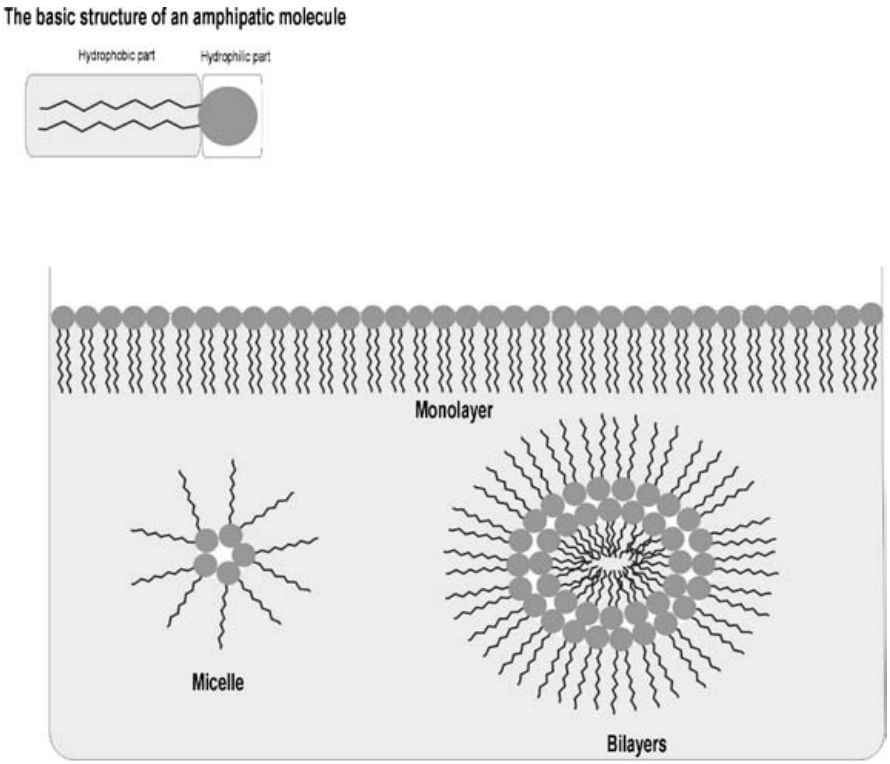


Fig. 3.7. When exposed to organic solvents and oils, amphipatic molecules self-assemble into monolayers, inverted micelles, inverted bilayers, and inverted vesicles with the hydrophobic (water repellent) part oriented toward the solvent and the hydrophilic (water attractive) part oriented away from the solvent

A wide variety of simulations of this stage in chemical evolution have been imagined, most of them based on enzyme-containing liposomes. True cellular life was not possible at this point in early evolution. Although the presence of such vesicles could have resulted in both kinetic alterations (i.e., changes in the speed with which reactions proceeded) and thermodynamic alterations (i.e., changes in the equilibrium point of a reaction), other important features of life were not yet present.

Step 5. During the next stage of prebiotic evolution, a variety of molecular additions resulted in increased stability of the primordial vesicles, which allowed them to withstand a wider range of physico-chemical stresses. Water-insoluble surfactant precursors binding to the surface of micelles or vesicles may result in more complex systems (Luisi 2002). Peptides rich in helix motifs and large hydrophobic components analogous to terpenoids, kerogens or sterols could have contributed to early membranes. Tolerance to environmental fluctuations allowed an increase in size. Sterols – like molecules from

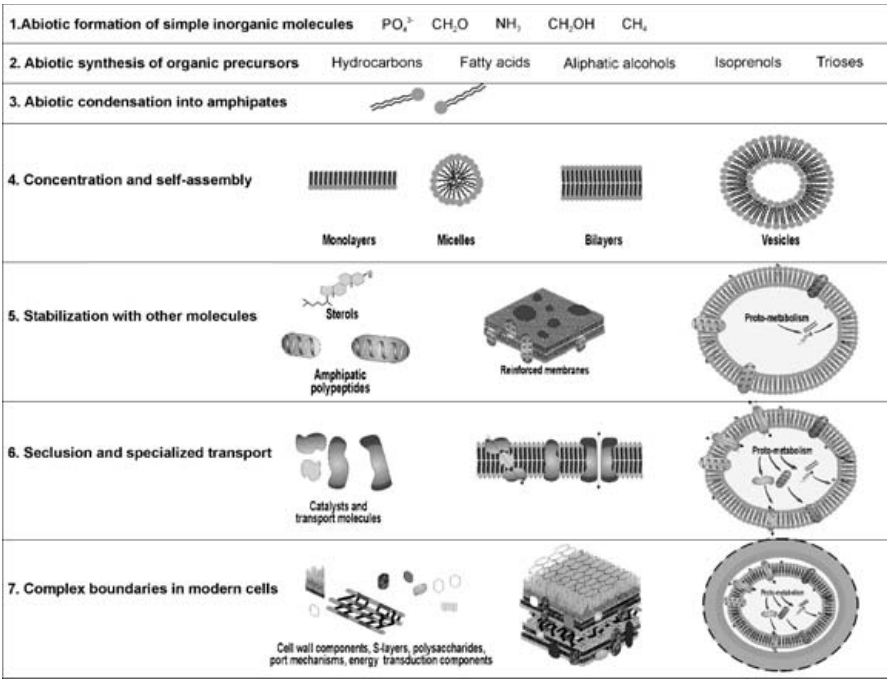


Fig. 3.8. Evolutionary steps in the accretion of early lipid-based membranes

modern organisms when inserted into its membranes allow a reduction in the fluidity of the membranes at high temperatures and prevent their solidification at low temperatures (see Fig. 3.10). As a result of this step, boundaries became for the first time reliable controllers of the internal volume.

Step 6. The insertion of specialized transporters and active catalysts into the boundary and/or inside the vesicles led to the creation and maintenance of internal disequilibria (see Fig. 3.8). As the internal chemistry became distinct from the external chemistry, the development of a metabolism also became possible, leading to the formation and localization of complex molecules. For the first time in prebiotic evolution, components required for the formation and stabilization of the system were formed and entrapped within the boundaries of a volume-stabilized system. The transition between non-life and life was probably closely related to this stage in early evolution.

Step 7. During the next 3.6 billion years of evolution, more components were added to the early membranes, eventually resulting in the development of the modern cellular design (see Fig. 3.8) (Margulis 1971, Liebl et al. 1984, Ourisson and Nakatani 1994, Hartman and Fedorov 2002).

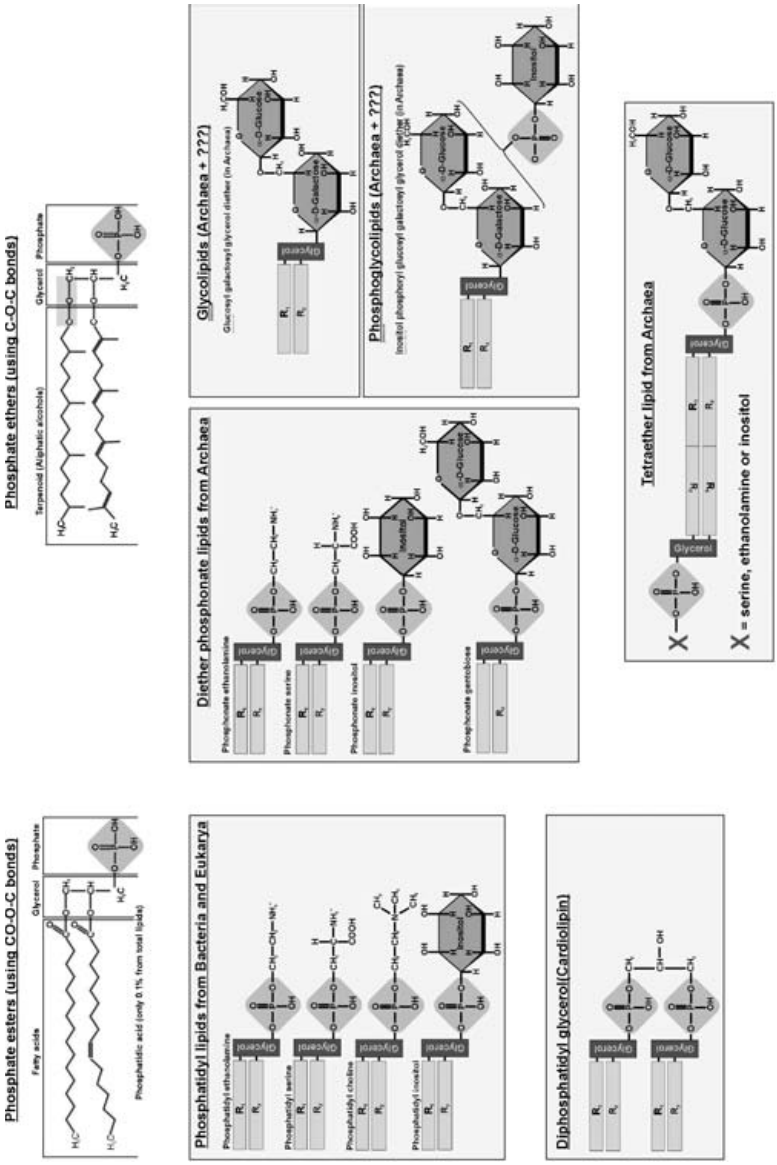


Fig. 3.9. A wide variety of lipids are present in the living world

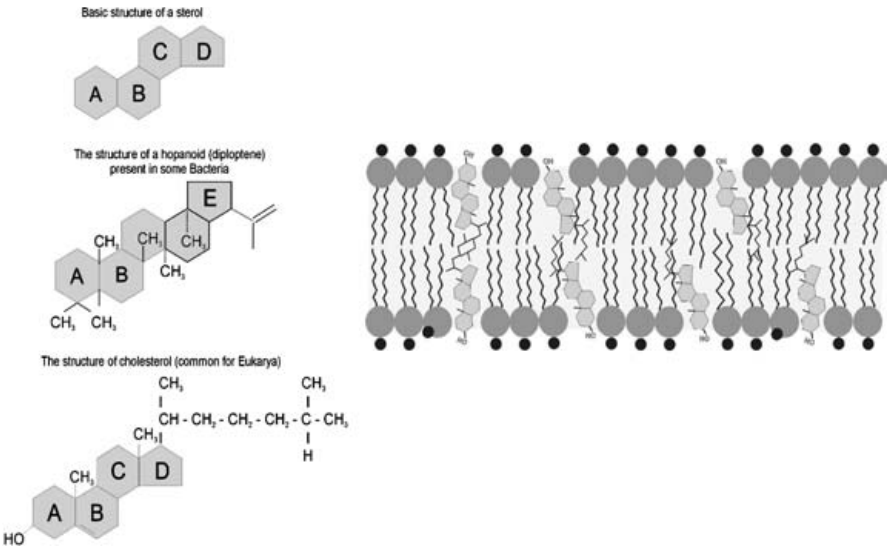


Fig. 3.10. Sterols present in Eukaryotes and hopanoids present in some Prokaryotes help reduce the fluidity of membranes at high temperatures and prevent solidification at low temperatures

One must be aware that in science "not everything that looks like a dog and barks like a dog is actually a dog", and that experimentation is preferable to any Occam's razor's-type of argument. We believe that lipid membranes are primeval mostly because they are so prevalent in modern organisms and because we have no better candidate. Yet, until more evidence will be offered, one theory is almost as good as another.

3.3 Outcomes of an Early Boundary

The formation of a boundary around a population of chemicals capable of dissipating energy by using catalysis, capable of reflexive activity and capable of energy transduction added a plethora of consequences (i.e., derived properties of life), that are otherwise hard to explain without a spatial sequestration. The addition of boundary-like features to any artificial life model is not expected to result in similar consequences because so far the determinism of the real world is different from the determinism of numerical algorithms. In order for any artificial life simulation to result in outcomes resembling the real world, a 'real-world-like determinism' has to be included into the program. But can we understand the determinism associated with real life forms? In order to gain insight into the boundary-related causality of life, one must investigate:

- the types of boundaries in the evolution of early life,
- the boundary-related derived properties of life,
- the possible connections between the evolution of early boundaries and other major topics concerning the accretion of life such as energy, metabolism, order, handedness and information.

3.4 Systems Without Defined Boundaries

Life forms cannot be fully merged with their environment without running the risk of 'becoming environment' and thus sharing its thermodynamic fate. Some degree of physical separation is essential for the existence of life. Systems without physical margins are exposed to unrestricted exchange of energy and matter with the outside world. They thus may display little if any control over their internal steadiness (specificity) and over their functional potential. Consequently, such systems are generally driven downhill toward a state of high thermodynamic equilibrium where all activity ceases and identity vanishes. For a physiologist, such systems have little chance of regaining their initial status (Quasi state). They are all but dead matter structures passively awaiting for their thermodynamic obliteration. It thus appears obvious why most scientists now believe that systems lacking a boundary cannot become alive. Oceans of life with non-discrete living units merging into each other to form a global super-being belong solely to the realm of science fiction (e.g., Stanislav Lem's *Solaris*).

3.5 Systems with Non-Specialized Boundaries

Numerous examples of energy-dissipative systems existing in nature display non-specialized boundaries. The physical separation of such systems may be based on phase transitions (liquid/gas interfaces, solid/liquid interfaces, plasma/gas interfaces), liquid/liquid interfaces or chemical interfaces (sharp salinity gradients), or they are based on geometrical motion patterns (such as in fluid vortices). The physical permanence of interface-based boundaries (e.g., fire, liquid droplets, a water surface or oily drops in water) is directly controlled through interactions between internal and external physicochemical forces. In the case of fluid vortices (waterspouts, dust devils, tornadoes, hurricanes, cyclones), the collective activity patterns provide the architectural means for maintaining the overall structure. The appearance of physical edges is merely a result of a motion pattern. Such systems and their 'virtual boundaries' only exist if an external energy resource is continually wasted. These systems do not store energy in alternative reserves while the energy resource they exploit is used as is without any energy transduction. Exchanges with the exterior are fully controlled by phenomena such as diffusion, convection and turbulence. Yet these systems show more compositional personality

than open systems and are useful analogies for understanding the transition between determinist systems and finalist systems (see Appendix C). Unlike the open systems, the systems with non-specialized boundaries are capable of withstanding some short-term changes (environmental fluctuations) and driving energy along paths that are not conventional to the outside world.

3.6 Self-Assembly of Specialized Boundaries

However appealing as analogies for life, fluid vortices and fire are not satisfactory models or even precursors for life (Owen 2002). The pathway toward a stable shape in early life forms did not occur via motion patterns or phase transitions, but rather through specialized boundaries emerging by self-assembly. Self-assembly is a phase transition phenomenon, a thermodynamically controlled creation of order, and represents one of the fundamental phase transitions toward life. Although self-assembly is an entirely probabilistic circumstance, it is not rare in nature. A wide variety of lifeless things may be able to self-assemble, including mineral crystals (Hurlbut 1971), molecular fences (Baggott 1994), surface mosaics (Bumm et al. 1999), paracrystalline S-layers (Brown et al. 1998), ice in snow flakes (Fassnacht et al. 1999), fractals (Richardson 1995, Kropman et al. 1998), metal–sulfur clusters (Berg and Holm 1982), enantiomeric capsules (Rivera et al. 1998), fullerenes or buckeyballs (Bagott 1994), nanotubes (Bagott 1994, Ohler et al. 2001), membranes and colloids (Deamer 1997, 1998, Ishihara et al. 1999) (see Fig. 3.11). Self-assembly is either based on self-complementarity or on mutual complementar-

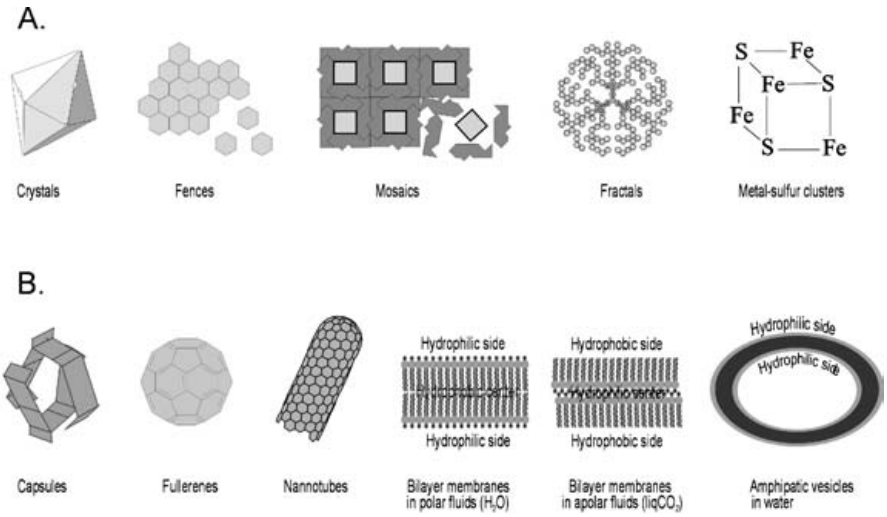


Fig. 3.11. Self-assembled and mutually assembled objects: (**A**) without creating physical seclusion, (**B**) creating physical seclusion

ity (Berg and Holm 1982, Mayer et al. 1997, Rivera et al. 1998, Ruiz-Mirazo et al. 1999). It may appear reasonable to assume that self-complementary assemblies were the simplest to emerge, yet nothing is known about the real nature of the earliest self-assembled structures to make such a speculation.

A general tendency exists in the literature to overrate the significance of self-assembly for early life. The fact that self-assembly has an energetic component to it is often ignored. The chances of generating life have often been misrepresented through the probability of obtaining abiotic micelles. Yet not all types of self-assembled objects are relevant as boundaries for life, while many derived properties of life appear independently of a self-assembled structure. Computer-based life simulators do not require self-assembly. Even if the file(s) containing the information required to build a 'cybernetic living form' are scattered throughout the hardware or in the RAM, their pieces do not hold together by themselves. They are traced, accessed and restored whenever needed by the file management system which is an inseparable part of the operating system. The introduction of a tendency to self-assemble into the properties of artificial life awaits more software development. In the real world, self-assembly plays an important role because little external assistance is required for the boundary to form.

Only those self-assembled structures capable of defining an internal space are relevant for life (see Fig. 3.11). Concerning the questionable possibility that life was ever bidimensional (McMullin and Varela 1997, Wächtershäuser 1988), two-dimensional structures such as molecular fences may also be of some relevance as boundaries. Still for life to form in fluid phases, only self-assemblages resulting in a three-dimensional separation of an inside from the outside appear appropriate. It is not self-assembly but rather the seclusion within a specialized self-assembled boundary that has to be considered as an essential boundary-related phase transition toward life (see Fig. 3.12).

3.7 Boundary-Derived Properties of Life

Although multi-molecular phases are capable of separating an interior from an exterior (Liebl et al. 1984), primordial vesicles must still have exchanged matter and energy very actively with the exterior, so that they must have behaved in a way not unlike open systems (Calvin 1969, Nicolis and Prigogine 1977). According to computational models of autopoiesis, early spatial separation led to a long series of beneficial consequences (Varela et al. 1974, Bresch et al. 1980, McMullin 1997, McMullin and Varella 1997), some of which are considered here (see Fig. 3.12):

- Thermodynamic Protection. During the formation of early membranes, the association of the early colloids with other macromolecules (Liebl et al. 1984) led to an increased ability to withstand hydrolysis (Turian 1999). This boundary-derived thermodynamic protection added to the

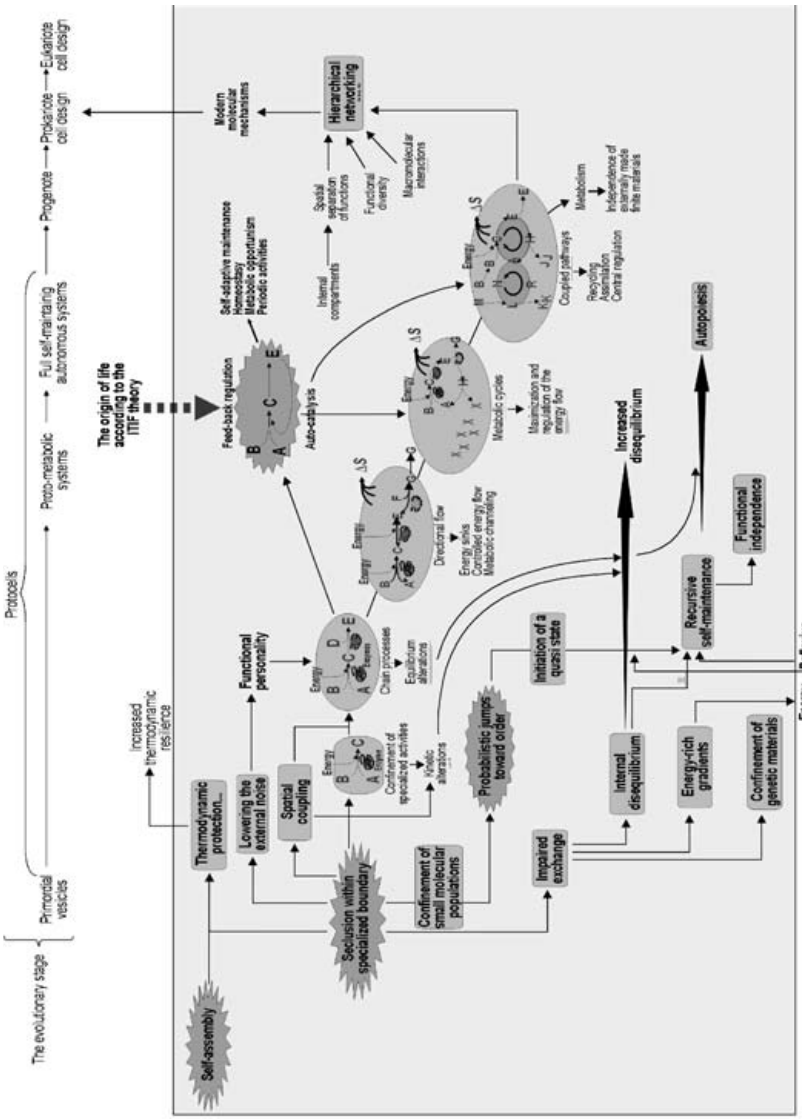


Fig. 3.12. Major boundary/metabolism-related transitions in the early history of life

overall thermodynamic resilience (the half-life) of the overall entity (see Fig. 2.5).

- Lowering the External 'Noise'. The open environment acts as an inhibitor for installation and maintenance of an internal chemical personality because random interference hinders reactions from becoming controllable. In the functioning of a deterministic system, this interference can be regarded as thermodynamic noise or random drift. In chemical forms of life, physical enclosures serve as a means of avoiding chemical hazards that are analogous to metabolic inhibitors, poisons and metabolic uncouplers. This eased pressure on the maintenance of internal functional personality was an essential prerequisite for the protection of the early metabolic network.
- Spatial Coupling and Confinement of Specialized Activities (Bresch et al. 1980, Turian 1999). The encasement of specialized activities (catalysts) inside the primary vesicles (Fishman and Citri 1975, Unden and Kröger 1982, Schmidli et al. 1991, Bachmann et al. 1992, Luisi 1993, Chakrabarti et al. 1994, Deamer 1997) and the subsequent coupling among the different reactions (Bresch et al. 1980) led to chain processes and to significant kinetic and equilibrium alterations (see Fig. 3.12). The chaining together of internal activities allowed a directional flow of energy and matter to be established and a network of reactions could then be formed with a well-defined chemical direction (directional flow). This in turn allowed the formation and use of energy sinks, controlling the energy flow, equilibrium alterations and metabolic channeling.
- Confinement of Small Molecular Populations (Turian 1999) had a beneficial probabilistic result, namely, the emergence of nonrandom catalysts via probabilistic jumps toward order. These probabilistic jumps resulted in the creation of a particular quasi-state for the first time during early evolution (Dyson 1997). The subsequent coupling between a quasi-state and a reflexive activity led to the emergence of recursive self-maintenance.
- Impaired Exchange with the Exterior (Turian 1999) has been mentioned by many authors and it is believed to have contributed to the creation of an internal disequilibrium (Ruiz-Mirazo et al. 1999). The limitation of the osmotic transfer created and maintained sharp (i.e., energy rich) gradients, used by life as energy currencies. The osmotic arrest of large molecules and tolerance of the osmotic swell also allowed the confinement of large anions (e.g., nucleic acids) which could serve as genetic materials (Nishihara et al. 1998).

3.8 Coupling of Spatial Seclusion with a Reflexive Activity

Chemical life forms exist in a delicate balance. On the one hand they need a fluid state to assist their three-dimensional interactions. On the other hand a reactive fluid phase comes with a thermodynamic price, because reactive

fluids are the vectors of the Nemesis of life that is thermal decay. One primary need of any self-sustained system is to manage its perpetuation (internal specificity), and this has to include control over the steadiness of its boundaries.

Experiments have shown that certain types of reflexive activity are correlated with the creation and stabilization of a boundary (Schmidli et al. 1991, Bachman et al. 1992, Luisi 1993). The hydrophobic interior of the bilayer membranes acts as a barrier against the free passage of ions. It has been posited that one of the main achievements of primitive membranes was the incorporation of electron transfer facilitators such as metal–sulfur clusters or metal impurities making the membranes selectively permeable (Koch 1985, Koch and Schmidt 1991). Because such specialized transporters allowed electrons to be more mobile than ions such as H^+, Cl^- and Na^+, membranes containing them can generate and maintain electro-osmotic gradients (Mitchell 1966, Skulachev 1992).

Later in evolution, after the addition of α-helix-containing polypeptides acting as ion selective channels (Paula and Deamer 1990, Oliver and Deamer 1994), specialized electron-transfer proteins, port systems and porin-like molecules (bundles of α-helices), these gradients could have been used to achieve assisted transport. After the addition of an ATP-ase, the first chemiosmosis-based energy transduction was also implemented. It is still unclear what the primeval mechanism for harvesting energy was. Was it based upon light-induced redox chemistry, chemiosmosis or substrate-level transduction? Anyhow the coupling of a primitive reflexive activity with a boundary led to a relative functional independence (Schmidli et al. 1991, Bachman et al. 1992, Luisi 1993). This coupling increased the stability of the boundary and allowed the emergence of internal control, one of the major characteristics of life (Nishihara et al. 1998).

It is generally believed that one of the most important consequences of a boundary is the entrapment of a population of catalysts within a diffusion-limited space. Catalytic polymers such as polypeptides enriched in hydrophobic amino acids could have been internalized by early liposomes (Luisi 2002). A variety of early energy-dissipative primordial vesicles could have been created this way (Luisi 1993, Deamer 1998). Although catalysis in narrow spaces leads to significant kinetic alterations, thermodynamic equilibration is also more rapid around confined catalysts, leading to a rapid cessation of activity in the absence of supplies. Thus competition always exists with external reactions which tend to be slower, but they have easier access to reagents.

The equilibrium between catalysis and the access to materials is one of the major hallmarks of life and a principal way of assessing its presence. For every given condensation reaction between two components, the survival of life forms at an early evolutionary moment may be represented through the equation of a diffusion-control mechanism (Alberty and Silbey 1992). Such a preliving system can only outcompete external processes if the gradient pro-

duced by the internal 'sink' is strong enough to drive reagents inside before they are wasted in outside processes. Most prebiotic reactions are endergonic and require assistance and energy to occur. However, other metabolic reactions are exergonic but require considerable activation energies. Their presence is not just a fortuitous occurrence for life but an achievement 'sought after' by life forms. Such processes proceed very slowly in the absence of a catalyst and external energy, but they offer the advantage of not occurring by themselves and of leading to final products with a high stability. The influence of life on reaction rates has often been discussed. A peculiar feature of life is its biased effect on the direction of a reaction (i.e., both its path and its finality).

Competition with external chemistry was not the only crisis faced by primordial vesicles. Having a boundary is almost as much a benefit as it is a curse. In order to function properly, living forms have to avoid their own waste. As 'metabolic garbage' accumulates, it has to be discarded through a boundary that is purposedly resilient to aqueous diffusion (i.e., opposed to passing solutes). Yet if they do not manage to eliminate these 'metabolic intoxicants', living cells run the risk of poisoning themselves with their own byproducts. It has been speculated that motion may have been invented for this very reason, i.e., to move away from a toxic area (Nealson 2002, personal communication). The accumulation of polyanions within primordial vesicles also resulted in an osmotic crisis (Ruiz-Mirazo et al. 1999). Without the ability to enlarge the boundary, the internal excess of hydrophilic radicals resulted in an osmotic swelling due to water uptake through leaks in the membrane, and this turgor pressure would eventually have broken the vesicles apart. All these crises represented selective tools for promoting further achievements of life, such as active transport, the development of a cell wall, controlled growth, motion, the internalization of the source of specificity, energy transduction and intermediary metabolism.

3.9 The Origin of Metabolism

One of the most important consequences of spatial seclusion is the confinement of chemical reactions that are rather distinct from each other within the same physical space. This imposed association led to reaction chains and, for the first time in prebiotic evolution, to alterations in the thermodynamic equilibrium of some internal reactions (see Fig. 3.13). Directional flow is essential for the development of metabolic cycles and coupled cycles, and for the emergence of early metabolism. It is difficult to assess to what extent these changes were controlled from the inside or from the outside. Some of their features are based on some of the components acting directly as rulers (constraints or attractants) upon other components of the network (Ruiz-Mirazo 1999).

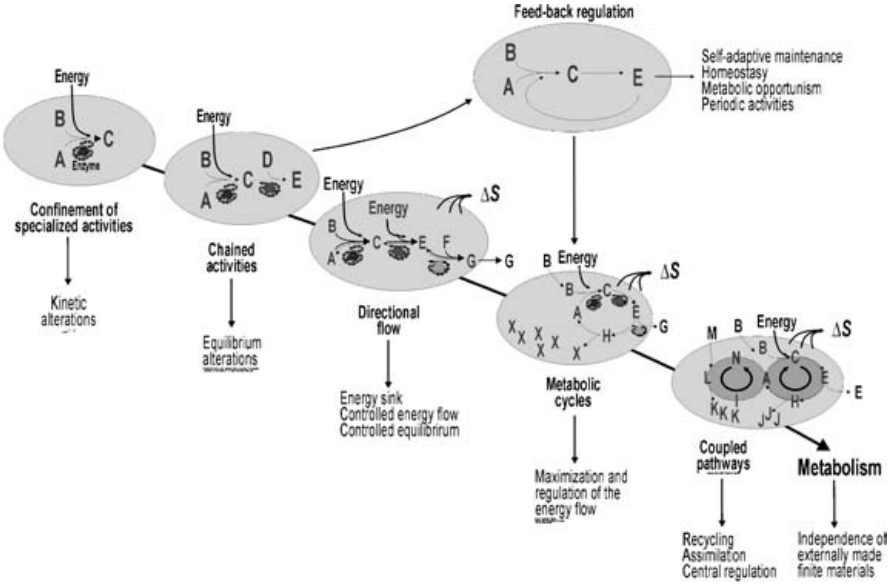


Fig. 3.13. Spatial confinement leads to changes in equilibrium and the evolution of the main features of metabolism

The creation of a reaction chain has the great advantage that the overall state of the system may be 'regulated' by a single lever (i.e., component), either pushing or pulling the system toward a well-defined out-of-equilibrium state. This strategy makes it possible to control the abundance of many parameters (e.g., chemicals) through a small number of levers. One obvious price to pay is that not every conceivable quasi-state is achievable and thus not every possible metabolic avenue may be explored. Yet it is worth paying this price to achieve one stable state more easily. For the first time in prebiotic evolution, an out-of-equilibrium state became stabilized through a stepwise regulation, as long as energy could be provided. The extension of a transformation chain coupled with a process for dissipating byproducts (e.g., the formation of a gas, a very small hydrophilic solute, or an insoluble mineral granule) could lead to a directional flow and gave an elegant solution for osmotic crises. This protocol only functions as long as the mechanism for transduction of the primary energy resource remains located on the inside. The coupling between a self-assembly process and a reflexive activity stabilizing it was probably one of the earliest cases of symbiosis during the molecular evolution of life.

Feedback mechanisms are mechanisms capable of regulating themselves and are of two types: negative and positive (Wiener 1947). Negative feedbacks self-inhibit themselves when they reach a certain threshold and are mainly used to maintain a defined functional level. Positive feedbacks en-

hance themselves once started and can be used to exacerbate the activity of a system once initiated, to deplete a resource very fast, or to create a periodic activity even when using a non-periodic resource. Because feedbacks are the backbone of functional steadiness, it is generally believed that they are a primeval requirement for the emergence of metabolism, and they are often presented as a fundamental phase transition toward life (see Fig. 3.13). According to the theory known as the Emergence of Template Information and Functionality (ETIF), the origin of life may be represented by the addition of the first feedback loop (Lahav and Nir 2002). When multiple feedbacks are applied to a set of reactions from distinct pathways within the same protocell, a state of self-adaptive maintenance may be generated, a tendency known as homeostasy. It was only after the addition of multiple feedback regulations to prebiotic evolution that enough 'pieces of the big puzzle' were achieved (i.e., high kinetics, out-of-equilibrium states, imposed energy flow and homeostasy), which could have been used to drive toward the irreversible genesis of finalist energy-dissipative systems (see Fig. 3.12).

Two of the most notable achievements of early life leading to the accretion of molecular networks are autocatalysis and metabolic cycles. Autocatalysis is generally defined as the ability of a component to accelerate its own formation. Positive feedbacks are the classic example of autocatalysis. Metabolic cycles are reaction chains organized in such a way so that one of their products is identical with one of their starting components (see Fig. 3.13). Therefore, an essential requirement for the emergence of a metabolic cycle is the existence of a multistep directional flow. A number of models have explored the consequences of autocatalysis and the importance of primeval cycles for early life (Eigen 1971, Eigen and Schuster 1979, Farmer et al. 1986). The enlargement of chemical cycles plus the coupling among different feedback-regulated cycles led to assimilatory metabolism and to a synchronization of different pathways (Maturana and Varela 1980, Luisi 1993, McMullin and Varela 1997). In chemical-based evolution (i.e., molecular Darwinism), metabolic diversity cannot increase too much, because arithmetical increase in the number of chemical components leads to exponential increases in the number of possible interactions among them. This evolutionary crisis is referred to here as the 'diversity crisis of molecular Darwinism'. The more complicated a metabolic network becomes, the more surely a system may poison itself through interference between distinct reactions and pathways. During early evolution, this crisis was kept under control in a number of ways:

- by limiting the number of monomers (20 amino acids in proteins and 5 major nitrogenous bases in nucleic acids),
- by using a limited number of metabolic pathways,
- by spatially separating interfering reactions inside each cell (cellular intracompartmentalization),
- by temporally separating interfering reactions within individuals (physiological ontogeny),

- by ecologically specialization (ecological compartmentalization), with different species housing different pathways.

One of the last major developments in the early history of metabolism was the development of hierarchical networking (see Fig. 3.12). Hierarchical networking required the preexistence of catalysis, directional flow, functional diversity and compartmentalization (DeLoof and Broeck 1995, Bresch et al. 1980) plus interactions between distinct pathways and molecules such as interactions between nucleic acids and proteins (Chakrabarti et al. 1994, Buiatti and Buiatti 2001).

The boundary-related properties of life are presented here as secondary to the energy-related features for various reasons:

1. Some types of reflexive activities such as heat-controlled and dehydration-controlled condensation may be achieved more easily than a selective boundary.
2. Some types of reflexive activity (such as group transfer) could be involved in the stabilization of a boundary.
3. Reflexive activity can exist without the need for a protective layer or any other form of physical separation.
4. Any low-entropy selective boundary in a fluid environment requires a reliable energy resource to form and maintain it.
5. Some lifeless negentropic energy-dissipative 'things' do form in nature (fluid vortices, fire, self-catalytic processes, speleothems, fractals and minerals), and these display reflexive activity but no specialized or selective boundaries.

It has been claimed that a primeval boundary may have been a disadvantage due to the lack of chemical communication with the exterior (i.e., import of useful monomers) (Orgel 2002). I would argue that boundaries were a very early achievement not only because they represented a minimal requirement for other processes, but also because they were protecting internal patterns from the hazards of the outside world. The exterior chemical world was so diverse and random, it acted as a universal metabolic inhibitor (poison) for the ability to direct any complex set of internal reactions. Eörs Szathmáry convincingly showed that a simple boundary is not enough to make life (Szathmáry 2002): "If the necessary building blocks are immersed in a sea of poison, side reactions tax the system so severely that the end result is always the same: the extinction of the replicators." As the transition toward life necessarily required the seclusion of energy-dissipative mechanisms from the exterior, either reflexive activity must have been intimately associated with the evolution of a territorial separation from the very beginning, or the emergence of reflexive activity and specialized boundaries were separated by a very short temporal gap. Reflexive activity must somehow have been directed toward the maintenance of the boundary as soon as a boundary

emerged (a primordial example of symbiosis or mutual assistance between two distinct features).

Some "origin of life" models use phosphatidyl choline (Schmidli et al. 1991, Luisi 1993) or photochemical formation of chemical components (Badahur and Ranganayaki 1970) to facilitate the maintenance of an early entity. One experimental analogy of a boundary-defined system also displaying reflexive activity has been demonstrated. It was based on colloid-mediated catalysis of the hydrolysis of ethyl caprylate (Bachman et al. 1992) (see Fig. 3.14). In this model a water-insoluble compound (ethyl caprylate) was mixed with a watery alkaline solution. Due to slow hydrolysis, a small amount of water-soluble caprylate formed first. This reaction is sluggish in its early steps, yet once a certain concentration of byproducts has been reached (0.1 M), micelles made of caprylate form. These micelles bind five molecules of ethyl caprylate each and catalyze their own hydrolysis. This mechanism can be used as an experimental analogy to demonstrate the replication and self-catalysis of micellar structures.

The most important phase transitions in the early history of life's boundaries (see Table 3.1) are self-assembly, seclusion within a specialized boundary, probabilistic jumps toward order and feedback regulation. Self-assembly is a conspicuous phase transition (Hazen 2001), considered as a basic property of life (Poglazov 2002). Self-assembly refers to both self-ordering and self-organizing events (Abel 2002). Its likeliness is hard to conceptualize apart from a well-defined physicochemical context. In an aqueous chemical world and in the presence of amphipatic molecules, the probability associated with the emergence of spatial seclusion can be mathematically derived from rates

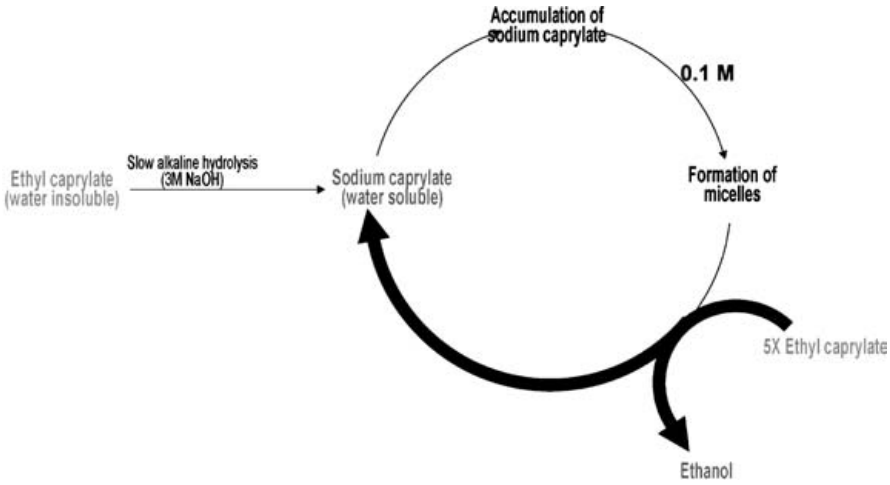


Fig. 3.14. Experimental model to demonstrate micelles replicating. Adapted from Bachmann et al. 1992

Table 3.1. Major achievements in the history of early boundaries

Minimal requirements	Major material-independent phase transitions	Major derived properties
Fluid phase	Self-assembly	Thermodynamic protection
Compositional diversity	Seclusion within a specialized boundary	Lowering external 'noise' Chaining internal activities Directional flow Impaired exchange
Degradation less than the difference between the entropic potential of the catalysis and the entropic potential of lifeless competitors	Probabilistic jumps toward catalytic specificity	Initiation of a quasi-state Recursive self-maintenance
The effect of the difference between the efficiency of internal catalysts and outside competitors must be larger than diffusion restrictions imposed by the semi-permeable barrier	Feedback regulation	Metabolic cycles Self-adaptive maintenance Hierarchical networking

of colloid formation (Edwards and Peng 1998). Probabilistic jumps toward catalytic specificity and novel quasi-states can be calculated as a function of vesicle size, chemical diversity, molar concentration and catalytic efficiency (Dyson 1997). Before life can emerge, feedback regulation has to occur to allow self-control in complex systems.

Most achievements presented in Fig. 3.12, such as quasi-states, chained activities, directional flow, feedbacks, homeostasy, metabolic cycles, and hierarchical networking, are derived properties that may be identified by using straightforward cause–effect reasoning. Due to cooperation and synchronization among independent autocatalytic systems, other derived properties were also possible. Metabolism is assumed to have emerged in a very early stage and never to have ceased to improve. Self-reproduction and spatial proliferation probably emerged after it, while mutation and evolution may have been added much later (Gánti 1974). Some of the minimal requirements for these boundary/metabolism-related events to occur are the existence of a liquid phase, chemical diversity, and a rate of degradation smaller than the difference between the entropic potential of the catalysis and the entropic potential of the lifeless competitors.

4 The Origin of Early Specificity. The Order, Complexity and Diversity of Life

> The physical problem of the origin of life can be reduced to the question: Is there a mechanism by which complexity can be generated in a regular, reproducible way?
>
> Eigen and Winkler-Oswatisch 1981

One of the key assets of life is its ability to maintain a low level of internal entropy while avoiding reaching minimal levels of it. Understanding the origin and evolution of this controlled equilibrium is complicated by the fact that more than one of the key properties of life such as order, complexity and diversity can relate to entropy and that one is often mistaken for the other. Order is often confused with organization, which is in turn confused with complexity, while complexity is often misinterpreted as diversity.

4.1 Order

Order refers to how consistent some particular arrangement is across space and time. Order often refers not only to arrangements of physical structures but also to functional relationships among them. The former meaning of order (i.e., periodic spatial arrangement) is over-emphasized here. Many ordered structures have high internal periodicity and can thus be predicted with remarkable accuracy using a low amount of information (see Fig. 4.1). The less information required to reconstruct a system, the higher its level of order. Hence one empirical way to express the order of a system is through the ratio between the amount of information needed to describe the system and its size.

The presence of order within a system may be automatically identified by using compression algorithms. The more predictable the distribution, the more ordered the system. The use of changes in entropy as a measure of changes in order is somewhat confusing because low entropy has a wider

R. Popa, *Between Necessity and Probability: Searching for the Definition and Origin of Life*, Adv. Astrobiol. Biogeophys., pp. 63–80 (2004)
http://www.springerlink.com/

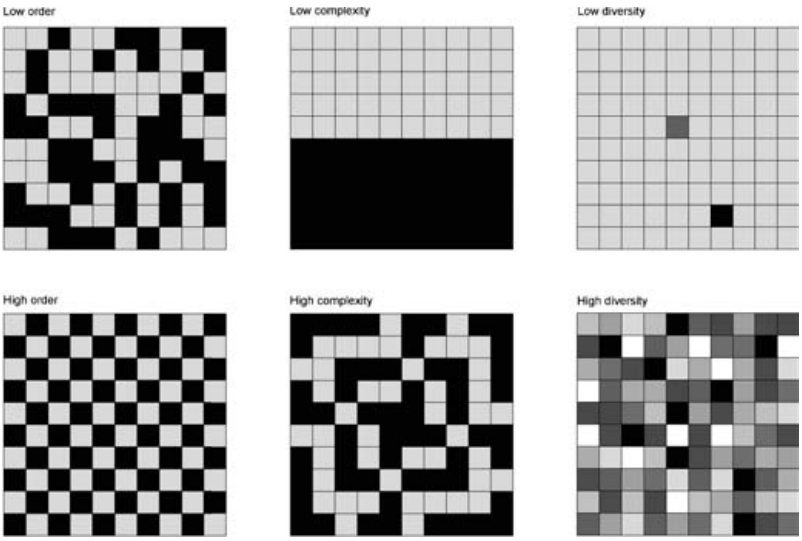


Fig. 4.1. Graphical representation of the difference between order, complexity and diversity

meaning than high order. Order remains a relative concept. According to one interpretation, ribosomes (cellular organelles used to make polypeptide chains) are highly ordered structures, because if a large set of ribosomes is studied, the same amino acids would be arranged in the same position with respect to the others. Yet if the architecture of one single ribosome is studied, its level of order is rather low, because the presence of one amino acid in one position in space has no predictive power upon the presence of another one in another position. Consequently, the likelihood that something is alive is not necessarily correlated with the amount of internal order. Highly periodic structures such as mineral crystals show a very high level of order and yet they are lifeless. While life as a general concept appears to evolve toward increased order, living entities tend to maintain whatever level of order works for them.

4.2 Complexity

Complexity refers to how intricate something is, and how elaborate its architecture or functioning is (see Fig. 4.1). Two general classes of complexity are known, static and dynamic (Abel 2002): "Static complexity pertains to physical and structural arrangements or states, while dynamic complexity reflects the degree of computational effort required to describe and know the uncertainty reflected by the object or state." Because complexity is long way afar from the periodicity of order and because repetitive patterns are often disrupted in the buildup of complexity, complexity is a parameter more re-

silient to predictability and to data compression than order. Thus, complexity may be measured through compressibility.

Negentropy can be used as an empirical measure of both the order and the complexity of a dynamic system. However, negentropy is imprecise in making the distinction between them. Some systems display a high level of order (periodicity) and a low level of static complexity and vice versa (see Fig. 4.2). A random distribution of internal components has low order and low complexity. The arrangement of white and black squares on a chess board shows high order and low complexity. A Christmas tree with all its ornaments is a very complex thing, yet contains little order. Fractals, snow flakes and the images in a kaleidoscope show high complexity, but they also show high order because they are generally based on simple algorithms repeated across space and time according to some overall pattern. The α-helix of proteins

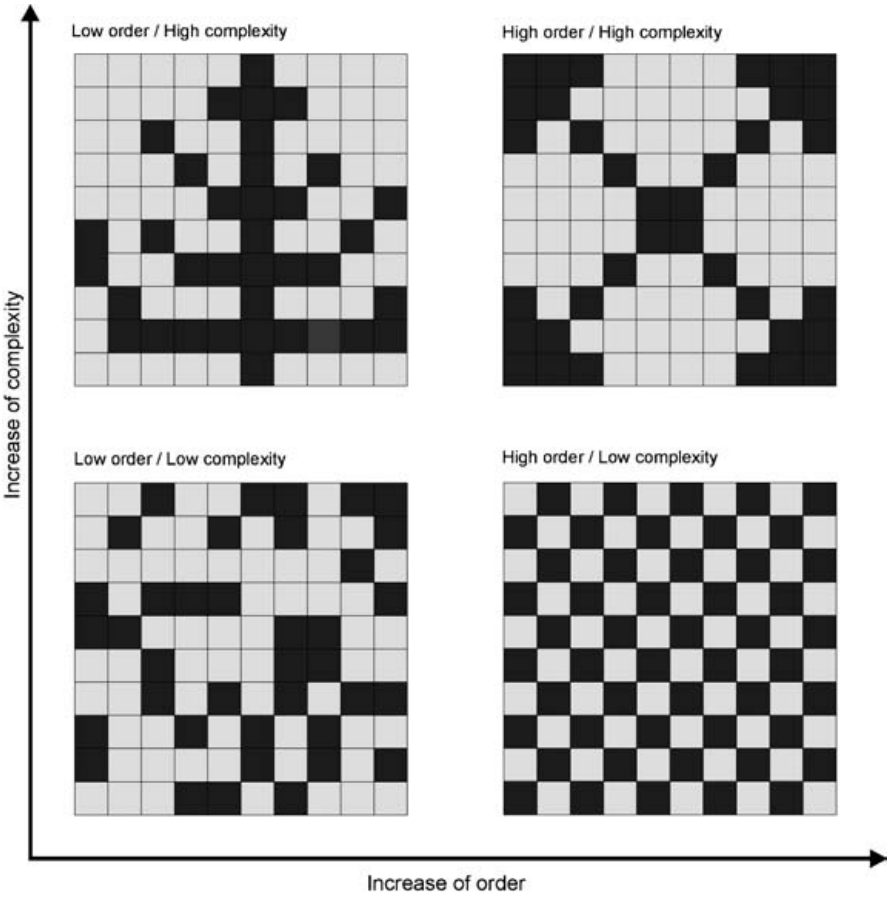


Fig. 4.2. Representation of systems with variable order and variable complexity

displays high order and low complexity, whereas the double helix of the DNA molecule shows less order and higher complexity than protein helices. While the secondary structures of proteins (α-helices, β-pleated sheets and collagen helices) show high order, their tertiary and quaternary structures show a considerable increase in complexity, yet not much increase in the level of order. Large protein assemblies such as ribosomes show some of the highest levels of complexity in the living world, but they are non-periodic structures.

A list of the most often perceived differences between order and complexity within a sequence or within a 2D or 3D structure has been provided by Abel (Table 4.1). The creation of order and complexity in life are related to the concepts of self-ordering and self-organization. According to Abel, significant differences exist between self-organizing and self-ordering events (see Table 4.2).

Table 4.1. Differences between order and complexity in molecular structures (after Abel 2002)

Order	Complexity
Regular	Irregular
Repeating	Non-repeating
Redundant	Non-redundant
Predictable	Non-predictable
Symmetrical	Asymmetrical
Periodic	Aperiodic
Monotonous	Variable
Crystal-like patterning	Linguistic-like patterning
Reducible	Largely irreducible
Compressible	Incompressible

Table 4.2. Differences between spontaneously self-ordering and self-organizing systems in nature (after Abel 2002)

Self-ordering	Self-organising
Increases redundancy	Decreases redundancy
Increases predictability	Decreases predictability
Increases symmetry	Decreases symmetry
Increases periodicity	Decreases periodicity
Increases monotony	Decreases monotony
Produces crystal-like patterns	Produces linguistic-type patterns
Decreases complexity	Increases complexity
Short-lived (highly dissipative)	Long-lived (minimal dissipation)
Produced by cause and effect	Still lacking natural process mechanism
Observed	Unobserved
Consistent with second law	Seems inconsistent with second law
Non-integrative	Integrative
Non-conceptual	Conceptual
Not particularly functional	Produces extraordinary function

4.3 Diversity

Diversity indicates the variety of internal components and their relative abundance within a system (see Fig. 4.1) and it is different from complexity. Although an increase in internal diversity often results in an exponential increase in complexity, many structures are highly complex but display low internal diversity, while rather simple structures may show a considerably larger internal diversity (see Fig. 4.3). As a numerical parameter, diversity increases in two directions: toward more types of subcomponents and toward a more balanced ratios among them (see Fig. 4.4).

Life on Earth has a very peculiar characteristic. Although cells contain a remarkable diversity of macromolecules, these polymers have a very low diversity of monomers. From more than 150 amino acids possible in nature only 20 are present in modern proteins. Yet they are combined in thousands

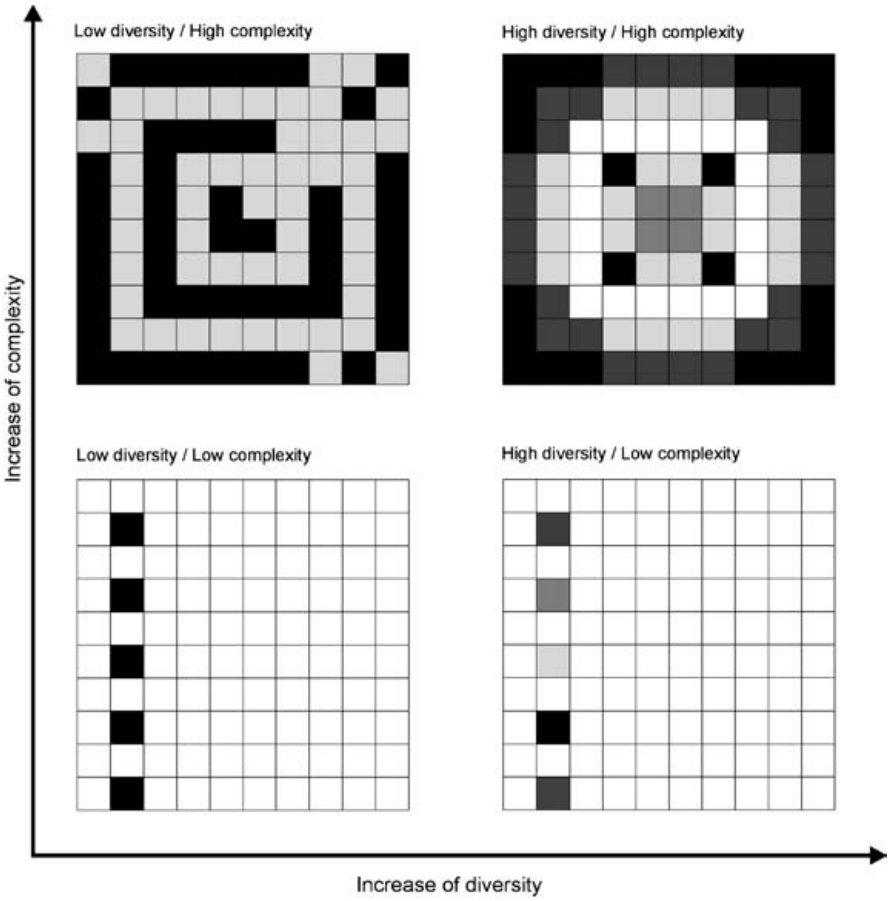


Fig. 4.3. Complexity and diversity are distinct features

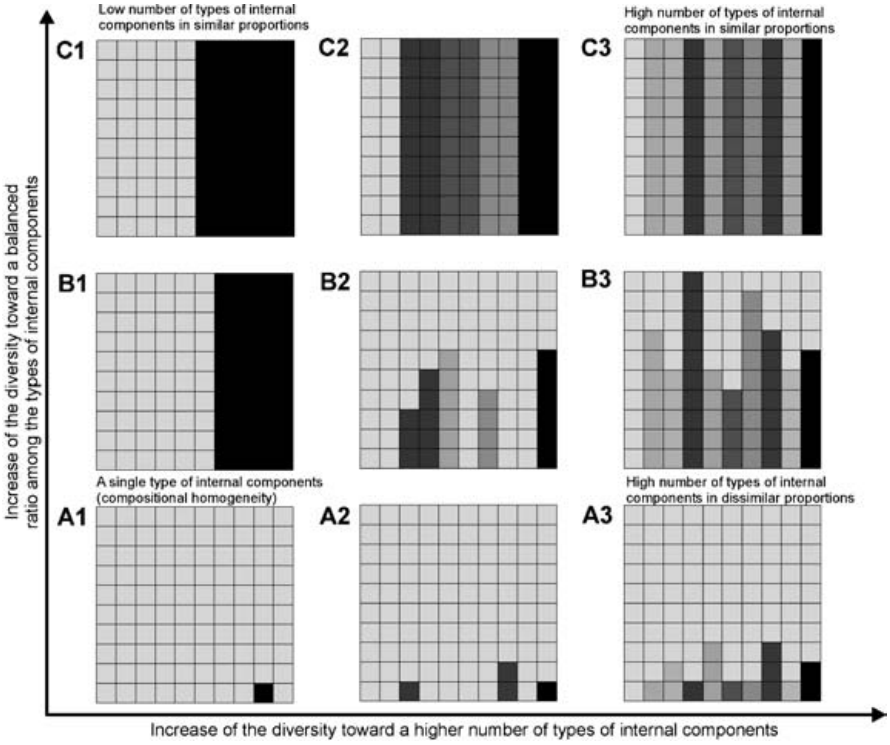


Fig. 4.4. The double meaning of compositional diversity. It increases in the direction of a higher number of types of internal component (1 to 3 on the *horizontal axis*) and toward a more balanced equilibrium between the abundances of internal components (A to C on the *vertical axis*)

of types of proteins in each cell. Although the DNA molecule only contains four types of nitrogenous base (adenine, thymine, guanine and cytosine), they can be arranged in an astronomically large set of combinations (genes). Yet from a chemical point of view, more than one hundred nitrogenous bases are possible. If the organic chemistry shows so much potential (environmental generosity), why do life forms only use a handful of monomer types as building blocks? This striking contrast between the relative simplicity of the primary structure of biological macromolecules and the tremendous diversity at their tertiary level might not be a random feature of life at all. A theory of life cannot ignore this particularity. The principles of cyclic chemistry and molecular coevolution have been used to try to explain this feature of life (Wong 1975, Wong and Bronskill 1979, DiGiulio et al. 1991, DiGiulio 1992, 1993, 1994, Boiteau et al. 2002).

It appears obvious that the combinatorial freedom among monomers dictates the relationship between the number of types of building block and the

number of possible arrangements. If no preference exists regarding which type of monomer can bind another monomer, considerable macro-architectural diversity can be achieved based on a very limited diversity of internal components. One should seriously consider whether the existence of the living state might not actually depend on a wide combinatorial liberty and on a small set of building blocks. This particularity of life can be used to interpret the patterns of its early evolution. I prefer to believe that the evolution of early life was not fully coupled with the external chemical evolution, but rather that life was an opportunistic phenomenon triggered by some favorable circumstances, and that once early life had been initiated, the biochemical and molecular network built itself in a way "purposedly" avoiding too many types of monomers.

As early life evolved, the compositional diversity of the organic polymers gradually increased so as to maximize their functional performance. However, because each new added monomer requires tremendous internal control (genes, regulators, synthetic pathways) and an exponential increase in the number of chemical interferences with preexisting pathways, the diversity of the monomers could not increase too much without becoming a managerial burden. Biochemical evolution was oriented toward achieving maximum polymeric diversity while using the minimum number of amino acids. The rigors of complexity dictate that the more amino acids are already present in proteins, the less likely other amino acids are to be added to the genetic code. Eventually, the number of amino acids from modern proteins became frozen at twenty. This diversity was established as a balance between the need to use as many types of radicals as possible, to accommodate some limited redundancy in the functional radicals, and to manage their biosynthesis with minimal interference. The same basic principle applied to the evolution of the nucleic acids. At the molecular level, early organisms tried to achieve the maximum information compression with the least number of nitrogenous bases.

4.4 Specificity

Modern life forms use genetic information to create and maintain structures of a remarkable order, complexity and diversity. According to Erwin Schrödinger (1944): "Life seems to be an orderly and lawful behavior of matter, not based exclusively on its tendency to go over from order to disorder, but based partly on existing order that is kept up." This ability of an entity to maintain itself was also referred to as homeostasy, 'steady state', 'quasi-state', 'the duration of similarity' or 'specificity'. This self-maintaining ability of modern organisms is controlled through complex molecular and biochemical mechanisms. But before such elaborate mechanisms were implemented, alternative controllers must have existed in nature. In the context of early

life evolution, specificity extends to more than cellular self-control and also indicates:

- how far a pattern is from being random,
- how consistent a pattern is across time,
- how unambiguous the interaction between distinct components is.

From a mathematical point of view, random sequences are the most complex of all. Yet life does not reach toward such complexity. For a living entity, the steadiness of its state is more valuable than achieving a high order and complexity or drifting from one highly ordered state toward another. Unless the environment changes significantly, living things tend to remain the same. This conservative tendency (stability) is one aspect of specificity that is similar to homeostasy. Yet while homeostasy represents the achievement of a steady level of some physical/chemical/functional parameter (such as temperature, pH, or the concentration of various chemicals), specificity also indicates the steadiness of large constructive components. The maintenance of glucose within a certain concentration range within the body is an example of homeostasy, while the ability to form insulin continuously with exactly the same composition, sequence, architecture and functional capabilities is an example of specificity. Self-imposed periodic activities also display high specificity.

Specificity is best defined as a material-independent feature of life, required for the maintenance of the constructive identity and for making the activity predictable and consistent. Yet specificity and homeostasy are interdependent. In modern organisms, high specificity is required in order to ensure homeostasy, while homeostasy is one of the preconditions for control over specificity.

4.5 Specificity of Polymer-Based Life

Order, complexity and diversity are not exclusively restricted to living entities. The formation of certain lifeless 'things' such as mineral crystals or fractals displays a high reproducibility (specificity). It is rather the ability to self-maintain a certain level of order, complexity and diversity that seems to matter in the formation of life. We do not know whether chemical forms of life can exist without polymers and therefore most models of early life evolution are based on polymers. Because polymers are at the same time building blocks, catalysts and memory units for life, a discussion of the evolution of prebiotic specificity has to refer to early control over the order, complexity and diversity of molecular chains. According to David Abel, the types of complexity associated with polymers are random (RSC), ordered (OSC) and functional (FSC). The classification used here is based on the increased complexity of early polymers and an increase in their functional performance.

Precondensation-Related Specificity (PreCondS)

This represents all events occurring before condensation:

- Primary specificity (DeNovoS) refers to the synthesis of monomers.
- Secondary specificity (SecS) represents any compositional changes occurring after primary synthesis.
- Spatial-heterogeneity-related specificity (HetS) refers to apparent compositional changes due to a heterogeneous spatial distribution.

Condensation-Related Specificity (CondS)

This represents all specificity-maintaining events occurring during condensation and affecting the primary structure of a macromolecule:

- The degree of condensation-related specificity (SizeS) refers to the number of monomers in a chain.
- Composition-related specificity (CompS) refers to the composition within a chain.
- Sequence-related specificity (SeqS) indicates how far a certain sequence is from a random expectation.

Conformation-Related Specificity (ConfS)

This represents the architectural constancy associated with the secondary structure of a chain:

- Handedness-related specificity (HandS) is represented by deviations from the racemic distribution.
- Architecture-related specificity (ArchS) refers to the consistency of architectural patterns.

Activity-Related Specificity (ActS)

Also known as performance-related specificity, this refers to the consistency in the performance of a population of molecules. Activity specificity is defined as the rate of legitimate reactions divided by the total rate of all (legitimate + side) reactions (Szathmáry 2002). Activity-related specificity is also known as functional sequence complexity (FSC) and defined as a succession of algorithmic selections leading to function (Abel 2002). One important type of activity-related specificity is catalytic specificity, which is believed to be the most fundamental type of specificity in the history of life (Arrhenius 2002).

Although a mathematical representation of most forms of specificity can be achieved, the relationships among the various types of specificity are not easy to represent and explain. Despite the fact that increases in low level specificities have positive effects on higher levels, these parameters are

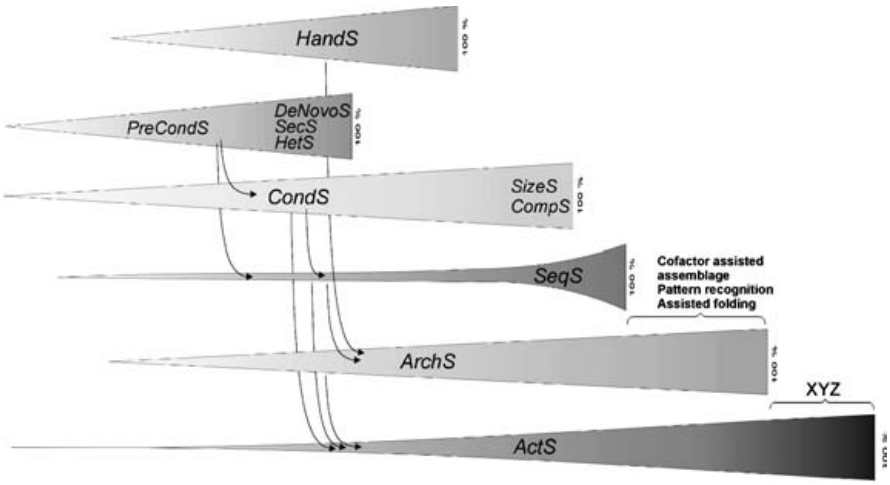


Fig. 4.5. Changes in specificity at different levels during the early evolution of life. Abbreviations are explained in the text

not always predictable from each other (see Fig. 4.5). Composition-related specificity can be calculated exactly from sequence-related specificity but the reverse is not true because monomers can arrange in a variety of ways along a sequence. Activity-related specificity is not fully predictable from sequence-related specificity because polymers with identical sequences can fold in a wide variety of ways. Activity specificity is not defined solely through architecture-related specificity because comparable activities can be achieved by a multitude of very close-related architectures. Monomer-related architectures are often not enough to define activity, because catalytic cofactors, pattern recognition and chaperones are often required for proper folding (see Fig. 4.5). Yet the computation of individual types of specificity is valuable in assessing which microhabitats or chemical pathways resulted in the largest and steadiest deviation from randomness during prebiotic evolution.

4.6 The Origin of Specificity

The specificity of modern life forms at both architectural and activity level is almost entirely controlled through genetic information which itself results from a long evolution. We generally assume that the specificity of early life came from abiotic nature, but we do not know how. It would be very convenient for models of early evolution if we could find a simple source of abiotic specificity that led to life, but reality is not this simple. Although the advantages of self-ordering processes as a way to simplify architectural build-ups are obvious, the use of abiotic self-ordering as the sole means to induce order can become a serious trap in building life, because it interferes

with the development of complexity and with the evolution toward functional sophistication (Abel 2002). Most instances of abiotic specificity are periodic structures, while the macromolecules used by life are fundamentally aperiodic crystals (see Appendix C).

According to Stuart Kaufmann, the level of order required for life to be initiated was relatively high (Kauffman 1993). However, early life catalysis must have been controlled by much simpler molecules, and activity-related specificity could have originated in a less elaborate source. Although for polymers, a high level of order cannot be separated from a source of information, for very short oligomers formed in small systems, order and specificity could have been achieved in a purely probabilistic manner. For reasons of simplicity, but also because order and information display some autonomous consequences, their evolutionary steps are discussed separately in this study (see Chaps. 5 and 6). The evolution of order refers to changes in its various types of expression and in its level, while the evolution of information refers to the type of protocols used to convey the message and to some extent to the material nature of the information carrier. Whether primordial conditions were enough to satisfy the minimal threshold of order required for initiation of pre-life cycles remains uncertain, because both the composition of the early environment and the minimal catalytic requirements are unknown, and also because not enough experimental evidence is available concerning this issue.

Pre-life specificity was probably initiated by a variety of independent yet interacting abiotic factors. It is likely that their influences overlapped with regard to:

- self-assembly (Edwards and Peng 1998, Brown et al. 1998),
- competing interactions (Muthukumar et al. 1997),
- external dissymmetry inducing non-randomness (Lahav and White 1980),
- probabilistic jumps (Dyson 1997),
- self-organizing events (Muthukumar et al. 1997),
- local scale order as a universal organizing principle (Hazen 2001) (see Fig. 4.6).

These primordial controllers of specificity were either external to the pre-life entities or based on constructive conjectures (built-in protocols). In either situation, they were not under genetic control and no elaborate subcomponent or functional routine was used to preserve them. They were a normal part of the way physicochemical nature functions. These sources of order have the advantages that they are accessible and ready to use, but the disadvantages of low precision, and being hard to own, inherit and manipulate.

In later steps during prebiotic evolution, other sources of specificity (means to control order, complexity and diversity) were used:

- Interactions with ordered physical realities such as mineral surfaces (Cairns-Smith and Hartman 1986).
- Polymer/polymer ordering effects (Weinberger et al. 1988).

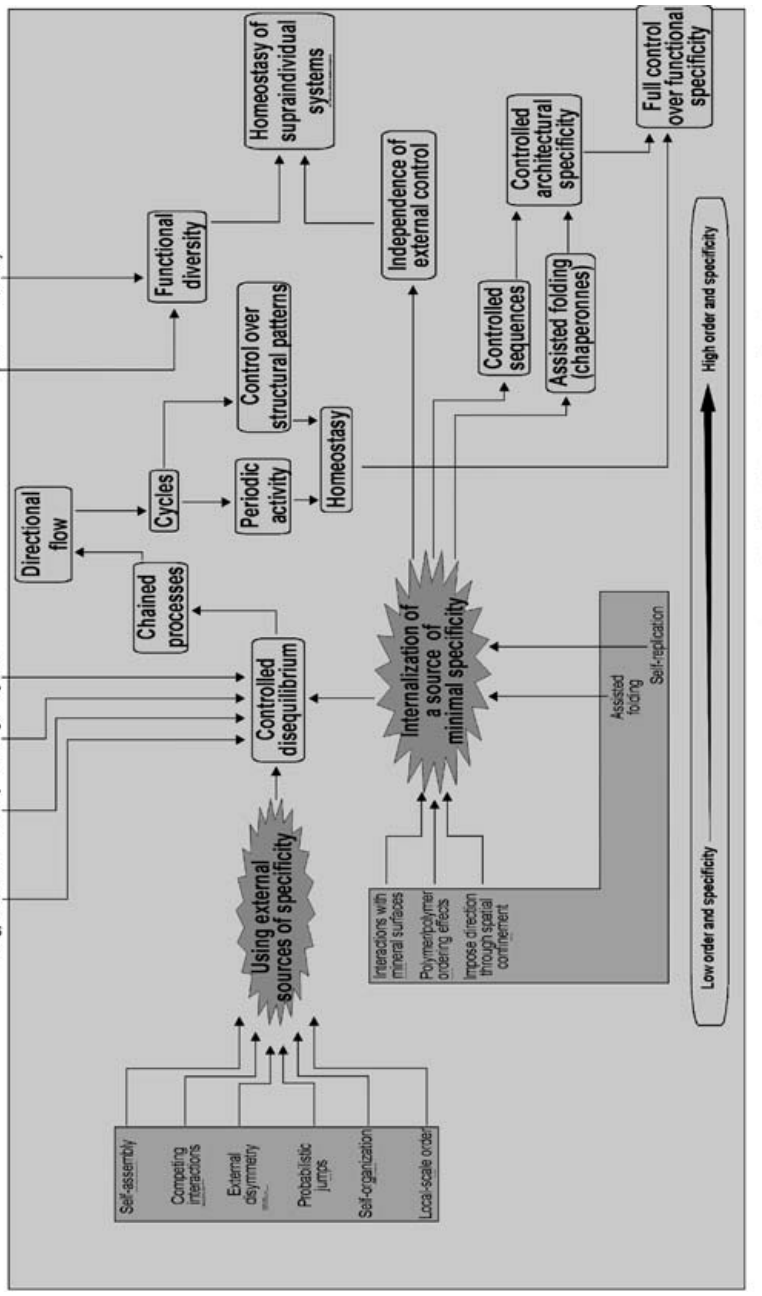


Fig. 4.6. Major events associated with the evolution of the specificity of order, complexity and diversity in the early history of life

- The imposition of a metabolic direction through spatial confinement (Kauffman 1993).
- Assisted folding (e.g., mutual assembly that later evolved into molecular chaperones and prions) (Prusiner 1997, 1998, Brown et al. 1998, Bagott 1994, Ohler et al. 2001).
- Self-replication (Eigen and Schuster 1977).
- Translation as one of the most advanced means of controlling internal specificity.

These sources were more elaborate and required an advanced biochemical and molecular 'technology', but had the advantage of allowing 'ownership over the patent' and providing greater precision and control over plasticity (memorizing variations).

4.7 Transition from External to Internal Control

Although according to Stuart Kauffman's theory the evolution of life-related events must have started with a relatively high level of order (Kauffman 1993) (and implicity, high specificity), most other theories are gradualist approaches starting from low levels. I am not going to use the dominance of this trend as supporting evidence because in science the popularity of an opinion is not proof of its correctness. Yet a gradualist approach seems more appropriate because it promotes the idea that the earliest activity-related specificity and architecture-related specificity were neither high, nor under elaborate control (see Appendix C for the appropriateness of the term 'control' in this context). These controllers are simple circumstances derived from the laws of quantum mechanics (Korzeniewski 2001) or from pure chance (Dyson 1997).

The self-assembly of early membrane systems is believed to have been followed by a continuous increase in order, complexity and specificity (Margulis 1971, Fox and Dose 1977). A variety of external factors with life-independent origins such as mineral-to-organic stereochemistry, thermodynamic selection in heterogeneous environments and photolytic stereoselection, have been shown to be capable of altering the distributional evenness of monomers and creating a nonrandom space distribution, occasionally with reproducible results (Cairns Smith 1982, Liebl et al. 1984, Mörtberg 1971). Certainly, not every abiotic asymmetric event was directed toward life. Most combinations are not significant for macromolecular life because, after condensation into polymers, they do not result in significant changes in the level of conformational bias or a significant increase in catalytic performance. Functionally, such cases lead to subliminal non-randomness.

However, since order could not have been 'controlled' by life-related mechanisms in the prebiotic era, it is hypothesized that at least one external source from the above list generated enough structural and functional distance from randomness to allow preliving units to function, before molecular mechanisms

took control. The evolution of functional independence meant harvesting energy and forming molecules inside, but it also meant that control over the level of order was gradually reassigned to internal mechanisms. Although the environment still has some influence over internal specificity, in modern life forms the specificity of order, complexity and diversity are predominantly controlled from the inside.

4.8 Major Events in the Early History of Specificity

The first major probabilistic phase transition event in the history of specificity was the use of an external source to achieve a minimal internal threshold, required to initiate the first functional cycle. A second major step was the internalization of a source of minimal specificity (see Fig. 4.6). Although the use of external sources of order could have been very early, the internalization of the source of order was probably historically subsequent to the use of a reflexive activity, because it takes energy to maintain any level of negative entropy in a dissipative environment governed by the second law of thermodynamics.

Because homeostasy is based on feedback mechanisms and spatially confined chained processes, high specificity also requires preexisting spatial seclusion (i.e., a boundary). This is the reason why, in this hierarchical representation of life, specificity-related achievements are presented as secondary to boundary-related achievements. The coexistence of internalized control with other achievements (such as reflexive activity, energy transduction, selective exchange, feedback regulation, and confinement of specialized activities), drove toward a 'controlled' disequilibrium and other derived consequences such as homeostasy, independence of external control, controlled SeqS, assisted folding, and controlled ArchS (see Fig. 4.6). Only after a level of internal control beyond a minimal threshold had been achieved could early entities operate satisfactorily at all levels of organization (overall composition, space distribution, sequence, architecture and activity). This allowed a better chance to withstand environmental pressure and, in the presence of variability, to further improve their performance and eventually allow functional independence.

4.9 The Origin of Feedback Mechanisms as a Source of Internal Stability

As stated in Chap. 3, feedbacks are considered to be important phase transitions toward life. A minimal feedback loop (Wiener 1947, cited in Korzeniewski 2001) represents the basis for the emergence of an adaptive system and for further metabolic developments (Gánti 1974, Fontana 1992). Negative feedbacks are very common in biochemistry. A feedback loop within

a metabolic pathway (see Fig. 4.7) might look like a rare phase transition event, but in certain cases it is relatively easy to explain.

Negative feedbacks have their origin in the tendency of products to interfere with their catalysts once thermodynamic equilibrium approaches K (see Fig. 4.7). The probability for such a mechanism is relatively high in a secluded environment where the products do not dissipate easily, such as in a watery space surrounded by a hydrophobic layer (e.g., a membrane). At this point the mechanism is still not a true feedback. Only when the complexity of the path increases so that a compound controls an earlier point in its chemical history does a significant phase transition occur. The emergence of feedback regulation (unless it is purely fortuitous) is related to the extension (fragmentation) of a previous path through the addition of an extra step. This fragmentation of a path is common when catalysts are used. The AB* product in Fig. 4.7 which was previously in 'control' of its own direct catalyst (enzyme 1) becomes a controller of an earlier step after another intermediary step is added (enzyme 2). By extension this can be presented as one of the simplest means to explain how the final product of a biosynthetic pathway can interact with an early enzyme from its synthetic path. Another alternative worth considering is undoubtedly the idea that the emergence of feedback regulation occurred by mere chance.

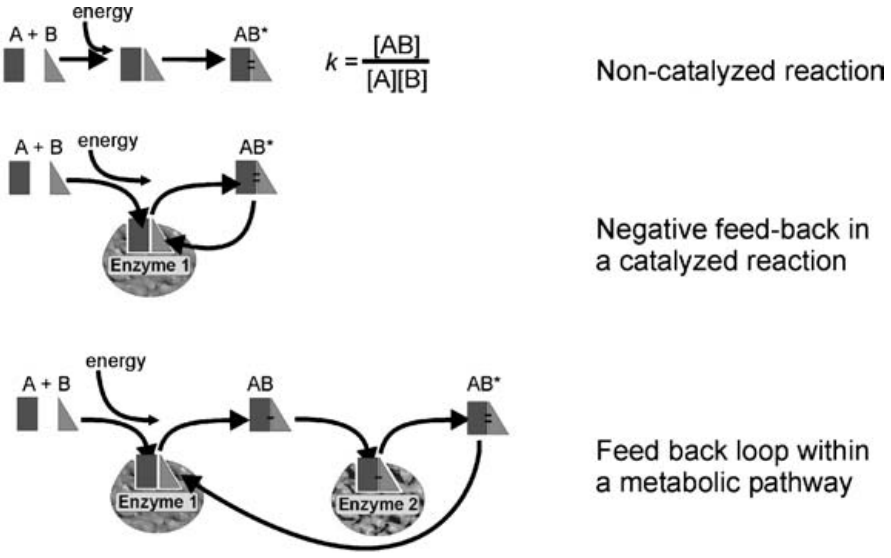


Fig. 4.7. A mechanism for the origin and early evolution of negative feedback regulation. A and B are reactants, AB and AB* are products, K is the equilibrium constant, [A], [B] and [AB] are the concentrations of compounds A, B and AB, respectively, in mol/L

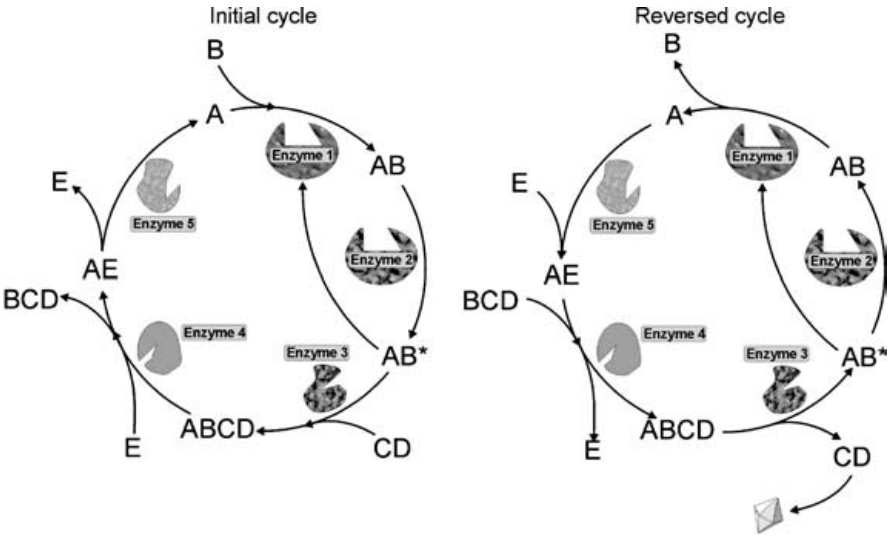


Fig. 4.8. A mechanism for the emergence of forward regulation through cycle reversal

4.10 The Origin of Forward Regulation

Some cyclic pathways are assumed to have been reversed during their ancient history. One example is the Krebs cycle, believed to have been used to fix carbon at one time, and only later as a respiratory cycle. The reversion of a cycle becomes possible in evolution when one path becomes coupled with another more exergonic process (such as another metabolic cycle, accompanied by the formation and escape of a gas or its coupling with the formation of a very insoluble byproduct). This mobilization pushes the initial equilibrium into the reverse direction (see Fig. 4.8). After the reversal of the cycle, the AB* compound which was initially a feedback regulator becomes a controller of enzyme 1, positioned further ahead in its path.

4.11 Consequences of Internal Regulation

Feedback regulation and forward controls have regulatory consequences that go beyond thermodynamic expectations and tend to push the system toward a steady state that is out of equilibrium with the exterior. When such effects expand to an entire system, a novel state is reached called homeostasy, which enables the system to be opportunistic and to buffer short term changes in environmental resources. Due to partially overlapping meanings and / interconnectivity, homeostasy and specificity enhance each other. Neither specificity nor homeostasy are fundamental phase transitions toward life (in the sense of their

probabilistic origin) but they are crucial derived properties of life. Although essential for understanding life and although they provide great life-related signatures, specificity and homeostasy cannot be used alone to demonstrate the existence of life. A variety of lifeless 'things' display internal control over their own internal patterns and over their future development such as fractals, growth of periodic crystals, fluid vortices and self-regulated cybernetic systems.

4.12 Forced Oscillations and Periodic Clocks

A very interesting development in the history of life was the emergence of oscillating reactions (Zaikin and Zhabotinsky 1970, Orbán 1980, Edblom et al. 1986, Cervellati 1995, Kurin-Csorgei et al. 1998, Orbán et al. 1998), forced oscillations (Pályi et al. 2002), and later an adjustable periodic clock (see graphs from Prigogine and Stengers 1984). Description of these achievements requires a specialized mathematical apparatus (Price and Stevens 2000, Purich 1979, 1980, 1982, 1995). Positive feedbacks are one of the major means to create periodic activity in the presence of a continual flow of energy. Although the exact molecular mechanism behind the biological clock is still under investigation, its existence is real (Alvarez and Sehgal 2002) and it gives the advantage of physiological anticipation (preparedness of a new quasi-state) for periodic resources, which is another potential signature of life.

4.13 Specificity-Related Phase Transitions Toward Life

The external source of minimal specificity represents the use of an external source to bring the level of internal specificity of order, complexity and diver-

Table 4.3. The main features related to the history of specificity of order, complexity and diversity

Minimal requirements	Fundamental phase transitions	Major derived properties
External non-randomness	External source of minimal specificity	Controlled disequilibrium
Self-organization	Internalization of the source of specificity	Functional diversity
Importable sources		
		Independence from external control
		Internal cycles
		Periodic activity and biological clocks

sity to a threshold compatible with the initiation of the first steady functional cycle and the creation of an out-of-equilibrium steady state (see Table 4.3).

Internalization of the source of minimal specificity is represented by the import of an external source of specificity or the assignment of control over internal specificity to an internal mechanism.

4.14 Specificity-Related Minimal Requirements of Life

The minimal requirements of life-related specificity are the existence of an external dissymmetry or a natural tendency toward self-organization and the existence of a source of specificity small enough to be hosted inside pre-life structures (see Table 4.3).

5 The Origin of Handedness

> Life is an inevitable consequence of the existence of optically active ... compounds.
>
> Klabunovsky 2002

Some physical realities such as fluid vortices, certain molecular architectures, circularly polarized electromagnetic waves, screws and some crystalline patterns can exist in two geometric states that cannot be superimposed on their mirror images (see Fig. 5.1). This property is known as handedness in reference to the word 'hand'. The word 'chirality' (from the Greek word *cheir*

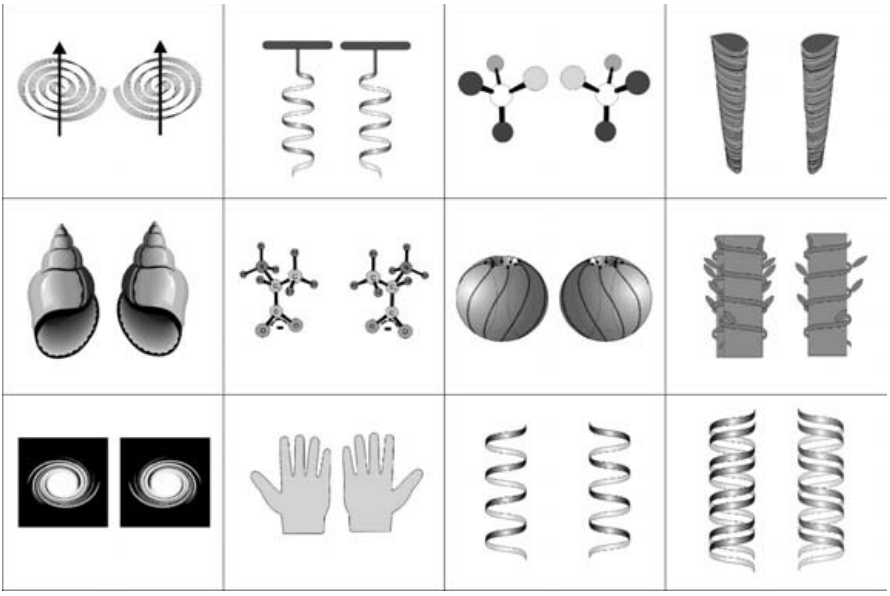


Fig. 5.1. Examples of handedness

R. Popa, *Between Necessity and Probability: Searching for the Definition and Origin of Life*, Adv. Astrobiol. Biogeophys., pp. 81–94 (2004)
http://www.springerlink.com/

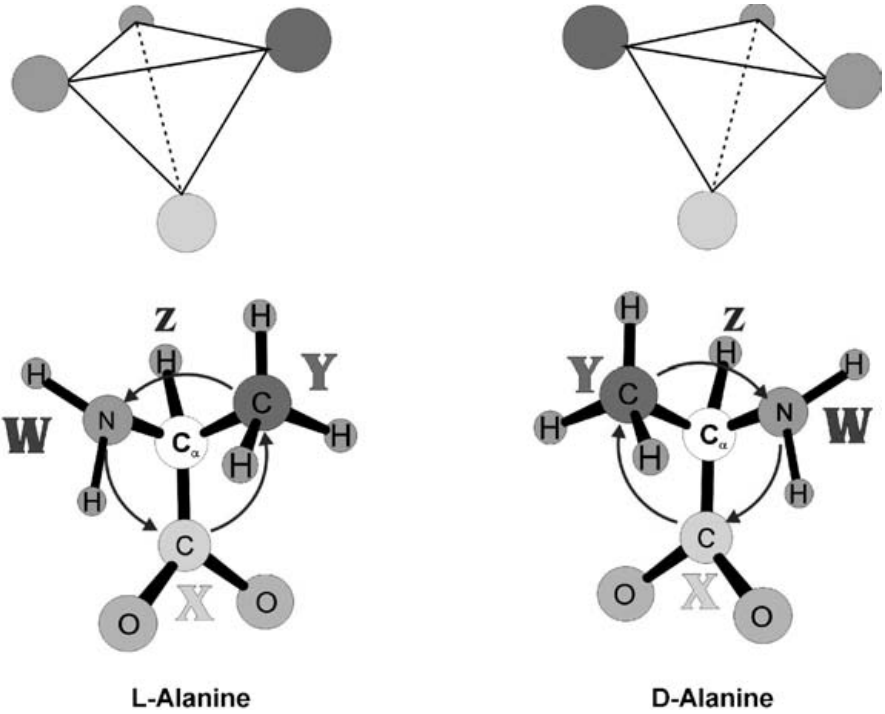


Fig. 5.2. AA steroisomers. Two arrangements (stereoisomers) are possible around a pyramid with four different corners. The atoms around an asymmetric carbon are labeled W, X, Y and Z. In alanine, the asymmetric carbon is C_α, W = NH_2, X = COO^-, Y = CH_3 and Z = H, with Z oriented away from the observer. If W, X, Y are arranged counter-clockwise, the molecule is an L-isomer, whereas if W, X, Y are arranged clockwise, the molecule is a D-isomer

meaning 'hand') was first used by Lord Kelvin in 1893 and is more frequently used to describe the handedness of molecular architectures.

Molecular chirality (stereoisomery) must not be confused with optical activity. Stereoisomery indicates the configuration of a molecule and it is labeled with L (or S) and D (or R), while optical activity indicates the direction of rotation of polarized light and it is labeled with l (for left, levorotatory or levogyre) and r (or d for right, dextrorotatory or dextrogyre). A mixture that contains 50% of the L-form and 50% of the D-form is known as a racemic state. In spiraling objects, the sign of the chirality (L or D) is established from the direction of rotation of the spiral as it goes away from the observer (clockwise means D and counter-clockwise means L). In organic molecules, chirality is often due to the fact that two possible arrangements can exist around an asymmetric carbon (i.e., an atom of carbon surrounded by four different radicals) (see Fig. 5.2). By convention the sign of the chirality in molecules with asymmetric carbons is determined as follows:

1. The four groups around the chiral center (i.e., the α-carbon in amino acids) are labeled with the letters W, X, Y and Z:
   - W has the highest atomic number, the NH_2 group in amino acids,
   - X is the COOH group in amino acids,
   - Y is the radical group in amino acids,
   - Z has the lowest atomic number, H in amino acids.
2. The Z group is pointing away from the observer.
3. If the W,X,Y sequence is counter-clockwise, the compound is an L-isomer. If the W,X,Y sequence is clockwise, the compound is a D-isomer.

Although D-pentoses (such as ribose and deoxyribose) and some D-amino acids (such as leucine, methionine, phenylalanine, tyrosine, proline, histidine, serine, threonine, asparagine) are d while their L-isomers are l, some D-isomers (such as valine, isoleucine, cysteine, alanine, lysine, arginine, glutamine, glutamic acid and aspartic acid) are l while their L-isomers are d.

5.1 Chirality and Life

Life shows a remarkable chirality. All amino acids from peptides are C1-L-stereoisomers (except for glycine which is not chiral and a few D-AAs present in some peptides and antibiotics), while all pentoses from nucleic acids are D-stereoisomers. Most proteinaceous α-helices and the B-form of the DNA chains are right-handed. Some protein helices and the Z-DNA form are left-handed.

Chirality is an important feature of the biological type of life, if not a condition of it, because higher-order structures are inherently connected with chirality. Stereoisomery has a great importance in stabilizing molecular helices and in establishing architectural specificity. The architecture of the biological macromolecules in which the arrangement of various monomers is correct but their chirality is wrong is unpredictable, and consequently, such molecules are not fit for performing steady functions.

The specificity of macromolecules varies at different levels of organization such as composition, space distribution, sequence, architecture and activity (see Chap. 4). A high degree of order at one level requires control from an upper level (more complex and richer in information), or it has to be controlled by other systems. The abundance of one organic chemical such as glucose in the blood is controlled by metabolic activity and requires at least two chemical agents (one creating glucose and the other degrading it), both agents being more complex than glucose itself. Furthermore, the existence, the activation and the correct functioning of each of these two agents is controlled by an even larger number of factors such as precise sequence, availability of cofactors, regulatory genes, hormones, assisted folding and ubiquitins. The regulation of glucose is performed through insulin, which is a polypeptide (i.e., considerably more complex than glucose). Insulin itself is regulated by

more complex enzymes , which are themselves controlled by even more complex apparatus (e.g., the translation machinery), which in turn depends on the existence of other, overriding molecular controllers.

In a hierarchical organization, behind each unit to be controlled, a pyramid of more and more complex requirements is created. For obvious reasons an increase in complexity cannot go too far without spiraling out of control and become either a cumbersome burden or too large for the organism itself. Managerial complexity tends to increase exponentially with arithmetic upgrades in functional diversity. Life's way to stop this avalanche of endless requirements while still allowing the functional diversity to increase is the uniformity-in-diversity approach. An important observation about the evolution of life is that the uniformity-in-diversity principle is consequent and not primordial. It was imperative but not built into the organization of the earliest pre-life forms.

In complex systems struggling for an efficient existence, the mechanisms that can be unified among different pyramids of functional interdependencies, and among different subsystems, become so and remain so. The early history of life was analogous to a competition among architects/builders, in which the participants capable of creating the most efficient and versatile constructions while using the fewest types of basic material survived the contest. The more common patterns are used to build functional hyperstructures, whilst the less complicated are reserved for the chains or pyramids of causalities. Fewer types of primary material are used to build a complex structure. The least complicated interferences among them are expected. There is less "paperwork", fewer errors, fewer architectural exceptions and simpler management.

The uniformity-in-diversity strategy is not a precondition for the initiation of a preliving system, but a consequence of the tendency of life to become self-organized after a certain complexity threshold has been reached. Because early life forms are believed to have been very simple, while the uniformity-in-diversity approach is complexity-driven, the purpose-like nature of life (i.e., the whys and hows of the functioning of life) must have existed before the uniformity-in-diversity approach. This is important because it allows the construction of a hierarchy of some molecular macro-evolutionary events. In modern life biology, examples of uniformity in diversity are:

- one common pathway to build all proteins (Ribosome-based translation),
- a limited number of energy currencies (ATP and membrane gradients) for all internal processes,
- the same chaperones to assist the folding of many polypeptides,
- a low diversity of monomers in NAs and proteins,
- two types of molecule (DNA and RNA) for all genetic information,
- a low number of histones to 'fold up' the entire DNA strand,
- one trigger to regulate an entire operon with many genes,
- one replication system to copy the entire body of genetic information.

The use of handedness is another example of a deliberate simplification. Whenever a racemic mixture of amino acids is used to build polypeptides, intolerably large configurational imprecision is produced. In a racemic world, the first amino acid in the chain (AA_1) has equal chances of being an L- or a D-isomer. After the second amino acid is added (AA_2) the probability that the chain has an L-AA_1-L-AA_2 sequence is $1/2^2$. For a chain made of 10 amino acids the number of possible combinations with respect to handedness alone becomes 10^{10}. It is obvious that, if amino acids are the monomers of choice, no control over architecture can exist without first controlling the handedness. In racemic mixtures, sequence has a non-significant influence over architecture, while in chiral mixtures, ArchS depends significantly on SeqS. Because ConfS determines ActS (especially in long polymers), without chirality, the genetic control over SeqS (however precise) would only bring microscopic advantages and hence would never have been selected.

One can certainly argue that not all polymers are made of chiral monomers and that life should have selected a less problematic monomer than amino acids to build enzymes. Many types of monomer must have existed in the waters of the pre-life era that were non-chiral. Life should have a chance to avoid chirality altogether by using achiral monomers. Why did life act otherwise? Is there anything special about chirality? It is hypothesized here that, if the handedness of the early amino acids could somehow have been controlled by a limited number of factors, both the architectural patterns and the activity could have become largely controlled through the sequence. In this particular case, higher levels of complexity (architecture-related specificity and activity-related specificity) could have been controlled through lower levels of organization (i.e., sequence). This would have considerably limited the endless pyramid of regulatory causalities described before and simplified the management of the overall system.

5.2 Natural Sources of Chirality

The abiotic origin of chirality is very hard to understand. Because only minor energetic differences exist between the two chiral states of a handed chemical (Salam 1991), the probability of the L-isomer forming is virtually equal to the probability of the D-isomer forming. Consequently, only insignificant deviations from the racemic state result during abiotic syntheses. When one chiral form can be naturally converted into another (L $\leftrightarrow$ D transitions), the thermodynamic similarity between the two states results in racemization and wipes out any chiral disruption. If more L-forms are present, L $\rightarrow$ D transitions are more frequent than D $\rightarrow$ L transitions, and the reverse happens whenever D-forms are in excess. The higher the chiral disruption, the stronger its racemization. Thus a close to 100% handedness of a 'racemizable' compound is almost impossible in the absence of life.

It would appear unlikely that the universe is globally handed with respect to any racemizable phenomenon or molecule. It is more likely that handedness should only be conspicuous in the form of local distributional disequilibria. In theory, if racemization is present and if enough time is available, there should be no overall handedness, while local heterogeneities should also gradually decrease. Yet chiral disruption is observed at many levels. This suggests that some handed forces are continually creating chirality *de novo*. Another possibility is that some handed disruptions are so slow to racemize that the universe is simply too young to have canceled them out yet. The sources of handedness can be classified into primary and secondary sources.

Secondary origin means transferring handedness from other handed causes. Handedness can either be transmitted vertically (inherited from pre-existing handed circumstances), or it can be transferred laterally (through interactions with other coexisting handed phenomena). Most chiral disruptive mechanisms described in the literature are examples of lateral transfer (Palache et al. 1962, Flores and Bonner 1974, Flores et al. 1977, Bada 1985, Collman et al. 1993, Zahn and Canary 2000). Chiral stabilization is a particular case of secondary handedness in which a system functions in a way that by definition preserves or amplifies an incipient chiral disruption (Salam 1991). Secondary sources cannot do more than transfer handedness from one physical reality to another, so they cannot be responsible for an increase in the overall chirality.

A primordial source is one that is capable of producing handedness without the pre-existence of a chiral disruption. Some potential examples are:

- Handedness may be the artifact of an intelligent culture. Although we use various technologies to produce chiral chemicals and other handed 'things' (Kagan et al. 1974, Knowless et al. 1982, Tani et al. 1982, Knowless 1985, 1986, Sharpless 1996, Noyori 2000), there is no evidence to support the idea that primordial chirality on Earth was in any way deliberate.
- Stochastic fluctuations are random events that result in spontaneous chiral disruptions and they can be followed by stabilization or amplification based on the way the system functions (Decker 1974, Thieman 1974, cited by Morozov 1978, Hegstrom and Konepudi 1990, Bonner 1992).
- An origin for handedness is proposed in the primordial heterogeneity, related to potential density fluctuations in the pre-Big-Bang era (Tegmark and Rees 1998).
- Transcopic interactions are handed interactions among phenomena (physical realities) occurring on very different scales. Because transcopic interactions are believed to have been relevant in the genesis of pre-biological chirality, they are explained in more detail.

The universe has no physical edge and is therefore infinite in all dimensional directions (time, volume and scale). For every given dimensional scale there is another scale that is above and many others that are below. Irrespective of

the scale to be described, the objects (phenomena or physical realities) from a smaller scale are called microscopic while the things from a larger scale are called macroscopic. Megascopic is a term more appropriate for describing 'things' situated at a very large scale with respect to a reference scale. Our galaxy is microscopic in relationship to its Big-Bang-generated universe while megascopic when compared to our planet. A microbial cell is microscopic in relationship to the Earth–Moon system, yet megascopic in relationship to one of its atoms. This scale difference results in a difference in physical perspective.

Megascopic objects can only 'observe' (i.e., experience the effects of) very large populations of microscopic objects, while most microscopic objects can only experience the physical effects of one megascopic object at a time. Using a parallel with Jonathan Swift's novel Gulliver's Travels (Swift 1726), a large crowd of Lilliputians cannot rotate Gulliver clockwise if half of them are left-swingers while half are right-swingers. Even if one group of 100 Lilliputians might manage locally to be totally composed of left-swingers (create a chiral heterogeneity), the overall effect would be the same. On the other hand, if Gulliver decides to rotate in one direction, all Lilliputians in contact with him will be affected by this asymmetry. Therefore, even if some handed microscopic objects display a heterogeneous distribution in the form of chiral patches across space, a megascopic 'observer' will only 'experience' them as a very large group. Therefore, the megascopic object is very likely to physically 'perceive' the microscopic objects as a racemic mixture and not be influenced by any minor local handed distribution.

In contrast to this, if some ants decide to climb along some vines to get to the top of a tree, even if half of the vines are left-handed and half are right-handed, each ant will only experience them one at a time and the ants from a chain will have a handed motion (either climb clockwise or counterclockwise). Although from a 'tree perspective', the world of vines is racemic, from an 'ant perspective' each trip is handed. Gulliver sees the world of Lilliputians as random while the Lilliputians see their universe as handed because they are so small most of them can only experience one Gulliver at a time.

Therefore, if handedness can be transferred (or experienced) among 'things or phenomena' situated on very different scales, micro-to-megascopic interactions are mostly racemic while the mega-to-microscopic interactions are handed. If megascopic objects are handed, even if they belong to a perfectly homogenous racemic mixture, they will be perceived as handed by all the microscopic objects they interact with. The principal postulate of transcopic interaction can be formulated as follows: "over time, a handed megascopic object (phenomenon or physical reality) capable of interacting with handed microscopic objects becomes surrounded by a region of handedness that stretches across space as far as the physical effect of

its own handedness and racemization rate allow." Certainly, the mega-to-micro interactions cannot make the universe more handed than it already is. Yet they can be responsible for local heterogeneities, thereby becoming relevant for the origin of a chirality-requiring phenomenon such as life.

Transcopic interactions have at least two theoretical consequences:

1. If our universe is overally handed, there are three probable explanations for this. Firstly, a significant early pre-Big-Bang bias or heterogeneity may have existed, thus creating a chirality in the distribution of the direction of rotation of the galaxies, which was further conveyed to lower scales of organization of matter. Secondly, other parallel universes beyond our 'physical sight' might influence the handedness of events in our universe. Finally, a physical reality on a scale beyond that of our own universe (a hyper-universe) may exist, inducing transcopic effects.
2. If the accretion of life required molecular chirality, it must have been easier for life to emerge in a place in space where a large scale asymmetry was also present (such as an asymmetric celestial circumstance). The advantage of a transcopic interaction is that it is long-lived and so can result in more than a minor short-term chiral disruption. It can also result in chiral maintenance or even chiral accumulation.

On a planetary scale, there exists a bias in the direction of rotation of fluid vortices (tornadoes, cyclones, dust devils, water spouts, water vortices, etc.). More counter-clockwise rotating vortices exist in the Northern Hemisphere and more clockwise vortices in the Southern Hemisphere. This phenomenon, the most popular example of handed transcopic interaction, is known as the Coriolis effect. One can also ask whether it is possible for some molecular-level chirality to have been created on the early Earth by a very large scale (e.g., celestial) asymmetry before life originated? One example is the chiral disruption of the asymmetric photolysis of amino acids during stereophotolytic exposure to sunset/sunrise UV-Circularly Polarized Light (Deutsch 1991). In this case the Sun itself represents the source of celestial asymmetry as slightly more Left-Circularly Polarized Light (LCPL) is produced at sunrise and slightly more Right-Circularly Polarized Light (RCPL) is produced at sunset, while the mornings are chillier than the afternoons. Consequently, just for celestial reasons, slightly more D-monomers would be degraded by stereophotolysis in the afternoon than L-isomers in the morning.

The particulars of the Moon's celestial orbit around 3.8 billion years ago (when life allegedly originated) have also been used to show how early chirality could have been amplified (Popa 1997). Earth's unusually large moon became locked into an orbital synchronization with the Earth rotation and this resulted in a tide-based resonant amplification of the photolysis of amino acids driven by circularly polarized UV light, thus creating a planetary excess of L-isomers (Popa 1997).

5.3 Evolutionary Steps Toward Biological Chirality

Understanding the origin of biological chirality is a challenge, because life as we know it cannot possibly function without a high level of chirality, and yet most abiotic mechanisms are not capable of producing and maintaining a significant chirality. At least four major steps were probably required to implement chirality in the terrestrial type of life (see Fig. 5.3):

- abiotic symmetry breaking,
- abiotic chiral amplification,
- biotic chiral amplification,
- chiral expansion.

Abiotic Symmetry Breaking (Chiral Disruption)

The exact source of early external handedness is hard to identify and has been attributed by different authors to a variety of factors such as stereo-interactions on quartz surfaces (Palache et al. 1962), preferential D-destruction by β-decay (Garay 1968, Petsko 1992), stereophotolysis with UV-Circularly Polarized Light (UV-CPL) (Mörtberg 1971) originating in neutron stars (Bonner 1992), stereo-absorption on kaolin (Flores and Bonner 1974), asymmetric polymerization on kaolin (McCullough and Lemmon 1974), pre-nucleoprotic metal complexes (Decker 1975), selective adsorption on calcite (Hazen 2001), photoresolution with Cr^{3+} (Norden 1977), weak-interaction-based parity violation (Konepudi and Nelson 1985, Szabó-Nagy and Keszthelyi 1999) leading to relative L-stabilization (Salam 1991), cross-interaction between D-pentoses and L-amino acids (Yarus 1988), asymmetric growth on crystals in the presence of magnetic fields (Thieman and Teutsch 1990), inhibition of D $\leftrightarrow$ L quantum tunneling as predicted by Hund's paradox (Goldanskii and Kuzmin 1991), enantiomeric crystallization from racemic mixtures of AAs (Viedma 2001), stereophotolysis with diurnal differences (sunset/sunrise) in the distribution of L-CPL and R-CPL (Kagan et al. 1974, Deutsch 1991) (see Fig. 5.3). The most important sources of early chirality are believed to be selective adsorption on mineral surfaces and asymmetric photolysis with UV-Circularly Polarized Light. While selective adsorption on minerals only had a local effect, asymmetric photolysis could have acted on a planetary scale.

Abiotic Chiral Amplification

Most of the abiotic chiral disruptive sources mentioned above did not produce large enough chiral disruptions to induce significant configurational effects in emergent polymers (see Fig. 5.3). Other abiotic mechanisms must have existed, increasing the disruption either through catastrophic changes from

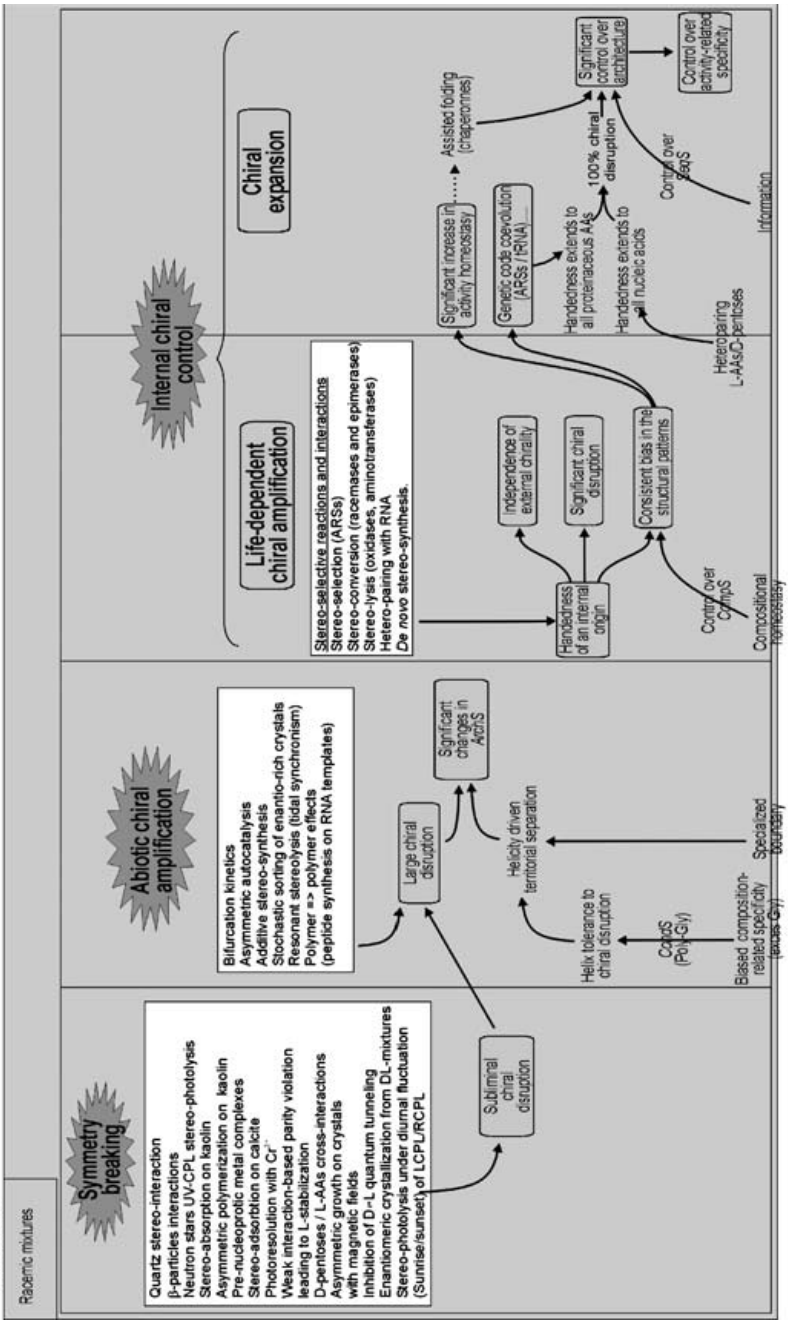


Fig. 5.3. Events associated with the origin of molecular chirality during the early evolution of life

a racemic state to homochirality or through gradual accumulation. Potential mechanisms are: bifurcation kinetics (Avetisov et al. 1991, Avetisov and Goldanskii 1993, 1996), asymmetric autocatalysis (Soai et al. 1995, 1999), additive stereosynthesis leading to gradual cumulative evolution (Mason 1988), stochastic sorting of enantio-rich crystals (Welch 2001), resonant stereolysis through tidal synchronism (Popa 1997) and polymer/polymer additive effects (e.g., peptide synthesis on RNA-like templates). On Earth, the synchronization between the lunar tides and photolysis due to UV-Circularly Polarized Light could have been one of the most important sources of planetary-scale amplification of chiral asymmetry (Popa 1997).

Particular attention has been paid to the possibility that chirality might maintain itself once it had emerged. Stereo-regular structures (polymers) favor more compact, regular conformations than random coils, and so are less exposed to degradation through hydrolysis (Boiteau et al. 2002). Upon exposure to periodic hydrolysis/condensation, this process results in an enhancement of stereo-regularity (local enantiomeric excess) (Boiteau et al. 2002). The excess of the amino acid glycine during abiotic synthesis (Harada and Fox 1964) resulted in a change in composition-based specificity and led to an architectural bias (more helices) after condensation. Helices have the ability to tolerate some level of chiral disruption (Morozov 1979) allowing early architectures to be stable even in the absence of 100% chirality. The addition of a hydrophobic boundary to a hydrophobic structure such as a helix would have acted as a mechanism for territorial separation of chirality (creation of a stable chiral heterogeneity) (see Fig. 5.3).

Life-Related Chiral Amplification

Life-related control over chirality followed a trend that focused on two evolutionary goals: the transition from external to internal control and the enhancement of chiral disruption. The major phase transition in the history of life-related chirality was the shift toward internal chiral control (see Fig. 5.3). This novelty is likely to have started with importation of a stereo-specific mechanism, i.e., mechanisms controlling chirality. After this historical moment, internally generated handedness must have reached a level of disruption high enough to make a significant configuration-related difference, ensuring functional steadiness and making external contribution obsolete.

These primordial mechanisms were in the form of helix-driven chiral stabilization and territorial separation (Blout and Idelson 1956, Harada 1970). As the internal chemistry became more complex, other control factors based on stereo-selective reactions and interactions were added (Lacey et al. 1993). Internal stereo-disruptive activities are now in the form of conformational stereo-selection operated by amino acyl tRNA synthetases (ARSs), stereo-conversion (racemases and epimerases), stereolysis (conversion of undesired isomers in other chemical forms performed by D-AAs oxidases and transaminases), and hetero-pairing with RNAs and *de novo* synthesis (see Fig. 5.3).

Irrespective of the specific mechanism, life-dependent chiral disruption has several important consequences: handedness of an internal origin, independence from external chirality, significant chiral disruption, and consistent bias in structural patterns. This bias was augmented by evolutionary upgrades in the control over composition-based homeostasy.

Chiral Expansion

The last major occurrence in the history of biological chirality was the expansion of chirality to other monomers. The early genetic code was much simpler in the beginning than it is now. Archaic codes were less precise and used fewer AAs and fewer nitrogenous bases (Goldman 1993, Hartman 1984). This may lead to the mistaken assumption that, since all modern AAs are L-stereoisomers, the composition of the outside world remained chiral throughout the entire history of the genetic code. However, strong physical coercions (environmental racemization) work against such a hypothesis.

A more reasonable alternative was suggested by studies of molecular phylogeny. The phylogeny of tRNA was found to be correlated with the biosynthetic distances among the AAs (DiGiulio 1994). This observation is in accordance with the 'coevolution hypothesis' (Wong 1975) which states that a coevolution existed between the biosynthetic pathways of the amino acids and the way the genetic code became organized. The first few chiral amino acids used by life (e.g., Ala, Pro, Arg) may have been imported from an environment where they were chirally disrupted by abiotic mechanisms. The rest of the proteinaceous amino acids were not imported, but rather biochemically-derived when the metabolism grew in complexity. Because the biosynthesis of amino acids (i.e., the transformation of one AAs into another) operates only on the side radical, the L-asymmetric configuration of the C_α is inherited along the biosynthetic pathway. This allowed the asymmetry of the earliest amino acids to expand to later ones.

Both the phylogeny of the tRNAs and the phylogeny of the AA-tRNA synthetases seem to support this scenario (DiGiulio 1992). The active site of the AA-tRNA synthetases involves both a precise recognition of the stereoisomery of the amino acids and recognition of their radical chain. Changes in the active site of the radical are not spatially dependent on changes in the L-recognition site. Therefore, mutant AA-tRNA synthetases will preferentially select the L-forms of the new amino acids. During molecular evolution, chirality is also believed to have expanded from amino acids to nucleic acids via the hetero-pairing between L-AAs and D-pentoses (Lacey et al. 1993).

Reaching almost 100% chiral disruption, the ability to control the sequence and assisted folding allowed, for the first time in evolution, full control over molecular architecture and activity-related specificity from lower levels of organization. This way the ultimate goal of molecular organization was achieved, i.e., an almost unlimited increase in complexity and functional diversity with little increase in 'molecular bureaucracy'. This integrated system

still has weaknesses because standardizing internal protocols leaves space for molecular parasites (selfish genes) whose activities speculate the way the host system functions. These cannot easily be avoided without compromising the performance of the entire system. Moreover, a centralized universal mechanism is like an Achilles' heel, making the system robust and hard to target but highly sensitive to a couple of minor factors such as the antibiotics that are interfering with translation. Furthermore, once a centralized hierarchy and genetic code establish their leading position over all cellular activities, they (the hierarchical organization and the code) become frozen in time with no possibility of changing or improving.

5.4 Handedness-Related Steps Toward Life

The major phase transitions during the evolution of biological chirality are as follows (see Fig. 5.4):

- Symmetry breaking, which consists in a chiral disruption of the natural racemic equilibrium. A variety of physicochemical mechanisms listed above could have been responsible for this transition.
- Life-independent chiral amplification which is required whenever chiral disruption is not large enough to have significant consequences on the architecture-related specificity.
- Internal chiral control, a mechanism which remains unclear. However, if life began with peptides, the internal chiral control was based on the inheritance of the L-configuration of the C_α during the biosynthetic transformation of one AA into another.

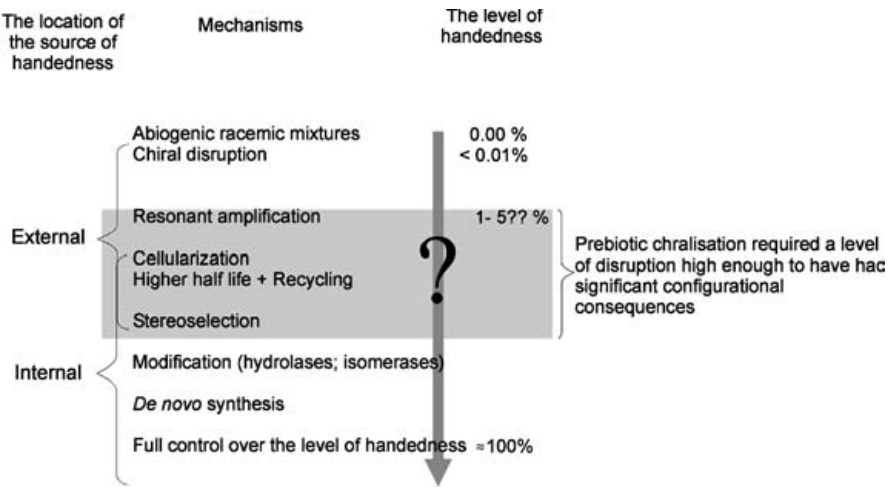


Fig. 5.4. Major evolutionary steps toward biological chirality

Our current knowledge only allows us to hypothesize that chirality applies to polymeric types of life only, where it brings significant managerial advantages (i.e., the simplification of architectural protocols). It has not yet been demonstrated that chirality is an absolute condition for life in general. While the types of life based on polymers would benefit greatly from the existence of chirality, other types of life might just as well be independent of it. However, chirality has played such an important role in the evolution of life on Earth that cannot be ignored by any theory of the origin of life. A minimal requirement for the existence of a terrestrial type of life is the natural emergence of handed monomers so that life cycles can be initiated at low complexity. If the amplification of abiotic chiral disruption was required, a large scale (planetary) and stable asymmetry was also required, acting as a condition for life.

6 The Early History of Bio-Information

> Not the good but their means of production must be inheritable.
>
> Cairns-Smith 1982

In a general sense, the information level of a structure equals the minimum number of instructions needed to specify the structure (Orgel 1973). The presence of a specialized system holding meaningful information (i.e., a genetic system) is essential for the existence of the living state, because any living entity must be capable of establishing, storing and maintaining a representation of its environment – a kind of encoded virtual reality (Lacey et al. 2002). This only refers to structures capable of containing information about themselves. Before competition among living forms, life had to face harsh competition with the random tendencies of the world. Therefore, for the early history of life, self-information may not be enough and it may only become meaningful (i.e., resulting in compositional specificity and steady function) if it can be shared among different entities (Yockey 1992, 2000). Consequently, bio-information "is much more than mere complexity or a decrease in comparative uncertainty in an environmental context" (Abel 2002).

Bio-information can be described as the amount of order, complexity and specificity that can be shared (or disclosed) among and about discrete systems. Shannon's information theory is purely mathematical and makes information oblivious to function, whence it cannot distinguish between meaningful (instructional) signals and noise (Abel 2002). Contrastingly, in life forms, no matter how many possible combinations a sequence has, there is no reason to call it information if it does not have the potential to induce something meaningful. Bio-information either has to possess some complexity that makes sense or it is random gibberish. The origin of bio-information is not the story of the development of some complexity that can be shared, but rather it is an instruction theory (Abel 2002), the story of some complexity that can be compressed and that makes sense when revealed. Any theory postulating that genes may have emerged randomly and then waited

R. Popa, *Between Necessity and Probability: Searching for the Definition and Origin of Life*, Adv. Astrobiol. Biogeophys., pp. 95–120 (2004)
http://www.springerlink.com/

to be used are fundamentally wrong, especially in a world dominated by the deleterious effects of the second law of thermodynamics. Genes had to have a functional meaning from the very beginning or they would have vanished soon after they emerged.

The advantage of using information is that the specificity of the activity can be stabilized at an optimal level which can stretch throughout the 'life' expectation of an individual. Moreover, the functional protocol (the knowhow or technological knowledge) can spread across time and space to other individuals. Both real life and artificial life are not random piles of genes but program-like things (genes or lines of codes or instruction, files connected to each other). Therefore, the information-related achievements of life must have occurred in a connected cascade.

This chapter focuses on the historical changes in the protocols used to convey information and the historical evolution of the material support of information in the particular case of life on Earth. The protocols used by modern cells to transfer information (replication, transcription and translation) are the result of an evolution. The ability to remember changes was as much important for the evolution of life as the ability to convey information from genes to catalysts. Early life forms must have had more primitive ways to 'remember' changes (variations) soon after they emerged and after they had proven to be useful. Without genetic memory, even the most fortunate or inspired biochemical novelties (or software protocols), instead of being progressive innovations, would be no more than short-lived and fruitless alterations.

One of the best known theories on the origin of bio-information was formulated by Manfred Eigen and his collaborators. They considered that a replicase (i.e., an enzyme capable of replicating RNA information) emerged first and that most of the complexity of life (quasi-species, hypercycles, metabolism, genome, adaptive evolution and others) were byproducts of this primordial event (Eigen and Schuster 1977, 1978a, 1978b, 1979, Eigen et al. 1981). Considering a spontaneous error rate during replication of 10^{-2} per nucleotide (Pörschke 1977), the first faithfully reproduced molecules should have been single-stranded RNAs not larger than 50–100 monomers. This model was expanded to the concept of hypercycle, the coevolution of spatially unlinked RNAs by cyclic functional coupling. In a hypercycle world, each RNA molecule encoded a polypeptide and supported the replication of the next member in the cycle (Eigen 1971). A hypercycle is an elaborate example of forward regulation.

Two general visions exist concerning early life: function-first hypotheses and replication-first hypotheses. The problem with a function-first hypothesis is its inability to explain the source of specificity that may be capable of preserving the function across time. The central problems of a replication-first hypothesis are: A replicative apparatus has to function almost perfectly if it is to function at all (Dyson 1997), and Replication means nothing without functional expression.

Many specialists (especially the supporters of the RNA world) now support the view that early molecules were at the same time catalysts and replicators. This issue remains unresolved from both a practical and a theoretical point of view.

A theory of early bio-information has to address issues such as the purpose of bio-information, the evolution of bio-information and the achievements of bio-information. The hypercycle approach is not the story of a simple life form, but an explanation of how later stages in life's evolution (after the apparition of translation machinery) may possess function. Despite its elaborate elegance, this approach does not make the problem of the origin of life any simpler, but adds more twists to an already complex puzzle. The hypercycle model only depicts a narrow segment of the history of information and it is unclear at this point if it would function at all. One potential problem with the hypercycle approach is its inability to overcome uncooperative mutants (Bresch et al. 1980). According to Manfred Eigen, the criterion for the survival of a system displaying replication of information is

$$N_\varepsilon < \log S \,,$$

where N is the number of bits of information that are replicated, ε is the probability of making an error when one bit of information is copied, N_ε is the number of bits of information lost by copying errors in each generation, and $\log S$ is a selection factor operated by the environment to penalize errors, equal to the number of bits of information supplied by the selective action of the environment.

In a stable environment, this condition is so stringent that it requires accurate (error-free) replication, otherwise errors accumulate over generations and eventually result in a progressive deterioration called an error catastrophe event (Eigen et al. 1981). In Manfred Eigen's model the jump of a population of N monomers from disorder toward order occurs with a probability of the order of

$$(1+\alpha)^{-N} \,,$$

where α is the chemical diversity of the monomers and N is the size of the molecular population. Calculations indicate that such a jump would only be statistically significant for $N \leq 100$ (Dyson 1997), which represents a very small population in chemical terms. Moreover, when thermodynamically sensitive polymers such as nucleic acids (NAs) are used to store information, incorrect replication is not the only source of mistakes. The overall variability is actually the sum of $N\varepsilon$, the non-replication-related mutations and the variation in the catalytic expression. If the total information loss (overall variability) is larger than the effect of selective pressure ($\log S$), a progressive degeneration becomes inevitable. Modern organisms have a tightly controlled variability with N of the order of 10^8 and ε of the order of 10^{-8}. As chemical mechanisms are far too simple to allow proofreading, prebiotic RNA replication could give error rates of 10^{-2} at best (Dyson 1997). This high

error probability makes such a system unsuitable for prebiotic evolution if $N > 100$, while no living system can be complex enough with 100 bits of inheritable information (Shannon 1949).

Computer simulations of Eigen's model for prebiotic evolution indicate that fluctuation and mutation also lead to three other potent perils (crises) of the hypercycle-based information packages (see Appendix C for descriptions) (Niesert et al. 1981):

- selfish RNA catastrophe,
- short-circuit catastrophe,
- population collapse catastrophe.

The probability of the selfish RNA catastrophe and the short-circuit catastrophe increase with the size of the molecular population (N), which must therefore be kept small while the probability of a population collapse increases in small populations and therefore requires much larger systems if it is to be avoided (Niesert et al. 1981).

Despite such numerical difficulties, one must agree with some of Manfred Eigen's postulates:

- One cannot talk about the full emergence of life without a mechanism to replicate information.
- Although variability may be detrimental to individuals, its necessity for the evolution of life is undisputed, whether evolution occurred in the form of random statistical fluctuations (Kimura 1970), or as a Darwinian selection (Dyson 1997).

Eigen assumed that life was organized in such a way that variability was always lower than the selective pressure. It is postulated here that the history of life-related information was much more gradual than the hi-fi replicase-based models imply. The $\log S$ selective term operates not on the results of replication, but on its functional expression as well. One cannot ignore the overall variability from this picture. The overall imprecision was probably larger in the past than it is now. Circumstantial and molecular evidence suggest that the history of bio-information had moments of considerably lower precision.

The genetic code is not totally random. It seems to hide an ancient rule of thumb and its molecular machinery appears to be the result of an evolution toward both complexity and precision (Kuhn 1972, Hartman 1975, 1978, 1984, Kuhn and Kuhn 1978, Barricelli 1977, 1979, DiGiulio et al. 1994, Ardell 1998). Even after 4 billion years of evolution, a certain level of informational imprecision still persists in the current genetic code. The obvious question is then: what would an early precise replication be good for, if its translation into active polymers was sloppy? One possible way to solve this conundrum is to assume that translation was a later event in life, and that it did not interfere with the invention of RNA replication. This theoretical option is

known as the RNA gene-enzyme conjecture and has often been used to imagine models of an ancient RNA world. However, a major rule in molecular macro-evolution is that the history of life could not have been regressive at any point. This means that the historical achievements of life could only have become stabilized if they brought some overall selective advantage. Adding sloppy translation (RNA → protein) into a world where RNA replication was allegedly perfect would have created a less competitive entity and not a better one. One possible alternative is that the RNA world may in fact have been an RNA/peptide world.

Another way to address this issue is by asking the question: is there more to information than the nucleic acids (NAs) display during translation? The internal information from any living entity has been traditionally separated into relevant (genetically purposeful) information:

$$I_{\mathrm{B}} = \log_2(n \times 2) \, ,$$

where I_{B} is purposeful information in bits, n is the number of negative feedbacks present in one individual, and 2 in brackets represents the minimal number of states a negative feedback loop can distinguish, and the total amount of information in the thermodynamic sense (Korzeniewski 2001). When messenger RNA molecules are translated into polypeptides, only part of the information (i.e., regarding their sequence) becomes a message embedded into output proteins. Newborn molecules do not hold enough information to allow a correct reconstruction of the parental RNA. Not only is the information in the sequence degraded, but no information regarding the molecular nature of RNA or its 3D architecture is ever translated. Consequently, the genetically purposeful information can be further divided into cryptic information (information that cannot be used as is and hence requires translation) and explicit information (functional knowhow).

When inherited information from modern DNA is discussed, we tend to forget the importance of all the explicit information contained in the rest of the cell. When a cell replicates, the daughter cells do not start on virgin territory with only the DNA–RNA information package at their disposal. They also inherit lots of explicit information (knowhow) in an already pre-organized way, 'telling' them how to handle things until the encrypted information gets deciphered (translated). Not even spores elude this absolute requirement. Spores carry a package of molecules that are used before they become active cells. In metazoans this issue is so important that the cell content (not only the NAs) is capable of defining what part of the genome is to be used with cyto-differentiation as a consequence. Depending on where they are located in space and with respect to each other within embryos, cells from our body become either skin cells, brain cells, or liver cells, using only one part of their full genome. This situation is analogous to a computer that is loaded with many possible programs yet becomes a MacIntosh, a PC, a Linux or a Unix the moment it is plugged into a specific location. This decision is fully controlled by the environment and not by the internal programming.

If modern life forms are not capable of initiating a living cycle with pure information (DNA), why should one believe that sloppy early life forms did so? If the principles of molecular evolution are right, in the beginning, the contribution of non-cryptic (non-NA-based) information must have been larger and not smaller as Manfred Eigen implies, and definitely never close to zero.

6.1 Early Sources of Bio-Information

The most important early sources of information acting even before replication were:

- probabilistic jumps toward order,
- environmental non-randomness.

Probabilistic Jumps from Disorder Toward Order

Such 'jumps' represent the probability that a random mixture of a types of monomer exposed to condensation will create a polymer that displays catalysis (an impaired reactivity b) in a space that is small enough to 'experience' its effects before the catalyst becomes thermodynamically obliterated (Dyson 1997). Due to the presence of the catalyst and an energy input, the system changes and becomes different, kept in an out-of-equilibrium state before an analogous catalyst (not identical but chemically related and performing in almost the same way) is statistically regenerated:

$$N(x) = \left(1 + ab^{-x}\right)^{-1} \ ,$$

where $N(x)$ is the probability of a right choice at each mutation inside one droplet containing a molecular population of size N (a right choice means a drift toward a certain quasi-stationary state), a defines the chemical diversity of the monomer units, and b is a quality factor describing the degree of discrimination of the catalysts. For a value $a = 8$–10, even if probabilistically one polymer might be catalytically appropriate ($b = 60$–100), its unique nature makes the probability of its emergence very low in large molecular populations ($N > 30\,000$) (Dyson 1997). Moreover, even if such an event might have happened, its effect was so 'diluted' and its repeatability so remote that it was not capable of making any informational difference, i.e., it could not influence its further occurrence.

This situation is somewhat analogous to Hardy–Weinberg equilibrium. Large populations without defined selective coercions are hard to push out of their state because small changes make little difference (they are subliminal). Analogously, large populations of solutes have no memory of randomly emerging catalysts and spontaneously ordered states. This is why it is believed that a phase separated system, i.e., any heterogeneous system creating some form of spatial seclusion such as pores within rocks, micelles, or bilayer vesicles, premerged a probabilistic jump toward order.

Environmental Non-Randomness (Dissymmetry)

This represents any gradual transition from disorder toward order directed by external ordering effects. This possibility was suggested by both the 'mineral-to-organic' theories (Odom et al. 1979, Coyne et al. 1981, Cairns-Smith 1982, Liebl et al. 1984, Coyne 1985, Cairns-Smith and Hartman 1986), and by the 'organic-to-organic' theories (Lohrmann and Orgel 1979, Orgel 1989). A variety of early polymers could have initiated life (Oró and Stephen-Sherwood, 1976, Hulshof and Ponnamperuma 1976, Ferris and Ertem 1992, Nielsen 1993, Eschenmoser 1994, Matthews 1995, Orgel 1995). When condensed in abiotic conditions, these polymers display a low specificity at all levels: overall composition, distribution of monomers along the chain, specific sequence, macromolecular architecture and activity (see Chap. 4). A totally arbitrary (random) composition is incongruent with the dependable activity required for the system to function away from equilibrium (Shannon 1949).

Because most physicochemical processes are random (blind choices), not every condensation protocol is meaningful with respect to the early control over order. According to the mathematical theory of communication, the conveyance of information must consider the consequences of three major communication problems: the technical problem, the semantic problem and the effectiveness problem (Shannon and Weaver 1963). These problems must somehow be addressed in every theory about early life information. A variety of interactions such as interactions between minerals and organics (Cairns-Smith 1982, Cairns-Smith and Hartman 1986, Arrhenius 2002), interactions between polynucleotides (Lohrmann and Orgel 1979, Rembold and Orgel 1994, Rembold et al. 1994), and interactions between polynucleotides and polypeptides (Orgel 1989, Barbier et al. 1993, Meleresch 1993, Zieboll and Orgel. 1994) have been shown to be non-random and to induce bias (order) during condensation (Liebl et al. 1984).

In the pre-life era, the two abiotic mechanisms presented above (probabilistic jumps and environmental non-randomness) generated distributional impairments in the early molecular chains that were based on statistical events, physical chemical gradients, local stereochemistry, thermodynamic stabilization, mutual transformations of monomers during condensation and mutual affinity (Liebl et al. 1984). This supports the idea that the most primitive source of order was not under the control of the 'pre-living entities' for at least part of their accretion. Later, as order became controlled from the inside, the information mechanisms also became more elaborate, more comprehensive and more accurate. Although one may grasp the overall picture of early bio-information, the chemical details remain unclear due to our lack of knowledge of the nature of the earliest bio-polymers (Hulshof and Ponnamperuma 1976, Ferris and Ertem 1992, Nielsen 1993, Eschenmoser 1994, Matthews 1995, Orgel 1995).

6.2 Contextual vs. Nominative Information and Explicit vs. Cryptic Information

It is generally assumed that the early catalysts required less information than modern enzymes. Isolated cations could have been involved in hydrogenation (e.g., Ni) and metal complexes such as FeS clusters could have mediated electron transfer, while organic monomers such as amino acids could have acted in a variety of catalytic ways (Bar-Nun et al. 1994). The existence of these catalytic performers did not require high precision and supports the idea that, in the beginning of life, there was no absolute need for an elaborate mechanism to control the specificity of the activity. A higher concentration of Ni in a prebiotic environment may have been enough to increase the probability of hydrogenating reactions. This biasing of the composition of 'newborn' molecules through environmental non-randomness is called contextual information (see Fig. 6.1). Contextual information is represented by alterations in the distributional evenness of the environment displayed as compositional heterogeneity or gradients. Contextual information may also be due to the overall properties of the physicochemical background, biased predecessors, vectorial interference from neighboring systems or impaired exchanges. The environmental bias so created resulted in a non-random composition of organic polymers after condensation (i.e., increased condensation specificity) (Liebl et al. 1984).

The next major step in the history of information was the switch to a nominative type of information (see Fig. 6.1). Using nominative information means 'empowering' discrete physical objects (such as mineral surfaces or molecular chains) with the ability to hold information. While using pH as a way to control reactions is an example of a contextual type of information, using RNA or DNA is a nominative type of information. Nominative sources of information are more compact (they show higher information density) than contextual sources. They are also more accurate and can be more stable if maintained in appropriate conditions. Using the principle of continuity (Lahav 1999), one may assume that the passage from contextual to nominative information occurred through using discrete objects such as mineral surfaces, capable of both local alterations in their physicochemical neighborhood and stereochemical effects. Minerals have a periodic structure but they can tolerate some level of crystal defects in their lattice (Cairns-Smith 1982). This aperiodic property may allow individual crystals to carry specific messages (Arrhenius 2002).

Nominative information can be of three types: explicit, cryptic and symbolic.

Explicit Information

Explicit information means that structural patterns or 'knowhow' may be conveyed or copied directly as they are without the need for translation.

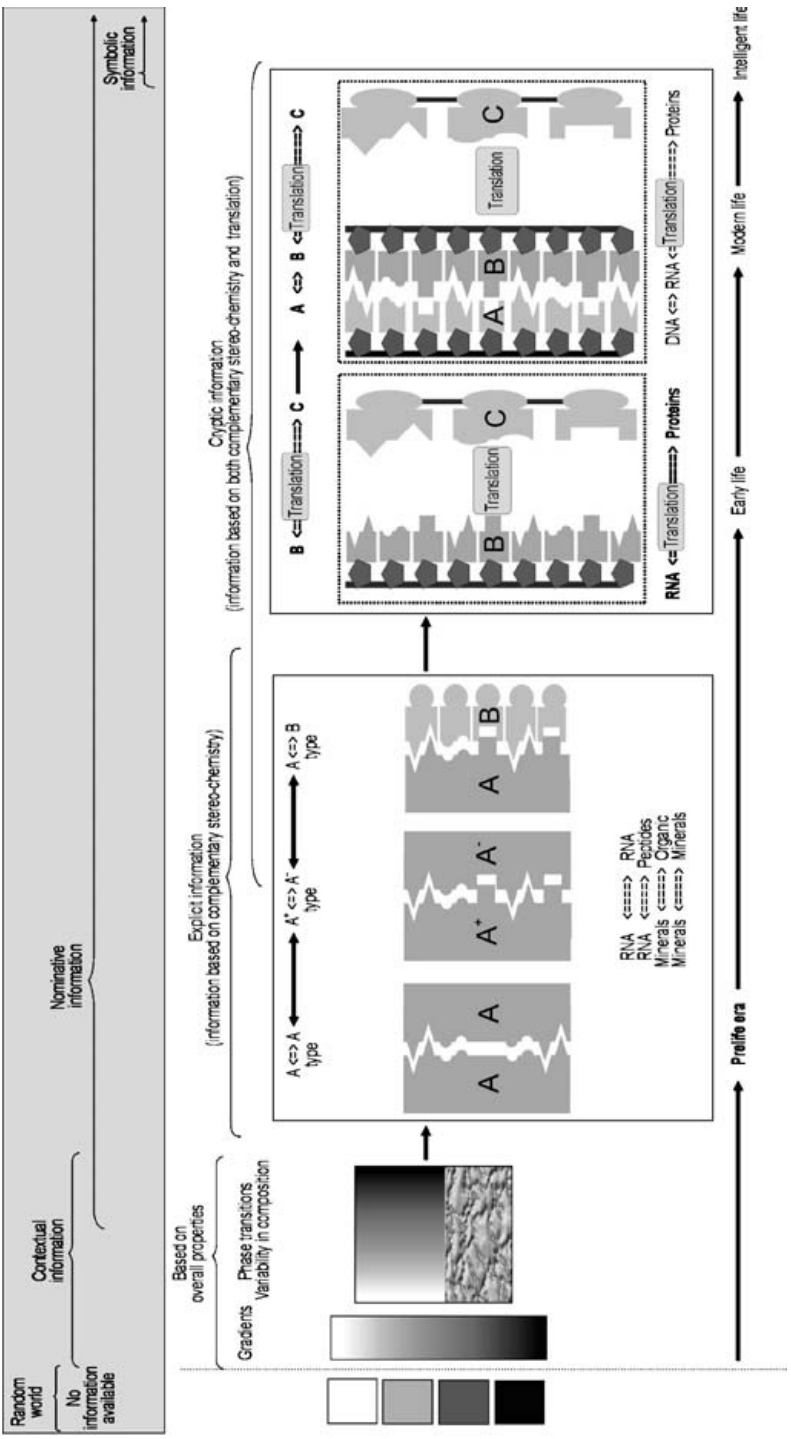


Fig. 6.1. Various types of information used during the early history of life

In a chemical world, explicit information is generally based on complementary stereochemistry, either as sequence complementarity (Milosavljevic 1995), landscape topography, or 3D arrangements. Examples of explicit protocols leading to non-random outcomes are the making of replicas (molds) from surfaces, DNA replication, DNA → RNA transcription and membrane heredity (see Fig. 6.2). Some examples of abiotic energy-dissipative systems containing explicit information regarding their own makeup and their own functioning are inheritance of handedness in replicated fluid vortices, crystal accretion, and fractal growth. In a world based on molecular chains, there are three possible types of explicit information conveyance that may have had some relevance to the early life forms on Earth (see Fig. 6.1):

- The A⟵⟶A type of explicit information means that the source and the byproduct (the donor and the recipient of information) are identical. They have the same physicochemical nature and their message is a palindrome (it reads the same from both directions).

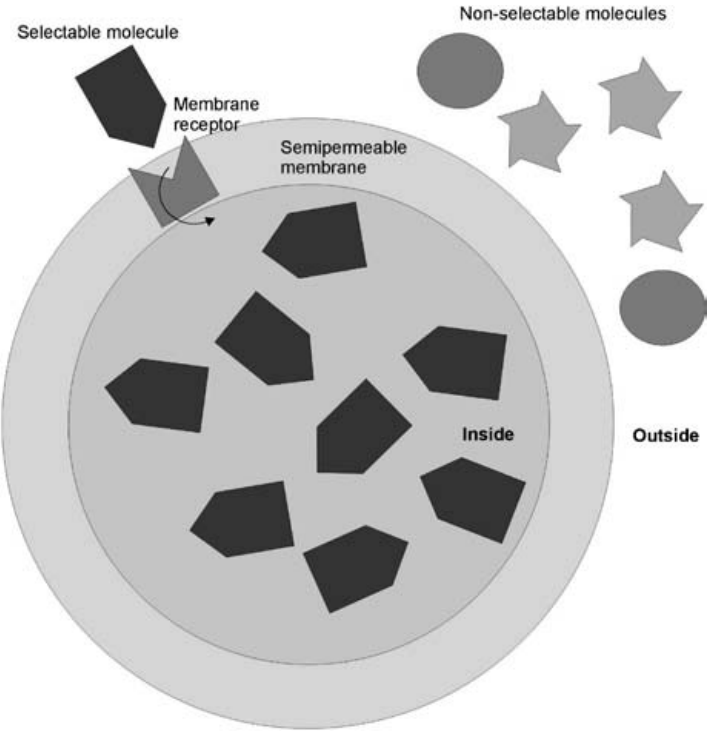


Fig. 6.2. Principle of membrane heredity. Specific receptors present in membranes may select the molecules entering the cells, thereby creating a distributional disequilibrium inside. Adapted from Cavalier-Smith 2000 and Szathmáry 2002

- The $A^+ \longleftrightarrow A^-$ type of explicit information is very similar to making a key using a non-identical but geometrically complementary mold. This type of sequence has the advantage of a defined direction of reading and higher diversity than the A⟷A type. It has the disadvantage that the sequence A^- is different from the sequence A^+, so that an extra copying of A^- is required to generate an A^+.
- The A⟷B type of explicit information means that, although stereochemical recognition exists, the donor and recipient have different chemical natures.

In modern life forms, all three types of explicit information are used. The A⟷A and $A^+ \longleftrightarrow A^-$ types are present in DNA replication, while the A⟷B type is present in DNA⟷RNA transcription.

Cryptic Information (or Encrypted Information)

Cryptic information means that the message is hidden in a specialized form and requires some form of language and a translation instrument to decipher (to be revealed). Building a house by copying another one piece by piece may be an example of an explicit protocol; building a house using a blueprint involves both explicit and cryptic information; but building the house from information contained solely in a detailed text (with no pictures or drawings) is a purely cryptic protocol. Computers use a cryptic mode for transferring information, performed by a processor which transforms the initial information into a succession of 0s and 1s according to a language or a binary code.

Symbolic Information

Symbolic information is represented by conveying a message that is structurally and functionally unrelated to the outcome. Examples are inducible and repressible genetic regulation, the use of hormones, cyto-differentiation factors, quorum sensing, and behavioral signals. The symbolic type of information is in itself ambiguous and hence cannot act alone. It has to be backed up by other forms of information. Symbolic information is not instructional knowhow. It is the trigger for initiating specific protocols. In the building of a house, an example of symbolic information would be the message: start building a house of type A.

In the early days of evolution information was explicit, then cryptic information was added, and only late in biological evolution was symbolic information used (see Fig. 6.1). The type of explicit information used first by life on Earth (A⟷A, $A^+ \longleftrightarrow A^-$ or A⟷B) is unclear and depends on the physical nature of the first information carriers.

Three basic groups of hypotheses addressing the composition of the earliest polymers may be used to understand the early information protocols:

- peptides-first hypotheses,
- nucleic-acids-first hypotheses,
- mineral world hypotheses.

Peptides-First World

According to the peptides-first hypotheses, peptide chains were the first catalysts used by life (Oparin 1924, 1968, Haldane 1929, Fox 1964, Calvin 1969, Bernal 1967). Besides catalysis, peptides may have served other functions as well (Dzik 2002). The belief that peptides were earlier polymers than polynucleotides is supported by the fact that the origin of nucleotide-related components (especially pyrimidines and nucleotides) is hard to explain (a near-miracle in prebiotic conditions), while amino acids and peptides are much easier to form (Guimarães 2002). This vision is also supported by the existence of unusual amino acids in non-ribosomal-made peptides. Against it is the need for chirality in amino acids, making the correlation between sequence and architecture possible. If peptides were indeed the earliest active molecules, then their non-randomness should have been relatively easy to control through environmental impairment (Liebl et al. 1984, Kunin 2000). Later confinement in small populations such as in vesicles further extended and expanded this heterogeneous distribution. In a subsequent step, a biased composition and biased distribution of various amino acids along the peptide chains was achieved through charge gradients or through stereochemically-controlled complementarity.

The complementarity among polypeptides (of an $A^+ \longleftrightarrow A^-$ type) later evolved into self-assemblage, protein–protein complex formation, protein–ligand complex formation and signal transduction (Buiatti and Buiatti 2001). Polypeptides contain considerable explicit information, but they are not the genetic material of life (i.e., physical support for its blueprint). Later in evolution, the NA-to-protein relationship was used to build a translation mechanism. The protein-first proposals are bottom-up theories and are essentially claiming that, in the origin of life, thermodynamic sustainability is more important than genes (Guimarães 2002). The major weakness of any peptides-first world theory is the implication that, at one point in evolution, information must somehow have been transferred from polypeptides to RNA in order to ensure functional continuity.

Although such a possibility has already been proposed (Lacey and Pruitt 1969) no compelling evidence exists for it. Neither has any molecular evidence yet been found to indicate that protein-to-NA information or any form of reverse translation may ever have existed. Moreover, nucleic acids such as DNA are made of a low variety of monomers (A, T, G and C) while polypeptides are made of 20 monomers. Thus the entropy of the DNA sequence is $\log_2 61$ while the entropy of the protein sequence is $\log_2 20$ (Yockey 2002). Consequently, a several-to-one code must exist to drive information from mRNA to proteins (Yockey 2002). The ambiguity of the reverse direction makes the

communication of information toward RNA questionable and this has been used to argue against a peptides-first world (Yockey 2002).

Nucleic-Acids-First World (RNA World)

Although the notion of an RNA world is quite popular in the current literature, considerable confusion exists about its intended meaning. Some authors suggest that the RNA world means that life started with a set of nucleic-acid-related chemicals, while others suggest that, at one point during the evolution of life, RNA was at the same time the major holder of genetic information and a key catalyst. Both theories are right on one issue: RNA-related molecules were (and still are today) important in both information-related and physiological activities. Personally, I believe that:

- life did not start with RNA,
- RNA-related molecules became very important tools in internal biochemistry,
- RNA became at one point the major genetic material,
- life invented and used the exotic chemistry of the nucleic acids to avoid 'interference from external chemical agents'.

According to some, specificity in an RNA world had an early period of environmental control when the information in polymers was assisted by mineral surfaces (Ferris et al. 1990, Ferris and Ertem 1992). This step was followed by the emergence of semi-conservative replication, a process based on a Watson–Crick type of pairing (Lohrmann and Orgel 1979, Eigen et al. 1981, Kanavarioti 1994, Orgel 1995). The semi-conservative replication was added to a selection that was based exclusively on thermodynamic stability. It led to a significant increase in specificity and to the emergence of the first gene (Gilbert 1986, Cech 1986, 1987, Eschenmoser 1994).

As in a peptides-first world, information was based on a 'mineral memory', before it became of an explicit $A \longleftrightarrow A$ type and/or an explicit $A^+ \longleftrightarrow A^-$ type. The emergence of an $A^+ \longleftrightarrow A^-$ type brought a significant increase in the genetic diversity and later allowed a separation of functions (one strand being used for storing information while the other was used for catalysis). According to the RNA world view, the $A \longleftrightarrow B$ type of explicit information is secondary to an $A^+ \longleftrightarrow A^-$ type, but in contrast to the peptides-first views, the $A \longleftrightarrow A$ type could have been acting much earlier. Ribozymes, which are enzymes made of RNA, supposedly evolved in complexity by gradual addition of polypeptide fragments (Dzik 2002). Finally, "the polynucleotide segments allegedly disappeared in most enzymes, with some biochemical living fossils preserved, like coenzyme A composed of one nucleotide and two amino acids" (Jeffares et al. 1998, Kyrpides and Ouzonis 1995, Dzik 2002).

Scientists have invested considerable effort to demonstrate that life may have been initiated by a gene (Muller 1966). The RNA-world-related views

consider that some early NA-like molecules were used as both information-containing units and as catalysts (Eigen et al. 1981, Guerrier-Takada and Altman 1984, Gilbert 1986, Cech 1986, 1987, 1993, Ferris 1993, Orgel 1995, 2000, 2001, Maniloff 1996, Schöning et al. 2000). Although appealing, this theory is not so easy to demonstrate because ribose is too sensitive to thermal degradation (Dzik 2002), because nucleotides are hard to form, and because NA-like polymers are complex structures. Most RNA-first theories are top-down approaches (Guimarães 2002), stating that the genetic material is the most important part of life. This vision suffers of a "selfish gene syndrome" and attributes the very existence of life to a self-perpetuating tendency. This tendency is not sufficiently supported by the laws of physics as we know them and thus using it as an explanation for life is essentially the same as believing in vital forces or entelechy.

Mineral-to-Organic World Hypotheses

The mineral-to-organic world hypotheses (Mekler 1980, Kuhn and Waser 1981, Cairns-Smith 1982, Fox and Matsuno 1983, Wächtershäuser 1988, Nussinov and Maron 1990, Clegg and Wheatley 1991, Arrhenius 2002) consider that the order associated with some natural periodic structures (such as minerals) or some aperiodic accidents on their surface represented the first source of transferable information. This view is supported by some experimental evidence which suggests that peptides or polynucleotides were formed by condensation on mineral surfaces (Ferris et al. 1996).

The mineral-world vision is based on inorganic-to-organic stereochemistry and it is used in both the peptides-first and the RNA-first hypotheses. Although the more efficient organic-to-organic stereochemistry later replaced this mechanism, this stereochemical connection between minerals and molecular architectures was never forgotten by life. Modern biomineralization which entails both crystallization at inorganic–organic interfaces and biomimetic synthesis (Mann et al. 1993, Brown et al. 2000) may represent molecular fossils of the mineral world era, preserved through eons of biochemical evolution. The translation of the message from mineral crystals to condensing assemblages of organic molecules remains "the prebiotic chemist's dream" (Joyce and Orgel 1999). "Although it should be experimentally verifiable this goal was not achieved yet" (Arrhenius 2002).

These three views: the peptides-first world, the RNA world and the mineral-to-organic world are consistent with the overall sequence presented in Fig. 6.1. In all these hypotheses, a contextual source of information must have been the earliest type of information used by pre-life entities and it was followed by a world dominated by a nominative source of information. Both the peptides-first world view and the RNA world view support the idea that an explicit source of information existed before the invention of encryption. Therefore, it is claimed here that the information-transferring protocol

changed over time, and that a gradual increase in specificity existed in the history of life. This vision is distinct from those theories supporting a grandiose phase transition toward life via a spontaneously emerged hi-fi replicase.

6.3 Postulates of the Early Evolution of Bio-Information

The following information-related postulates are emphasized here:

1. Information is not the most appropriate way to explain the very beginning of life. Other achievements (such as those related to the conservation of energy, the maintenance of the level of negentropy and the maintenance of specialized boundaries) must have happened before information, and acted in ways that increased the probability for the organization of modern informational protocols.
2. Life did not start with the self-replication of information (Eigen and Schuster 1977), nor with the assisted replication of information (Kunin 2000). Other more primitive information protocols were used in the very beginning, controlling the specificity of the catalytic oligomers. Instead of being replicated, early information was rather preserved, shared and enhanced.
3. A hi-fi replicase and the translation machinery were late achievements in the accretion of life (possibly emerging after the control over handedness).
4. Before being of a nominative and cryptic type (e.g., DNA, mRNA, genomic RNA), early bio-information was of an explicit type. The early explicit information was in the form of consistent chemical biases and later in the form of inheritance of specialized catalysts and molecular packages. The early explicit type of information did not disappear, but remained forever associated with life's inheritance, and it is still present in modern life forms.
5. Life on Earth could not start with one single gene or one single function, much as artificial life cannot start with one single line of code.
6. For the accretion of a molecular type of life, the use of explicit information is more important than the use of cryptic information. If the explicit information is lost and the life cycle is severed, the purely informational molecules of life (such as DNA) cannot restart life by themselves.
7. In the presence of explicit information, variation was not as risky an endeavor for early life forms as cryptic information was. Thus, pre-life entities that were primarily based on explicit information were more robust to variation than the $\log S$ term assumes, because changes in explicit information protocols do not result in severe consequences that are so common when using cryptic information.
8. Although information may be directed from RNA-like molecules toward DNA, it is not clear whether this type of molecular Lamarckism ever applied from polypeptides toward NAs.

Assuming that the vision presented here is reasonably close to reality, I will next draw a picture of the overall evolution of early bio-information on Earth.

6.3.1 The Contextual Information Era

A variety of physical attractors (efficiency-directed crises) drove the evolutionary changes in the information protocols. In a large and random aqueous solution, a statistical jump toward an ordered state has an extremely low probability. Developments along other main issues of life such as boundary or energy plus environmental heterogeneity may have significantly increased this probability. Due to a non-homogeneous distribution of monomers and to nearest-neighbor preferences, some peptides may have been more abundant than others on the early Earth (Tyagi and Ponnamperuma 1990). Entrapment within a closed space led to a decrease in population size ($N = 10^4$ in a spheroid with $\varnothing = 1$ μm at 1 mM). The presence of reflexive activity extended the temporal existence of energy-dissipative entities while the use of contextual sources of information based on natural asymmetries (such as gradients and local chemistry) biased the compositional distribution.

These factors are not necessarily causal because they do not induce a transition toward order with a probability that is close to 100%. Therefore, probabilistic jumps must be considered as phase transitions of the history of life and not as emergent events. Even in the presence of such probabilistic jumps, the activity specificity remained relatively low ($b = 60$–100) (Dyson 1997). The resulting 'away from random' distribution can be expressed either as an energy term (thermodynamic distance from the outside equilibrium), or it can be described as a quasi-stationary state inside an attraction basin (a matrix representation based on the concentration range, the mean and the variance of each component).

If physical separation persists (such as in the presence of a stable boundary or an impaired exchange), this situation extends over time beyond its expected limits because subsequent chemical developments bias the outcome. In a tridimensional space this can be suggested by a deeper basin of attraction for the steady state or as a higher transition boundary (taller saddle-passes), and requires a high energy to transit the system into another state. Even if it represents a source of specificity, a contextual source of information is not the most advantageous option because it has little genetic memory (it is hard to restore after equilibrium is overrun). A contextual source of information only exists as long as a natural heterogeneity (e.g., a chemical gradient) exists. This form of asymmetry is local, and so cannot be carried across space.

6.3.2 The Mineral-to-Organic Era

One of the earliest achievements of life associated with the history of information is variability. In a prebiotic world using contextual information,

variability is synonymous with environmental heterogeneity and has little adaptive value. Later, in a world of nominative information based on external sources of information (such as mineral surfaces), variability was represented by the architectural tolerance to compositional plasticity. Later still, when information was assigned to specialized carriers such as NAs, variability was represented by inheritable sequence alterations. The selection of the right genetic material was based on the structural robustness of the carrier to alterations in its sequence. This may as well be the very reason why in NAs the units involved in stereo-recognition (nitrogenous bases) are remote from the part binding the nucleotides from the same strand (the phosphodiester bridge C3′–O–P–O–C5′).

In a further phase of prebiotic evolution, the transition was made from using a contextual source of information to using a nominative source of information (see Fig. 6.1). The simplest type of nominative information in nature is the use of physically stable asymmetries (aperiodic or periodic events on the landscape topography of mineral surfaces). This particular type of A⟷B protocol (where A is a mineral surface and B an organic mixture or polymer) was derived from a contextual source, which consisted in the participation of mineral surfaces in alterations of the local physical chemistry (e.g., acid/base, hydrophobic/hydrophilic, hydration/dehydration, coordinative chemistry and magnetic effects). The basis for this type of information protocol is the differential affinity of various monomers with surface anisotropies, and results in a non-symmetric distribution of monomers. This mechanisms works better with amino acids than with nucleotides because amino acids have a wider variety of side radicals. Based on judgments regarding chemical complexity and thermodynamic stability, most researchers now believe that RNA-like molecules were less likely than peptide chains as the very primordial catalysts. The chemical diversity of the AAs, plus their relative ease of condensation in prebiotic conditions, the higher stability of the amide bond and their simpler chain make them better candidates for mineral-to-organic interactions. Would this mean that prebiotic information flowed first from minerals to peptides?

The mineral-to-peptides world was represented by spatially localized activities resulting only in low non-equilibrium consequences. Although polypeptides are more stable and interact better with mineral surfaces, experimental evidence indicates that mineral surfaces can also interact with nucleotides and nucleic acids (Odom. et al. 1979, Lawless et al. 1985, Ferris et al. 1990, Ferris and Ertem 1992). This supports the view that at some point two worlds may have coexisted: a mineral-to-peptide world and a mineral-to-NA world. Although mineral sources are not rich in genetically useful information, they are better than contextual sources. Micro-crystallites of nm size may have been moved along being trapped inside vesicles and they may even have served as a way to harvest energy (e.g., solar light, redox energy or hydration/dehydration). Despite considerable theoretical and experimental

effort (Cairns-Smith 1982, Coyne 1985, Cairns-Smith and Hartman 1986), the replication of this type of information has not yet been demonstrated and more progress is required to understand its alternative uses.

6.3.3 The Organic-to-Organic Era

The use of mineral surfaces as memory holders has both advantages and disadvantages. Mineral surfaces are very stable and require little or no biochemical mechanism to form. They are also self-ordered periodic structures, thus requiring little or no external information to assemble. However, most mineral crystals have little variability and are hard to copy. Moreover, the information is only available at the surface and crystals are often parts of larger objects (rocks) that cannot be moved along. One of the greatest risks for the early cells was poisoning themselves with their own waste products (Nealson 2002). To avoid this and to identify fresh resources, early cells needed to move (or be moved) to other areas, which meant losing contact with any external sources of information (e.g., mineral surfaces). Therefore, the next step in the evolution of information must have been represented by information derived from organic-to-organic interactions.

It is not possible to know which type of nominative information (A$\longleftrightarrow$A, $A^+\longleftrightarrow A^-$ or A$\longleftrightarrow$B) came first, because the nature of the first biomolecules is unknown. Not all types of explicit information listed above were necessarily present. Entrapment inside vesicles served not only as a means to promote a probabilistic jump toward order but also to facilitate organic-to-organic stereochemistry as well. It is assumed that organic molecules took charge over their own assembly using self-organizing principles (Muthukumar et al. 1997) and that the mineral information gradually became obsolete. A new organic-to-organic world era began (see Fig. 6.3). Evolution moved from simple to complex and from general to particular, as specificity was conveyed first on the basis of overall chemical properties, then on the basis of overall geometry, and later through precise sequences.

At this point in early evolution, a revolutionary technology also emerged, namely replication, a feature with considerable significance for life. Replication provided the means for 'remembering' and spreading meaningful biochemical novelties. Although the self-replication of peptides has been demonstrated experimentally via a simple thioester-based activation (Lee et al. 1996), a peptide-to-peptide replication model of life is still in its theoretical infancy. A similar limitation applies to a peptide-to-NA world (Nielsen 1993). The emergence of a way to replicate RNA led to what is called the RNA world, in which RNA-like molecules served a dual role: informational and catalytic (Eigen 1971, Bass and Cech 1984, Pace and Marsh 1985, Sharp 1985, Gilbert 1986, Orgel 1986, North 1987, Joyce 1989, Lamond and Gibson 1990, DeDuve 1991, Cech 1993, Unrau and Bartel 1998). There are two possible ways to replicate NAs: self-replication based on strand complementarity (Cech 1986, Joyce 1989, Rembold and Orgel 1994, Rembold et al. 1994, James

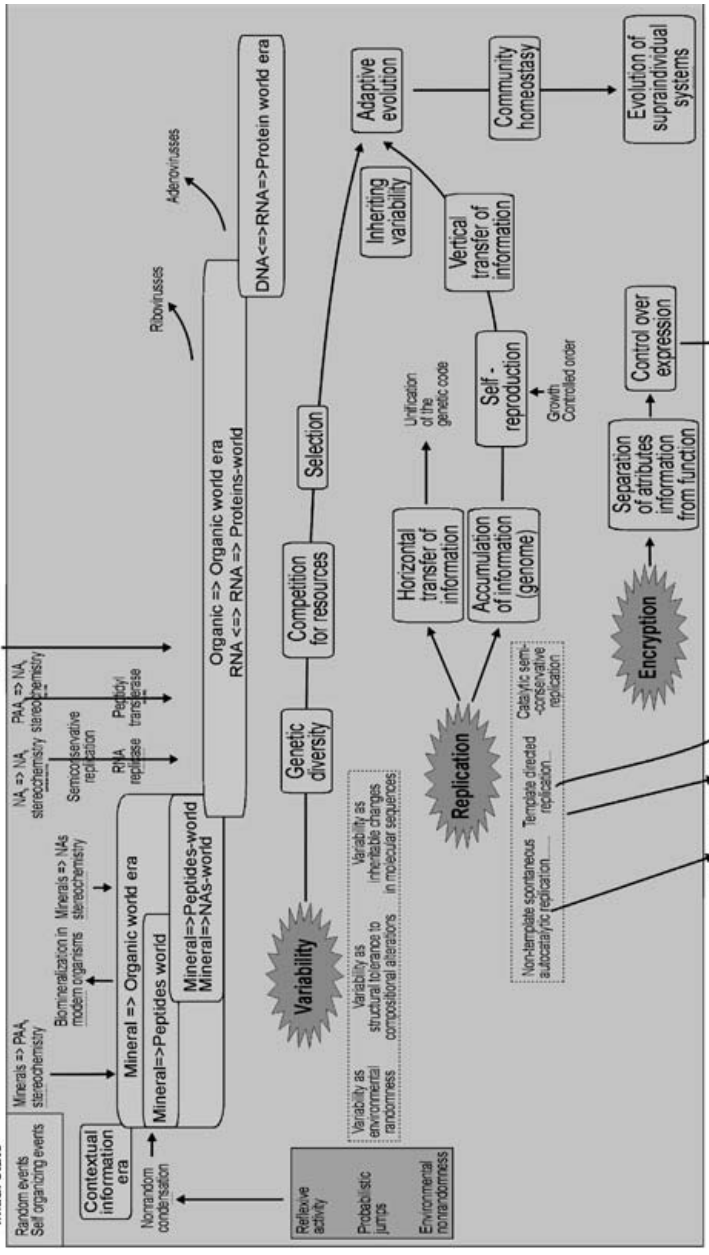


Fig. 6.3. Upgrades of information protocols during the early evolution of life

and Ellington 1995), polypeptide-assisted replication (Eigen 1971, Biebricher et al. 1986, Orgel 1989, Meleresch 1993). RNA may not have been the most primitive type of nucleic acid (Joyce et al. 1987), but no molecular fossils of its predecessors exist in modern life forms to provide a clue to the past.

Although an organic-to-organic-based stereochemical protocol has advantages over a mineral-to-organic protocol, such as compactness, reproducibility, better stereochemistry, mobility and the possibility of replication, organic-to-organic protocols are far from being simple. Therefore, they should not be used lightly when attempting to explain the origin of life.

One limitation of linear information is that it may develop in two possible directions in space. One possible solution for the directional imprecision of the A⟷A protocols is the use of palindromes. If an A⟷A type of explicit information was ever used by life, palindromes could not have been too relevant, because the diversity of palindromes does not increase greatly with a (where a is the monomer diversity). This rigor leaves little space for variation, because one mutation at one end has to be backed up by a similar mutation at the opposite end in order for the sequence to remain a palindrome. Another disadvantage of the A⟷A type of information is that, if no chemical difference existed between information holders and catalytic performers, each biomolecule must have served a dual role (Gilbert 1986). Such sources of information cannot be used selectively inside protocells and a time-dependent regulation (either homeostasy or programmatic activity) could not therefore be implemented. The parts of such a primeval metabolism could not be initiated or shut down whenever required because all the molecules present inside (genes or catalysts) acted at their full reactive potential at all times. Holding a collection of alternative pathways (physiological backups) would also have been impossible. Therefore, it may be speculated that an A⟷A control (if it ever existed) preceded homeostasy, metabolic cycles, discriminate expression and metabolic ontogeny.

The A^+⟷A^- type of explicit information appears to be more convenient for early life than the A⟷A type of information. The A^+⟷A^- type of information has higher diversity (i.e., better information content) for the same number of digits. This allows larger variation and metabolic diversity. Yet such an information protocol was far from being the ideal solution for various reasons:

- It is hard to implement in the absence of a configuration-based pairing. (In fact, it has to be based on NAs because peptides do not display a Watson–Crick type of pairing.)
- It requires some form of assistance to control the reading direction.
- It requires non-symmetrical bonding of the assistant in order to discriminate the direction along the chain. It is possible that this need for spatial orientation of the message may have led to another molecular fossil; the selection of those monomers that were bound through asymmetric bonds (C–NH–CO–C in polypeptides and 5′C–O–P–. . .–P–O–3′C in NAs).

- Information and expression had identical chemical substrates, thus complicating the internal management of early pathways.

6.3.4 The Emergence of Encryption

On the basis of what is known about modern biopolymers, the early mineral-to-organic world could have been populated by at least two major types of entities: mineral–peptide preliving units and mineral–NA preliving units. The mineral–peptide units had higher thermodynamic stability, higher complexity, deeper steady-state-related attraction basins, higher catalytic diversity and a lower propensity for complementary self-pairing than the mineral–NA units. The association of two such units may have been the second primeval symbiosis after the association between self-assembled vesicles and reflexive activity. This symbiosis was only possible if the interference between the biochemistry of the two types of units was not too strong. Such a relationship requires more than mutual tolerance. It requires specific chemical recognition between peptides (or amino acids) and nucleic acids (or their monomers). This recognition has been intensively explored experimentally (Behmoaras et al. 1981, Orgel 1989, Barbier et al. 1993, Lacey et al. 1993, Melleresch 1993, Connell and Yarus 1994, Zhang and Egli 1994, Zieboll and Orgel 1994, Yarus 1988, 1998). The RNA-like partner in this symbiosis was specialized in maintaining the accuracy of replication, while the peptide partner was specialized in metabolic expression (catalysis, material exchange, energy harvesting) and in the thermodynamic protection of the NAs.

Early polypeptides were simple and synthesized by a mechanism other than ribosome-based translation (Dzik 2002). The translation machinery, i.e., the decryption of information, is believed to have emerged as a byproduct of the structural landscape complementarity between peptides and nucleic acids (Buiatti and Buiatti 2001). Experimental evidence shows that chiral hetero-pairing also existed between peptides and nucleic acids (Gabbay 1968, Gabbay et al. 1968a, 1968b, 1972, 1976, Gabbay and Kleinman 1970, Adawadker et al. 1975, Sheardy and Gabbay 1983), which increased the thermodynamic stability (Guimarães 2002) and internal control over handedness.

Gradually, the early nucleic-acid-based world took charge of:

- the condensation of amino acids (peptidyl transferase),
- the stereo-specificity of codon–anticodon recognition.

The peptides reciprocated the symbiosis by performing:

- the catalytic activation of amino acids on the carboxyl group (AA adenylation) (Orgel 1989),
- the semi-conservative replication of nucleic acids (RNA replicase),
- the specific association between early tRNAs and adenylated amino acids [it has been shown that modern AAtRNA synthetases belong to two families of proteins believed to have been initially encoded by complementary strands (Rodin and Ohno 1995)],

- energetic metabolism and thermodynamic stabilization of nucleic acids (histone-analogous functions) (Hasegawa and Yano 1975, Ishigami and Naguno 1975, Hartman 1978, 1984, 1995, Lacey and Staves 1990, Knight and Landweber 2000).

In time, the importance of minerals as information carriers decreased until they became 'technologically' obsolete.

The details of the early peptide–nucleic acid association are unclear. It has been hypothesized that RNA replication was preceded by the attachment of the amino acids to the $2'(3')$-termini of RNA templates that favored the initiation of replication at the end of the template rather than at internal positions. It has also been hypothesized that protein synthesis was preceded by the association of pairs of charged RNA adaptors in such a way as to favor non-coded formation of peptides (Orgel 1989). Polar and stereochemical requirements seem to have played an important role in the establishment of the genetic code (Goldman 1993, Hartman 1995). The emergence of a proteinaceous hi-fi RNA replicase requires a high level of chirality (Bonner 1995). This requirement allows an alignment between the history of handedness and the history of information-related protocols.

The coupling between an RNA replicase and a peptidyl transferase has so many derived consequences that it has been assumed to be the starting point for biological life (Kunin 2000). This event also signals the emergence of encryption, considered in this theory as the last fundamental phase transition step required for the assemblage of life. Although the genetic code may always have been a triplet (Maddox 1994), the modern translation machinery is the result of a long evolution toward complexity and toward error minimization (Orgel 1968, Kuhn 1972, Hartman 1975, 1978, 1984, 1995, Hasegawa and Yano 1975, Ishigami and Nagano 1975, Barricelli 1977, 1979, Kuhn and Kuhn 1978, Kocherlakota and Ackland 1982, Konecny et al. 1993, DiGiulio 1994, Ardell 1998).

Because all amino acids used by life are of an α-type, because they are all L-stereoisomers and because some environmentally-common abiotic amino acids such as α-amino-n-butyric, norvaline and norleucine are not present in proteins (Weber and Miller 1981), it is assumed that primitive life forms started by using a limited number of simple chemically-synthesized AAs such as Gly, Ala, Asp, Val and Glu (Hartman 1975, Ishigami and Nagano 1975, Ivanov 1993, Baltcheffsky et al. 2002). The genetic code evolved gradually (Lacey et al. 1975, Barricelli 1979, Guimarães and Moreira 2002), not by importing novel amino acids from the environment, but rather by mobilizing amino acids of a biosynthetic origin into the genetic code (Wong and Bronskill 1979). The evolution of the AAtRNA synthetases seems to support a coevolution between the genetic code and the development of biosynthetic pathways (Wong 1975, DiGiulio et al. 1991, DiGiulio 1992, 1993, 1994). Wong's coevolution theory was later characterized as non-viable (Ronnenberg et al. 2000), yet, it has never been demonstrated as such. A more refined picture of the

evolution of the genetic code has been published recently (Guimarães and Moreira 2002).

The great advantage of encryption is that it allows the existence of a chemical difference between information holders and catalytic performers. It is not unlikely that proteins from modern ribosomes may represent more than a scaffold for protein synthesis. They may also represent a shell protecting the RNA-based peptidyl transferase from architectural stress or from a 'poisonous' chemical entourage. The inside of the ribosomes might represent one of the last chemical fossils of the late RNA world, a representation of how the early environment was. The ancient environment allowed RNA both to exist and to catalyze the formation of amide bonds. The topographic complexity of the explicit information carriers is almost endless and requires a multitude of ways to read, create and maintain it, while encryption maximizes the variability of the output yet minimizes the compositional diversity of the information holder.

Other benefits of encryption were a decrease in the dependency between information and its thermodynamic stability, larger data bases, minimization of redundancy, the unification of the information-reading protocol, temporal control over expression, storage of information that is infrequently used but worth remembering, controlled reproducibility, genetic memory, vertical transfer of information and 'patent secrecy'. Encryption restricts information from phylogenetic strangers or from competing entities that do not have compatible translation machinery, thus allowing the maintenance of the 'technological' secrets inside each genetic family. The NA–protein relationship is credited with a great many derived consequences and is frequently used as a sine-qua-non explanation of how life emerged or even as a way of defining life (Kunin 2000). It is appealing to consider the onset of encryption as the beginning of biological life. Following encryption, other derived properties were added such as full control over internal information, the creation of a genome, self-reproduction, Darwinian evolution and horizontal gene transfer.

6.3.5 The Rise of the DNA World

It is commonly assumed that the early RNA world was followed by a DNA world (see figure in Robertson and Ellington 1998), and that translation is a process older than DNA (Dzik 2002). The addition and use of DNA required three preconditions:

- a ribonucleotide reductase,
- a reverse transcriptase,
- a DNA polymerase (Léon 1998).

Some scientists favor the view that an RNR (an enzyme converting ribonucleotides into deoxyribonucleotides) emerged very early, possibly before translation (Benner et al. 1993, Lazcano et al. 1992, Lehningher et al. 1993).

The metabolic transformation of uracil into thymine preceded the DNA world. The transition to a DNA world was not straightforward because every biochemical novelty interferes somehow with the fitness of the precursors, e.g., modern deoxyribonucleotides have been shown to inhibit ribozymes (Léon 1998).

One possible scenario is that the first changes in the RNA-to-DNA transition were represented by the biosynthetic emergence of thymine and deoxyribonucleotides. The monomers dAMP, dCMP, dGMP and dTMP did not form continuous DNA sequences from the very beginning. They rather represented rare positions inside RNA chains, acting either as termination signals, stabilizing double helices, or termination palindromes, by forming stable double helices at the end of the chains. As the RNA replicase could not read across such unfamiliar positions and regions, a new reading-assistance complex (the primordial DNA polymerase) was invented, acting as a helper of the RNA-polymerase. As the density of DNA-related monomers inside the RNA chains increased, so did the length of the DNA-like contiguous insertions, the complexity of the DNA polymerase, and the need for its overexpression.

The addition of an RT allowed storage of information into the more stable DNA chains, while RNA kept the role of transiting information and providing for catalytic functions. This scenario does not support a sharp switch from the RNA-dominated world to the DNA-dominated world, and so obeys the principle of continuity in molecular evolution (Lahav 1999). The present DNA world is seen here as having its origin in a period when the genome was a mosaic of RNA and DNA. The intervening sequences present in modern Eukaryotes and viruses can either represent defective transposons (Cavalier-Smith 1985) or 'living fossils' from a former RNA world (Robertson 1996). The coexistence of RNA-based organisms with DNA-based organisms supports an endosymbiosis between an RNA-based cell and DNA-based cells leading to the eukaryotic nucleus (Hartman and Fedorov 2002). According to this hypothesis, the cytoplasm of the modern Eukaryotes is the remnant of RNA-based proto-organisms (a Chronocyte) while the nucleus was a DNA-based symbiont (such as an Archaean Prokaryote) (Hartman and Fedorov 2001). The existence of deviations from the universal genetic code in mitochondria and in nuclear systems (Ueda and Watanabe 1993, Yokobori et al. 1993), as well as the dual specificity prolyl-cysteinyl-tRNA synthetases used by some Euryarchaea (Bunjun et al. 2000), suggests that the unification of the genetic code was not fully completed by the time RNA endosymbiosis occurred. The greatest advantages of the DNA world were the high thermodynamic stability of the information and the separation of information carriers from catalysts. The high stability of the DNA and the ability of its double strands to assist enzymatic repair may also have contributed to its selection (Martell 1992).

6.4 Information-Related Fundamental Phase Transitions Toward Life

The features considered as fundamental phase transitions in the history of information are described here.

Variability

Variability is most frequently defined as a sequence alteration (Fontana 1992). In this study, however, variability is applied to a variety of types of information (from contextual to nominative, from explicit to cryptic, and from material to symbolic), and to a variety of types of information carrier (physicochemical gradients, mineral surfaces and organic polymers). Therefore, variability is defined here as the ability to make (and withstand) continual alterations in the information carriers. In order to keep pace with the changes, the potential for variability has to be higher than the long-term environmental pressure. If a lateral exchange of information and reproduction exists, the variability of the entire population and not the variability of the individual must be higher than the environmental pressure.

Replication

Replication is the ability to make copies of an information carrier. Early replication was not necessarily precise, but the specificity of the replication must always have been greater than the overall variability. According to one interpretation, the sudden, fundamental transition into a system characterized by replication, variation (i.e., variability) and selection represented the transition from the inanimate to the living (Kuhn 2002). Replication is such a remarkable feature of life that it has even been proposed that life may be characterized through replication (Csanyi and Kampis 1985). However, it appears obvious that life is more than replication and that a clear distinction has to be made between concepts such as replication, heredity, reproduction, copying, multiplication and inheritance (Szathmáry 2002). Copying a material can lead to replication but this does not equal reproduction. Copying information requires that no overlap should be present between parental material and the offspring's material, while reproduction does imply an overlap. Multiplication is considered as being less than either replication or reproduction. Multiplication exists whenever one unit is produced in multiple copies (analogous to a photocopy machine), without each one of the offspring participating in the process. Because of a lack of replication, heredity is impossible when only multiplication is present (Szathmáry 2002). Inheritance refers to the possibility of acquiring functional capabilities as a result of reproduction. The chemical basis for replication is autocatalysis (Orgel 1992, cited in Szathmáry 2002). A variety of models of enzyme-independent artificial

molecular replications (self-replicators) has been proposed in the literature (Bag and von Kiedrowski 1996, von Kiedrowski 1999).

Encryption

Encryption may be the ultimate fundamental phase transition step required to generate life. Encryption is related to the emergence of a subcomponent exclusively specialized in carrying information and having a composition that differs from most of the catalysts. Encryption allows the separation of internal functions and temporal regulation and is therefore the basis for maintaining a steady state and a quasi-state in variable environments. The decryption of genetic information in the living world is based on the process of translation, which is assisted by ribosomes. A question not addressed yet in the bibliography (at least to my knowledge) is the possibility that ribosomal translation was derived from an inverse process, i.e., a process in which polypeptides were used to build up polynucleotides.

6.5 Minimal Requirements for the Emergence of Bio-Information

These are (see Table 6.1):

- an environmental non-randomness influencing the functioning of preliving units,
- a source of information that can be both imported inside preliving structures and transported,
- environmental diversity and environmental heterogeneity,
- robustness of information carriers to compositional alterations.
- replication of information must be thermodynamically possible.

Table 6.1. Major features related to the history of information

Minimal requirements	Fundamental phase transitions	Major derived properties
Environmental non-randomness	Variability	Control over expression
Importable information	Replication	Genome
Diversity and heterogeneity	Encryption	Genetic diversity
Robustness to change		Self-reproduction
Replicable information		Darwinian evolution

7 The Purpose-Like Nature of Life

> It is absurd to suppose that purpose is not present because we do not observe the agent deliberating. [...] Nature is a cause that operates for a purpose.
>
> Aristotle

According to Jeffrey Tze-Fei Wong (2002): "A living system is one capable of reproduction and evolution, with a fundamental logic that demands an incessant search for performance with respect to its building blocks and arrangement of these building blocks. The search will end only when perfection or near perfection is reached. Without this built-in search, living systems could not have achieved the level of complexity and excellence to deserve the designation of life." The reader must understand that a distinction exists between how the common language defines purpose and what the theory of early life means by it. In Dexter's dictionary the word 'purpose' is anthropocentrically associated to performing an action with a conscious (deliberate) aim, intention and/or design. Yet life displays elaborate and suitable features that resemble the results of a purpose-like action (Szathmáry 2002). In fact any material–energetic order has the potential for a 'tendency' (or 'purposefulness') directed in the opposite sense to entropy (Valenzuela 2002). Because the 'purposefulness' of their function is such a vigorous and typical property of living organisms (Korzeniewski 2001, Szathmáry 2002), the search for a theory of life is more productive if it focuses on the best explanation for life (Bedau 1998).

Whatever driving factor pushes (or pushed) life to emerge independently of its material nature has been gathered under notions such as the reason of life, the purpose-like nature of life, the essence of life, the meaning of life, raison d'être, a 'built-in search for performance', or the basic process of life. The word 'purpose' is interpreted in this study to mean a target-oriented temporal development and not an intentional (i.e., conscious or divine) action. The purpose-like nature of life is also interpreted as something far from esoteric

R. Popa, *Between Necessity and Probability: Searching for the Definition and Origin of Life*, Adv. Astrobiol. Biogeophys., pp. 121–137 (2004)
http://www.springerlink.com/

and theological speculation, but rather as a logical (straightforward) physical puller. In a conscience-absent world, 'purpose-like' indicates the general direction processes are pulled toward and not a deliberate (intentional) action toward a pre-defined or a pre-envisioned objective. Using this semantic representation, purpose can be identified in many aspects and levels of life. The purpose of a catalyst is to accelerate the formation of a product; the purpose of a negative feedback regulation is to preserve the compositional identity of the entity (Wiener 1947, Bernal 1967); the purpose of an entire living individual is to reproduce itself in as many copies as possible (Korzeniewski 2001), while the purpose of a group of individuals is the permanence of a genetic package over extended periods of time (Purves et al. 1995). But what about the purpose of all living individuals as a collective, or the purpose-like nature of life in general? Does life have a built-in overriding reason that pushes evolutionary events in any specific direction?

The belief in an overall direction is not the only philosophy concerning the origin of life. In several quite popular interpretations, life is the result of a chance-like event during chemical evolution, something that just happened to emerge and it remained so thereafter. Yet this vision precludes the possibility of a guiding principle or of a central tendency without which long-lasting prebiotic geochemical evolution is hard to explain.

One of the most interesting aspects of dissipative structures is their coherence (Prigogine and Stengers 1984): "The system behaves as a whole, as if it were the site of long-range forces. In spite of the fact that interactions among molecules do not exceed a range of 10^{-8} cm, the system is structured as though each molecule were 'informed' about the overall state of the system." The multitude of derived properties of life plus this so-called 'inner awareness' has led some to believe that there is more to life than what can be actually measured (Pfeffer 1897, Putter 1923). According to old vitalist interpretations, living entities exist and become what they are due to a mysterious force called a perfecting principle, entelechy, vital force, or mneme. Although such concepts no longer dominate the modern philosophy of life, we are still so much influenced by Aristotle's ultimate causality that we never fully deny the possibility that some less obvious primeval force or forces might push living things into the physical existence. Although it has been theorized that life might have no unified explanation, or that lifes "purpose" is too hard to understand, or even that the question of the nature of life has no interesting answer, many scientists believe that living phenomena must have a unified explanation that is worth exploring (Boden 1998, Bedau 1998).

The question of the essence of life has two aspects: achievability and implementation. It is believed that "one can separate the logical form of an organism from the material basis of its construction, and that its capacity to exist and to reproduce is a property of the form and a property of the matter" (Emmeche 1992). Various attempts have been made to explain this purpose-like nature of life (Morán et al. 1999, Buiatti and Buiatti 2001), and

the variety of opinions regarding this subject is so broad that it is almost impossible to make a comprehensive and objective review of them all. Some of the most notable examples are:

- "The vital forces are molecular forces" (Huxley 1868).
- The essence of life can be found as a "deep intertwinement between the relational-constructive logic of a basic biological system and the logic of its thermodynamic implementation" (Bergareche and Ruiz-Mirazo 1999).
- According to the 'selfish gene' concept, the function of a gene (its purpose) is to reproduce or repair itself and thus to withstand time (Dawkins 1989). This concept puts the event that created the physical permanence ahead of its own purpose while in a normal world causes should precede effects and purposes should precede developments and not vice versa. If a physical reason is to be found in the accretion of life, it should be something that existed in nature as an overall tendency before life itself emerged as its physical substantiation.
- "The essence of the primary form of life is based on the notion of supple adaptation, defined as an automatic and continually creative evolutionary process of adapting to changing environments, a process more rooted into the fabric of life than the natural selection itself" (Bedau 1998).
- "An intrinsic tendency toward self-organization exists in nature and in life" that is also related to the origin of life (Hazen 2001).
- The essence of life can be described as "a network of inferior negative feedbacks (regulatory mechanisms) subordinated to (being at the service of) a superior positive feedback (potential of expansion)" (Korzeniewski 2001).
- Life is based on "the organization of feedback loops where given N component molecules the result is the iterative cooperative reproduction of the whole loop" (Buiati and Buiati 2001).
- The essence of life is related to its intrinsic search for "perfection or near perfection" (Wong 2002).

Most interpretations regarding the essence of life belong to two general clusters:

- order-based interpretations,
- energy-based interpretations.

Order-based interpretations are generally harder to accept than energy-based interpretations because some of the central tenets of the order-centrist opinions may appear to some as logic independent. What most order-based theories essentially say is that:

1. an intrinsic tendency toward order exists in nature,
2. life emerges as a byproduct of this natural bias,
3. no other attractor is needed to drive the emergence of life and its subsequent evolution.

Yet if no effort is invested in explaining the source of this ordering tendency, a large portion of the evolution of life becomes very hard to explain, in particular the optimization of the energy flux. A tendency toward higher order does exist in some instances in nature (see the self-assembly issue in Chap. 4), but it is not solely responsible for life. Moreover, ordering effects are not the main attractors for the accretion of life. As for the notion of supple adaptation, I see no special strength in this alleged fundamental property of life and in its claim to address the problem of the essence of life. The way this concept was formulated allows too little insight or revelation into the force(s) pushing life into physical existence.

The present theory considers that energy-related principles drove life into existence and thus relate more closely to the purpose-like nature of life than any other explanations. The biochemical 'innovations' required to achieve the thermodynamic upgrades of life (its polymeric nature, high energy density and low entropy) are so elaborate that according to Francis Hoyle, their spontaneous emergence (probabilistic assemblage) was an impossible event. The hierarchical vision has the disadvantage of requiring more than one step (see Fig. 7.1). One complication of any stepwise origin (including a hierarchical one) is that that the accretion of life is multidimensional. During the origin of

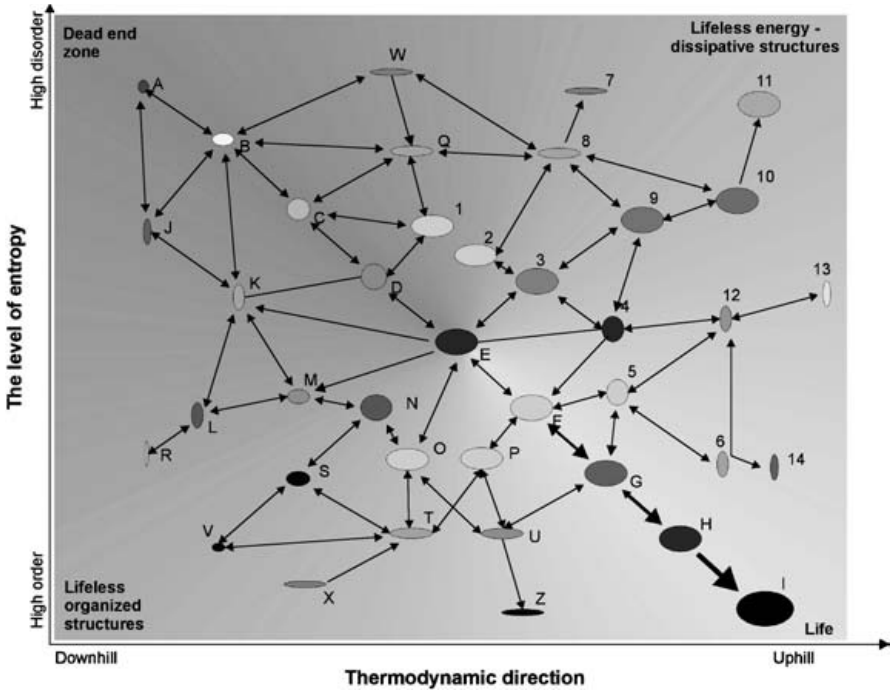


Fig. 7.1. Because the origin of life is a development across a multidimensional landscape, a multitude of virtual directions were possible in its history

life, a variety of states were possible (labeled A to Q and 1 to 14 in Fig. 7.1). Some states such as A, V, X or W require phase transitions to emerge and are therefore probabilistic, while others such as J, K, L, or M are caused by preceding factors and are therefore deterministic. Because many intermediary steps are required to achieve the living state (I), rerouting can occur at so many points in its evolutionary history that the chances of the system I ever emerging approach zero. Put simply, the laws of physics as we most commonly understand them should push chemical evolution toward a dead end and not toward life. Many states such as Z, 7, 11, 13 and 14 can no longer advance toward life and represent evolutionary dead ends. If the sequence that leads to life has to be long (e.g., A → B → C → D → E → F → G → H → I), bifurcations could occur in too many points, obliterating any reasonable probability. There is a severe problem with long reaction chains: deadly side reactions drain it out to such an extent that ultimately the terminal intermediates of the chain disappear (Szathmáry 2002b). One may speculate that the shorter a path the better the chances for its propagation (King 1982, 1986).

Addressing the question of the purpose-like nature of life is no different from asking why the general path toward the living state was directed this way against the thermodynamic laws that govern our universe (higher entropy and dissipated energy). It seems that life not only emerged successfully but still exists today despite four billion years of environmental pressure. This might suggest that at least one hidden tendency (a purpose-like aspect) assisted the accretion of life and applied throughout its entire history. One way to address this issue is to answer questions such as:

- Should life be considered the ultimate tendency of natural physicochemical evolution? If so, why do we not see life all over in the outer space or life forming *de novo* in our chemical simulators? If not, is life a chance-like event, and what is the probability of its emergence?
- Is it possible for the emergence of life to have had both deterministic and probabilistic aspects?
- Can life emerge by itself with no 'deliberate' assistance? Is it reasonable to expect life to emerge spontaneously in a random experimental simulation or should some minimal conditions be provided?
- Should these preconditions for life be provided simultaneously or are they needed in some special sequence?

The emergence of life must have had a probabilistic component. One cannot totally exclude the possibility that life emerged by chance simply because the odds are against it. The probabilistic unlikeliness of an event does not make it impossible. Although the odds are against them, a couple of people may still win the lottery even though their "well-informed" friends may have articulately demonstrated that it was impossible for them to win. The phase transition achievements of life all involve some probabilistic component.

Life is more than a self-maintaining phenomenon. It is also a self-building phenomenon. The emergence of life is certainly different from winning the

lottery. Once the lottery is won, the event remains frozen in time and the lucky winner starts over-spending the prize. Statistics shows that many lottery winners go bankrupt in 4 to 5 years. Only a few invest smartly in their future. At any given time, only a handful of rich winners can be identified and most of them are winners from the last few years. Life is different. Due to a reflexive activity, life resembles the most cautious lottery winners, because it invests heavily in its development and permanence. There is no special force that dictates how much energy a reflexive system should invest in its future. Yet those forms of life investing too little cannot withstand the scrutiny of the future while those that overdo it cannot compete with the present. The types of preliving systems that use the right balance between earnings (catalysis) and investments (reflexive activity) 'live to see another day', spread over space and withstand the test of time. At any given moment, an observer should see all the types of life that ever emerged and have not vanished yet.

A winning number at the lottery has no effects over the following numbers in the series. In contrast, life creates new order that biases the result of the 'lottery' in its favor by promoting life-directed phase transitions. Certain life-directed performances increase the probability that other phase transitions will occur and be driven in the same general direction. The development of biosynthetic pathways and environmental homeostasy results in a remodeling of the environment that makes it more suitable for the maintenance of life. This idea was brought to a planetary scale in the Gaia concept (a planetary-level homeostasy).

The emergence of life was certainly not a one-step event and it was more than a stepwise development. The emergence of life was hierarchical (Hazen 2001). A big difference exists between stepwise and hierarchical. Stepwise means that various features were added one by one in a complexity-dictated order, while hierarchical means that the features of life were added in a sequence controlled first by their functional significance and second by their level of complexity. The chance-controlled analogy used by Francis Hoyle (Hoyle and Wickramasinghe 1993) are often interpreted as temporal coincidences and are therefore calculated as products of individual probabilities. In reality, the parts required for one single microbial cell to function are so complex (see Fig. 7.2) that the probability of them getting assembled directly from a primordial 'soup' seems non-existent. This observation is due to a basic rule in probability theory, namely that the probability of a group of chance-like events happening simultaneously equals the product of their individual probabilities. Therefore, if five events must occur simultaneously (with individual probabilities of the order of 10^{-5}) the simultaneous emergence of all of them might be a number too small to be meaningful. For features as complex as cell components to emerge by chance and simultaneously, the entire universe might not be large enough and old enough.

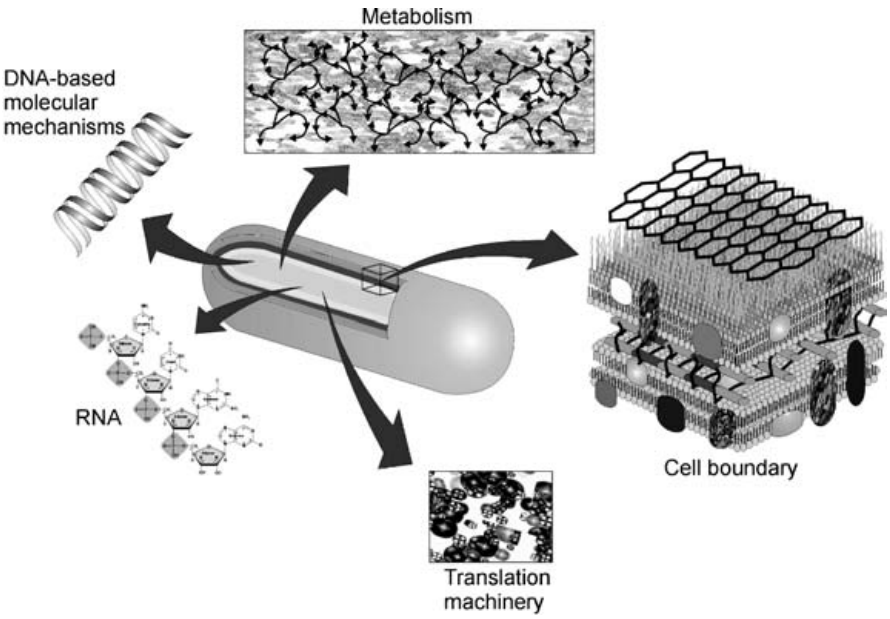


Fig. 7.2. The complexity of the mechanisms required for the functioning of a living cell is so large that a simultaneous emergence by chance seems impossible

Most scientists now believe that life originated in a number of smaller and probabilistically likelier steps. Instead of being one big chance-like event, life might actually be an accretion of a series of events emerging at different moments in time (see Fig. 7.3). Life never won the lottery. The odds were against it. The first winning number was used as an investment to provide survival until the next 'draw' and to influence the second winning number, which then promoted the third, and so on. If this was indeed the case the overall probability for the origin of life is not a straight product of individual probabilities but more like a sum of individual probabilities.

A stepwise emergence is not enough to clear this problem, because the environmental challenge was conspicuous for every given step, so that a fully functional entity was continually required. With so much competition around, the early pre-life entities did not have the luxury to rest (to lay down evolution) and enjoy their achievements. Not only did life emerge in steps but the steps were arranged in a precise sequence (a scaffold or a meaningful hierarchy) of priorities. In a hierarchical accretion the simplest and most necessary requirement emerges first, while the more elaborate and dispensable (or replaceable) ones join later. A stepwise option does not come without a price. One should still ask how so many events could occur in such an unlikely direction (see Fig. 7.1). This question brings up the issue of an overall tendency in life.

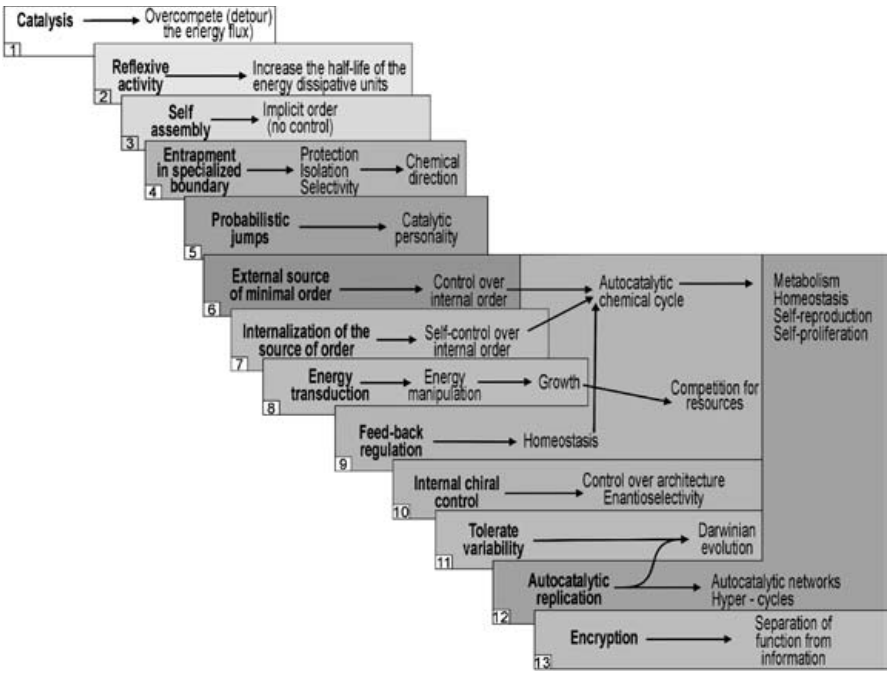


Fig. 7.3. Life accreted as a sequence of successive layers and was functional at every moment in its history. Fundamental probabilistic events are represented in *black*. Emergent (derived) properties of life are represented in *grey*. *Arrows* indicate causal influences

The survival of life is partly based on complexity and redundancy and its functioning cannot be modeled as a linear set of events. Imagine a computerized system made of 10 million components. If the function of the system only allows one straightforward path to exist, the overall system only operates if all the components function precisely within their specifications. Even if all the components are reliable (say a one in a million chance for each one to break down on any given day), the system itself has little chance of functioning for more than 24 hours. The system is made of so many components that statistically at least one of them will break every day. Some man-made systems such as elevators, highway exits and receptors function this way with no built-in backups. Once one of their major components fails, their entire function is obliterated. Aware of such risks (and whenever possible), engineers construct redundant subsystems for use in emergencies. The constructive principles for their subcomponents might also vary. Some of them are more robust (yet less efficient) and are only used during crises, while others are faster and more efficient but less reliable. This constructive diversity can keep a complex system working with numerous internal parts damaged. This is why straightforward probability is difficult in judging the actions and survival of complex systems.

Life does not require a standard composition. In theory life forms can be made of a variety of material substrates. The Drake-type equations used to calculate life's chances in the universe, as well as the concept of habitable zones, must be used with reservation because they put severe and unverified constraints on what life should be based on, without a satisfactory check on the validity of its premises. When one discusses life on a cosmic scale, a 'cosmic diversity' should also be considered. Why should water be the only possible fluid? Why not ammonia, or sulfuric acid or liquid CO_2? Why only carbon chains and not sulfur or silica? Why chains and not chainless 3D architectures? The universe is so vast and so diverse that the number of rare events and rare combinations of events leading to life has to be much larger than the Drake equation allows.

The emergence of life is conditioned by a specific sequence of events. Many experimenters (including Pasteur) have tried to verify whether life can emerge spontaneously from a mixture of organic chemicals, with no success. More promising results have been obtained in artificial life simulations (Adami 1998). It has been demonstrated that certain properties of life can emerge (condensation, growth, replication, evolution), but never life itself. The deduction seems straightforward: how can nature make life if we failed with all the experimental conditions controlled? Though disappointing, these results must be considered for what they actually are – a couple of chemical combinations out of myriads of others never verified. What if life not only required a special combination of parameters but a defined sequence of them as well? The design of such an experiment must start with the basic principles and priorities that regulate the accretion of life. In most life simulation experiments, the caveat lies in the experimental design. Pasteur's boiled soup was 'almost' the same from the beginning of the experiment to the end of it. No extra energy resource was intentionally provided during the incubation, no periodic activity was applied to induce internal cycles and condensation reactions, no source of order was provided and no environmental crisis was introduced to promote upgrades. It would have been a miracle for life to emerge in such conditions. The creation of artificial life can be used as an analogy to what might have happened in reality, but the failure of such an 'experimental demonstration' cannot be used to prove that such an event is totally impossible.

The difficulty in anticipating the development of complex systems does not make their behavior chance-like. Many large energy-dissipative systems (the most notorious are fluid vortices) are made of a multitude of small identical components that are represented as acting purely probabilistically for mere mathematical convenience. Yet the system they are part of behaves collectively. The overall action cannot be explained as a sum of the random activity of the individual components. This aspect of dissipative structures is known as coherence. As cited previously, although the molecules only interact on a short range (10^{-8} cm), a coherent system behaves as a whole, as if it

were the site of long-range forces (Prigogine and Stengers 1984). The system is structured or functions as though each molecule were 'informed' about the overall state of the system. For energy-dissipative coherent systems, mathematical models representing the activity of the micro-components as solely statistical seem inappropriate. From an experimental standpoint, the behavior of these micro-components might seem random, but in reality it is not. "Physical objects come into being that behave as if they have a purpose, an intention, and an aim"(Kuhn 2002).

Due to microscopically similar and cumulative dissymmetries that are beyond our experimental 'perception', the micro-components of a group behave as a group with properties differing from the sum of the individuals. The location, shape, size, speed and extension in time of fluid vortices, tornadoes, volcanic eruptions, earthquakes and even of planetary magnetic inversions are imprecise and very hard to predict on the basis of limited, local, discrete measurements, e.g., the distribution probabilities derived from the theories of gases cannot explain the formation of a vortex. Yet their components must obey some intrinsic collective rules to realize such large coherent structures. In reality, it is not the individual behavior of the molecules in a fluid that is probabilistic, but our perception and representation of them. The imprecision in anticipating their behavior is but a reflection of limitations in our exploratory technology, computational capabilities, and theoretical maturity. From a purely physical point of view, complex and coherent events cannot just appear from nothing without a reason; a judgment that is scale independent and thus applies from a quantum level to a celestial scale. Overall forces and/or collective tendencies situated often at unforeseeable or unmeasurable (to us) scales and ranges dictate such collective behavior regardless of our ability to anticipate them.

Current knowledge seems to suggest that the emergence of self-assembled energy-dissipative systems such as living entities has a random component, but at the same time life could also have been 'pushed into existence' by some driving forces and/or by collective properties. The explanation for the 'purpose-like nature of life' [i.e., its driving force(s)] must be something that obey(s) some minimal set of requirements:

- It must have acted as a driving force from the early steps toward life to modern life forms.
- It must be displayed from the simplest form of life (the earliest protocell) to the largest supra-individual level (the biosphere) (Patten et al. 1997).
- It must be general enough to apply to every possible type of life, independent of its physical or chemical substrate.

Although many approaches are possible, two general questions are addressed here:

- What is life doing in its very essence that makes a difference in nature?

- What fundamental tendency of life applies during its entire evolution and to all types of life?

Since the beginning of the 20th century, scientists have claimed that life began with abiotically-formed catalysts (Oparin 1924, Haldane 1929). Yet as shown in Chap. 4, catalysis is not always an advantage for the system. Because every action has a thermodynamic reaction, catalysis results in an increase in the overall entropy (system plus surroundings). Most of the early catalysts must have been deleterious, i.e., they resulted in a rapid entropization with severe consequences for the fate of prebiotic systems. Such activities were further aggravated by variations in the availability of resources and especially for organic polymers; their catalytic abilities were limited in time, as they were controlled by their degradation rate.

One universal property of life is that the entropy of the system should remain constant (or decrease) at the expense of an increase in the external entropy. Given this constraint, it would seem logical to claim that the primeval step toward life was not the emergence of a catalyst but rather a reflexive activity associated with catalysis that channelled the catalysis in a self-preservation direction (see Fig. 2.4). This type of activity has 'thermodynamic advantages' for the entities showing a catalysis/reflexive activity based energy–dissipative strategy. If more types of energy-dissipative strategy are present and competing for the same resource, the relative contribution of those displaying reflexive activity is larger because their half-life is also longer.

During the origin of life, as phase transitions were gradually added, more derived properties followed (growth, heterogeneity, chained processes, metabolic cycles, competition for resources, molecular clocks, and Darwinian evolution), bringing improvements in the ways that energy got dissipated. The evolutionary sequence that led to life suggests that the earliest outcome of life-directed energy-dissipative entities must have been to increase their energy-dissipation efficiency (intensification) toward a broadening of their energy resources (extension) and an increased spatial coverage (expansion), plus the regulation of the energy flow (leveling of energy spikes). It is hypothesized here that this trend was followed throughout life's accretion and evolution and is intimately related to what may be perceived as the purpose-like nature of life.

The experimental analogy presented in Fig. 7.4 uses as a control a sealed and sterile system (A) containing water, sediments and organic molecules exposed to a day–night cycle. The system A is an analogy of a planet situated in the same position in space as Earth, with the same initial chemical composition but with all life forms absent or inactive (an A-type planet). In A as well as in an A-type planet, although all the organic molecules are present, they are either not catalytically active or not spatially connected. Thus they cannot function as a coherent energy-dissipative entity. Another system (B), analogous to a biosphere (a B-type planet), contains bacteria, algae and some

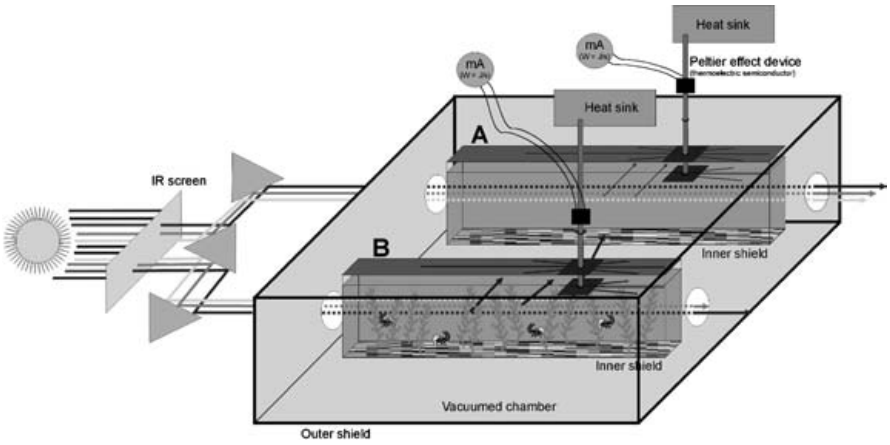


Fig. 7.4. A hypothetical experiment used as an analogy to show the thermodynamic consequences of a biosphere. A is the control and B the sample

shrimps. The two systems A and B are closed (i.e., they have no material exchange with the exterior) and adiabatic (i.e., there is no heat exchange with the sides due to a vacuum chamber). A heat conductive device is installed on top of both systems, leading the heat produced inside toward a heat sink through a thermoelectric semiconductor (i.e., a Peltier-effect device used to measure how much heat passes through a wire). The initial calorific content of the internal materials is identical in A and B.

Light is the only energy resource provided. If energy (light) is applied periodically to simulate a day–night cycle, a community develops in B producing biomass (organic carbon = C_{org}) by carbon fixation and oxygen (O_2) by photosynthesis during the light phase. In the dark the C_{org} is oxidized using the available O_2. In the beginning there is no equilibrium between C_{org} formed in the light phase and the C_{org} oxidized in the dark phase, but after a number of cycles, a steady state is reached between production and destruction. The system B becomes organized in such a way that the C_{org} and O_2 formed in the light phase is enough to withstand the dark phase and to reinitiate the cycle from where it started.

The system A also displays some periodic transitions and heat exchange but they are considerably lower and have a lesser inertia. In an A-type planet, this energy dissipation is mostly based on surface fluorescence, greenhouse effects, tidal dissipation, meteoric bombardment, volcanic activity, thermal convection, and the photo-oxidation/reduction of certain chemical species. Some of the solar energy might even be stored abiotically in the form of a redox differentiation. During the dark phase of the cycle, most of this energy is liberated quickly through diffusion and electron transfer and the system gradually returns to its initial state. In time, an irreversible hydrolysis occurs and the overall calorific content of the organic materials in A decreases. The

energy trapped in the living entities from B has larger day–night-related fluctuations but the C_{org} never reaches the low values from A. The maximum amount of living material in B never increases above an upper limit controlled by the minimal material resource (most frequently carbon, nitrogen, phosphorus and iron). The dissipative efficiency of B can be represented as a ratio between the heat produced and the average C_{org}. The two systems (A and B) differ from two major points of view:

- the scale of the energy dissipation (B has a larger energy-dissipative potential than A),
- the buffering capacity (B is self-adjusting).

The observations from this 'experimental analogy' can be extrapolated to a large scale such as an entire planet without significant changes in the logical operators.

Due to an in-flow of solar energy, entropy is displaced outside the biosphere (Polishchuck 2002). A planet such as Earth can be viewed as a relatively closed system with a considerable input of solar energy and a limited input/output of matter (meteorites/gas escape) relative to the planet's size, while the available space for the development of life (ecological niches) remains roughly the same over time. In the absence of life, only a small fraction of the incident light that hits the Earth is degraded into heat due to phenomena such as fluorescence, redox differentiation or fluid flows. In the presence of a planetary biosphere, a larger portion of the solar energy becomes heat. "Sunlight is high-quality energy that can do work. After 'striking' Earth, much of this energy is used by living systems to create islands of order, transforming this energy into heat that departs into space as infrared" (Brin 2002, Anbar 2002). All natural ecosystems display a standing stock (biomass) and a productivity (energy inputs) which, over many cycles, fluctuate around a central value. The science of ecoenergetics shows that a built-in tendency exists in all ecosystems to reach a level of complexity and to achieve protocols that maximize the overall energy flow. Even if "the amount of living matter is preserved during evolution" (a principle promoted since 1901 by N.A. Umov, Mirzoyan 1997), the energy flow increases continually. Considering life as a strictly negentropic or anti-entropic 'thing' is a mistake. "To order or to organize themselves, living beings contribute more to the disorder of the environment (or to the whole system) than if they do not exist" (Valenzuela 2002). Due to the presence of life, the Earth has been viewed as a B-type planet (see Fig. 7.4) that "acts as a photon multiplier, and generates entropy radiated to the universe at large" (Patten et al. 1997).

A common misconception about life is that it somehow disobeys the second law of thermodynamics. In reality life is only locally 'flouting' the second law of thermodynamics while globally it is strictly obeying it (Yockey 1973, Lifson 1987). "The living surface of Earth is like a water wheel, dipped into a flowing river" (Brin 2002). "Living systems swim upstream in a rapid flowing stormy river" (Anbar 2002). It is "the flow, not the water as such, that

makes the difference" (Brin 2002). To me life looks much like a sailing boat that uses the power of the wind to cross the ocean in directions that appear sometimes against the overall current and wind (using the wind itself as its driving force).

This emphasizes the fact that it is the overall tendency of the second law and not the material nature of the universe that promotes (drives) life into physical existence. On a universal scale, life is just a minor local phenomenon acting in a way that shortens the path toward the overall 'thermal death'. A 'life-rich' universe is a shorter living universe than a 'lifeless' universe. Not only is a living planet a 'warmer place' than a dead planet, but the fluctuations in the energy inputs are also more efficiently buffered. Moreover, in the presence of life, more forms of energy are degraded into heat than would be expected in the absence of it. Even at their highest evolutionary peak, on their largest scale and at their highest complexity level, life-related systems perform the same physical function: they speed up and regulate the overall energy flow. This promotes the following function-related interpretation: life is a localized self-reproducible and self-adjustable strategy to speed up the second law of thermodynamics and regulate the overall energy flow.

Most certainly, life is not the only way to speed up entropization and it is not the ultimate physicochemical development. Life is not on the list of 'things to do' in the physicochemical evolution of the universe. Thus abiotic evolution does not necessarily drive toward life. It is therefore mistaken to credit life with an ultimate causality. Although life has a causal component to it (many of its achievements are deterministic), its emergence has probabilistic crossroads and it is hampered by various limitations such as:

- environmental conditions fluctuating beyond its physical stability,
- energy present in hard-to-transduce forms,
- conditions favoring rapid degradation rates,
- hard-to-dissipate thermodynamic byproducts (e.g., heat),
- local complexity too low to allow intricate enough mechanisms to form.

Due to these restrictions, even if the origin and existence of life has some deterministic components, life cannot be expected to emerge *de novo* in random biomimetic (early Earth simulation) experiments. Therefore, the identification and quantification of the minimal conditions required for the emergence of life and the probabilities associated with its essential phase transitions are a major objective for future studies in exobiology.

Is there any mysterious purpose deliberately driving the physicochemical evolution in the universe toward the genesis of life? The answer seems to be categorically ... NO. While there is no supernatural, hidden and deliberate intention in the physical world that targets life, life is a physically purposeful[1]

[1] The term purpose is used here in its physical vectorial connotation and not in similitude with intention or deliberate and anticipative intelligent action.

phenomenon. In most environments the physicochemical laws point away from life and toward an increased entropization. Therefore, the accretion of life, (i.e., the chance that probabilistic events will join and stabilize a reflexive energy-dissipative mechanism with further increase in the internal complexity and efficiency) is only possible if certain environmental conditions are met (see Fig. 7.5).

When the first mechanisms merged with each other, e.g., primordial vesicles engulfing catalysts or self-replicators, the stability of the association was loose due to limited backups. This is consistent with a normal physicochemical evolution driving action in both directions of the entropy range with an entropization bias dictated by the second law. However, by the time the last fundamental achievements of life were added to pre-life systems, e.g., the internalization of the source of order and internal chiral control, this forward tendency became so vectorial that the last steps toward life, such as encryption and its derived consequences, almost acted as an adaptive necessity and might therefore leave the impression of being pushed into 'physical reality' by some mysteriously directed force.

These events only appear to happen in a defined direction because only the few that did so became selected and could be observed later. Imagine a fish tank and an automatic feeder on top of it that periodically drops particles of food in the tank. If the mixture that falls into the tank contains 99.99% food particles and 0.01% glass marbles the fish from the tank will perform a selection based on feeding preference and a predominance of glass marbles will eventually accumulate at the bottom of the tank. Just because we observe many glass marbles in the sediment and no food particles does not mean that they are the most common physical occurrence. They are in reality the remnants of a selective process and any inferences concerning their likeliness is misplaced without analyzing their initial abundance, the selective forces and time.

Despite vitalist assumptions, the origin of life is not driven by any special 'life-generating force'. The reality is actually trivial. The last probabilistic events to be added in the early history of life (the replication of information and encryption) (see Fig. 7.5) have a very pronounced functional meaning. They become the sole solutions to a defined managerial crisis. However, their mobilization or even their invention remains probabilistic. The issue of the purpose-like nature of life can be contemplated from both a philosophical and an evolutionary prospective. From the point of view of the last phase transitions and the last derived developments toward life, changes can be perceived as deterministic, but from the point of view of the earliest steps (geochemical evolution, prebiotic syntheses, random catalysis, self-assembly and formation of vesicles), developments toward life seem either random or purely physicochemical causalities. To the inquiry as to whether or not life is a natural consequence of the universal laws of physics (Guerrero 2002), the answer appears to be ... YES!

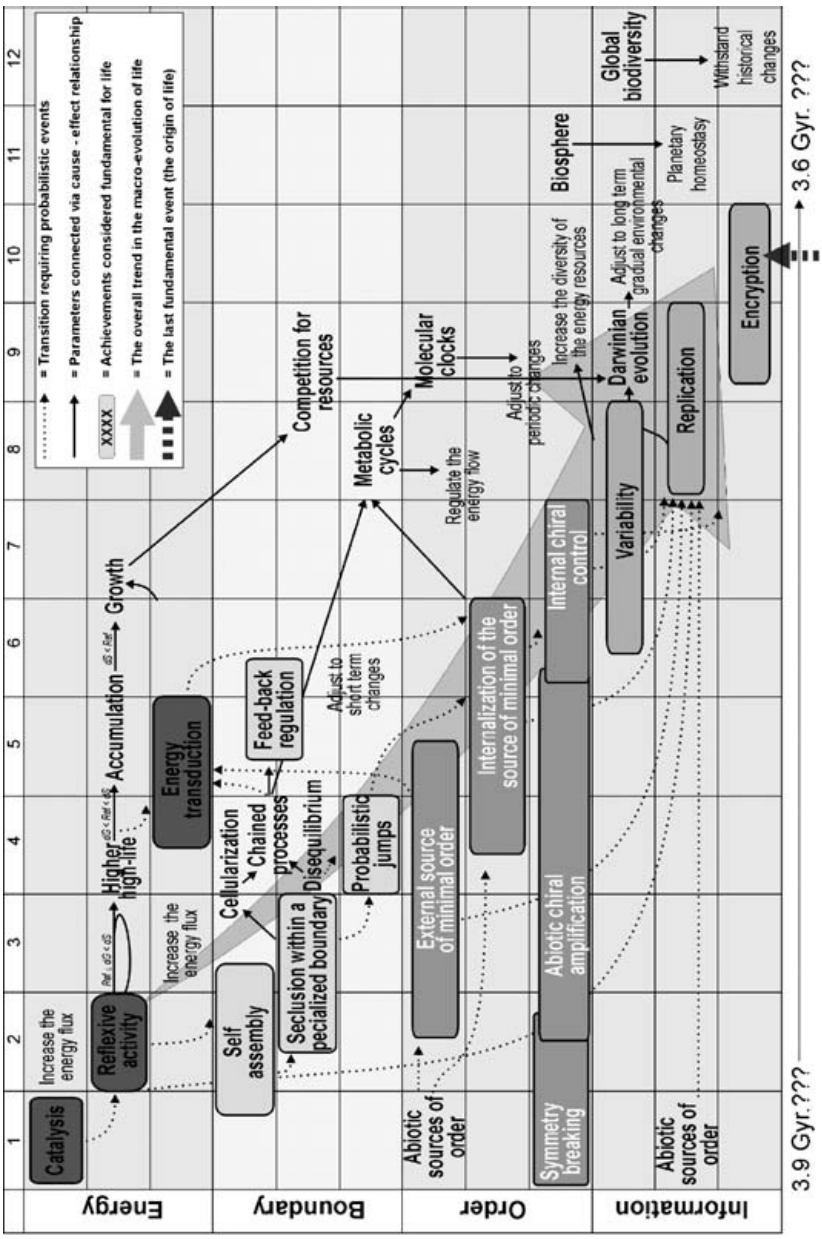


Fig. 7.5. Features of the evolution of life. The maximization of the energy flux as an overall trend during the emergence of biological life

Life is not the result of any ultimate causality. There is no such thing as a life-oriented universal determinism. The successful construction of artificial life will not occur as the result of miracle outputs in random experiments but upon the application of the right environmental requirements at the right moment, consistent with theoretical expectations. The truly scientific experiments in exobiology and artificial life are not those based upon a 'let's do that and check what happens' strategy, but those based upon a 'let's think it over and give it the right circumstances so 'we can make it happen' strategy.

8 Assembling the Early Puzzle of Life

> The origin of life cannot be discovered,
> it has to be invented.
>
> Eschenmosher 2001

So far no theory, no approach, no set of formulas, and no blackboard scheme have been found satisfactory in explaining the origin of life. The absence of solid experimental evidence is the largest drawback in the theoretical models of early life. Therefore, no one may claim the 'ultimate model'. At the current level of knowledge, this remains impossible. The theory presented here gathers what I consider the most compelling data into a model based on the principles of hierarchical accretion. The challenge to any hierarchical theory about the origin of life is to organize the early achievements of life in a sequence that is thermodynamically meaningful and provides temporal direction (overall increase in stability and efficiency). Some of the major assumptions of this model are as follows:

- The origin of life is not purely conjectural nor is it purely additive. Life is hierarchical at both the constructive level and in its ontogeny. Thus the accretion (origin) of life must have been more than stepwise and probabilistic; it was also hierarchical. During the 'assembly' of life, the various achievements ('technological novelties') were not added as soon as they emerged as pure environmental opportunities. They were added (mobilized into the pre-life forms) in a meaningful order that was based on their usefulness and functional rank.
- The story of the origin of life is an all-or-nothing type of scenario. It cannot be truncated without a loss. It has to be a comprehensive description as no part can be left behind without misunderstanding the big picture.
- The early history of life contains both parametric (criterion-based) and causal (function-oriented) components.
- Life and living entities are not equivalent as philosophical categories. Life is a self-maintaining strategy to maximize the overall entropization, while living entities are the physical units performing this function. Apart from

R. Popa, *Between Necessity and Probability: Searching for the Definition and Origin of Life*, Adv. Astrobiol. Biogeophys., pp. 139–158 (2004)
http://www.springerlink.com/

their interdependence, life and living entities are expected to show some distinct properties and different evolutionary coercions and to result in different consequences. This is particularly important in the field of artificial life, where life might be achieved and yet the living entities may remain symbolic.

- Life is recognizable by what it does, and not necessarily by its form or composition (Farmer 2002). Although the material nature of different types of life may be different, they are all based on common universal principles (properties) such as reproduction, evolution, order, metabolism and energy flow.
- Some of the achievements of life are derived properties while others are phase transitions:
  - The derived properties of life are causal and therefore deterministically derived from preliminary circumstances. Some derived properties may be traced back to natural physicochemical phenomena and hence explained through life-independent causes, while some derived properties require life-related circumstances.
  - The phase transitions are probabilistic changes.
- The natural history of early life was not a continually smooth improvement but a case of punctuated coevolution. The five major themes followed by life forms during their accretion (energy, boundary, metabolism, specificity and information) gradually coevolved over time, their history punctuated by various phase transitions. The major visions dominating the models of the origin of life are:
  - Cells first, metabolism second, and genes third (Oparin 1924, Haldane 1929).
  - Replication (information) first, metabolism second, and cells third (Eigen et al. 1981).
  - Order first (Kauffman 1993).
  - Metabolism first, cells second, and genetic mechanisms third (Wächtershäuser 1988).

  It may not have been the intention of their authors, but these models are often interpreted as sequences of discrete achievements. This type of interpretation leads to irreconcilable theoretical paradoxes due to a built-in ranking of achievements that are too advanced to have evolved independently of each other. It is rather likely that neither cells, nor metabolism, nor information (in their modern forms) had historical priority. No such thing as replication first or cells first or metabolism first was ever possible. Because these features coevolved at a concerted pace (dependent on each other), their significance for life cannot be ranked or separated from each other.
- The sequence of events that led to life was not random but was dictated by managerial coercions with regard to its overall energetic efficiency. The energy and entropy requirements dictated the direction of the accretion of

life-related events, and are the primordial requirements for understanding the origin of life.

- On a cosmic scale, life is but a minor player that uses energy heterogeneities as a means of endurance. Everyone comparing living forms with watermills pushing water uphill into irrigation canals, or with sailboats navigating against the wind, must keep in mind that there will always be more water in the river than water in the canal and more waves on the ocean than sailboats.
- Life-centrist visions such as 'life viewed as a cosmic imperative' are overstated. The connection between nature and life is not reciprocal. Life forms might only exist within a natural framework of operating physical laws, yet the existence of natural laws does not guarantee that life has to appear.

Some scientists believe that life emerged as a symbiosis (mutualism) between independently developed mechanisms. It has been hypothesized that life emerged when a system capable of replicating information (Schrödinger 1944, Eigen et al. 1981) became coupled with another system capable of metabolism (Von Neuman 1948, Dyson 1997). The 'double origin hypothesis' (Dyson 1997) considers that life emerged twice: once as a creature based on polypeptides capable of metabolism, and another time as a creature based on nucleic acids capable of replication. I believe that symbiosis-related events played a smaller part in the history of life than a gradual accretion of achievements. The symbiosis mentality is generally supported by the spectacular phase transitions produced by symbioses. The pre-symbiosis 'participants' claimed by all these theories had too little functional independence and too little 'technological' maturity to exist by themselves. Symbiosis did play a role in the origin of life but its role was episodic rather than dominant. The early life pre-symbionts were not autonomous entities capable of metabolism or replication. They were simple mechanisms imported by the early life forms, which became more complex this way.

Most scientists believe that the origin of life is not a spontaneous process but an evolutionary process, which should legitimize the question: Did the accretion of early life follow the rules of Darwinian evolution? Although Darwinian evolution must have emerged at some point in the history of life and become conspicuous, some differences exist between the Darwinian evolution of the living forms and the punctuated coevolution toward life. Darwinian evolution means qualitative changes in the fitness of a genetic line while punctuated coevolution means technological upgrades toward becoming alive. The force behind Darwinian evolution is the crisis between the tendency of a genetic line to survive and environmental pressure, while its mechanisms are variability and selection. The force behind punctuated coevolution is maximization of the energy flux, while some of the mechanisms added were not gradual improvements but revolutionary technological upgrades (phase transition events) and their derived consequences.

The relative distances among the events on the diagram of the accretion of life (see Fig. 7.5) do not indicate equal time intervals. Some of them may have taken days to achieve while others may have developed over thousands of years. Furthermore, this diagram is not necessarily a representation of the historical genesis of probabilistic phase transitions outside in the lifeless physicochemical world. Some features such as the potential for self-assembly, abiotic sources of order and molecular recognition were already present in lifeless nature (in the form of precursor mechanisms) before they became part of the prebiotic evolution. The diagram presented (Fig. 7.5) is not a ranking of their abiotic likeliness, but a ranking of their mobilization in what later became life.

Even the most elaborate technological achievements of life are rooted into abiotic processes. The early metabolism originated in processes occurring at mineral–organic interfaces (Wächtershäuser 1988, Clegg and Wheatley 1991); energy transduction might have originated in periodic events (Boiteau et al. 2002) driving to group transfer reactions; photosynthesis originated in photo-oxidation, while the early translation could have been initiated in abiotic confinements (Kuhn and Waser 1981).

It is certainly easier to talk about the steps of the origin of life than to demonstrate them. The biochemical and molecular mechanisms present in modern life forms are so complex, so numerous and interconnected that during their accretion a multitude of directions was possible at every given point in their evolution. Due to the fact that the laws of thermodynamics rule most physicochemical events in life's environment, the evolution toward life could easily have gone another way or even backward, toward disassembling the life-related mechanisms into random chemistry. Thus, if life emerged, events should have proceeded toward accretion and not toward degradation, for some defined reason and for at least one of the possible paths. Identifying this path requires some guiding principles concerning the accretion of life, principles capable of addressing the purpose-like nature of life.

8.1 The First Step Toward Life: Coupling Catalysis with Reflexive Activity

Catalysis and reflexive activity can emerge in nature as chance-like events (see Fig. 7.5). Although physical factors and chemical composition may alter the probabilities, these events remain purpose-independent. The probability associated with catalysis and reflexive activity is hard to estimate because these features depend on numerous details. Their likeliness varies considerably among different models of prebiotic life and artificial life simulations. The complexity of most energy-dissipative entities makes catalysis and reflexive activity more appropriate for experimental simulations than tools in predictive models.

The probability of a catalytic event may be expressed in particular cases (chemical systems) as a change in reaction kinetics, and in more general cases (chemical and non-chemical systems alike, including artificial systems) based on the overall energy-dissipating potential or as a change in the rate of entropy increase. Because reflexive activity is a measure of how much of the energy processed by the system is invested in restoring the state of the system, the presence of reflexive activity may be identified either as a negative correlation between the extra energy-dissipation performance and the entropy variation within the physical domain of the catalyst, or as a positive correlation between the energy-dissipation performance of the catalytic unit and the distribution heterogeneity of the entropy in the overall system.

8.2 Self-Assembly

Self-assembly is the physical result of self-complementarity (Rivera et al. 1998), which is primarily a probabilistic circumstance. The forces behind self-assembly are usually short-range forces while their dynamics is local (Mayer et al. 1997). The computational applications of self-assembly that are best studied and most relevant for the type of life on Earth are:

- modeling structural features of polar solvents,
- cluster formation of hydrophobic monomers in polar environments,
- self-assembly of polymers (Mayer et al. 1997).

Self-assembly is an order-generating phase transition that is advantageous for pre-life forms because it requires no programmatic control. A numerical simulation of the order that may be created through self-assembly has been based on the changes in the entropy level in lattice molecular automata (LMA), which is an extension of the lattice polymer automata (Mayer et al. 1997). The formal definition of the entropy of such a system is:

$$S = k_{\text{system}} \ln Z \ ,$$

where k_{system} is an LMA intrinsic constant corresponding to the Boltzmann constant k_{B} for a physical system and Z is a partition function over the states of the molecules in the simulation. Because Z is not known explicitly, an approximation to the entropy S_{LMA} can be calculated from

$$S_{\text{LMA}}(t) = \ln \frac{\sum_{i=1}^{n} D_i}{n} \ ,$$

where n is the number of molecules on the lattice and D_i is an occupation pointer of molecule i with the value 0 if the molecule does not change its location (i, j) on the lattice between times t and $t+1$, and 1 otherwise. This formula is a representation of the partition function of n molecules in space (Mayer et al. 1997).

8.3 Seclusion Within Specialized Boundaries and the Origin of Metabolism

Some particular cases of self-assembly can result in phase separation at the molecular level. A variety of organic and inorganic materials can form structures with phase boundaries (Tanford 1978) and separate from the solution as microvesicles through multi-molecular aggregation (Turian 1999, Deamer 1986, Luisi 1993). Once a space is isolated behind a semi-permeable barrier, such as in vesicles or in surface mosaics, another set of derived features results, the most relevant of them being: thermodynamic protection, isolation from external noise, impaired exchange, compositional personality and entrapment of small molecular populations. The existence of a selective boundary required some control over compositional specificity but must have emerged before internally controlled disequilibrium and before metabolic homeostasy.

A selective boundary allowed the existence of an 'osmotic drive' (Kauffman 1993), believed to be responsible for the emergence of internal negentropic processes (Skulachev 1992) and the introduction of a spatial heterogeneity that led to vectorial activity inside protocells (Lyubarev and Kurganov 1995). The maintenance of low internal entropy is one of the most remarkable features of life, a primordial expression of self-maintenance. The vectorial activity (whether in the form of target-oriented chemistry or spatial organization of chemical reactions) was directly responsible for the origin of a large number of life-related processes such as chemiosmosis, assimilatory biochemistry, homeostasy and sensitivity. One notable derived property of life is the existence of cycles, which are a convenient way to speed up and regularize the energy flux while keeping valuable materials inside.

A numerical model for studying the principles behind autocatalytic cycles and their kinetics within proliferating chemical systems was explained in Tibor Gánti's chemoton model and used in computer simulations of life-like processes (Békés 1975). When cycle-based energy dissipation is initiated, the ratio of energy flux to material flux is maximized. An ideal organism or super-organism (such as a biosphere) is able to recycle all its building materials inside and only exchanges energy with the outside world. Therefore, the ultimate physical achievement of a biosphere appears to be that it becomes the perfect recycling-based energy-dissipating machine.

There appeared a cooperative and synchronous functioning of three subsystems confined within an enclosed space:

1. an autocatalytic chemical cycle,
2. the replication of polymers,
3. the ability to incorporate molecules into membrane shells.

This cooperation drove life toward other general achievements such as: metabolism, self-reproduction, spatial proliferation, mutation, and evolution.

8.4 Probabilistic Jumps Toward Catalytic Specificity

An important consequence of entrapment is the acquisition of a stable spatial heterogeneity leading to nonrandom reactions (a way of achieving chemical direction without a controlled program). This 'jump' toward order represents the probability of a random mixture of monomers condensing into a polymer that displays catalysis in a space that is small enough to experience its effects before hydrolysis leads to its thermodynamic obliteration (Dyson 1997). Correct choice means a drift toward a desirable quasi-stationary state. The probability of a correct choice at each mutation inside one droplet containing a molecular population is

$$\phi(x) = \left(1 + ab^{-x}\right)^{-1} ,$$

where x is a fraction representing the active monomers in a mixture, a is the chemical diversity (the number of types) of monomer units, and b is the specificity of the emerging catalysts.

In an open fluid, even if a catalyst formed, its effect would be so 'diluted' in space and its repeatability so remote, that it would not make any difference (i.e., influence the overall chemistry) or promote its further occurrence. Large populations of solutes have no memory of early randomly emerging ordered states. This observation supports the hypothesis that during the accretion of life, spatial seclusion was historically positioned ahead of Dyson's probabilistic jumps (see Fig. 7.5).

8.5 Feedback Regulation

Feedback regulation is one of the most relevant events in the history of metabolism. This process was allegedly associated with a chemical direction inside early cells and contributed greatly to their physiological determinism. Feedbacks represented the basis for internal regulation and led to a generalized homeostasy. In conjunction with gaining chemical direction and control over order, feedbacks provided the tools for the emergence of autocatalytic chemical cycles (Gánti 1974, Békés 1975). Because feedback regulation and especially forward regulation require the pre-existence of a chained process and a minimal catalytic specificity, it was historically situated after seclusion within a specialized boundary and after the early probabilistic jumps (see Fig. 7.5).

8.6 Internalization of Minimal Specificity

This evolutionary transition (the internalization of minimal specificity) somewhat overlaps the concept of 'spontaneous emergence of autopoietic organization' (Varela et al. 1974, McMullin and Varela 1997). The difference is that

the internalization of minimal specificity is a looser yet more general concept: it is stepwise, and considers that internal order could have originated via the direct import of an external source of specificity (mainly order). Although external sources of order were used very early during the accretion of life, their internalization must have followed spatial seclusion. The later development of internally generated order must also have required the manipulation of energy.

8.7 Control over Chirality

Some authors consider that the existence of chirality is related to the particular materials that the terrestrial type of life is made of and hence that chirality may be an unavoidable secondary feature on Earth. Chirality is used by life as a simplifying management tool, providing the ability to control high levels of organization (such as architecture and activity) through lower levels (such as composition and sequence). Our current knowledge regarding molecular evolution does not support a scenario in which a few amino acids of a chemical origin were first selected for chemical convenience and in which only subsequently were living forms compelled to solve the chiral problem (and then only because amino acids were handed). It is more likely that chirality was approached from the beginning as a managerial advantage. The entities to become alive selected a variety of molecules from their environments, but those that were stereoisomeric were particularly advantageous and therefore sought-after. Instead of amino acids being the option and chirality their consequence, chirality was the strategy and amino acids the instruments of its implementation.

The details of the origin of biological chirality are unclear. The theory of the biological 'big-bang' states that the spontaneous breaking of the mirror symmetry in initially racemic mixtures led to a chance-like chiralization and enzyme-like activities, and that these in turn led to enantio-selectivity, which further enhanced chiral disruption (Avetisov and Goldanskii 1993). The present theory assumes that no such catastrophic event was ever possible. The history of chirality displayed at least three phase transitions toward life:

- symmetry breaking,
- life-independent chiral amplification,
- import of a chiral control mechanism.

None of these events was strong enough to bring chirality fast enough to the 100% level. A low, yet architecturally significant chiralization must have preceded life. The control over chirality could not have been invented *de novo* by early life while it was emerging because:

1. its absence leads to severe stereochemical inconsistencies in the architecture of the macromolecules,

2. racemic states impaired all types of stereo-specificity-based catalysis,
3. it is much easier to import such a mechanism and to adjust it than to invent it *de novo*.

The addition of chirality to biochemical evolution represented a probabilistic phase transition, but its gradual improvement (qualitative upgrade) remains deterministic and was efficiency-driven. The internalization of control over chirality allowed the amplification and expansion of homochirality to other life-related monomers based on enantio-selectivity. Although life-independent amplification of the symmetry breaking existed before architectural specificity was initiated, the life-related chiral amplification was a byproduct of it. Despite the fact that the chiral expansion and 100% internal chiralization required an advanced specificity and an early form of control over information, advanced chiralization must have preceded the enzymatic control over semi-conservative replication, which is hard to envision without chirality.

8.8 Inheritable Variability

Variability in modern life forms is due to multiple causes such as import of information, learning and, most important, mutation. The most general description of a mutation is an alteration along a molecular chain responsible for the preservation of information. Yet variability was not always represented by sequence alterations in molecular chains. The earliest and simplest forms of variability were represented by environmental drift, later by structural tolerance of mineral surfaces to compositional and distributional alterations, and only later became represented by inheritable changes in molecular sequences.

The first rule dictating the appropriateness of a material as a genetic substrate is structural resilience to the variability of the sequence. This requirement is one of the most important factors driving the emergence of the DNA molecule. Because variability is so ancient, so non-specific and so pervasive in nature, it is hard to tell where exactly it became a part of the history of life. The mutation rates in modern microorganisms can be as low as 10^{-8}–10^{-6} base mutations per site per generation but it must have been much lower (10^{-3}–10^{-2}) in early life forms (Valenzuela 2002). This alleged high early variability was used as a tool to demonstrate mathematically that neutral fixation was not likely in the beginning and that major features of life such as self-organization, self-maintenance, repair mechanisms and the initiation of the genetic code and reproduction were acquired in less than one million years (Valenzuela 2002). After that, evolution was much slower and the weight of each mutation in evolution decreased with some drift becoming possible, while later evolution became combinatorial, i.e., based on rearrangements (Valenzuela 2002). At its evolutionary apex (with variability present as inheritable changes in molecular sequences), a considerable interdependence existed between sequence architecture and function and this step is thus believed to have required an advanced chirality (see Fig. 7.5).

8.9 Replication

According to Leslie Orgel (1992): "The chemical basis of replication is autocatalysis" (Szathmáry 2002). This is certainly true in the case of modern life and in an early RNA-dominated world, but it was not necessarily so at the beginning of life. One can also envision an assisted replication in which the template is catalytically passive and a different system acted as a catalyst. Paraphrasing Eörs Szathmáry (Szathmáry 2002): the origin of life required the emergence of natural replication whatever its type was.

Autocatalytic replication can be realized either through self-catalytic units or through autocatalytic sets (mutual catalysis) (Farmer et al. 1986). One of the simplest analogies of a self-replicating cycle is the formose reaction discovered in 1861 by Butlerov (see Fig. A.6) (Szathmáry 2002). Other cycles have also been proposed (Bag and Von Kiedrowski 1996, Von Kiedrowski 1999, Wächtershäuser 1988). Although the kinetics of assisted replication and self-replication of nucleic-acid-related molecules is relatively well understood (Eigen et al. 1981, Von Kiedrowski 1986, Von Kiedrowski et al. 1989, Nowick et al. 1991, Rebek 1990, 1991, Tjivikua et al. 1990), its early performers are unknown. The replication of nucleic-acid-related compounds is commonly presented within the framework of template reactions with one molecule serving as a pattern (model, guide, template) for the formation of others.

The need for accurate template-directed replication is a vision influenced by the way the modern biological world is constructed. However, this was not always true during the early history of life. Replication may not always have been template-directed. Unlike the modern catalysts that are derived from a translation process, the earliest protocols to convey information were not cryptic but explicit. According to Maynard-Smith and Eörs Szathmáry, evolution went from holistic toward digital replicators (Szathmáry 2002a and 2002b). From a replication point of view, these concepts (holistic information and digital information) overlap with the concepts of contextual information and cryptic information described in this study. One great advantage of explicit information is that it does not require high accuracy. This is important because it emphasizes the fact that, historically, the emergence of encryption came after the invention of accurate replication.

A catalytic component (participant in a mineral-to-organic world, a peptide-based world, or an RNA world) can tolerate considerable sequence deviation as long as the overall structure at the catalytic site resembles the topography of its predecessors. In most mathematical models of early life, the need for accurate replication is implicit in the initial postulates. This results in the replication errors of the offspring being inherited and amplified with each successive generation, until information becomes obliterated. These models are not accurate representations of the real early world. Death was often regarded as an essential factor of life (Engels ca. 1880). Yet death might have played a different role in the beginning of life. Accurate replication is absolutely required only if death is a normal part of the life cycle,

so that the offspring must always provide a template for future generations. But in a world where living entities 'live forever', with offspring 'budding' continually from the same source (i.e., from a template mother) while also sharing explicit information, there is no need for replication to be absolutely accurate.

As an analogy, not all seeds of a tree end up as reproducible offspring. Many never germinate while many become alive but their leaves never make it to the canopy thus showing lesser reproductive potential. Even if one in a million seeds becomes a mature tree, the life of the parent ('the genetic queen') is so long and the number of seeds so large that the overall forest endures. Therefore, in a deathless early world, only the replicates that resemble the parental strands need to make it to the next generation, while all others may exist as sterile offspring. This particular situation gives lots of space for imprecise replication to exist, without the risk of an error catastrophe (see Appendix C). The early biosphere may have functioned with a smaller number of such 'genetic queens', and with a myriad of physiologically potent 'genetic mules' which were either reproductively defective or could not afford more replication mistakes. Yet if an ample lateral gene exchange existed, a collective strategy for survival could be implemented and the equilibrated replication of many alleles became possible.

Although the template amplification part of the replication process is deterministic, the initiation of replication is a probabilistic feature with the synthesis of a template molecule as the first rate-limiting step (Eigen et al. 1981). This template-directed process is believed to be a rather late stage in the evolution of replication. An alternative numerical model for an earlier spontaneous emergence of autocatalytic replication has been constructed which avoids the need for early templating (Farmer et al. 1986). When the initial set of chemical components reaches a certain diversity, autocatalytic reactions are initiated (Kauffman 1971) with a reasonable probability if the diversity is not excessive (Farmer et al. 1986).

The mathematical problem was formulated as follows (Farmer et al. 1986): If a number of oligomers of a defined length L (up to 1000) are present in a mixture, with $R \sim 0.5$ (where R is a measure of their interconnectivity), how many species are needed in order to generate an autocatalytic set? The critical value P situated between the subcritical $P_{\min}$ corresponding to exponential decay and the supercritical $P_{\max}$ where there is exponential growth, is expressed as

$$P \approx B - L \,,$$

where B is the size of the alphabet, L the radius of a firing disk, (i.e., a starting list of oligomers containing all chains shorter than a given length L), and BL the number of molecular species. Although P is difficult to estimate in real systems and becomes complex when kinetics is considered (Farmer et al. 1986), the model is still valuable to illustrate the probabilistic nature of autocatalytic replication and to exclude the need for a template. After

the initiation of autocatalytic replication, the system potentially explores an infinite-dimensional space (Farmer et al. 1986) and grows in complexity becoming an autocatalytic network (Rossler 1971, 1974, 1983). Later, hypercycles formed (Eigen et al. 1981), interpreted here not as probabilistic phase transitions but as a derived achievement.

8.10 The Last Step Toward Life: The Emergence of Encryption

Encryption is an achievement that separates information carriers from catalysts. Encryption allows the regulation of catalytic expression as a function of physiological needs and not as a function of environmental opportunities. Hence, while prior to encryption the response to environmental stress was straightforward and internal activity largely depended on external opportunities, the environment played a much lesser role in dictating the internal steady state after the emergence of encryption. Encryption also made possible the maintenance of 'phenotypically silent pathways' (in the form of silent genes), which in turn became a basic tool in the emergence of physiological ontogeny.

Encryption also provided the opportunity for discontinuous expression and storage of huge amounts of information in small spaces. A significant consequence of the existence of stored/silent information in the form of sequence motifs is the possibility of programming cellular activity. This programming is considered crucial for the installation of the vital state and for allowing the laws of Darwinian evolution to proceed (Arrhenius 2002). Modern Darwinian evolution might therefore have appeared after (or be contemporaneous with) encryption. Situating the emergence of encryption before replication would lead to substantial difficulties (Eigen et al. 1981, Niesert et al. 1981, Dyson 1997), because early and less precise encryption increases the need for accurate replication. It is also suggested here that precise encryption followed replication in the history of life.

8.11 The Non-Life-to-Life Transition

It has already been theorized that the last or the crucial steps toward life were represented by one of the following:

- The coupling between self-replication and a membrane-bounded structure (the minimum protocell) (Morowitz et al. 1988), which also led to chemical autopoiesis (Bachman et al. 1992, Luisi 1993).
- The replication of information which became related to the metabolism (Von Neumann 1948).

- A 'double origin' hypothesis which considers that life emerged twice: as one creature capable of metabolism and based on proteins and another creature capable of replication and based on nucleic acids (Dyson 1997).
- The symbiosis between an RNA-based peptidyl transferase and a peptide-based nucleic acid polymerase (Kunin 2000).

Because no universally accepted opinion exists on what life represents, there is little consensus on where to draw the line between lifeless phenomena and life.

The nucleic acid/protein relationship is credited with so many derived consequences that it is also frequently used as a sine-qua-non explanation of how life emerged or even as a way of telling life apart from non-life (Kunin 2000). Even if the nucleic acid/protein relationship has many obvious beneficial consequences for the biological type of life, it is not appropriate as a non-Earth-centric discriminator of life, because the living state is independent of any particular molecules.

Replication has been interpreted as a significant transition toward the living state (Csanyi and Kampis 1985). Replication has numerous consequences but it is probably insufficient to make something alive because replication is not the same as reproduction (Szathmáry 2002). Moreover, the variety of types of replication described so far, such as phenotypic replication, modular replication, processive or holistic replication (Szathmáry 2002), and semi-conservative replication, plus the possibility of generating specificity by using templates that are unlike their products (e.g., the translation of mRNA into proteins), make replication less relevant as a starting point for life.

A popular opinion is that Darwinian evolution is the achievement separating non-life from life (Miller 2002). This opinion is partly based on the great success of Charles Darwin's legacy. However, it is well known that Darwinian evolution is not a phase transition but a consequence of preliminary circumstances such as selection, competition, and inheritable variability. Using the principle that it is not the output but the cause that defines the consequences, I argue against using Darwinian evolution as a transition point toward life. When the history of already-present-life is discussed, Darwinian evolution may seem like a cause of most features. However, when the non-life-to-life transition is discussed, Darwinian evolution is a consequence and not a cause or a non-life/life boundary.

It appears obvious that, in order to identify a transition toward life, a set of features rather than one single feature is required. "Molecular-informational feedback loops in utilization of materials and energy from the environment for the purpose of growth, reproduction and evolution" has been considered as the threshold to assess whether something has become alive (Lahav and Nir 2002). Bio-functionality and natural selection based on fitness is also believed to have represented the point where a system emerged from chaos into a Darwinian selection regime (Arrhenius 2002). The transition into a system characterized by replication, variation (i.e., variability) and selection has also

been interpreted as a transition from the inanimate to the animate state (Kuhn 2002).

Considering that certain preliminary achievements are present, as discussed in early chapters, the last event required to judge that something is on an irreversible route to becoming alive is the onset of encryption (and decryption) of genetic information. This boundary is proposed here as a way to identify the beginning of life. Like self-assembly, encryption is not a property commonly included in computer-based simulations of life because computer programs do not perform their own assembly and their own decryption from a blueprint to physical realities. The task of decryption is performed by the system manager. In this theory, the universality of the modern genetic code is explained by many codes emerging in the beginning (Kuhn and Kuhn 1978), followed by a competition that eliminated all the 'clones' unable to share information through horizontal gene transfer. This early selection is defined here as the 'war of the codes'. The main outcome of this genetic war was: if cryptic information is exchanged laterally more than it is transmitted vertically, there can be no more than one language (one genetic code) remaining.

After the formation of life, a biosphere was created with significant consequences for global homeostasy, considered in the Gaia theory as a planetary self-regulating entity (Levine 1993). It has alerady been suggested that a fundamental condition for the formation of a biosphere is the ability to evolve (Szathmáry 2002).

8.12 Cosmochemical and Geochemical Requirements for the Origin of Life

Many scientists claim to have simulated or theoretically addressed the prebiotic conditions that led to life. It is imperative to know that only some specific cosmochemical and geochemical circumstances are appropriate for the emergence of life. According to Ricardo Guerrero (Guerrero 2002), besides water in the liquid state, the self-maintaining behavior of life seems to have absolute requirements for:

- self-bounded cellular organization,
- a source of energy flow where the system intervenes,
- a source of electrons (an electron donor) from chemicals such as hydrogen, hydrogen sulfide or carbon chemicals,
- a reversible sink of electrons (an electron acceptor) to complete redox reactions,
- a source of carbon, usually carbon dioxide, or carbon–hydrogen compounds of molecular mass under 250.

Although in general one may agree with this list, from the perspective presented in the present study, the list still requires more quantitative assessments and consideration of the following questions:

- How much energy is enough?
- Can there be situations with too much energy?
- Is there a relationship required among the available energy, the entropization and the efficiency of energy conversion?
- Is the presence of an electron sink enough and is the force of the attractor also important?

Thus a quantitatively-oriented (although not yet exobiological) set of cosmochemical and geochemical requirements has been suggested (Arrhenius 2002). In this interpretation the origin of life requires:

- a sufficient rate of supply of source molecules to compete with the destruction rate,
- selective concentration of desired reactants with the exclusion of interfering species,
- selective condensation, utilizing natural mechanisms to form sequence-specific libraries of nucleotides, peptides or other macromolecules assumed to have formed prebiotic scaffoldings,
- random interaction and growth of such species to reach the large sizes that eventually permit bio-functionality.

According to another interpretation (Benn 2001), the key requirements for organic life to emerge on an Earth-like planet are believed to be:

- a source of organic precursors,
- solid surfaces where precursors can condense,
- long-term maintenance of temperature within a range suitable for organic reactions,
- protection from hazards (e.g., meteoric impacts).

Although this set of requirements is more appropriate for an exobiological approach, it is still too Earth-centric to have generalist virtues and to represent the requirements of life independently of its composition. Based on the major properties of life, this study suggests that the general requirements for the emergence of life are as follows:

- An overall tendency capable of pushing events toward fruition must be present in the real world. This force is related to the second law of thermodynamics, but in an artificial type of life, an analogous alternative attractor may be used.
- The energy density of at least one convertible energy resource must be larger than the overall ΔS of the components that are coupled with the use of the irrespective resource.
- The difference in activity (catalysis minus the efficiency of the competitors) must be higher than the effect of the restricted access to building materials resulting from the presence of a boundary.
- A fluid state must be available to allow 3D combinatorial connectivity.

- Enough diversity is needed to provide the major catalytic processes, e.g., polycondensation, polymerization, degradation and group transfer.
- A potential for spatial seclusion must be present in the environment.
- In order to provide an open field for evolution, the environment must not be homogenous, the stability of the preliving entities must be larger than the variability, which in turn should be larger than zero and large enough to offer solutions to environmental crises.

8.13 Major Trends During the Early History of Life

A variety of specific features of life can be used to identify the general patterns during early evolution (Boiteau et al. 2002):

- self-regulation,
- molecular economy (the use of a few classes of molecules with a high combinatorial probability),
- molecular recognition (allowing cross-interactions),
- self-assembly,
- the use of specialized energy holders,
- the presence of cyclic pathways,
- the use of specialized holders of blueprint and functional information (heredity).

Five major themes have been followed in this study regarding the emergence and early evolution of life: energy, boundary, metabolism, order and information. The polymeric types of life are likely to be characterized by another feature (handedness) which might not be a condition for the existence of extraterrestrial types of life. All these major issues are directed toward the same goal: the maintenance of the physical identity of living entities.

Throughout the history of these themes, a variety of steady and transitional states were present. The states characterizing the different major issues are (and were) not independent of each other. According to this theory, in order to understand what life is in general and in order to identify the universal signatures of life (and solely for these purposes), the nature of its physical expression as well as the particular mechanisms must be treated as irrelevant. Only the major themes, their driving forces, the fundamental phase transitions and their universal derived consequences are important.

The list of features of life cannot be fully comprehensive for two reasons. First, an excessive number of features of life (and interpretations of their meaning) exist, making an objective and comprehensive review difficult. Second, a large number of life-related fingerprints that are common to the biological type of life have been purposefully avoided in order to focus the issue on life as a general concept. The evolution of chirality was added to this scenario only because of its crucial importance in understanding the

evolution of the terrestrial type of life, and also because it might be important for other types of life as well, if they are chemical and polymeric.

8.14 Differences Between Early Life and Modern Life

Apart from being simpler, early life differed from modern life in many respects:

- The energetic metabolism has two antagonistic components: assimilation and dissimilation. It is generally believed that one major skill of life forms is to keep assimilation plus repair (i.e., reflexive activity) to a high enough level to counteract the damaging effect of hydrolysis. However, such a strategy only works as long as the intensity of hydrolysis remains constant. Random environmental fluctuations generally result in lethal consequences because the state of the entire system is difficult to monitor. It is hypothesized here that the strategy followed by the early forms of life was to couple assimilation with self-imposed dissimilation and to deliberately increase the value of dissimilation to a point significantly larger than the environmental hazards. Consequently, the variability of the hydrolysis could be 'smoothed'. This biochemical strategy might represent the origin of the tendency of modern life forms to over-respire. In modern life forms, assimilation and repair systems functio n together with others capable of controlling the quasi-state of the system and adjusting the reflexive activity accordingly.
- Although all early living forms contributed to the overall energy and material flux, not all of them were capable of self-reproduction. The early populations were composed of a mixture of individuals:
  - A small number of them ('genetic queens') were 'lucky enough' to have an accurate replication and thus were capable of self-reproduction. These 'genetic queens' transmitted information both vertically and horizontally.
  - Most organisms, although derived from a vertical transfer of information, were functionally and reproductively different from the parental entities due to imprecise replication or a flawed information-inheritance protocol. Their survival required considerable lateral exchange of information. The early world was numerically dominated by these mutant 'genetic mules', otherwise capable of a variety of ecophysiological activities.
- External chemical richness and high activity were inhibitory (disruptive) for the maintenance of chemical personality (steady state) within early, poorly-confined vesicles. It was neither chemical diversity nor environmental richness that was mostly missed by preliving systems, but rather the avoidance of too much of them. Many modern organisms live in a 'cornucopia' of organic materials, but they can easily manage this by being well

isolated from outside molecules. This gradually evolved into a total dependence of external organics and ecological parasitism, culminating with human cells using a package of tens of thousands of genes, yet unable to synthesize 10 of their 20 proteinaceous amino acids.

- Death was not always the 'Nemesis of life'. Genetically programmed cell death (apoptosis) played no role in the early evolution of life. Programmed death is a 'luxury' of an advanced genetic line, invented late after life originated in a period dominated by Darwinian evolution and accurate replication.
- Presenting early evolution as a set of successive generations is totally wrong. The alternative proposed here minimizes the possibility for an information catastrophe (see Appendix C), because variation was mostly transmitted horizontally and replication therefore occurred in the form of small packages rather than a genome.
- The early world was dominated by molecular Darwinism and not by Darwinian evolution. The offspring inherited what was possible (in terms of precision) given their 'current technology' and improved it later through other means. Unlike in modern life, most information required for the functioning of early life was gathered after reproduction via lateral exchange of information. Lateral gene transfer was more important in functional determinism than the vertical transfer of information.
- Gene replication was a collective attribute and not an individual skill. Only some individuals (replicators) of a population display this ability. Early life forms existed as a genetic clan or not at all, with some individuals capable of replicating genes, some capable of various metabolic performances, all of them exchanging information actively and all of them speaking the same genetic language (i.e., the same genetic code).
- For a long time during early evolution, genetic information was explicit and not cryptic. This explains how early living forms could function without using a 'hi-fi' replicase.
- As soon as the thermodynamic conditions on Earth allowed, life emerged in a variety of types and used a variety of functional principles and building materials. Even among life forms made of the same materials, a variety of genetic codes existed.
- Only organisms with compatible informational packages could play the game of surviving by exchanging information. The ample lateral gene exchange (driven by imprecise replication) led to a severe inter-clone competition which favored those 'genetic clans' capable of greater functional plasticity and greater information sharing. The present genetic code is universal, although it is not the only one possible, the only one present or the most stable.
- In most modern theories, the universality of the genetic code is explained on the basis of a unique primordial protocell referred to as the protobiont, minimal cell, last common ancestor (LaCA, LCA), last universal ancestor

(LUCA), cenancestor, first living being (FLB) (Altstein 2002), urgenote (Guimarães 2002) or preprokaryote (Baltscheffsky and Jurka 1984). Some theories explain the uniqueness of the genetic code through panspermia (life seeded from the outer space). According to the present theory, the universality of the genetic code is explained by many codes emerging in the beginning, followed by a competition (a war of the codes) that eliminated all clones unable to share and understand shared information. Paraphrasing Hucho and Buchner (1997), who refer to signal transduction, the import of extraneous information was as fundamental a feature of early life as metabolism and self-replication.

- Late in evolution, the complexity and size of the informational package required to build an individual became so large that the vertical transfer of information became dominant and overrode the horizontal gene transfer.
- The early life forms did not have one chromosome. They had many internal genes in many copies. The formation of a genome, gathering all the information of one individual in a single continuous package (an RNA-made chromosome) was dictated by a variety of coercive effects such as the need to inherit all the information after the physical separation of the offspring, the need to have at least one offspring functional after separation in the event of an incorrect replication, the need to separate information-related molecules from function-related molecules, avoiding genetic selfishness and the need for temporal control over gene expression.

8.15 Early Life and Artificial Life

A common motif in modern science is the creation of analogical models of early life and the exploration of their derived consequences. The field of artificial life is a guiding star of modern science (Langton 1989, Emmeche 1992, Adami 1998, Bergareche and Ruiz-Mirazo 1999, Standish 1999). One of the most insightful consequences of this field is to postulate that the logic behind life is beyond its material nature (and thus to admit that life may be made of a variety of materials). Unfortunately, energy considerations and territorial issues are often misrepresented and even considered as less relevant for the logic behind the accretion of life in most artificial life simulations. Although this may seem reasonable for a computer world where little if any second-law-directed entropization is present, such logic fails when applied to the real world. Not only is entropization an important issue for life, but the need for an attractor, a purpose-like nature of life, becomes more stringent the more complex the early life forms become.

Only a couple of simulation models (computational metabolism) take into consideration the energetic/thermodynamic and spatial requirements of the living state such as dissipation, irreversibility, couplings, currencies, and con-

tacts (Bergareche and Ruiz-Mirazo 1999, Ruiz-Mirazo et al. 1999). This deliberate avoidance is attributed to two major factors:

1. Most mathematical models are by construction symbolic simplifications, while the mathematical representations of energy requirements and the consequences of a boundary are not trivial.
2. It is easy to ignore these issues because software-based information suffers little (if any) from time-scalable degradation (the equivalent of thermodynamic extermination). At first glance it might seem nonsensical to introduce an extra variable (random degradation) to a model already burdened by complexity; a variable with consequences that are hard to anticipate but surely negative.

Models of artificial life have been constructed displaying mutation, self-replication, evolution (Pargellis 1996) and communication in neural networks (Maynard-Smith and Szathmáry 1999, Hertz et al. 1990, Vank et al. 1997, Rolls and Treves 2000). Even now, software-based artificial life is merely an analogy of a molecular parasite and not an example of a physical form of life, and thus teaches us too little about how life actually originated.

8.16 The Definition of Life

From a functional perspective life is defined as any strategy using internally replicated information to build and maintain negentropic energy-dissipative entities and to gradually adjust their functioning to spatial and temporal fluctuations. This parametric definition considers that living entities are either physical or virtual realities and describes them independently of their size, time frame or material nature.

From a function-related perspective, life is a self-reproducible (dependable) strategy to speed up and regulate an environmental downhill energy flow. Because this activity is consistent with an overall universal tendency (the second law of thermodynamics) and because a reflexive activity existed that was coupled with the energy dissipation, life as a phenomenon was promoted into existence by the same forces that regulate the overall direction of the second law. The 'purpose' of living entities is to become the ultimate energy-dissipating machines considering their material make-up. It may appear contradictory but in the particular case of life, function did not precede its temporal fruition. First there was the need, then there was the means, and later the function got perfected. Systems that can mutate freely and reproduce their mutations can evolve in directions that ensure their preservation (Horowitz 2002). Given sufficient time, such a system will acquire the complexity, variety, and apparent purposefulness that we recognize as 'being alive' (Horowitz 2002). Although this purpose-like characteristic of life cannot be used as a way to distinguish life from non-life, it still remains a powerful reminder that an outside attractor has to be present to drive life's origin.

9 The Material-Independent Signatures of Life. Forensic Tools of Astrobiology

> In astrobiology, apply common sense, postulate the general, search for the obvious, and expect the unexpected.

9.1 Rules of Thumb in Astrobiology

Biological life is intimately related to the geochemical conditions on Earth and is fit for this planet's energy flux. It has often been suggested that life was also built in accordance with the particular local conditions offered by the early Earth. Common sense dictates that the constructive details of life on another planet should also be a reflection of the particular local conditions. Moreover, the collective activity of all life forms on a planet should have some measurable consequences on the global geochemistry. Comparison with the Earth-bound type of life is certainly inspirational but only up to a point. One central rule in astrobiology is: life can be made of many things and can have many forms. The search for extraterrestrial life cannot be limited to the search for Earth-like examples. Despite the common sense of this guideline, a manifest tendency exists today to judge the geochemical conditions from other planets through Earth-colored glasses. Much too often we hear expressions such as 'conditions too hostile to harbor life', or 'the search for Earth-like planets as potential hosts of life', or 'chemistry appropriate for life', or 'water as the fluid of life', or 'terra-formation of another planet to make it appropriate for life'. Irrespectively of how hostile another planet might appear to our Earth-based metabolism, we cannot state with certainty that life cannot be present before a comprehensive investigation is performed which includes the search for life's material-independent signatures.

In our space exploration, we have only reached the Moon and we envision ourselves as explorers of the Universe. Yet the extreme size of the Universe and the eons behind us guarantee that other life forms and cultures are ahead of us. The only thing that separates us from getting in direct contact with

R. Popa, *Between Necessity and Probability: Searching for the Definition and Origin of Life*, Adv. Astrobiol. Biogeophys., pp. 159–172 (2004)
http://www.springerlink.com/

them is the enormous distance. Although intelligent life might be common in the Universe, information theory shows that the probability of finding complex systems decreases with their complexity (Anbar 2002). This means that we might explore thousands of worlds populated with microscopic forms of life before we have the "big third degree encounter". The notion of terraforming other planets to make them 'suitable for life' is a good example of this mentality and brings us to another important rule in astrobiology: do not try to change the experiment just to fit the model – explore it, try to understand it and adjust to it. Life might be there already in an unforeseen form.

Over the years, the idea has occasionally emerged that Earth has been so much bombarded by meteoric showers (tens of tons every day) that if any extraterrestrial life is out there, a contamination would have been inevitable. It follows that since this has apparently never happened in recorded history, we can assume that there is nothing to worry about and that we can safely bring samples from outer space back to Earth. Fortunately, most astrobiologists are cautious on this issue. A good example was the treatment of the Apollo sample returns (both humans and samples). Planetary protection is the branch of astrobiology concerned with the quarantine of the Earth from outside contamination and equally with the protection of other planetary bodies against unwilling contamination with terrestrial life. This discipline functions on the healthy principle 'better safe than sorry'.

All examples of life forms we know so far are discrete entities. An interesting model was presented by Stanislaw Lem in his novel *Solaris*. In this model life was presented as a living ocean; as something homogeneously distributed over an entire planet. Would we be capable of identifying such a form of life? Even if this hypothesis is correct, an external energy resource must always exist and be transduced, which would give us the tools to identify it.

Space exploration has provided no evidence of life on the planetoids our instruments have probed so far (the Moon, Venus and Mars). We have not explored enough of the Universe to make any categorical statement about the diversity and distribution of life out there. But when we set about exploration, have we been asking the right questions? Did we obey the implications of the basic rules of astrobiology? Astrobiology is the perfect example of a science in which the absence of evidence is not evidence of absence. After every mission reporting the absence of life, the job of the astrobiologists is to ask whether there is any caveat in the exploratory strategy that could have led us to miss something important. Do we understand the mistakes and are we able to avoid them in future explorations?

In the future, it may be possible for humans to land on Mars. We do not want our emissaries to be armed with knowledge based purely on Earth-like examples of life. We want them to be open-minded and aware of the most general characteristics of life. Otherwise they might report just dust and rocks and pebbles and the cold deserts, declaring the place sterile although they might be stepping every day on the very thing they seek. One of the

main rules in exploratory astrobiology is: When searching for ET life, address the essential more than the particulars, look for the obvious, yet expect the unexpected.

9.2 The Main Questions in Astrobiology

The two most common predicaments in astrobiology are:

- Can we implement an exploratory protocol with no articulate model of life?
- Is life measurable if the material basis of life is ignored?

In order to build a reliable strategy when searching for non-biological life forms in outer space, we must discuss the implications of the following questions:

- Is life a discrete phenomenon?
- How many parameters are required to define life?
- What are the most common false premises and fingerprints in exobiology?
- What principles should be used to define the general fingerprints of life?

Once we have answered these questions:

- What are the general signatures of life?

Is Life a Discrete Phenomenon?

This question can also be formulated as: Is life qualitatively different enough from lifeless phenomena or is life always spatially distinct? Can life be identified with any measurable precision? It has been asked whether a continuum exists between chemical systems that are unambiguously non-living and those that are unambiguously alive (Hazen 2001). If the transition toward life was a smooth upgrade, then we face the experimental problem of differentiating black from white on a scale that has all the grays in-between. If only a gradual transition existed, differentiating life from non-life is a matter of statistical interpretation and requires some artificially established conventions.

Controversial interpretations of where life should start are inevitable on a gray scale. Even if life on Earth is unitary (using a single genetic code) there are still heated arguments about whether viruses are alive or not. And if viruses are found to be alive, will the same argument apply to prions? And if prions are alive ... then what about crystal growth? Where should the line be drawn between non-life and life? This question has no easy answer and requires more thought and more experimental models to address it properly.

It is suggested here that the origin of life was not precipitous, and yet that it was not a smooth transition. Although the accretion of life displayed some gradual improvements, it was also stepwise. Probabilistic phase transitions

(qualitative jumps) in the history of life resulted in a 'bumpy' evolution. These sharp events can be used as transition markers to define life. If the transition marker for life is established to be the self-control of specificity, both prions and viruses can be considered as alive (Altstein 2002). But if the boundary is established to be the encryption of information, then viruses are alive, while prions are not. This approach allows one to estimate how far something is from becoming alive, because all the gradual changes between non-life and life can still be used as markings on a virtual ruler of life. It seems that life is a discrete 'thing' with observable properties and thus measurable.

9.2.1 How Many Parameters are Required to Identify Life?

Many phenomena can be experimentally identified using a limited number of parameters. Is life one of them? Is one single feature (parameter, achievement, or mechanism) enough to demonstrate that life is (or was) present? If more than one parameter is needed to identify life, how many parameters are minimal and sufficient. Science supports the claim that life is not one thing but a circumstance of many mechanisms acting together for the fulfillment of a common and more general goal. No single parameter appears to be enough to define life (Buiati and Buiati 2001, Kompanichenko 2002). Therefore, no experimental procedure or criteria can unequivocally differentiate between non-living matter and life (Buick 2002). There can be no such thing as a universal and simple life-detecting instrument (like Captain Kirk's tricorder). The Universe is so complex that the job of a life-searching 'detective' is not to find the smoking gun but to define life in a particular physicochemical context and amass relevant evidence until the major requirements of life are satisfied. Because more than one parameter is required to define life, the true question is: What set of parameters is needed to demonstrate its existence?

9.2.2 False Premises and Misguided Fingerprints in Astrobiology

The tendency to portray life as based on the particular features of life from Earth is the foundation of what is called the weak anthropic principle or anthropic cosmological principle (Barrow and Tipler 1986), the Earth-centric vision or geocentric vision (Conrad and Nealson 2001). The most frequent error when looking for non-biological life is to overemphasize the importance of water, carbon chains, bilayer membranes, proteins, nucleic acids, and isotopic fractionation, and to use a narrow view of the meaning of energy.

Water and Life

Considerable resources are invested today into finding whether other planetary bodies are rich in water. Although water displays very convenient properties for life on Earth (Benn 2001, Rizzoti 2002), the inference that all life

in the Universe is water-dependent is probably wrong. According to most theoretical interpretations, it is not the chemical nature of the liquid water that is the most important for life, but rather its liquid state between 0 and 100°C. It is not the hydrophilic character of water that counts the most for the spontaneous aggregation of colloids and the maintenance of biochemistry, but rather the fact that it has a pronounced chemical character at all (which may be hydrophilic or hydrophobic). It can be envisaged that the bilayer membrane of a form of life developed in a very hydrophobic fluid must have a hydrophobic interior and a hydrophobic exterior and that lipophilic catalysts should dominate its metabolism. In a hydrophobic world, the major source of molecular inactivation will not be hydrolysis but solvation in the surrounding fluid and aglutination. The macromolecular aggregates would be packed through hydrophilic attraction and through short-range electrostatic bonds.

The future of extraterrestrial exploration for life does not belong to the remote sensing of water but to *in situ* study of local fluids, whatever their chemical nature. If the local thermodynamic conditions are tolerant enough to maintain the stability of the catalytic macromolecules, a variety of fluids should be appropriate for life. Certainly, one very helpful condition would be for the fluid to be either very hydrophilic or very hydrophobic in order to allow a strong phase separation in the presence of amphipates. Sampling a volume of liquid (say CO_2 or methane) from another planet and bringing it to a research station at temperatures and pressures in which it might volatilize would wipe out most evidence of potential life. Aliens living at 800°C and 10 mbar will never find any trace of life in samples taken from our oceans and brought into their conditions. The detection of new and unknown life forms is something that requires considerable *in situ* observation and less remote sensing.

Carbon Chains and Life

The importance of carbon chains for life has been so heavily emphasized in biology (Benn 2001, Rizzotti 2002) that an exaggerated perception has been created that life is likely to consist of carbon-based chains (Delsemme 2002). It has even been deduced that we might be able to find life in outer space if we look for spectroscopic signatures of C–C and C–H bonds. Experimental data shows that large molecular structures can also be made using other atoms such as silica, phosphorus, or sulfur. Even in biological life, complex molecules such as nucleic acids can form with a backbone requiring P and O, besides H and C. Although carbon has great advantages for life, there is no absolute need to condition the existence of life on an abundance of carbon.

Another frequent misconception is that molecular chains are necessarily required for life. Indeed, genetic information in the biological type of life is linear and written onto sequences of nucleic acids. Most enzymes function as tridimensional architectures but they are not made by accreting bricks

(monomers) directly in a final 3D configuration. They appear first as a linear string followed by a secondary 'wrapping'. However, in an early world dominated by information in the form of chemical events spread over a mineral surface, the information would not have been linear but bidimensional. A hypothetical living world can be envisioned that is capable of building 3D architectures directly, without a secondary folding of preexisting chains.

In this hypothetical world the monomers of life have at least three active radicals involved in the formation of the architecture. These monomers can be derivatives of amino acids, some of them di-aminated and some of them di-carboxylated at the C_α. Alternatively, half of the monomers can contain three amino groups and an active radical while the other half can contain three carboxyl groups and a radical. As in modern day proteins, in this hypothetical world the amino and the carboxyl groups from the C_α position are used for making amide bonds while the side radical is used to confer local chemical personality. Instead of the residue having 2 chemical connections between the C_α and its neighbors (as in modern day proteins), each monomer residue from the 3D world has 3 connections with its neighbors.

In this 3D world, molecular structures would be built directly in two or three dimensions. For this world, information would not be linear and decrypted from a sequence, but bidimensional (stored at the surface). It could be displayed as a rolling surface (or by rolling the nascent macromolecule over a surface) that matches its stereochemistry. In the 3D world, secondary folding would probably be less important and temperature fluctuations could become the tools of translation. As in conventional proteins, the monomers of this 3D molecular construct would be held together by amide bonds, disulfide bonds and H bonds. Unlike in the conventional protein world, an SDS treatment trying to unwrap the molecular complex would not result in polypeptide chains, but in molecular fences because each amino acid derivative is connected with three neighbors and not with two.

From an experimental point of view, we are lucky that the enzymes of our world were not built as in a 3D world, because it would have taken us forever to figure them out. Consequently, although they are essential for the terrestrial type of life, carbon chains are not necessarily related to the very essence of the living state. In chemical forms of life, the living state is dependent on atoms capable of participating in the formation of complex and reproducible architectures. Life on another planet will probably be revealed first through a chemical complexity analysis and not through its similarities with life on Earth.

Bilayer Membranes and Life

In our world the most common boundary that protects the internal chemical 'personality' from a random exterior is a bilayer membrane made of lipids and consolidated by 'rivets' made of proteins or of long ethers, diether- and tetraether-type polar lipids (Morii and Koga 1994). This type of boundary is excellent for an aqueous environment within reasonable temperature and

pressure ranges (between −10°C and +130°C and between 1 and 200 bars). Yet even in our world, living forms may exist in very extreme conditions such as in deep sea habitats, at high temperatures, in supercritical water, and under tremendous pressures. Remaining stable in these conditions requires more than a thin plasmalema (cell membrane). Most prokaryotes have other layers besides the lipid membranes, including cell walls, glycocalyx, LPS and S-layers. We know of no case of a living cell that does not contain lipid bilayers. Yet there is no reason to reject the idea that, in a world dominated by other fluids, the structure of the membrane might be different (see the above discussion about inverted membranes in hydrophobic fluids).

Moreover, boundaries can also be made by self-accretion not only from amphipates but from other components as well (see Chap. 4). The search for living entities in the fluids of another world will not be a search for vesicles made of lipid bilayer membranes in watery fluids, but rather a search for a phase separation or enclosed spaces (demonstrated through a distributional heterogeneity) a search with structures, with a distinct chemical personality (demonstrated as a steady state in disequilibrium with the exterior).

Energy and Life

All chemical bonds, all chemical components and ultimately all structures of life contain a certain amount of energy and a temporal stability that depend on the basic materials and external conditions. Knowing these conditions it should be possible to project limits for the minimal energetic cost of life and the quantity of energy required to build life forms. The minimum available energy required by life forms can be established primarily from the price of its activity and the yield of energy transduction:

$$\Delta E_{\mathrm{i}} = \frac{\Delta R}{B_{\mathrm{i}} \chi_{\mathrm{r}}} 10^2 \ ,$$

where E_i is the energy required within the system to keep it functional, i.e., 'alive', R is the energy dissipated through minimal maintenance processes, e.g., respiration, B_i is the energy embedded ino the structure of the system, e.g., biomass, and χ_r is an efficiency term showing how much of the imported energy is wasted as heat during minimal maintenance processes.

If the external energy is imported (transduced) with an efficiency χ_{i}, the minimal energy used in the living state is expressed as

$$\Delta E_{\mathrm{u}} = \frac{\Delta E_{\mathrm{i}}}{\chi_{\mathrm{i}}} 10^2 \ ,$$

where ΔE_{u} is the minimal energy used to maintain the system in a functional (i.e., living) state, and χ_{r} is the efficiency of energy transduction. Substituting ΔE_{i} from the previous equation, the minimal available energy required to maintain the living state can be determined as

$$\Delta E_{\mathrm{av}} > \Delta E_{\mathrm{u}} = \frac{\Delta R}{B_{\mathrm{i}} \chi_{\mathrm{r}} \chi_{\mathrm{i}}} 10^4 \ .$$

By measuring the half-life of some alleged living structures, it should be possible to estimate whether the available energy on a planet is sufficient. Although the mere existence of such structures should be a proof of energy sufficiency, the existence of active life involves more than just withstanding degradation. Life also deliberately keeps the energy degradation high, a feature that can be very helpful in its identification. The energetic requirements for life on a planet are not direct functions of the distance from its closest star. The planets that are closer to their sun are exposed to more energy per unit surface, but molecular structures are also degraded faster. The opposite is also true for more remote planets. Some theoretical energy limits have been established by balancing these factors (Sertorio and Tinetti 2001).

Unlike lifeless energy-dissipative systems which display a straightforward correlation between fluctuations in the energy resource and energy-dissipative performance, living entities display self-control, assisted transduction, anticipative cycles and energy reserves. Moreover, all living entities function according to some energy guiding principles, also called the laws of bioenergetics (Skulachev 1992) reformulated here to fit astrobiology requirements:

- First Law: Avoid direct utilization of external energy sources in the performance of useful work.
- Second Law: Possess at least two interconvertible energy currencies, one fluid-related and the other structure-related.
- Third Law: All energy requirements can be satisfied if at least one of the energy currencies is produced at the expense of an external energy resource and if sufficient external energy is available.

It has often been claimed that the Sun is an excellent source of energy because its light is abundant. Yet many life forms make a living with rather trifling energy resources. In the deep hot biosphere scenario, more biomass is stored underground than above ground. It appears that the relative energy potential of one energy source is important but not critical, if alternative sources can be used. From an astrobiological point of view, life does not require lots of energy to be present, but rather a transducible source of energy with a flux density sufficient to counteract internal entropization.

The energy-related existence of life must be demonstrated not solely on the basis of energy dissipation, but also through parameters such as reflexive activity, assisted transduction, the use of energy currencies, making energy reserves, buffering energy peaks, withstanding energy gaps and anticipative energy cycles.

Are Proteins and DNA the Only Polymers of Life?

One extreme consequence of the Earth-centric vision is to associate life with proteins and DNA (Yockey 2002). The search for proteins or DNA or for their monomers as a proof of life is a classic example of a misguided experiment. Assume for the moment that a PCR-performing instrument will be added to

a Mars mission as a way to check whether life is or has been present. Certainly, there is nothing wrong with asking if DNA-based life exists on Mars or if the Mars lander itself has introduced any Earth-related contaminants. What is wrong with this experiment is overrating its significance instead of addressing the real question: Is there (or was there) life of any type on Mars?

A PCR-based experiment is inappropriate because, from a cosmic perspective, DNA does not mean life, it only means complex chemistry. In the early history of life on Earth, DNA was selected as an easily manageable molecule under Earth conditions. Even on Earth, life might have existed for many millions of years before DNA was invented. It seems likely that on another planet, under different conditions, other structures should satisfy genetic functions. It appears that screening for any specific macromolecule in the outer space as an indication of extraterrestrial life (either proteins, amino acids, DNA, lipids or PAHs) is not so much a priority but an experimental luxury once the real questions have been addressed, and if there is still some room left in the spacecraft and money in the mission program. The real priority of a mission is to characterize the local geochemistry and to find macromolecules in general and heterogenous energy dissipation in particular.

Isotopic Fractionation

Atoms with different neutrons in their nuclei but with the same numbers of photons of each other are called isotopes. Some isotopes such as tritium (a superheavy isotope of hydrogen) or ^{14}C are in a relative quantum disequilibrium and over time tend to expel excess neutrons as radiation. These isotopes are called radioactive isotopes. Other atoms such as deuterium (a heavy isotope of hydrogen) or ^{13}C have an excess of neutrons but not enough to make them unstable. These isotopes are called stable isotopes. Mainly for kinetic reasons, heavier atoms are just a little less active in chemical reactions. A common consequence of using a long sequence of catalysts is the preferential use of lighter isotopes in final products, a phenomenon known as fractionation.

The enrichment of organic molecules in light stable isotopes is frequently interpreted as a sign of life. Enrichment in light carbon has been reported in organic compounds from 3.8 billion year old rocks (banded iron formations from Greenland). No fossils were found as these rocks are intensely metamorphosed sediments, yet the stable isotope signature remains. Despite some enthusiastic preliminary interpretations, this type of data is still inconclusive. First, it is hard to demonstrate that these organic molecules have the same age as their host rocks. They might be material infiltrated within the pores of the rocks millions or billions of years later. Second, life-independent processes such as the Fisher–Tropsch process leading to hydrocarbons can also result in significant fractionation (Dzik 2002), especially if repetitive cycles are applied.

Non-Earth-Centric Signatures of Extraterrestrial Life

Studying the history of early life has great benefits for exploratory astrobiology. If a general interpretation of life can be found, the identification of extraterrestrial life forms can be approached solely in terms of its material-independent fingerprints. In this theory the origin of life has been presented as a hierarchical accretion (a sequence of ranked achievements). The most important achievements involved in maintaining the momentous physical identity (thermodynamic resilience and maintenance of internal negentropy) were added first. The other achievements of life (e.g., long term maintenance, adjustment of the steady state to variable conditions and inheritance) were added later. Most of the characteristics displayed by life are derived and only a small subset contains phase transitions, actually responsible for the main changes toward life.

In modern life forms, all mechanisms are intricately tied together in a complex web that seems to have no clear causal beginning and no overall purpose. This complexity makes the discrimination of the essence of life from its form very difficult without creating circular arguments. But during the first accretionary steps of life, the level of complexity was lower than in modern life. Therefore, during the origin of life, its functional principles, its causal agents and its 'purpose' were more obvious. Simpler mechanisms and a lower interconnectivity among the functional systems should allow an easier identification of what is important about life as opposed to what is secondhand conjecture and derived complexity. Because the field of chemical simulation of life (biomimetics) is still in its infancy, numerical simulations and artificial life forms are yet the most valuable tools in understanding life.

Life has an intrinsic tendency to expand itself to an upper tolerance limit controlled by the available space, constructive materials and energy resources. Life appears as one of the most efficient means to dissipate energy for its composition, but it is not an ultimate natural development. Physicochemical events are not driven toward life by causal necessity. Therefore, although life is conspicuous whenever present, even if the local conditions might seem appropriate, life is not a necessary occurrence. It follows that the emergence and existence of life-like phenomena is not mandatory for a planetary body, because some of the prerequisites for the emergence of life such as environmental complexity, thermodynamic stability and reflexive activity are probabilistic and not always achieved. Expecting life to emerge by itself in early life simulation experiments is statistically flawed if the probability of the major phase transitions are not addressed, and legitimately sanctionable as nonscientific.

Observations indicate that only one type of life exists on our planet. This result has been used to support the hypothesis that life originated in a singular event in the geochemical history of Earth. The present theory alleges that life is alone on Earth because it competitively eliminated all other (informationally incompatible) forms of life. Because the evolution of life is oriented toward efficiency, the emergence and success of a novel (and less efficient)

type is very difficult in the presence of a preexisting competitor that wipes out all the material resources, uses energy more efficiently and fills the living space. The current form of life on Earth occupies all available ecological niches and only its catastrophic elimination on a global scale could create grounds for another type of life to emerge.

Before the entire surface of the planet became filled with one biosphere, numerous types of life could have coexisted. The stabilization of one type of life over the entire planet was probably driven by both the competition for resources and the strategic advantages derived from a horizontal flow of information. The apparent contradiction between the unity of life (the almost universal genetic code) and the deterministic character of life is not the result of a historic genetic singularity but the byproduct of a selective circumstance.

One cannot deny the experimental usefulness of some material-dependent signatures of life such as biominerals, redox differentiation or morphology. Yet the 'fingerprints' that seem most useful in experimental exobiology and artificial life are the ones that are material-independent. The present theory puts forward the view that, when exploring outer space for life, parameters such as water, carbon chains, bilayer membranes, energy richness, proteins or nucleic acids are not the best signatures. The true material-independent conditions and signatures of life are based on principles such as:

- the existence of a fluid state,
- macro-dimensional architectures with structural plasticity,
- spatial seclusion behind semi-permeable boundaries,
- the existence of a transducible energy resource, its flux density higher than the entropization of the transducer,
- the existence of an internal 'vehicle' for information.

Although understanding the overall consequences of life for the geochemistry of another planetary body might be generally foreseeable (if the local geochemical conditions are satisfactorily known), the form in which living entities would reveal themselves as well as the details of their mechanisms are not. The emergence of life is neither chaotic nor meaningless, but its material expression is not predictable.

The Scale Problem

As once suggested by Carl Sagan, living entities on other planets might live faster or slower and might be smaller or larger than we would expect here on Earth. While on Venus a second might represent an entire generation, on Pluto a hundred years might represent the equivalent of a blink of an eye. While on Earth a living entity ranges between 0.5 μm and 100 m, on a gaseous planet such as Jupiter, a living entity might spread over 500 km. If the length/size of an exobiology observation is not adjusted to local kinetics and mass density conditions, the measurement might be irrelevant. Therefore, before measuring life, an appropriate scale must be established that is related

to the particular environmental conditions on each planet. A preliminary study must be performed to establish how fast and how strongly the natural processes are occurring in the absence of the entities that are questioned as being alive, and to ascertain their life-independent stability. This will allow us to establish the frequency, amplitude, sensitivity (resolution), variability and number of measurements required for statistical confidence.

9.3 The Material-Independent Signatures of Life

None of the subsequent signatures of life has been found to be sufficient in itself as a proof of life. Yet they are relevant as a group.

1. Catalysis is the ability to speed up processes, yet it should not be interpreted as exclusively chemical. Although living entities have an overall tendency to speed up dissipative processes, catalysis is often the property of one sub-unit. The identification of a catalyst is based on the fact that, when separated from its host, it speeds up processes without changing the overall equilibrium.
2. Reflexive activity represents how much of the energy lost by the system is recovered through performing work on itself (see Chap. 4). Although it is controlled by specific mechanisms, reflexive activity is measured as an overall property and identifiable as a correlation between the intensity of the activity and changes in the state of the living system. Reflexive activity is not restricted to individuals. Symbiosis is an example of reflexive activity in which two living units are involved in negentropic reciprocation. In some cases the particular mechanism for reflexive activity is difficult to locate because many components can be involved in it, or because many intermediary steps might be used before the actual negentropic effect is implemented.
3. Energy transduction represents the deliberate conversion of some energy forms into alternative forms. Life forms use this energy to control their internal entropy level. The existence of energy transduction can be demonstrated from the ability to synthesize energy currencies that are too unstable to exist alone. Although their actual concentration might be low, their production rate can be very high (high turnover). One way to demonstrate the presence and the target-oriented nature of energy transduction is to disrupt it experimentally (inhibition or uncoupling) without altering the energy resource. This intervention should lead to the total collapse of currency production and the obliteration of the function of the system.
4. Using alternative energy resources for a common purpose must have subliminal consequences on the state of the system. Experiments must demonstrate that energetic alternatives are possible. Life-independent systems are usually unable to use other sources than those explicit in their design, and accordingly change completely when the resource changes.

5. Increased overall energy dissipation is the capacity of living forms to induce energy degradation (e.g., conversion into heat) faster than it might be expected on the basis of life-independent kinetics (Anbar 2002). Because energy dissipation is a molecular collective property, it must be demonstrated at an individual level or supra-individual level.
6. Internal regulation of the energy means that the energy uptake is controlled by more than external factors. This feature can be identified from the existence of an energy flux that depends not only on the available energy, but also on the state of the system and is directed toward maintaining its quasi-state.
7. Accumulation of energy is a derived consequence of life that can be measured from an increase in the overall energy content of the system. The existence of energy reserves can be identified from a time lag under fluctuations in the energy resource or even as functional resilience after long periods of total shut down of the energy resource.
8. Growth is a derived consequence of life that can be measured as an increase in size over time.
9. Death is the irreversible obliteration of the functioning of a living system. A living system can only exist as long as its minimal negentropic level is maintained. Therefore, because life cannot emerge spontaneously, disrupting the energy resource long enough must lead to the obliteration of its functionality, even if the energy resource is restored. Programmed death (apoptosis) is one of the most remarkable properties of modern life and can be shown as a resource-independent half-life whenever reproduction is impaired. Apoptosis is implemented and regulated at the individual level, yet its emergence is dictated by supra-individual needs.
10. Competitive energy subtraction means that, even though the system is physically separated from the exterior, the efficiency of internal catalysis is such that the energy flux density through the system is larger than the energy flux density through life-independent competitors.
11. The existence of an internal phase separated from the exterior by a selective boundary can be demonstrated by direct observation of shape patterns and by complexity analysis.
12. Impaired biased exchange with the exterior may be studied through distribution heterogeneity.
13. The existence of a quasi-state (homeostasy) can be demonstrated on the basis of a compositional steadiness that is more or less independent of the level of the energy resource, unless sub-minimal or excessive levels are reached. Most life-independent systems tend to change their state significantly as a result of changes in the amplitude of the energy resource.
14. Homeostatic resonance refers to the fact that, unlike lifeless energy-dissipative systems which display self-destructive resonance in certain conditions due to an unregulated accumulation of energy, living systems are able to adjust to periodic fluctuations in energy and to use them (i.e., accumulate energy) in a non-destructive manner.

15. Internal clocks are self-imposed periodic activities that exist by virtue of the functional principles of each system. Internal clocks (periodic activity) can be present even under steady energy sources.
16. Anticipative periodic activity is the phenomenon in which the existence of internal periodicity in the quasi-state, adjusted to periodic fluctuations in the external energy resources, is used by life forms for anticipative metabolic preparedness. This property of life is very common in green plants but less well studied in micro-organisms, which rarely show periodic activities. Such activity consists in deliberate (programmatic) changes in the quasi-state made with energy expenses in the anticipation of incoming energy peaks.
17. Adjustable clocks are another feature, in which the internal complexity of living systems allows them to adjust the periodicity of their internal state to match steady changes in the rhythm of periodic energy sources. Little is known about this interesting feature of life and more research is needed to understand it.
18. Internalized control over the steady state. A significant proportion of the state of the system is controlled by factors located inside the system. Therefore, the disruption of external patterns has limited effects over the stability of living forms.
19. A measurable variability smaller than the limits established by stability constraints ($0 <$ variability $<$ stability). The variability of living forms must be positive by design to allow evolution, yet below the threshold required to maintain collective stability. This condition is hard to measure directly, but evolution can be identified as one of its derived consequences.
20. Reproduction can be identified from replication, seedling effects and the capacity for multiplication.
21. The use of cryptic information is hard to demonstrate. However, encryption is a material-independent property of life that is worth exploring because it defines the transition toward life.

The alleged universal signatures of life described above are selected to be independent of the particular material composition. Taken alone, none of these features reveals life, yet not all of them are required to demonstrate that life is present. The number of signatures that can be assessed depends on the particular conditions and the physical nature of the subject under investigation. Using all these signatures to identify life would probably be costly and redundant because many of their derived consequences overlap. Moreover some of them might prove difficult to measure in some conditions, or the result of measuring them might be questionable. The best strategy to determine whether life is present or not is to use the overall representation of life's accretion as a basis, and to add experimental procedures one after another in order of experimental feasibility and numerical significance until the existence of a sufficient range of derived features of life can be demonstrated.

A Models and Theories of Life

> The simplest explanation is probably the correct one.
>
> Occam's razor principle

A variety of numerical and chemical models have been proposed as analogies and simulators of the evolution and functioning of early life. Because each model emphasizes a different aspect of life and because they were followed by a variety of clone models, their interpretation and relevance is continually debated and no review is truly comprehensive. The present material is not an exhaustive list of all models of life, nor is it a detailed discussion of each one of them. It is rather organized to display a wide variety of models and objectives. I start by presenting an interpretation listing the most general features of early life evolution and continue with a couple of better known models of early life.

A.1 Major Steps Toward Life

In the analysis presented by Robert M. Hazen (2001), the origin of life is viewed as a sequence of 'emergent events', each of which added to molecular complexity and order. The living state is viewed as a naturally emerging complex chemical system capable of self-reproduction. The early history of life is divided into four major steps:

1. Emergence of biomolecules. The first organic molecules are of an abiogenic origin (earthly chemistry plus extraterrestrial materials). The early oceans are viewed as a dilute solution of organic molecules.
2. Emergence of organized molecular systems. The origin of life was preceded by the formation of organized structures. Microscopic environmental heterogeneities were exacerbated under a constant energy flow leading to abiotic order. This may have been due to the effect of an alleged fourth law of thermodynamics: The flow of energy from a source to a sink through an intermediate system tends to order the intermediate system (Bak 1996).

R. Popa, *Between Necessity and Probability: Searching for the Definition and Origin of Life*, Adv. Astrobiol. Biogeophys., pp. 173–195 (2004)
http://www.springerlink.com/

Abiogenic patterns and dissymmetry might have represented the earliest source of order and information for life. The origin of chirality is also attributed to molecular interaction with enantio-selective surfaces.

3. Emergence of self-replicating molecular systems. Molecular metabolism is interpreted as assembling large functional units (e.g., macromolecules) from smaller components, and it is believed to have somehow preceded inheritance. The early macromolecules could have been of two types: molecular chains and molecular surfaces. Molecular chains self-replicated via self-complementarity. Molecular surfaces self-replicated at the edges. Eventually, cross-catalytic molecules formed self-replicating cycles or networks. Systems of organic molecules are not considered to be alive because they do not have the ability to self-reproduce.
4. Emergence of natural selection. Natural selection is not restricted to individual-based Darwinian-adaptive selection. Self-replicating molecules forming catalytic networks can also compete for resources and evolve. Variability can make some molecular networks more efficient allowing faster growth at the expense of less well performing networks and resulting in gradual evolution.

Although this interpretation points out a possible attractor behind the emergence of abiotic order (ordering effects from an energy flux through intermediate systems), it is less clear what the physical attractor pushing life into physical existence might be. From a chemical point of view, encapsulation is regarded as just a step in an increasingly complex set of stages between geochemistry and biochemistry. However, encapsulation appears to be more than a mere step in the evolution of life. It is an achievement with spectacular consequences for the living state that include maintenance of a quasi-state, alterations in the chemical direction and chaining activities.

A.2 The (M,R)-System Model

The (M,R)-system was developed by Robert Rosen and used to define the general properties of sets of abstract metabolic networks leading to self-maintenance (Rosen 1958, 1959, 1963, 1966, 1967, 1971, 1973). The simplest (M,R)-system is a catalytically mediated metabolic net (Morán et al. 1999). In this model metabolic systems are defined through a mathematical mapping between inputs and outputs (see Fig. A.1). The minimal model includes a set of substrings (components) S_0, S_1, S_2 and S_3 and six reactions (1 to 6). The components S_1, S_2 and S_3 have an external origin. The origin of S_0 is not specified.

The cooperation of two cyclic subsystems is required:

- The M unit transforms inputs (S_1 and S_2) into products (S_1–S_2). The M unit recycles the S_1–S_2–S_3 complex and it is considered to be the equivalent of a metabolic cycle.

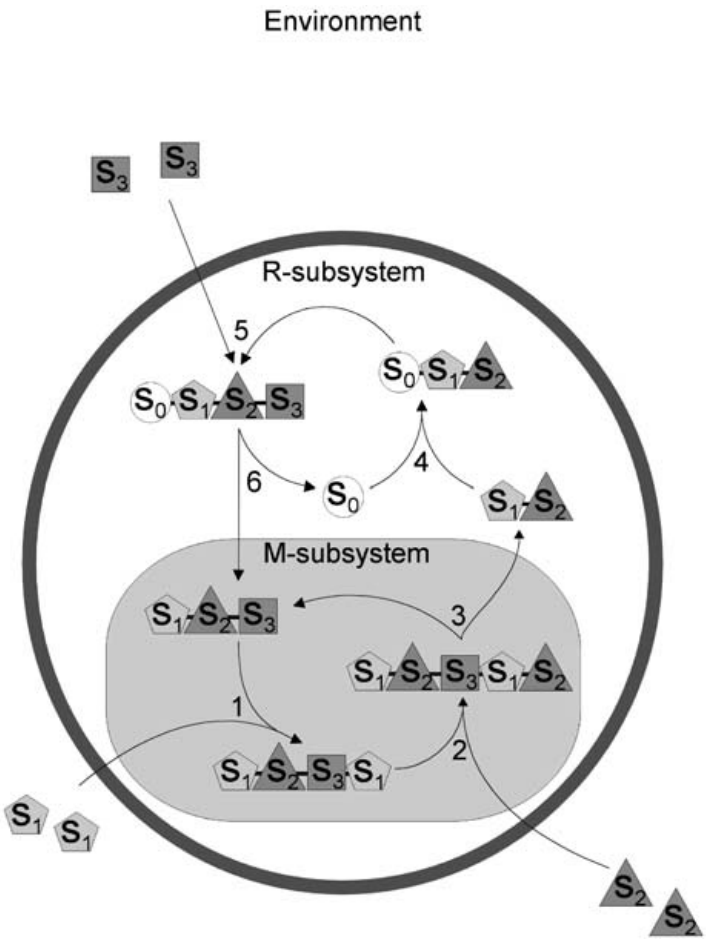


Fig. A.1. A representation of Rosen's minimal (M,R)-system. This model includes four distinct substrings S_0, S_1, S_2 and S_3 and six reactions $1, \ldots, 6$. (Adapted from Morán 1999)

- The R unit introduces the component S_3 into the system. Because the R unit transforms the products of M, it is sometimes interpreted as a repair unit for M.

According to Rosen, for the (M,R)-system to function, (i.e., to drive energy through itself), the reaction inside the M box must be more rapid than the reactions from R, which must be more rapid than any external reactions: "Whenever one M unit ceases to function (due to degradation or depletion), it is repaired or replenished by an R unit." Therefore, the repair components R may be regarded as the equivalent of a genetic control. According to Rosen's interpretation, this makes the distinction between metabolic and

genetic somewhat subjective (Rosen 1971). The first (M,R)-systems were imagined as catalytically-mediated metabolic nets without any special genomic component. A later extension of Rosen's model is the (M,R,G)-system which includes a genomic level as well (G).

The origin of the S_0 component is unclear, as is the origin and stability of the catalysts mediating any of the six internal reactions. In one interpretation, the entire M unit may be considered to act as a catalyst. The source of the energy driving the system is not discussed but it may be speculated that one of the input units S_1, S_2 or S_3 is in disequilibrium. In its simplest form the (M,R)-system is capable of self-maintenance. However, the system is unable to grow, being limited by the amount of S_0. The system cannot exist forever if the regeneration of its boundaries is not addressed. If boundaries are sensitive to degradation, one of the products of the cycles has to be useful as a boundary repair unit. Otherwise the system would gradually decay beyond repair.

The metabolic level (M) is generally described by the mapping

$$f : A \longrightarrow B \ ,$$

where f is a set of catalysts (M units), A is a set of substrates and B and set of products. The repair level (R) is described by the mapping

$$\Phi : B \longrightarrow H(A, B) \ ,$$

where Φ is the mapping which yields from inputs B, Φ represents the set of repair units the machinery within R which produces the repair catalysts (Morán et al. 1999), and $H(A, B)$ is the set of all possible mappings from which f is drawn. In the (M,R,G)-system, the genomic level is described by a mapping

$$\beta : H(A, B) \longrightarrow H\big(B, H(A, B)\big) \ .$$

Any system that requires supervised control, risks entering into an upward spiral of iterative recursions. Infinite recursion, i.e., the need for an ever-increasing number of controllers, is avoided in the (M,R)-system by building all the elements of A, B, f, Φ and β from the same materials (set of molecules) (S_0, S_1, S_2 and S_3 in Fig. A.1). This deliberate simplification strategy was described in this study as one of the driving forces for the use of handedness. Each internal cycle controls other cycles and is controlled by the other internal cycles. The network functions as long as all types of subsystem are always present and interdependent, one system (B) catalyzes the activity of the other (M) and as long as external materials remain available.

According to Rosen's initial model (Rosen 1971): "There is no essential distinction between 'metabolic' and 'genetic', but only a conventional and subjective one." This equivalence seems inappropriate because 'metabolism' means 'actual activity' while 'genetic' means 'virtual activity'. The physical implementation (the expression) of the genetic potential is the subject of molecular biology. Rosen's model has also been extended to spatial considerations using reaction–diffusion parameters (Rosen 1981).

A.3 The Two-Polymerase System

This model was proposed by Victor Kunin as an alleged triggering circumstance for the beginning of life (Kunin 2000). The model tries to settle a long debate on the nature of the first molecules of life – RNA or proteins. The observation was made that, in contemporary cells, two key enzymes control the synthesis of all RNAs and proteins. While the molecule creating protein chains is an RNA-based structure, the molecule creating RNA polymers is a protein-based structure. The two-polymerase model suggests that the self-dependency of life was triggered by the mutual catalytic dependence between two kinds of biopolymers (one proteinaceous enzyme and one ribozyme) (see Fig. A.2). Victor Kunin suggested that this dependence is a probabilistic event, a 'frozen accident', molecular fossil, or remnant from the first living system.

The specificity of the protein synthesis is thought to be achieved by the composition of the surrounding medium, while the specificity of the RNA synthesis is attributed to Watson–Crick base-pairing. It is assumed that, although primitive, this system possesses the potential to evolve into a primitive ribosome and then into modern life. The model explains this primeval mutualism as the origin of the ample interaction between nucleic acids and

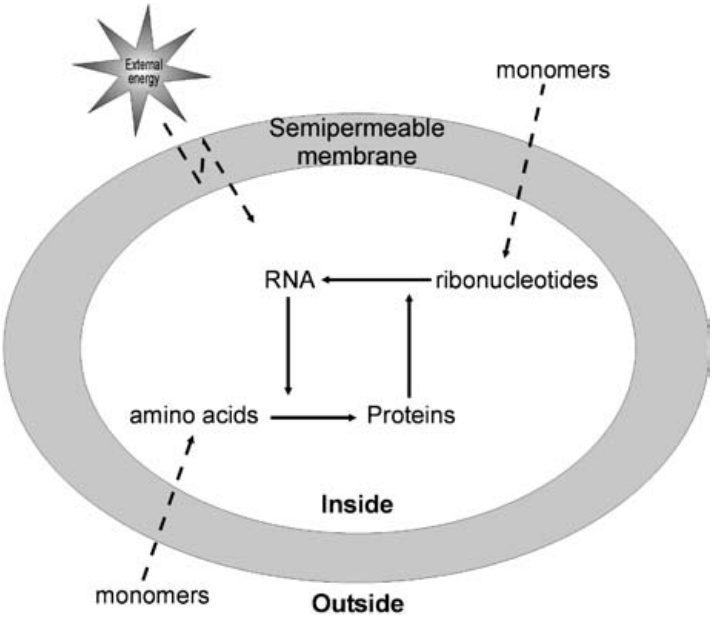


Fig. A.2. A hypothetical primeval life form based on a mutual catalytic dependence between an RNA-based peptide polymerase and a protein-based RNA polymerase. (Adapted from Kunin 2000)

proteins. The two-polymerase system still requires more theoretical development to answer some important questions:

- Although it provides some experimental inspiration, this model lacks a long-desired connection among different models of life and support from relevant experimental evidence.
- Despite its initial claim, the model is not a representation of the origin of life but the origin of one of the required interdependency loops within life forms (i.e., the connection between the condensation of nucleic acids and the condensation of polypeptides). Other internal loops that are crucial in allowing a protein/RNA type of life to exist, such as energy transduction/boundary stability, hypercycles, and handedness/configuration-based specificity, are not included in this model.
- In modern life forms, RNA participates only in a limited way with regard to peptide formation (i.e., catalysis of amide bond formation). The rest of the process, i.e., activation of monomers, transport of monomers and architecture of the ribosome, requires dozens of proteins besides RNA. No ribozyme has ever been experimentally shown to form peptides alone.
- The model is not backed up by a mathematical theory or a computational simulation proving its validity.
- The model does not satisfactorily explain the origin of the high specificity of early polymerases. Claims that high protein specificity originates in environmental dissymmetry have never been substantiated in the literature.
- Although the author of the model points out the need for a constant supply of monomers, the model does not address the side reaction and competitive inhibition problems.
- The need for energy and protection is claimed without linking it to any particular chemistry or another model.
- The interconnections among the major systems required for the very existence of the living state (genetic system, metabolic system, energy transduction system, boundary control) are not discussed.

A.4 The Hypercycle Model

A simple autocatalytic cycle based on one level of control may be able to self-maintain and even grow, but it is not capable of evolution. The hypercycle model elaborated a solution to this problem (Eigen 1971), based on a two-level autocatalytic system designed in an attempt to identify the conditions leading to the evolution of molecular populations. The lower level is represented by autocatalytic cycles corresponding to single-stranded RNA (ssRNA) loops of complementary nucleotides (see Fig. A.3). These molecular pairs $(I_1, \ldots, I_6)$ mutually reproduce by template replication. Each molecular pair codes for an enzyme catalyzing the formation of another pair. The higher-level cycle is represented by all the enzymes $(E_1, \ldots, E_6)$ joining the RNA cycle. The

hypercycle has been compared to an extended (M,R)-system constrained into a cyclic form (Morán et al. 1993). Unlike Rosen's (M,R)-system, the hypercycle model is genomic with RNA-like molecules being used as a source of information.

Manfred Eigen anticipated that in a stable (stringent) environment, errors would accumulate in the absence of error-free replication and lead to a progressive deterioration called error catastrophe (Eigen et al. 1981). If enzymes participate in the hypercycle, the functioning of the entity might be maintained and the lower level (RNA-based) cycles can mutate and become more efficient. Due to this process, the entire hypercycle can evolve. According to Eigen's interpretation, in a hypercycle, the self-reinforcing loops add more stability to the system, making it robust with regard to mutations during replication and less vulnerable to error catastrophes.

Computer simulations indicated three other crises within Eigen's model besides error catastrophes: the selfish RNA catastrophe, the short-circuit catastrophe and the population-collapse catastrophe (Niesert et al. 1981) (see Appendix C). While the probabilities of the selfish RNA catastrophe and short-circuit catastrophe increase with the size N of the molecu-

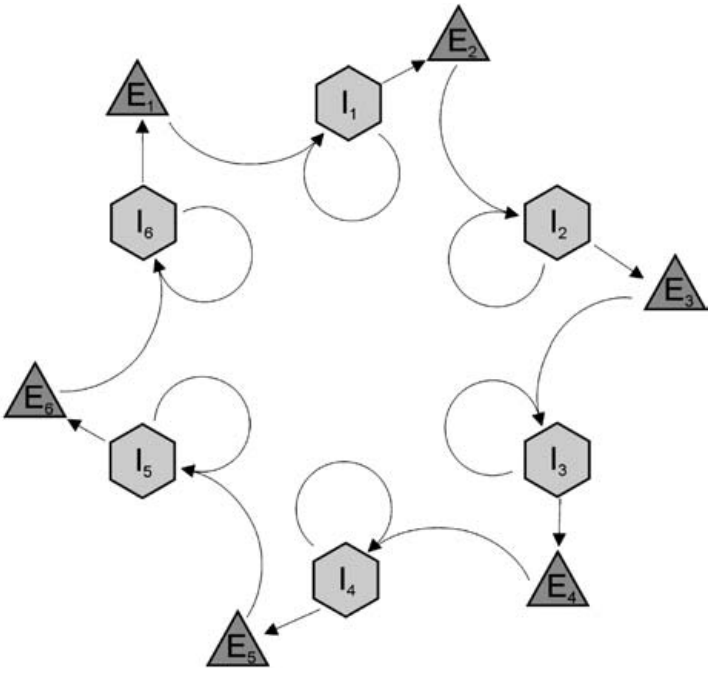


Fig. A.3. The inner level is represented by RNA-like molecules $I_1, \ldots, I_6$ that can be reproduced by template-assisted replication in the presence of enzymes $E_1, \ldots, E_6$ produced by previous loops in the hypercycle. (Adapted from Eigen 1971)

lar population, thus requiring small populations, the probability of a population collapse is higher for small populations, thus requiring large systems. A very serious caveat of Eigen's hypercycle model is the assumed availability of the translation machinery without explaining its origin and precision. The significance of compartmentation to the hypercycle model has also been discussed (Eigen and Schuster 1979). Eigen's hypercycle is a valuable model for understanding the intricacies of the replication of information.

One property of a hypercycle which does not exist in the real world is the use of one replicase for each individual molecule. This configuration does not obey the unity-in-diversity principle of life and makes hypercycles paradoxical because more gene products have to be involved in the functioning of unity than in its replication. The hypercycle is organized for a single grand purpose: replication. However, it seems obvious that life is much more than 'genetic selfishness'.

A.5 The Autocatalytic Network Model

The autocatalytic network model was developed by Stuart A. Kauffman (1986, 1993). The objective of this model was to investigate whether some minimal diversity and complexity is required for the emergence of self-maintenance. The following reasoning was used. Assume that some specific polymers can form by combinatorial condensation of smaller molecules. When the polymers are small (chemically simple), only a limited number of chemical interactions among them is possible (see Fig. A.4). A linear increase in the size M of the polymer means an exponential increase in the number of possible polymeric products, and hence more diversity. As the complexity of the polymers increases, so does the number of possible interactions among them. If each polymer is a catalyst, as the ratio of reactions to polymers also increases, it must eventually become so large that the number of catalyzed reactions is larger than the number of polymers that require catalysis to form. In the autocatalytic network model, catalytic probability represents the likelihood of a given substance to catalyze other reactions. When all polymers have at least one step in their formation catalyzed, an overall self-sufficiency becomes possible. In Kauffman's view "any sufficiently complex set of catalytic polymers will be expected to be collectively autocatalytic" (Kauffman 1993).

According to this model life emerged as "a connected metabolism, like a self-reproducing set of catalytic polymers, emerging spontaneously as a phase transition when a sufficient number of potentially catalytic polymers were mixed with a sufficiently complex set of organic molecules." Life is a collective property. The origin of life does not mean that each molecule reproduces itself, but a collection of molecules has the property that the last step in the formation of each molecule is catalyzed by some

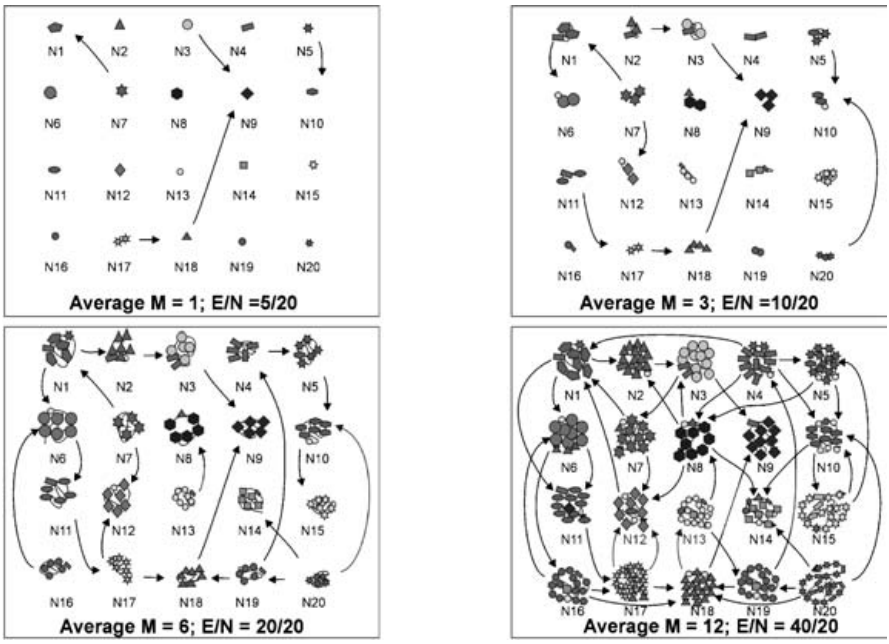


Fig. A.4. The principle of an autocatalytic network. Twenty nodes $N1, \ldots, N20$ of size M are randomly connected via an increasing number of edges $E_1, \ldots, E_n$. Nodes represent virtual polymers, each comprising M monomers. Edges represent possible catalytic reactions among them. As the average size of the polymers increases ($M = 1, \ldots 12$), so does the number of possible connections among them. For large values of M, as E/N increases past a threshold of 0.5 to more than 1.0, most points become connected in one gigantic self-sufficient network. (Adapted from Kauffman 1993)

molecule in the system (Kauffman 1993). Life is not improbable. Life is "an expected, emergent, collective property of complex systems of polymer catalysts."

Kauffman's model is presented as a series of computer simulations of large sets of randomly combined ligation and cleavage reaction networks, followed by the statistical characterization of their ensemble. Two types of networks are considered by this model:

- Non-genomic catalytic networks (autocatalytic sets of polypeptides). In Kauffman's model, peptides represent both catalysts and modules of the network.
- Quasi-genomic autocatalytic networks (enzymatic RNA or ribozymes).

Results indicate that, if the initial variety of substances and the catalytic probability are below a critical threshold, the network could never grow, while above that threshold, the network grows without bound.

The autocatalytic network is one of the most profound approaches to date, yet it is not an accurate representation of the realities of the living world. The model postulates that the early accretion of life (at the metabolic level) could not make progress without a very high diversity/complexity of an abiotic origin. Yet because high diversity/complexity imply a steady negentropic development, it is unlikely in abiotic nature. Stuart Kauffman claims that evolution does not require a genome. The network is built entirely of interacting polymers/catalysts. Yet it is known that the composition of life is much more diverse and many simple molecules are acted upon without reciprocating the catalysis. The potential of one catalyst to act on many others is misrepresented. In the real world some catalysts are able to interact with many others and significantly influence the entire network (e.g., ATP-ases, nucleic acid polymerases, ubiquitins, peptidyl-transferase). Consequently, in the autocatalytic network the need for enough interactive potential claims a larger network than is actually required. In Kauffman's model the catalytic properties are assigned randomly with no spatial considerations meant to result in phase transition events and in molecular associations in micro-environments. As a consequence, an arrangement of elementary reactions capable of realizing an autocatalytic net while maintaining thermodynamic consistency is extremely unlikely (Morán et al. 1999). This is believed to result in an overestimate of the actual minimal limit diversity. According to Eörs Szathmáry (2002), the model developed by Stuart Kauffman displays some other problems as well:

- The number of different reactions that can be catalyzed by peptides is unrealistic. Not only beneficial reactions can be catalyzed, but side reactions are also possible, driving the system out of a safe state. Aware of this potential weakness, Stuart Kauffman pleaded that, as the system becomes more complex, many side reactions would not actually drive the system out of its state, because different systems would provide for each other even with a low specificity. This creates a dependency between life and a high level of complexity. According to the autocatalytic network model, life cannot be too simple.
- It has been argued that the probability P of a peptide sequence catalyzing only one reaction depends on the length M of the molecule (Lifson 1997). Because the number of combinations increases exponentially with M, so does the chemical diversity and the catalytic potential. It is still unclear how valid this argument is (Szathmáry 2002).
- The system proposed by Kauffman has a limited heredity (Maynard-Smith and Szathmáry 1995). However, due to their modular nature, there can be a higher diversity than with holistic replicators (Szathmáry 2002).

Despite these points, the autocatalytic network model remains a useful tool for a quantitative assessment of the critical diversity (and complexity) required for the accretion of life (Farmer et al. 1986).

A.6 The Chemoton Model

The chemoton model proposed by Tibor Gánti (1971) is one of the most elaborate models of primitive life. Since its initial publication, this theory has undergone a number of revisions and became considerably more sophisticated (Gánti 1974,1975,1979a, 1979b, Békés 1975, Szathmáry 2002). The chemoton model tries to describe the simplest imaginable living cell as a chemical supersystem, a network formed of three independent autocatalytic subsystems or replicators (see Fig. A.5):

1. One autocatalytic chemical cycle (the equivalent of a metabolic cycle).
2. A system consisting of a template macromolecule pV_n able to replicate by a polymerization process in the presence of the compound V' according to template kinetics and carrying genetic information.

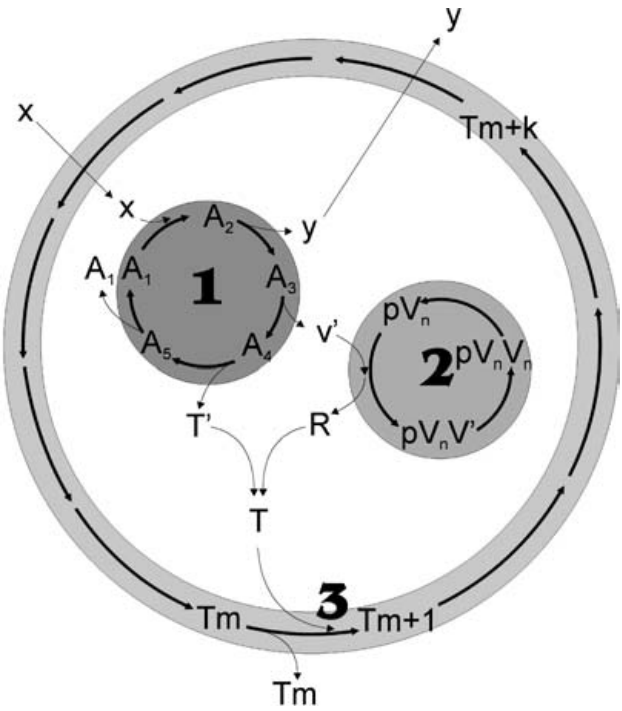


Fig. A.5. The chemoton model of an abstract chemical network. Cycle 1 is an autocatalytic metabolic cycle producing raw materials V' and T' for two other cycles 2 and 3. Cycle 2 is a template replication cycle. Cycle 3 is a membrane growth cycle. x is a raw material for a catalytic metabolic cycle, y is a byproduct of the autocatalytic metabolic cycle, $A_1, \ldots, A_5$ are intermediate molecules of the autocatalytic metabolic cycle, pV_n is a template molecule made of n molecules of V, T_m is a membrane made of m copies of the membrane forming the T molecule. (Adapted from Szathmáry 2002)

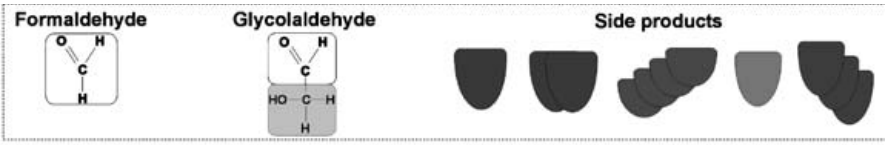
3. An encapsulating membrane T_m made of m copies of the monomer T capable of spontaneously incorporating itself into the membrane by self-assembly, and resulting in the growth of the membrane surface.

The autocatalytic cycle (1) requires the chemical x as a raw material and it produces the molecule y (a useless byproduct), the molecule V′ [a monomer used in the template-based replication cycle (2)], the molecule T (a compound with self-assembling properties) used to form a membrane (3) and resulting in two identical copies of the molecule A_1. The fact that two copies of A_1 are formed at the end of the cycle is viewed as a means to limit the random effects produced by side reactions and represents the material basis for growth. A special stoichiometric coupling is believed to exist between these systems providing an internal balance among their products. Consequently, these systems (1), (2) and (3) function synchronously and cooperatively, and as an emergent property, the entire chemoton becomes autocatalytic.

In theory (under fairly general conditions), if raw materials (x) are provided and if byproducts (y) are constantly removed, the system should be able to grow and reproduce (Szathmáry 2002). Although none of (1), (2) or (3) is alive, the three subsystems together are considered to fulfill the criteria of living systems as required by Tibor Gánti, (i.e., metabolism, self-reproduction and spatial proliferation). The more complicated chemotons also have the ability to mutate, evolve and reproduce by spatial separation (Gánti 1974). A numerical representation of a chemoton was given by Békés (1975). According to the chemoton theory, one can distinguish between absolute life criteria and potential life criteria (Szathmáry 2002). The absolute life criteria are characteristics present in any living system while the potential life criteria are required for evolution only.

The chemoton model is a virtual chemical network and it does not promote any specific chemistry. Due to its theoretical approach, this model can be applied to a wide range of possible self-replicating chemical cycles, yet it was never implemented in practice and shown to result in a new form of life. One condition for the existence of a chemoton is the maintenance of the identity (the quasi-state) of its 'metabolic cycle' (1). The formose reaction discovered in 1861 by Butlerov is one of simplest self-replicating process and is sometimes used as an analogy of an internal cycle (Szathmáry 2002) (see Fig. A.6). The formose reaction has five steps (1 to 5), five intermediates (A_1 to A_5), and two inputs (formaldehyde), and after each cycle two intermediate components (A_1) are produced. For an autocatalytic cycle with n steps, the specificity of a reaction at step i is S_i. Specificity in this case corresponds to the activity specificity (ActS) defined earlier and is measured as the rate of legitimate reactions divided by the total rate of all (legitimate + side reactions) (Szathmáry 2002).

A chemical system has little possibility of adjusting itself (maintaining its state). Systems that are dynamically stable, known either as attractor-based systems (Hageweg 1998) or steady-state systems (Jablonka and Lamb



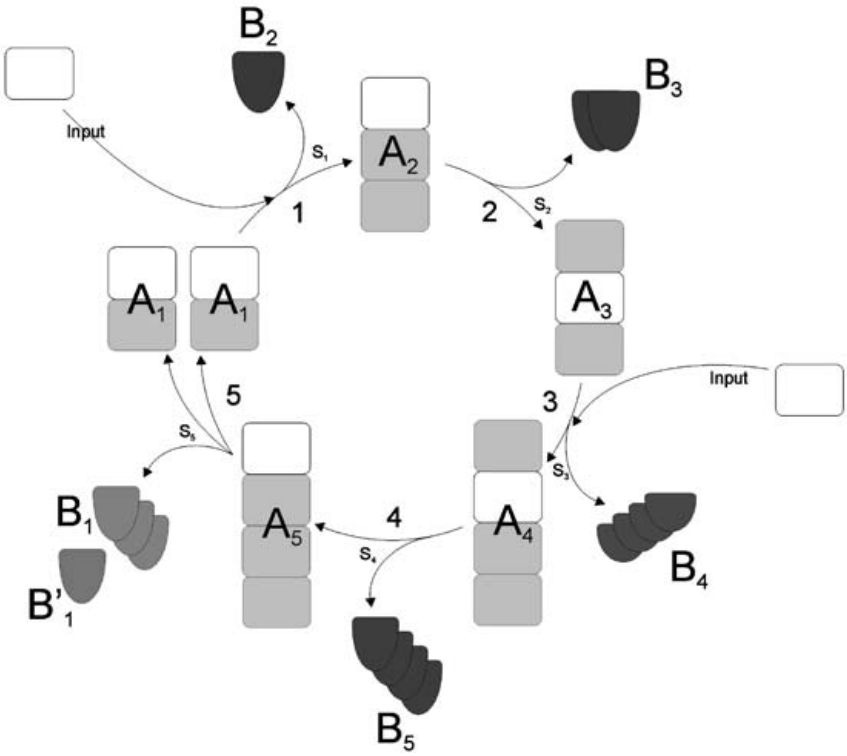


Fig. A.6. The formose reaction is an example of an autocatalytic cycle. (Adapted from Szathmáry 2002)

1995), are a requirement for something to be defined as alive. In the case of a chemoton, it has been estimated that the deviation from the steady state can be calculated as a function of the occurrence of illegitimate reactions and the number of steps:

$$2\prod_{i=1}^{n} S_i > 1 \ ,$$

where n is the number of steps in the cycle and S_i is the specificity of step i (a measure of how much of the product of a reaction is part of the cycle). If

other factors apart from specificity and the number of steps are ignored, the steady-state (SS) equilibrium of a system must be 1, while for growth, a value greater than 1 is required for the chemoton to be stable (Szathmáry 2002).

This formula only indicates two of the factors controlling the stability of the system (i.e., the specificity of the reactions and the number of steps). Side reactions are one of the weakest points of the replicating cycles (see Fig. A.7). When the number of side reactions increases, the stability of the system decreases considerably. Very high reactive specificity (95% legitimate reactions) is required for such a system to function. The logic behind this result is very simple – if too many byproducts are formed, not enough A_1 intermediates remain available at the end of the cycle to reinitiate it. The second factor controlling the system is the number of steps. It has been demonstrated that the larger the cycle, the harder its propagation (King 1982, 1986). The graph in Fig. A.8 indicates that, as the number of steps in a cycle increases, so does the negative effect of the side reactions. For the formose reaction (5 steps), the average error cannot be larger than roughly 10%. This stringent requirement is a heavy burden for any prebiotic system living in a chemically complex and uncontrollable world. With such high specificity requirements, no chemical cycle or combination of reactions of any reasonable length should ever become alive without some assistance.

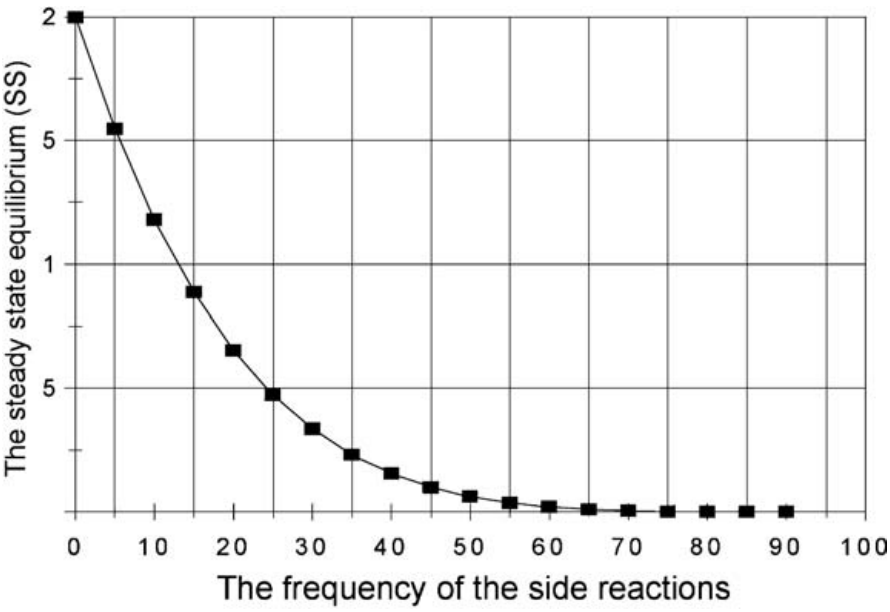


Fig. A.7. Effect of side reactions in the maintenance of the quasi-state of a replicative cycle. The system is considered stable and capable of growing when the value on the vertical axis is greater than unity

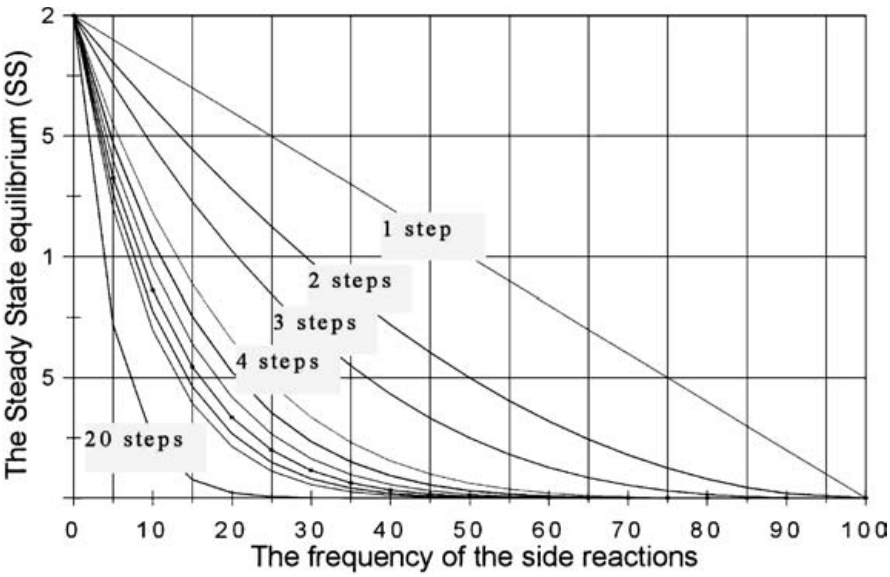


Fig. A.8. The effect of the number of steps on the maintenance of the quasi-state of a replicative cycle. The system is considered stable and capable of growing when the value on the vertical axis is greater than unity

It has been suggested that the intervention of enzymatic catalysts might add a significant trend (overall specificity) to the cycle and lower the decay rate (Szathmáry 2002). This issue adds another complication to the model, because one must explain where the specificity of the catalysts comes from. Moreover, if catalysts emerged by chance, e.g., probabilistic jumps (Dyson 1997), how could their specificity be maintained in the absence of any selective pressure? The size of the cycle and its specificity are not the only factors controlling its existence. Other factors are the half-life of the intermediate components, the participation of intermediates in alternative reactions, coupling with the import of input materials, the fluctuation of resources, and the way byproducts are eliminated.

It has also been observed that one of the characteristics of the autocatalytic cycles is that they are composed of small molecules and so have limited diversity and heredity. The limited number of units and the limited number of functional combinatorial possibilities makes the number of types smaller than the number of individuals and brings evolution by natural selection to a halt. In modern organisms (even in viruses), the number of possible combinations of sequences is much larger than the possible number of individuals. The entire Universe does not contain enough matter for all possible combinations of 200 bases into RNA sequences (i.e., $4^{200} \approx 10^{120}$). This limitless list of possibilities is required because in theory (if the conditions do not change too much), unlimited heredity allows evolution to go on indefinitely. Due to

the presence of cycle (2), the chemoton system was defined as an inheritor (Szathmáry 2002). Modern nucleic acids are made from modules added one at a time. This modularity is not present in most simple chemical replicators.

In the chemoton model, the relationship between the metabolic cycle producing the intermediate A_1 and the membrane-forming activity requiring the chemical T is stoichiometric. Because the increase in spherical volume is not linearly related to the increase in surface area, the volume:surface ratio increases with the increase in volume. Without a regulation of the 1:3 stoichiometry, more membrane is continually formed than what is actually required. This circumstance can become one of the means for spatial reproduction.

Despite its elaborate nature, the chemoton model still requires advances concerning the following:

- Identification of the natural source maintaining the stoichiometric coupling between the three subsystems.
- Identification of the attractor that pushes the chemoton to form (i.e., what makes it tick). Such forces can be represented by a driving off of chemical side products and/or a catalyst-based increase in specificity and overall exergonic effects.
- Although some experimental models already exist coupling membrane growth with division and template replication (Bachmann et al. 1992, Szostak et al. 2001), more simulations are required.
- The genetic cycle within a chemoton is informationally selfish and does not provide information for the functioning of the other cycles. Although it performs as a thermodynamic driving agent, it does not otherwise reciprocate the symbiosis. Could this have been the primary role of replication? In this case the existence of template self-replication is not really necessary for the specificity of the system. Can a living system driven by a strong enough attractor function as well with just two metabolic cycles and a membrane?
- "Replicators need a requisite milieu that allows them to replicate despite the adverse effects of side reactions. The admissible number of steps in non-enzymatic metabolic cycles increases hyperbolically with the logarithm of the specificity at each reaction" (Szathmáry 2002).

A.7 Pargellis's Model of Artificial Life

This computer model simulates self-replicating computer organisms (Pargellis 1996). Using mutations in a sequence as a source of variability and competition for a limited resource (i.e., computer memory and CPU time), this model was designed to explore the behavior of biological life forms during the early stages of evolution. The simulation starts with a primordial soup composed of randomly generated sequences of computer operations selected

from a basis set of 16 'opcodes'. The author found that large and inefficient self-replicating 'organisms' were spontaneously generated with a probability of about 10^{-4}. The efficiency of the 'protobiotic ancestors' is first increased by eliminating unnecessary code and later by increasing complexity.

A.8 The Autopoietic Model

This model is a computational approach indicating the ability of systems to create and self-maintain themselves by assimilation and boundary formation (Maturana and Varela 1973, Varela et al. 1974, McMullin 1997, McMullin and Varella 1997) and an attempt to define 'minimal life'. In its general principles the autopoietic model is not far from the chemoton model (Szathmáry 2002). In the autopoietic model, life is cellular and, although in principle other life forms are possible, they are ignored (Luisi 1993). A cell has a boundary and life is presented as a metabolic network. Although initially designed for biology, this model was later extended into a broader theory used in other fields such as cognitive science, management strategy and mathematical logic (Morán et al. 1999). According to this model, an autopoietic system is defined as being capable of continual self-regeneration (see Appendix C) and self-perpetuation (Luisi 1993). When applied to biogenesis, the model involved computer simulations of catalytic reactions and diffusion events.

The simplest autopoietic system (see Fig. A.9) is based on the following components, processes and principles:

- an external metabolite A capable of crossing the boundary,
- a component C capable of self-assembling into a boundary,
- a product of the decomposition of C,
- a unimolecular reaction $A \longrightarrow C$ with rate $k_{\mathrm{gen}}[A]$,
- a decomposition reaction $C \longrightarrow P$ with rate $k_{\mathrm{dec}}[C]$,
- a rate of diffusion of A through the boundary,
- a rate of diffusion of P through the boundary,

with

$$v_{\mathrm{gen}} = \frac{\mathrm{d}[C]}{\mathrm{d}t} \,, \qquad v_{\mathrm{dec}} = -\frac{\mathrm{d}[C]}{\mathrm{d}t} = \frac{\mathrm{d}[P]}{\mathrm{d}t} \,.$$

Mathematical models of autopoiesis have been presented elsewhere (An der Heiden et al. 1985, Schwegler and Tarumi 1986). In the simplest case, the reactions are considered irreversible (back reactions are neglected) and they only occur inside the boundary. In addition, all diffusion processes and the self-assembly are more rapid than the reactions. C is replaced in a process measured by $v_{\mathrm{dec}} = k_{\mathrm{dec}}[C]$. The system regenerates its own structure with the rate $v_{\mathrm{gen}} = k_{\mathrm{gen}}[A]$. The fate of the system can be represented through the ratio between v_{gen} and v_{dec}:

- If $v_{\mathrm{gen}} < v_{\mathrm{dec}}$, the system is unstable (implosion) because decay is larger than buildup.

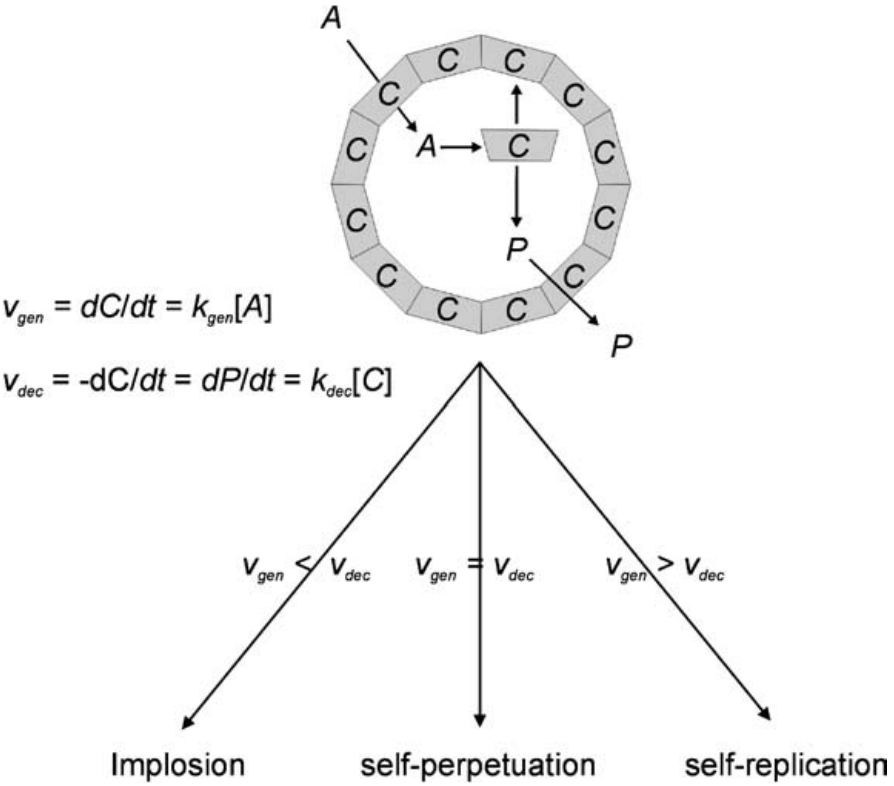


Fig. A.9. Autopoietic system. The boundary components C are represented as polygonal objects to underline their tendency to self-assemble. A is an entering metabolite, P is a metabolic product, $A \longrightarrow C$ is a self-generation reaction, and $C \longrightarrow P$ is a decomposition reaction. (Adapted from Luisi 1993)

- If $v_{\mathrm{gen}} = v_{\mathrm{dec}}$, the system self-perpetuates in a steady state (homeostasis) at the expense of A.
- If $v_{\mathrm{gen}} > v_{\mathrm{dec}}$, the system will grow at the expense of A.

If the system is monodisperse (thermodynamically stable only at one size), self-replication is also possible. In this model replication is used in synonymy with reproduction. The autopoietic model has two remarkable emergent properties:

- It produces its own boundary.
- It self-generates.

In a more elaborate example, five types of molecule were used (substrate, catalyst, and three bonds of different stabilities) together with seven events (production, two types of bonding, two types of disintegration, and two types of diffusion). Some parameters are also used to represent production, decom-

position and diffusion. The essential condition of the model is the presence of a catalyst able to induce polymerization of a substrate within a closed membrane which permits the diffusion of the substrate but not the catalyst. As a logical consequence, a vectorial transfer of the substrate toward the inside is achieved and the activity becomes directed.

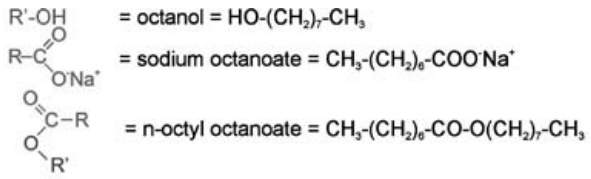
The chemoton model was used to explain the principles of chemical examples of self-replicating micellar systems (Luisi and Varela 1990):

- Sodium octanoate in isooctane/octanol in a ratio of 9:1 (v/v) (see Fig. A.10). Octanoate-reversed micelles are formed with octanol acting as both co-surfactant and solvent. Reversed micelles have the hydrophobic side toward the outside. The reaction between octanol (an alcohol) and octanoate (a carboxylic acid) results in *n*-octyl octanoate (an ester) plus water. This ester has little amphipatic character and tends to stop the formation of micelles. In the aqueous basic milieu formed within the vesicles, Li^+ ions become active in hydrolyzing the ester into octanol and octanoate, which further adds to the colloidal surfaces. As a result the system catalyzes its own 'self-replication'.
- The oxidation of octanol into octanoate with sodium permanganate (Luisi 1993).
- Ethyl caprylate alkaline hydrolysis, used as an analogy for the transition from micelles to vesicles, is illustrated in Fig. 3.14.
- The model proposed by von Kiedrowski and the model proposed by Rebek have been used to demonstrate autopoiesis on the basis of template-based replication (Luisi 1993).

These systems are considered autopoietic because the products of the reactions are the consequence of the boundary constraint and the products self-assemble within the boundary.

Autopoiesis is not considered to be a property of a structure or one molecule but of a whole system. No genomic control is used, while Varela even considers that "reproduction is not intrinsic to the minimal logic of the living system" (Varela 1996). Although the interpretation of autopoiesis remains controversial (Fleischacker 1989, Morán et al. 1999), this model still remains an insightful and instructive representation of the accretion of life. The most important achievement of the autopoietic model is the discussion of the emergence and consequences of a self-maintaining border between the system and its environment. This 'membrane' produces a confinement which allows the realization of a network that constitutes the system. The model considers that, without a boundary (membrane), there is no basis for distinguishing the living system from its surroundings, and thus no autonomous system at all (Varela 1996).

Computer models of autopoiesis are based on hypothetical particles moving freely and interacting with each other (forming bonds). The program called Substrate-Catalyst-Link (SCL) has been implemented using the



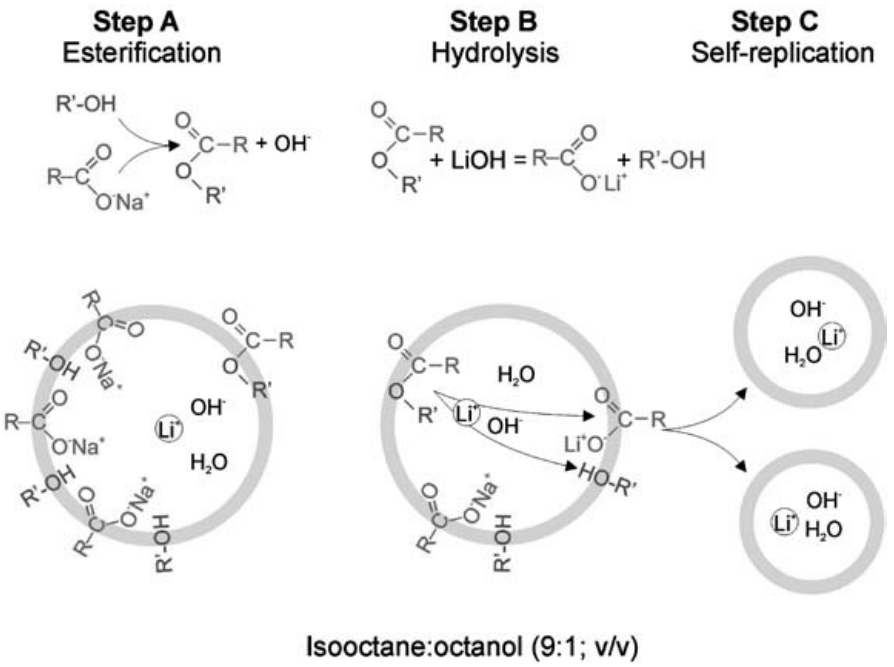


Fig. A.10. Li octanol micelles. Example of a micellar self-replicating system. In hydrophobic solutions (isooctane:octanol 9:1 v/v), reversed micelles are formed from octanol and octanoate. The reaction between the octanol and the octanoate (step A) results in the ester *n*-octyl octanoate, which tends to stop the formation of micelles. In the aqueous, basic internal milieu, Li^+ can be used to catalyze hydrolysis of the ester (step B), which further increases the micellar surface and results in self-replication. (Adapted from Luisi 1993)

SWARM simulation system developed at the Santa Fe Institute[1] (McMullin 1997). The complete source code relating to SCL v0.05.01 is available at: `ftp://ftp.santafe.edu/pub/swarm/users-contrib/anarchy/` under the

[1] `http://www.santafe.edu/projects.swarm`

name `scl-0.05.01.tar.gz` (McMullin and Varela 1997). The model is certainly not perfect, but it is very instructive and inspirational for somebody trying to understand the steps toward closed systems and their derived consequences.

A.9 The Algorithmic Chemistry Model

This theoretical model promoted by Walter Fontana is used to spontaneously generate self-maintaining systems or 'grammatical structures' (Fontana 1992, Fontana et al. 1994, Fontana and Bus 1994a, 1994b). This model presents minimal biological organization based on two abstractions from chemistry:

- The collision of molecules generates specific new molecules.
- Many different reactants can yield the same stable product.

Symbolic entities and abstract chemistry are used to build other symbolic entities. The language used is a version of LISP. A couple of functions in one variable are defined and used to represent primitive operators. The model is built by defining the following:

- A universe is defined using a λ-like language, with functions representing material objects from the real world.
- Asymmetric interactions among different objects. In a first step the objects are represented by functions of one variable, e.g., $f(x)$ and $g(x)$. The interaction between objects, e.g., $f\big(g(x)\big)$, can result in other objects, e.g., $h(x)$.
- Collision rules establish three aspects, the consequences of interactions. The objects, e.g., f and g, may or may not survive the interaction:
  - What happens to the products of the interaction?
  - In the presence of products, the interaction is called reactive. Otherwise it is called elastic.
  - Computational limit. To avoid infinite recursion, computational limits are imposed.
- Systems define what happens with ensembles of functions (particles).
- Kinetics can be induced through a stochastic process called 'turning gas'.

This model shows that initially random functions self-organize into ensembles of specific functions displaying cooperative interactions. The system displays extremely long transients with stabilizing interaction patterns that include hierarchical organization and self-maintaining sets. The system self-organizes in a closed web of mutual synthesis reactions. Fontana's algorithmic chemistry model demonstrates how populations of individuals interacting with each other can give rise to complex, stable and adaptive interactions. Walter Fontana argues that some features of life could be expected to reappear 'if the tape were run twice':

- hypercycles of self-reproducing objects,
- self-maintaining organizations,
- a considerable increase in internal order and complexity.

In its early forms this model did not consider spatial requirements, genetic mechanisms and rate constants. Notably, the model shows that self-maintaining organization arises as a consequence of features of chemistry, without appeal to natural selection.

A.10 Chemical Reaction Automata

Chemical reaction automata are open, non-equilibrium, chemical reaction systems performing autonomous tasks studied by the field of metachemistry (Bro 1997). While conventional chemistry deals with individual atoms, molecules and reactions, metachemistry studies chemical reaction systems, networks and hierarchies. The field of metachemistry uses a specialized set of theoretical concepts. Generalized chemical species are defined as any members of a hierarchy, and generalized chemical reactions as any transformations within and between hierarchies. The chemical operator concept is used to identify the functional building blocks of autonomous reaction systems. One chemical reaction represents an elementary operator. Two or more reactions can be used to construct several classes of operators such as sensors, transducers, electronic shuttles operators, recyclers, actuators, drivers, controllers, transporters, molecular wires, etc. (examples are given in Bro 1997).

According to Bro, the inclusion of chemical reaction networks into a micellar structure can lead to a primitive chemical reaction automaton (chemical reaction organism). Chemical reaction organisms are autonomous input/output-type systems, analogous to chemical plants and living cells (see Fig. A.11). The functioning of such a system includes (see Fig. A.12):

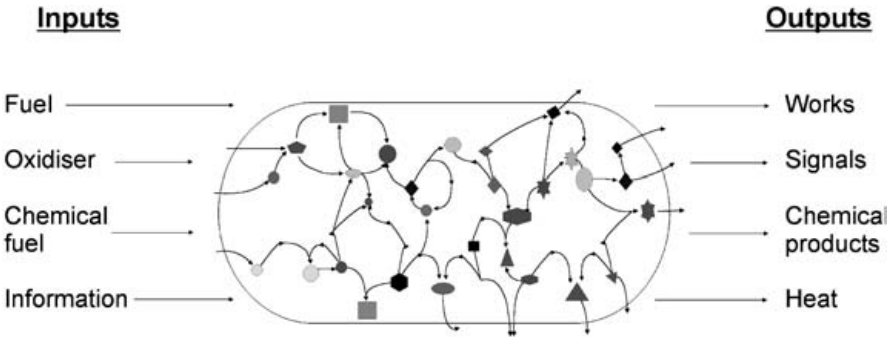


Fig. A.11. Diagram of an input/output system. (Adapted from Bro 1997)

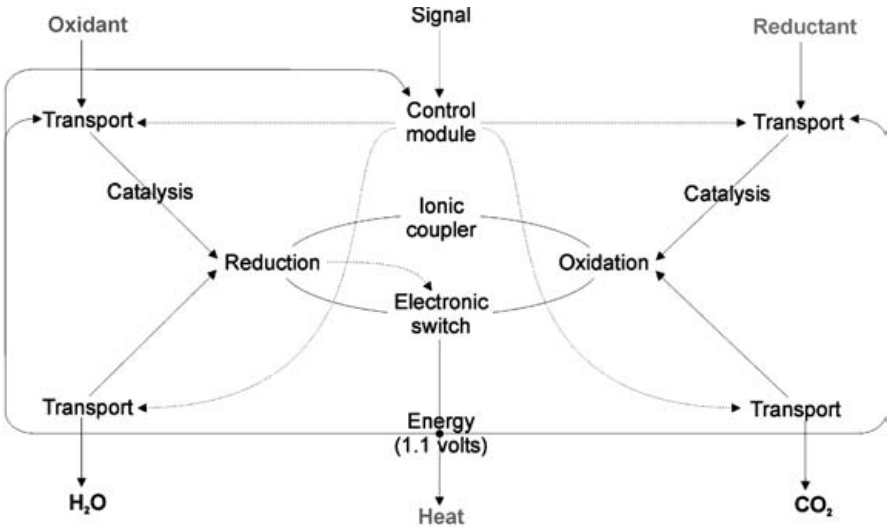


Fig. A.12. Diagram of a simple chemical operator network. (Adapted from Bro 1997)

- inputs of energy, chemicals and information (signals),
- outputs such as work, chemicals, signals and heat,
- an interface – semi-permeable enclosure, transport systems, sensors and actuators,
- an interior – a reaction medium (a fluid), internal partition and operator network.

The internal networks are described as topotactic structures. Within the network, the reaction nodes are physically separated and constrained to specific locations providing reactive order (Bro 1997). In an autocatalytic network, most reactions are controlled (self-regulated) and directed toward the minimization of side reactions and extraneous products. The conditions required for the existence of the system are a supply of external energy, a supply of input chemicals, and exergonic reactions coupled with endergonic reactions acting as drivers. Chemical reaction automata are not designed to explain how life originated, but rather to understand better how a basic metabolism functions. They are a representation of the living world without reproductive and adaptive capacities.

B Chronology of Definitions and Interpretations of Life

There are a large number of definitions and interpretations of life. The definitions presented here only serve a general bibliographic purpose as no attempt has been made to classify or discuss them. For a more extensive bibliography and discussions of various definitions of life, the reader is directed to the excellent bibliography already published (Luisi 1998, Lahav 1999, Korzeniewski 2001, Pályi et al. 2002).

1855 Life will always remain something apart, even if we should find out that it is mechanically aroused and propagated down to the minute detail (Virchov 1855).

1871 Life is a power, force, or property of a special and peculiar kind, temporarily influencing matter and its ordinary force, but entirely different from, and in no way correlated with, any of these (Beale 1871).

1872 Living things are peculiar aggregates of ordinary matter and of ordinary force which in their separate states do not posses the aggregates of qualities known as life (Bastian 1872).

1878 Life is neither a principle nor a resultant. It is not a principle because this principle, in some way dormant or expectant, would be incapable of acting by itself. Life is not a resultant either, because the physicochemical conditions that govern its manifestation cannot give it any direction or any definite form. [...] None of these two factors, neither the directing principle of the phenomena nor the ensemble of the material conditions for its manifestation, can alone explain life. Their union is necessary. In consequence, life is to us a conflict (Bernard 1878a).

1878 If I had to define life in a single phrase ... I should say: Life is creation (Bernard 1878b).

1880 No physiology is held to be scientific if it does not consider death an essential factor of life. [...] Life means dying (Engels ca. 1880).

1884 The broadest and most complete definition of life will be the continuous adjustment of internal relations to external relations (Spencer 1884).

R. Popa, *Between Necessity and Probability: Searching for the Definition and Origin of Life*, Adv. Astrobiol. Biogeophys., pp. 197–205 (2004)
http://www.springerlink.com/

1923 It is the particular manner of composition of the materials and processes, their spatial and temporal organization which constitute what we call life (Putter 1923).

1933 A living organism is a system organized in hierarchical order [...] of a great number of different parts, in which a great number of processes are so disposed that by means of their mutual relations with wide limits, with constant change of the materials and energies constituting the system and also in spite of disturbances conditioned by external influences, the system is generated or remains in the state characteristic of it, or these processes lead to the production of similar systems (Von Bertalanffy 1933).

1934 Life has the following characteristics: 1. Character of animal or plant manifested by the metabolism, growth, reproduction, and internal powers of adaptation to the environment; 2. vital force distinguished from inorganic matter; 3. experience of animal from birth to death; 4. conscious existence; 5. of being alive; 6. duration of life; 7. individual experience; 8. manner of living; 9. life of the company; 10. the spirit and 11. a duration of similarity (Webster's International Dictionary 1934).

1944 Life is replication plus metabolism. Replication is explained by the quantum-mechanical stability of molecular structures, while metabolism is explain by the ability of a living cell to extract negative entropy from its surroundings in accordance with the laws of thermodynamics [reformulated by Dyson (Dyson 1997) from Schrödinger 1944].

1948 The essential criteria of life are twofold: (1) the ability to direct chemical change by catalysis; (2) the ability to reproduce by autocatalysis. The ability to undergo heritable catalysis changes is general, and is essential where there is competition between different types of living things, as has been the case in the evolution of plants and animals (Alexander 1948).

1948 Life is not one thing but two, metabolism and replication, [...] that are logically separable (Von Neumann 1948).

1952 Life is a potentially self-perpetuating open system of linked organic reactions, catalyzed stepwise and almost isothermally by complex and specific organic catalysts which are themselves produced by the system (Perrett 1952).

1956 Life is the repetitive production of ordered heterogeneity (Hotchkiss 1956).

1959 The three properties mutability, self-duplication and heterocatalysis comprise a necessary and sufficient definition of living matter (Horowitz 1959).

1961 Any system capable of replication and mutation is alive (Oparin 1961).

1967 Life is a partial, continuous, progressive, multiform and conditionally interactive, self-realization of the potentialities of the atomic electron state (Bernal 1967).

1968 Life is a hierarchical organization of open systems (Von Bertalanffy 1968).

1972 Life is a structural hierarchy of functioning units that has acquired through evolution the ability to store and process the information necessary for its own reproduction (Gatlin 1972).

1973 Life is made up of three basic elements: matter, energy and information. [...] Any element in life that is not matter and energy can be reduced to information (Fong 1973).

1973 Life is a metabolic network within a boundary [Maturana and Varela 1973, reformulated by Luisi (Luisi 1993)]. All that is living must be based on autopoiesis, and if a system is discovered to be autopoietic, that system is defined as living, i.e., it must correspond to the definition of minimal life (Maturana and Varela 1973).

1973 Living organisms are distinguished by their specified complexity (Orgel 1973).

1974 The criteria of living systems are: metabolism, self-reproduction and spatial proliferation. The more complicated kinds also have the ability to mutate and evolve (Gánti 1974).

1975 We regard as alive any population of entities which has the properties of multiplication, heredity and variation (Maynard-Smith 1975).

1979 Life is the property of plants and animals which makes it possible for them to take in food, get energy from it, grow, adapt themselves to their surroundings and reproduce their kind. It is the quality that distinguishes an animal or plant from inorganic matter. Life is the state of possessing this property (Webster 1979).

1979 Life is that property of matter that results in the coupled cycling of bioelements in aqueous solution, ultimately driven by radiant energy to attain maximum complexity (Folsome 1979).

1980 Living units are viewed as objects built up of organic compounds, as dissipative structures, or at least dynamic low entropy systems significantly displaced from thermodynamic equilibrium (Prigogine 1980, Prigogine and Stengers 1984, cited and reformulated by Korzeniewski 2001).

1981 The sole distinguishing feature, and therefore the defining characteristic, of a living organism is that it is the transient material support of an organization with the property of survival (Mercer 1981).

1982 A living organism is defined as an open system which is able to fulfill the following condition: it is able to maintain itself as an automaton. [...] The long-term functioning of automata is possible only if there exists an organization building new automata (Haukioja 1982).

1984 The uniqueness of life seemingly cannot be traced down to a single feature which is missing in the non-living world. It is the simultaneous presence of all the characteristic properties [...] and eventually many more, that makes the essence of a biological system (Schuster 1984).

1985 Replication – a copying process achieved by a special network of interrelatedness of components and component-producing processes that produces the same network as that which produces them – characterizes the living organism (Csanyi and Kampis 1985).

1986 Life is synonymous with the possession of genetic properties. Any system with the capacity to mutate freely and to reproduce its mutation must almost inevitably evolve in directions that will ensure its preservation. Given sufficient time, the system will acquire the complexity, variety and purposefulness that we recognize as being alive (Horowitz 1986).

1986 Life is characterized by maximally-complex determinate patterns, patterns requiring maximal determinacy for their assembly. [...] Biological templates are determinant templates, and the uniquely biological templates have stability, coherence, and permanence. [...] Stable template reproducibility was the great leap, for life is matter that learned to recreate faithfully what are in all other respects random patterns (Katz 1986).

1986 A living system is an open system that is self-replicating, self-regulating, and feeds on energy from the environment (Sattler 1986).

1987 Just as wave–particle duality signifies microscopic systems, irreversibility and trend toward equilibrium are characteristic of thermodynamic systems, space-symmetry groups are typical for crystals, so do organization and telemony signify animate matter. Animate, and only animate matter can be said to be organized, meaning that it is a system made of elements, each one having a function to fulfill as a necessary contribution to the functioning of the system as a whole (Lifson 1987).

1989 The characteristics that distinguish most living things from nonliving things include a precise kind of organization, a variety of chemical reactions we term metabolism, the ability to maintain an appropriate internal environment even when the external environment changes (a process referred to as homeostasis), movement, responsiveness, growth, reproduction and adaptation to environmental change (Vilee et al. 1989).

1992 To biologists, life is an outcome of ancient events that led to the assembly of nonliving materials into the first organized, living cells. 'Life' is

a way of capturing and using energy and materials. 'Life' is a way of seeing and responding to specific changes in the environment. 'Life' is a capacity to reproduce; it is a capacity to follow programs of growth and development. And 'life' evolves, meaning that details in the body plan and functions of each kind of organism can change through successive generations (Starr and Taggart 1992).

1993 Life is the ability to communicate (de Loof 1993).

1993 Life is an expected, collectively self-organized property of catalytic polymers (Kauffman 1993).

1994 Life is a self-sustained chemical system capable of undergoing Darwinian Evolution (NASA working definition of life, Joyce 1994, 2002).

1994 Life is like music; you can describe it but not define it (Lazcano 1994).

1997 Life may [...] be described as a flow of energy, matter and information (Baltscheffsky 1997).

1997 It is suggested that the existence of the dynamically ordered region of water realizing a boson condensation of evanescent photons inside and outside the cell can be regarded as the definition of life (Jibu et al. 1997).

1997 Living organisms are systems characterized by being highly integrated through the process of organization driven by molecular (and higher levels of) complementarity (Root-Bernstein and Dillon 1997).

1998 First we give the definition of a biosystem as an adaptive, complex, dynamic system that is alive to some degree (Clark and Kok 1998).

1998 A living entity is defined as a system which, owing to its internal process of component production and coupled to the medium via adaptative changes, persists during the time history of the system (Luisi 1998).

1999 Life is seen as a recursive (self-producing and self-reproducing) organization where dynamic and informational levels are mutually dependent (Bergareche and Ruiz-Mirazo 1999).

1999 To Schrödinger's (1944) mother of all questions 'What is life', biologists can therefore answer today that they do not consider it some magical force that animated lifeless materials, but rather an emergent property based on the behavior of the materials that make up living things (Turian 1999).

2000 Life is defined as a material system that can acquire store, process, and use information to organize its activities (Dyson 2000).

2000 Life is defined as a system of nucleic acid and protein polymerases with a constant supply of monomers, energy and protection (Kunin 2000).

2001 A potentially useful conceptual approach to the question of life's definition is to consider the origin of life as a sequence of 'emergent' events, each of which adds to molecular complexity and order (Hazen 2001).

2001 Life on the Earth [...] seems to possess three properties (strongly related to each other and in fact being different aspects of the same thing) which are absent in inanimate systems. Namely, life is (1) composed of particular individuals, that (2) reproduce (which involves transferring their identity to progeny) and (3) evolve (their identity can change from generation to generation). A living individual is defined as a network of inferior negative feedbacks (regulatory mechanisms) subordinated to (being at the service of) a superior positive feedback (potential of expansion of life) (Korzeniewski 2001).

2001 We adopt this weak definition of life. A living system occupies a finite domain, has structure, performs according to an unknown purpose, and reproduces itself (Sertorio and Tinetti 2001).

2001 The characteristics of artificial life are emergence and dynamic interaction with the environment (Yang et al. 2001).

2002 Ignoring the misgivings of those few life-origin theorists with 'mule' fixations, life is the 'symphony' of dynamic and highly integrated algorithmic processes which yields homeostatic metabolism, development, growth, and reproduction (Abel 2002).

2002 Life is the process of existence of open non-equilibrium complete systems, which are composed of carbon-based polymers and are able to self-reproduce and evolve on the basis of template synthesis of their polymer components (Altstein 2002).

2002 Any living system must comprise four distinct functions: 1. Increase of complexity; 2. directing the trends of increased complexity; 3. preserving complexity; and 4. recruiting and extracting the free energy needed to drive the three preceding motions (Anbar 2002).

2002 Life is defined as a system capable of 1. self-organization; 2. self-replication; 3. evolution through mutation; 4. metabolism and 5. concentrative encapsulation (Arrhenius 2002).

2002 Life is defined as a self-sustained molecular system transforming energy and matter, thus realizing its capacity of replication with mutations and anastrophic evolution (Baltcheffsky 2002).

2002 Life appears as a set of symbiotically-linked molecular engines, permanently operating out of equilibrium, in an open flow of energy and matter, although recycling a great deal of their own chemical components, through cyclic chemistry (Boiteau 2002).

2002 Life is a chemical system capable of transferring its molecular information independently (self-reproduction) and also capable of making some accidental errors to allow the system to evolve (evolution) (Brack 2002).

2002 In order to be recognizable life must: 1. be a non-equilibrium chemical system; 2. contain organic polymers; 3. reproduce itself; 4. metabolize by itself; 5. be segregated from the environment (Buick 2002).

2002 We consider to be alive any homo- or heterotrophic cellular irreversible heat engine, or their assembly, that carries instructions for its function, reproduction, topical location, individuality and life cycle (Eirich 2002).

2002 The living organism is a multilevel open catalytic system achieved in its evolutionary development of maximal catalytic activity in basic process and possessing the property of self-reproduction. Life is a process of functioning of living organisms (Erokhin 2002).

2002 Paraphrasing Theodosius Dobzhansky: Life is what the scientific establishment (probably after some healthy disagreement) will accept as life (Friedman 2002).

2002 Life is matter that makes choices, binds time and breaks gradients (Guerrero 2002).

2002 Living beings are complex functional systems. Life is an abstract concept describing properties of cells, concrete objects. Life is the process manifested by individualized evolutionary metabolic systems. The functions, which are called life, are: metabolism, growth, and reproduction with stability through generations (Guimarães 2002).

2002 Life is an energy-dependent chemical cyclic process which results in an increase of functional and structural complexity of living systems and their inhabited environment (Gusev 2002).

2002 Life is simply a particular state of organized instability (Hennet 2002).

2002 Life is synonymous with the possession of genetic properties, i.e., the capacities for self-replication and mutation (Horowitz 2002).

2002 Life is a system which has subjectivity (Kawamura 2002).

2002 Life is metabolism and proliferation (Keszthelyi 2002).

2002 Life is an inevitable consequence of the existence of optically active organic compounds (like proteins) (Klabunovsky 2002).

2002 Life is a new quality brought upon an organic chemical system by a dialectic change resulting from an increase in the quantity of complexity of the system. This new quality is characterized by the ability of temporal self-maintenance and self-preservation (Kolb 2002).

2002 Life is a highly organized form of intensified resistance to spontaneous processes of destruction developing by means of expedient interaction with the environment and regular self-renovation (Kompanichenko 2002).

2002 Any system that creates, maintains and/or modifies dissymmetry is alive (Krumbein 2002).

2002 Life is the form of existence of a substance capable of self-reproduction and maintenance of permanent metabolism with the environment (Kulaev 2002).

2002 To be alive is to be a degradable, semi-isolated system which has survived because it was able to generate a molecular memory both of its environment and how to respond to it. Life can be defined by the following list of characteristics (Lacey et al. 2002):

1. It must be isolated from an external environment but still be able to exchange materials with it (must possess individuality).
2. It must be susceptible to degradation by the environment.
3. It must be small enough so that rates of transfer of materials into and out of the isolated system will be rapid.
4. It must be able to generate a molecular representation of its internal and external environments (i.e., it must have a molecular memory of its environments).
5. It must be able to sense the environment and respond to it, i.e., it must be able to synthesize active molecules capable of utilizing materials it encounters in the environment.

2002 A terrestrial living entity is an ensemble of molecular-informational feedback-loop systems consisting of a plurality of organic molecules of various kinds, coupled spatially and functionally by means of template-and-sequence directed networks of catalyzed reactions and utilizing, interactively, energy and inorganic and organic molecules from the environment. A living entity is an uninterrupted succession of ensembles of feedback-loop systems evolved between the emergence time and the moment of observation (Lahav 2002).

2002 A living entity is an ensemble of molecules which exhibit spatial organization and molecular-informational feedback loops in utilization of materials and energy from the environment for its growth, reproduction and evolution (Lahav and Nir 2002).

2002 It's alive if it can die (Lauterbur 2002).

2002 From a chemical point of view, life is a complex autocatalytic process. This means that the end products of the chemical reactions in a living cell (nucleic acids, polypeptides and proteins, oligo- and polysaccharides) catalyze their own formation. From a thermodynamical point of view, life is a mechanism which uses complex processes to decrease entropy (Markó 2002).

2002 Life is an attribute of living systems. It is continuous assimilation, transformation and rearrangement of molecules as per an in-built program in the living system so as to perpetuate the system (Nair 2002).

2002 Any definition of life that is useful must be measurable. We must define life in terms that can be turned into measurables, and then turn these into a strategy that can be used to search for life. So what are these? a. structures, b. chemistry, c. replication with fidelity and d. evolution (Nealson 2002).

2002 Life is a system which can reproduce itself using genetic mechanisms (Noda 2002).

2002 Life is a structurally stable negentropy current supported by self-correction for the biological hereditary genetic code [...] providing an energy inflow (Polishchuck 2002).

2002 Life is instantiated by the objects that resist decay by means of constructive assimilation (Rizzotti 2002).

2002 We propose to define living systems as those that are: (1) composed of bounded micro-environments in thermodynamic equilibrium with their surroundings; (2) capable of transforming energy to maintain their low-entropy states; and (3) able to replicate structurally distinct copies of themselves from an instructional code perpetuated indefinitely through time despite the demise of the individual carrier through which it is transmitted (Schulze-Makuch et al. 2002).

2002 Life is a form of matter organization that is energetically and informationally self-supported, with a good capacity of self-instruction and creation (Scorei 2002).

2002 Life is the ability of an organism to formulate questions (Soriano 2002).

2002 Life is a historical process of anagenetic organizational relays (Valenzuela 2002).

2002 Life is a population of functionally connected, local, non-linear, informationally-controlled chemical systems that are able to self-reproduce, to adapt, and to coevolve to higher levels of global functional complexity (Von Kiedrowski 2002).

2002 A living system is one capable of reproduction and evolution, with a fundamental logic that demands an incessant search for performance with respect to its building blocks and arrangement of these building blocks. The search will end only when perfection or near perfection is reached. Without this built-in search, living systems could not have achieved the level of complexity and excellence to deserve the designation of life (Wong 2002).

2002 The existence of a genome and the genetic code divides living organisms from non-living matter (Yockey 2002).

C Dictionary

Absolute life criteria. Traits that are present in all living systems (Szathmáry 2002) (see Potential life criteria).

Amphipates. See Amphipatic molecules

Amphipatic molecules. Amphipates; molecules with one end hydrophobic and the other end hydrophilic, e.g., soaps, detergents, phospholipids (see Fig. 3.5). Amphipatic molecules have a tendency to arrange in micelles, forming layers and even enclosed systems (see Figs. 3.6, 3.7).

Anagenesis. Living things are viewed as anagenetic beings capable of generating, maintaining and receiving organization (Valenzuela 2002). Examples are self-repair, self-maintenance, self-change and generation of similar organization.

Anapleurotic pathways. To 'fill up'; metabolic reactions in which new carbons enter into the composition of life-related molecules.

Anastrophe. Concept developed by Herrick Baltcheffsky to contrast the term 'catastrophe' (Baltcheffsky 2002). Anastrophes are major qualitative jumps (upgrades) during the early development of life. Anastrophes are sudden, drastic constructive changes in biological evolution. It has been estimated that, in cosmic evolution, anastrophes are generally more important than catastrophes.

Aperiodic crystals. Structures containing a variety of components but the overall ensemble is built on some precise periodic principles. Unlike minerals (periodic crystals), aperiodic crystals are not periodic on the atomic scale (`www.lassp.cornell.edu/lifshitz/quasicrystals.html`).

Apoptosis. Programmed cell death. Unlike death due to an unforeseeable and irreversible malfunction, apoptosis is part of the genetic program of a cell. Apoptosis is common in the living world and serves a variety of functions such as morphological differentiation of fingers in vertebrates, physical elimination of 'obsolete' individuals or of individuals too old to serve a reproductive function.

R. Popa, *Between Necessity and Probability: Searching for the Definition and Origin of Life*, Adv. Astrobiol. Biogeophys., pp. 207–223 (2004)
http://www.springerlink.com/

Archaea. Group of micro-organisms distinct from bacterial described. Their phylogenetic position was established by Carl Woese. The membranes in Archaea contain lipid ethers instead of lipid esters. Because many Archaea have an extreme life style (hypersaline, hyperthermophiles, methanogens) it has been suggested that they are somehow primordial. This claim is now generally dismissed.

Astrobiology. Exobiology; discipline studying life outside Earth, non-biological types of life, the basic principles of life in the Universe, the early emergence of life, the conditions for the emergence of life and the overall consequences of the presence of life on a planetary scale.

Attractor. Any physical agent (e.g., a force) pulling a process or a system into a defined direction, or toward a defined state.

Attractor-based systems. Inheritance systems that are dynamically stable (Hageweg 1998). Another term used to describe it is 'steady state' (Jablonka and Lamb 1995).

Autocatalytic process. A process in which the end products catalyze their own formation (Markó 2002).

Autonomous system. A system in which a set of processes self-constitutes recursively through functional/adaptive interactions with its environment (Ruiz-Mirazo 1999). In this theory, a system initiates autonomy at the moment when contextual or explicit information is deliberately used to maintain the state of the system. In time, improvements in the autonomy are made through biosynthetic additions and novel control mechanisms, until a fully self-maintaining state is achieved.

Autopoiesis. Self-generation, from the Greek 'auto' (self) and 'poiesis' (formation). Concept used since 1974 in computer simulations to serve as an integrative characterization of the nature of living systems (Varela et al. 1974). "An autopoietic system (sometimes considered as a minimal living system), is a network of processes of production (synthesis and destruction) of components such that these components: (i) continually regenerate and realize the network that produces them, and (ii) constitute the system as a distinguishable unity in the domain in which they exist (Morán et al. 1999).

Avidan organisms. Digital organisms present in the Avida artificial life simulation system. Avidan organisms have a particularly simple phenotype, accreting information (or complexity) as they evolve. In Avida, the organisms reproduce and accrete information (or complexity) as they evolve but do not interact with each other, as opposed to Tierran organisms which interact with each other, giving rise to an ecology of phenotypes.

Bioid. Term used by to indicate pre-life forms (Decker 1973).

Biological type of life. The type of life present on Earth, based on liquid water, carbon chains, phospholipidic membranes, ATP, protein-based catalysis, and nucleic acid memory. Hypothetical types of life based on other material substrates or using different mechanisms are defined as non-biological types of life. The artificial life forms are classified as non-biological types of life.

Biomimetic. Type of experimental approach attempting to demonstrate the emergence of early life-related molecules and events based on simulation of hypothetical primeval conditions (Arrhenius 2002).

Catalysis. A process to speed up chemical reactions without altering their final equilibrium.

Central dogma of biology. In earthly life forms, information flows only from DNA to mRNA and from mRNA to proteins and not in the reverse direction (Crick 1968).

Chaotic systems. Systems are chaotic if they are deterministic through description by mathematical rules and have mathematical descriptions which are nonlinear in some way (from The Interactive Textbook of PFP 1996, `dept.physics.upenn.edu/courses/gladney/mathphys/Contents.html`).

Chiral. A chiral object cannot be placed over its mirror image in such a way as to cover it exactly (Gilat 2002).

Chreod. Concept introduced by Waddington (1957) to describe the stabilized paths of ontogenetic development in embryo development. According to Waddington, the embryo grows in an 'epigenetic landscape' where there coexist stable segments and segments in which a choice among several developmental paths is possible (Prigogine and Stengers 1984) (see also Entelechy).

Chronocyte. Hypothetical primitive life form based on RNA as genetic material. The cytoplasm of modern eukaryotes may be a molecular fossil of an RNA organism (a chronocyte) after the endosymbiosis of another (DNA-based primordial cell) which in turn became the nucleus (Hartman 2002).

Closed system. A system that exchanges energy but not matter with its surroundings (see also Open system and Isolated system).

Coevolution. Evolving at a concerted pace.

Coevolution hypothesis. In the context of the genetic code, coevolution might have existed between the biosynthetic neighborhoods of the amino acids and the way the genetic code was organized (Wong 1975). According to this theory, only a few amino acids may have been imported from the outside world in the very beginning of life. The rest of them were synthesized inside rather than imported. Each new tRNA and the corresponding AA-tRNA synthetases were added only after each new amino acid was synthesized inside

early cells. Although this hypothesis has been strongly criticized (Ronnenberg et al. 2000), its insightful elegance makes it a distinct possibility for early molecular macro-evolution.

Coherence. Property of dissipative structures. "A coherent system behaves as a whole, as if it were the site of long-range forces. In spite of the fact that interactions among molecules do not exceed a range of 10^{-8} cm, the system is structured as though each molecule were 'informed' about the overall state of the system" (Prigogine and Stengers 1984).

Communication problems. The technical problem, the semantic problem and the effectiveness problem; issues related to communication in the mathematical theory of communication (Shannon and Weaver 1963).

Compartmentalization. The enclosure of a space into cohesive and interactive semi-open or semi-closed structures (Guimarães 2002). Compartmentalization also refers to the formation of spatial separations (compartments) within a single unit cell.

Condensation. Type of chemical reaction (see Polycondensation).

Consortium. In microbiology, an association of micro-organisms from different species living in metabolic interdependence.

Contextual information. Contextual information refers to alterations in distributional evenness, measurable as a compositional heterogeneity or as gradients. Contextual information is due to the overall properties of the physicochemical background, to biased predecessors, to vectorial interference from neighboring systems or to impaired exchanges.

Control and controlled systems. Control is a relative term. In order to avoid misunderstandings it preferable to explain the term 'control' in each particular context. If one system X contains a chemical A that influences the concentration of another chemical B, then A controls B. This does not imply that X is a controlled system. When the system X is compared with another system Y that uses a genetic mechanism to gain autonomy, only Y is considered a controlled system. During mineral growth, the arrangement of the atoms in the nucleation point controls the accretion, but when crystal growth is compared to systems that display feedback-based homeostasy, only the latter are described as controlled systems.

Cryptic information. Information is considered cryptic whenever the message is hidden in a specialized form and requires a language and a translation instrument to be revealed. Explicit information somehow overlaps with the concept of 'digital replicators' (Szathmáry 2002a, 2000b).

Crystal. According to most textbooks, crystals are structures with a periodic repeating pattern of atoms. Not all specialists agree with this definition because it creates confusion when discussing quasi-crystals and aperiodic

crystals. Periodic crystals are defined as periodic on the atomic scale, while aperiodic crystals are not.

Death. Interpreted in cellular history as an irreversible transformation of the state. A change of state so ample that the normal function can no longer be restored solely on the basis of resources and mechanisms available inside. From a life-cycle point of view, primitive organisms and most modern prokaryotes are immortal. Their death is based on thermodynamic/material starvation and the accumulation of deleterious mutations. Programmed death (see also Apoptosis) appears to be associated especially with multicellular organisms.

Determinist systems. Systems functioning according to rigid rules and having no probabilistic options. In theory, the behavior of determinist systems is predictable once the relevant conditions are established. Deterministic systems conform to the ideal of a machine in which wear and tear, mechanical failures and unreliability are absent. Modern computers are conceived as deterministic machines (Krippendorff, from the Web Dictionary of Cybernetics and Systems). Determinist systems act passively (and precisely) along the direction imposed by external coercions. A determinist system cannot alter (control) its fate in any way. In contrast, a finalist system tends to become something or to take a certain precise direction (state) despite outside attractors.

Earth-centric representation of life. Approach considering that life elsewhere has properties similar to life on Earth (same temperature ranges, also based on water and carbon, made of cells, containing proteins and nucleic acids, etc.). 'Earth-centric' is not identical with 'geocentric'.

Emergent. Arising unexpectedly or as a new development. Emergence represents the rise of a system or property that cannot be predicted or explained from antecedent conditions. Emergent phenomena are inherently unpredictable from observations or their isolated constituent parts (Hazen 2001).

Encryption. The act of hiding information by using a code.

Energy transduction. The deliberate conversion of one energy form into another that is easier to store and manipulate.

Energetic opportunism. The ability of energy-dissipative structures to withstand shortages in the external energy supply by making energy reserves whenever external energy is available.

Entelechy. An immaterial source regulating embryo development. This concept was promoted at the beginning of the 20th century by the German embryologist Hans Driesch (Prigogine and Stengers 1984). See also Chreod.

Error catastrophe. A functional crisis during the evolution of an information-driven system due to a progressive deterioration over the generations of the genetic information through less than perfect replications (Eigen et al. 1981). See also Population collapse catastrophe, Selfish RNA catastrophe and Short-circuit catastrophe.

Essence of life. A generalist explanation of why life exists and why it emerges, independent of what the life forms are made of. The essence of life can be interpreted as an explanation of the attractor (overall force) that pushed life forms into physical existence. The expression 'essence of life' is somewhat similar to others such as the meaning of life, the role of life in nature, and the purpose-like nature of life.

Eukaryotes. Complex organisms whose cells contain nuclear membranes, non-circular chromosomes, and organelles such as mitochondria and chloroplasts, larger ribosomes appearing probably from prokaryotes by endosymbiosis.

Evolution. Temporal change in the structure, organization and/or the acting toward a specified thermodynamic, kinetic and/or informational direction (Valenzuela 2002) (see also Structure and Organization). A Carnot cycle is an example where, even if changes are observable for fragments of the cycle, there is no overall evolution as the system cycles from one state to another and becomes what it was before.

Exobiology. Scientific field studying the origin of life, alternative forms of life, the functional principles of artificial life, and life outside Earth.

Explicit information. The explicit type of information is embedded into the nature of the system by virtue of its structure or its way of functioning, or it is a part of the particular environment the system exists in and does not require secondary processes (translation). Explicit information is obvious as given. Explicit information somehow overlaps with the concept of holistic replicators (Szathmáry 2002a, 2002b).

Feedback regulation. A control over the rate of a process based on its products. Two basic types of feedback regulation can exist: positive and negative. In negative feedbacks, the products inhibit one or more steps leading to them. This type of regulation maintains the activity or concentrations at a certain level. In positive feedback regulation, processes are accelerated. Positive feedbacks speed up processes once started, until the resources are exhausted in creating periodic activities. Feedback regulation should not be confused with (or used to mean something similar to) reflexive activity. Although feedback regulation and reflexive activity have some common properties and can influence each other, they are distinct things. Reflexive activity involves putting work into a system as a result of its activity while feedback regulation refers to regulating the intensity of the activity through its

products. Reflexive activity is about energy being directed backward, while feedback regulation is about information being directed backward. Because the system feeds energy back into itself during a reflexive activity, it is better to use the expression 'feedback regulation' when speaking about regulatory feedbacks, thus avoiding circular arguments.

Finalist systems. These are systems that tend to become something or take a certain (precise) direction (or state), irrespective of outside attractors. In contrast, a determinist system acts passively (and precisely along the direction imposed by external coercive effects).

First living being (FLB). This is the most primitive complete open system emerging and existing under prebiotic conditions, consisting of carbon-based polymers, able to self-reproduce and evolve to modern cells on the basis of template synthesis of its polymer components (Altstein 2002).

Fisher–Tropsch synthesis. Technology used to make gasoline from CO and H_2 at 200–250°C under catalysis, often claimed to mimic the prebiotic synthesis of organic molecules.

Full self-maintaining state. Represents a culmination of the evolution of protocells toward autonomy (Ruiz-Mirazo 1999). A state of full self-sufficiency is a state at which all the required building blocks are provided through assimilation pathways and full control over reproduction has been achieved.

Gaia. A concept of planetary-level homeostasy (Lovelock 1996).

Geocentric representation of life. Geocentric mentalities relate life to a planetoid object. In a geocentric approach, the properties of life are related to the properties of its celestial host. Geocentrism is a concept broader than Earth-centrism. Freeman Dyson (Dyson 2002, presentation at JPL) elaborated a hypothesis on the properties of ET life forms living at low gravity in outer space far from any planetoid.

Gordian knot. According to an ancient legend, a very complicated knot was tied to the end of a car by Gordius. Legend said that whoever succeeded in untying the knot would become the king of the entire world. When challenged to untie the Gordian not, Alexander the Great (the ruler of Macedonia) cut it with his sword.

Habitable zones. Concept used in astrobiology to define general zones in our Galaxy where life may exist.

Heredity. Heredity in general means that life begets life while reproduction is the material process of begetting.

Holistic replication (replicators). Synonymous with processive replication (replicators). Holistic replication is a process where it cannot be said (as

for DNA) that replication is half-complete. In a holistic replication, a whole series of chemical transformations is required until two individuals of the initial type are formed (Szathmáry 2002). The formose reaction and the reductive citric acid cycle are examples of holistic replications (also see Phenotypic replicators).

Holistic view. An interpretation, opposed to the reductionist interpretation, in which an integrated whole has properties independent of and greater than the sum of its parts. A holistic description is centered on the most general properties of a thing and not on its particular mechanisms, composition or structural details.

Homeostasy. Represents the ability to maintain an appropriate internal environment even when the external environment changes (Vilee et al. 1989).

Hydrolaze. Enzyme catalyzing a hydrolytic process (degradation in the presence of water). Proteases, amylases, cellulases, lipases and nucleases are examples of enzymes that degrade proteins, starch, cellulose, lipids and nucleic acids, respectively.

Hydrophilic. Chemical component with affinity for water (see also Hydrophobic).

Hydrophobic. Water repellent or lipophilic; chemical component with affinity for lipids and organic solvents (see also Hydrophilic).

Hypercycle. Concept elaborated by Manfred Eigen (Eigen 1971). In this model the coevolution of spatially unlinked RNAs occurs by cyclic functional coupling. In a hypercycle world, each RNA molecule encodes a polypeptide and supports the replication of the next member in the cycle (a typical example of forward regulation) (see Fig. A.3).

Imitators. See Phenotypic replicators.

Inheritors. Individuals with a hereditary system. Inheritors must carry information regarding the system as a whole (Szathmáry 2002).

Intelligent life. Life capable of taking decisions based on anticipating causal connections without direct preexisting experience. Intelligent life is not an inevitable end product of the evolution of life (Benn 2001), but an outcome of enough complexity to generate self-awareness.

Isolated system. A system that exchanges neither matter nor energy with its surroundings. See also Closed system and Open system.

Kinetic. Concerning chemical reactions, the speed of the reaction (see Thermodynamic).

Last Common Ancestor (LCA). The last common ancestor of any group of species. LCA is the most recent species from which all of them descend (Castresana and Saraste 1995).

Last Universal Ancestor (LUA). The universal ancestor is defined as the last common ancestor of the three domains of life: Archaea, Bacteria and Eukarya (Castresana and Saraste 1995). If the early evolution was a phylogenetic 'bush', no individual cell or phylogenetic line may be considered as the LUA. The LUA is often offered as an explanation of why the genetic code is universal on Earth. The theory presented in this book argues that the LUA never existed, and that the universality of the genetic code is due the unification of the genetic codes during a period of very active horizontal information exchange.

Laws of bioenergetics. Laws describing the use of energy by life (Skulachev 1992):

1. Living cells avoid direct utilization of external energy sources in the performance of useful work.
2. Any living cell possesses at least two energy currencies, one water-soluble (ATP) and the other membrane-linked ($\Delta\mu_{H^+}$ and/or $\Delta\mu_{Na^+}$).
3. All the energy requirements of the living cell can be satisfied if at least one of the three convertible energy currencies is produced at the expense of external energy sources.

Laws of thermodynamics. The four laws of thermodynamics are:

1. When one form of energy is converted into another, the total energy is conserved.
2. Whenever processes occur, the entropy of the Universe increases. An increase in disorder (overall) is therefore spontaneous. If the volume and energy of a system are constant, then every change to the system increases the entropy. If the volume or energy changes, then the entropy of the system can actually decrease.
3. The entropy of each pure element or substance in a perfect crystalline form is zero at absolute zero (Planck 1913, Alberty and Silbey 1992).
4. The flow of energy from a source to a sink through an intermediate system tends to order the intermediate system for the 'purpose' of ever-increasing the overall energy flux (Bak 1996).

Life form. Term most commonly used to depict variations around one type of life, e.g., a bacterium species is another life form than an algae which is another life form than a fly, which in turn is another life form than a human). Sometimes 'life form' refers to one living individual.

Limit cycle. In a limit cycle, no two instants are equivalent. The chemical reaction acquires a phase similar to that characterizing a light wave, for example (Prigogine and Stengers 1984).

Living individual (living entity). Term referring to a physical entity that is relatively autonomous and has all the information required to complete its functioning.

Life and living entities. Although these terms are frequently used interchangeably, they are distinct from each other and have distinct properties, tendencies and priorities. "Life is a power, force, or a property of a special and peculiar kind, temporarily influencing matter and its ordinary force, but entirely different from, and in no way correlated with, any of these" (Beale 1871). "Life is what is common to all living beings" (DeDuve 2002). "Life is an attribute of living systems" (Nair 2002). Unlike living entities, life is an abstract concept (Guimarães 2002), a process (Anbar 2002, Guimarães 2002), a strategy (a way of approaching a problem), while living individuals are physical units performing it (Altmann 2002). Living beings are complex functional systems. "Living things are peculiar aggregates of ordinary matter and of ordinary force which in their separate states do not possess the aggregates of qualities known as life" (Bastian 1872). A living entity is an ensemble of molecules which exhibits spatial organization and feedback loops in utilization of materials and energy from the environment for its growth and reproduction (Nir 2002). For the individual, reproduction is neither necessary nor sufficient (Gánti 1971). The main purpose of one living unit is self-preservation, while the main purpose of life is to promote the second law of thermodynamics (see Essence of life). The properties of life change much more slowly during evolution than the properties of living entities. Although both life and living entities aim toward self-preservation, the priorities of life always override the priorities of living individuals. The main mechanism for the adjustment of living entities is homeostasy while the main mechanism for the adjustment of life is adaptive evolution. Although semantically discrete, life and living entities are conditional on each other. No life can exist outside of living individuals and vice versa. Therefore, understanding the physical meaning of being alive can only be intellectually explored if the very essence of both life and living entities are studied.

Lipophilic. Hydrophobic.

Liposome. A particular case of a vesicle in which the surfactant is a glyceride (Luisi 1993).

Meaning of life. See Essence of life or Purpose-like nature of life.

Megascopic. Event, phenomenon or physical reality situated at a considerably larger scale than a reference dimension. A means to emphasize macroscopic.

Metabolism. A set of activities whereby molecular systems interact with the environment, taking up matter and energy, transforming these into internal proper elements and other products, and excreting wastes (Guimarães

2002). Metabolism can be universally defined as the recursive self-maintenance of controls upon the energy flows necessary for the physical realization of an operationally-closed component production system (Bergareche and Ruiz-Mirazo 1999). According to Haruhiko Noda, metabolism does not need to be included in a definition of life (Noda 2002). Life can exist without metabolic transformations if the right components are provided externally at the right times and concentrations.

Metachemistry. The study of chemical reaction systems, networks and hierarchies (Bro 1997).

Modular replication. Replication achieved through the addition of somewhat standardized modules (Szathmáry 2002). Adding homologous pieces to a chain one after the other (e.g., formation of DNA or RNA) is an example of modular replication.

Mule paradox. An expression often used when defining life. In essence, if life is explained through the ability to reproduce, then the mule (which like a worker honey bee is unable to reproduce) should not be considered alive. This paradox was addressed through concepts such as units of life and units of reproduction (Szathmáry 2002). See Units of life and Units of reproduction.

Multimers. Term invented by Christian DeDuve to describe the early molecular chains. Christian DeDuve considers that neither oligomers or polymers are appropriate terms for early life because they "evoke images of regularity and homogeneity" (DeDuve 1995).

Mutual affinity (mutual information). Represents the affinity between different ordered structures. The difference between independent and joint minimal encoding lengths of objects. A central concept in algorithmic information theory, used in Chaitin's mathematical definition of life (Chaitin, 1919).

Neutralism. As opposed to selectionism. Neutral evolution is a theory promoted by Kimura (1968). The neutralist theory claims that most genetic variation observed at the molecular level is not to be explained in terms of selection, but rather as a consequence of mutation and random drift.

Negibacteria. Gram-negative bacteria. See also Posibacteria (Szathmáry 2002).

Nominative information. Information held by a well-defined carrier.

Non-Earth centric. Only referring to the fundamental properties of life and to none of the particular fingerprints of Earth-based types of life such as DNA, amino acids, ribosomes, genes, chromosomes, lipids and so on.

Occam's razor. The simplest explanation is probably the correct one.

Open system. A system that exchanges both matter and energy with its surroundings. See also Closed system and Isolated system.

Organization. The relationship (distance, distribution, contiguity, specificity, succession, interactions) or parts of a being (Valenzuela 2002). See Structure.

Orthologous evolution. Vertical evolution, as opposed to paralogous or horizontal evolution.

Palimpsest. A manuscript on which an earlier text has been wiped off and the vellum or parchment reused for another.

Palindrome. A sequence, e.g., a word or sentence, that reads the same in both directions (e.g., rotor, madam, ATGCCCGTA, Was it a rat I saw).

Periodic crystals. Structures having a repetitive pattern of atoms. See also Aperiodic crystals.

Phase transition. A significant qualitative change in the nature of an entity, a sequence of events or a protocol.

Phenotypic replication. Type of replication in which the template provides a landscape for the architectural reshaping of other molecules (Szathmáry 2002). This type of replication is characteristic of introns. Phenotypic replicators are also called Imitators.

Polycondensation. Type of reaction leading to the formation of a longer molecular chain. Polycondensation must not be confused with polymerization (see Polymerization). The polycondensation of the molecules of life (lipids, nucleic acids and proteins) involves the extrusion of water between participating monomers.

Polymerase Chain Reaction (PCR). Procedure used in molecular biology to amplify genetic material such as DNA.

Polymerization. A reaction putting together monomers for the formation of oligomers and polymers without the loss of any atoms between participating monomers. The macromolecules of life are polymers but they are formed in a process of polycondensation (see Polycondensation). A common mistake is to reason that, if the macromolecules of life are polymers, they must have been formed through polymerization. It is incorrect to say that peptides (or proteins) are chains of amino acids. Because polycondensation of peptides involves the loss of some matter (water), it is correct to say that peptides (or proteins) are chains of residues of amino acids.

Population collapse catastrophe. Probabilistic event identified during computer simulations of Manfred Eigen's replicase-first model. A population collapse catastrophe is the result of a statistical fluctuation in the population of molecules when one essential component of a hypercycle falls to zero, an

event which leads to a rapid collapse of the entire hypercycle (Niesert et al. 1981). See also Error catastrophe, Selfish RNA catastrophe and Short-circuit catastrophe.

Posibacteria. Gram-positive bacteria. See Negibacteria (Szathmáry 2002).

Potential life criteria. Characteristics required for evolution only (Szathmáry 2002). See Absolute life criteria.

Primary pump. Concept developed to explain the evolution of metabolism as a set of coupled cycles (Boiteau et al. 2002). According to this hypothesis, prebiotic polypeptides emerged through a permanent, evolutive molecular engine, the primary pump driven by repeated wetting/drying alternations and fed by high-energy molecules (cyanic acid, nitrogen, oxides, etc.) (Boiteau et al. 2002). As a cautionary note, evaporation was described as inefficient for the accretion of early polymers because of the large initial dilution expected and the destructive effects of the energy source, solar radiation or thermal vaporization (Arrhenius 2002)

Prion. Pathogenic agents discovered by Prusiner (1997). Prions are proteins with identical sequences that can fold in a variety of ways with comparable free energies. In practice there are so many ways to fold a protein that the active form is not necessarily the most stable configuration. Prions are proteins that are folded in a naturally uncommon architecture, able to induce the refolding of normal proteins in a shape that is identical to the shape of the inducer and physiologically wrong. Some diseases such as bovine encephalopathy and Kourou are due to prions. This way of multiplying information is called phenotypic replication (Szathmáry 2002).

Processive replication (replicators). Synonymous with holistic replication (replicators).

Progenote. A pre-cellular entity with a rudimentary, imprecise linkage between its genotype and phenotype (Castresana and Saraste 1995). The progenote allegedly existed before the Last Universal Ancestor. If early evolution was a phylogenetic bush, no individual cell or phylogenetic line may be considered as the progenote or the Last Universal Ancestor.

Prokaryotes. Simple unicellular organisms with no nucleus, no mitochondria and no chloroplast. Bacteria and Archaea are examples of Prokaryotes. See also Eukaryotes.

Protocell. Primitive compartmentalized system which enclosed self-replicating gene-protein alliances (Turian 1999). The 'age of the protocell' is a concept formulated as the third stage toward life (DeDuve 1995, vital dust, cited by Hill 2002). The protocell was described as the first living unit surrounded by a membrane that was capable of requiring a number of key

properties leading to the emergence of the common ancestor of all life on Earth (Hill 2002).

Pump (primary). See Primary pump.

Punctuated coevolution of life. Unlike Darwinian evolution, which became over time a unitary concept, punctuated coevolution is not a single unitary concept but a description of the way prebiotic events accreted. The accretion of life would be better described as both a punctuated transition and a coevolving process (set of events). The entities that eventually became alive achieved their fundamental phase transitions and their derived properties one after another in a a punctuated transition. The emergence of life may also be described as a coevolving process (set of events) because the changes associated with the various aspects of life (energy, boundary, metabolic function, order and information) changed at a concerted pace.

Purpose-like nature of life. See the Essence of life.

Q-beta replicase. Enzyme able to make copies of an RNA template.

Quasi-species. A population of molecules with non-identical yet related sequences capable of self-replication and evolution. Although the concept of quasi-species developed by Manfred Eigen and his collaborators was first applied to RNA molecules, it can also be used for other types of molecule and in computer simulations.

Quasi-state. A defined succession of steady states. A quasi-state represents all the states an organism may pass through in its normal development.

Racemic mixture. An equal mixture of L and D isomers.

Recursive self-maintenance. The ability of a system to control its own maintenance (Ruiz-Mirazo et al. 1999).

Reflexive activity. Acting in a way that confers thermodynamic benefits to the performer. Reflexive activity is present whenever part of the energy resulting from a process is used to lower the degradation rate of the unit that is inducing the process. An activity restoring the integrity of a catalyst or reinitiating the process with energy or matter resulting from the process itself. Reflexive activity must not be confused with feedback regulation. See Feedback regulation.

Reproduction. The "material process of begetting life and required material overlap between the parents and the offspring" (Szathmáry 2002). If heredity in general means that life begets life, reproduction is the material process of begetting.

Ribosomes. Cellular organelles made of many proteins and ribonucleic acids arranged in a complex architecture. Ribosomes are cellular machines used to make proteins in a process called translation. During cellular trans-

lation, the information from a sequence of a nucleic acids (mRNA) is used to make a chain of amino acids (a polypeptide).

Ribozyme. Enzyme made from an RNA-related structure.

Right-handed structure. A spiral structure that turns clockwise in moving away from the observer.

Self-assembly. The tendency to aggregate into large ordered structures. Self-assembly is a thermodynamically controlled creation of order (Luisi 1993).

Selfish RNA catastrophe. Probabilistic event identified during computer simulations of Manfred Eigen's replicase-first model. A selfish RNA catastrophe is the situation when a single molecule learns to replicate itself faster than its competitors but forgets its function as a catalyst, becomes a parasite and chokes the rest of the population to death (Niesert et al. 1981). See also Error catastrophe, Short-circuit catastrophe and Population collapse catastrophe.

Short-circuit catastrophe. Short-circuit catastrophe occurs when an RNA molecule, which is supposed to be a link in the chain of hypercycle reactions, changes its sequence in such a way as to catalyze (poison or otherwise significantly interfere with) a later reaction in the chain. As a result of this circumstance, the chain is short-circuited and the hypercycle contracts to a simple hypercycle or even to a single cycle unable to provide a minimal functional complexity (Niesert et al. 1981). See also Error catastrophe, Selfish RNA catastrophe and Population collapse catastrophe.

Specificity of activity. Activity specificity is defined as the rate of legitimate reactions divided by the total rate of all (legitimate + side) reactions (Szathmáry 2002). Activity specificity is a measure of how consistent the activity of one molecule or a series of molecules is across time.

Speleothem. Structure made in caves primarily by water-based solvation or precipitation. Examples of speleothems are stalactites, stalagmites, draperies, crystals and cave pearls.

Supple adaptation. Concerning the essence of life. An "automatic and continually creative evolutionary process of adapting to changing environments, a process more rooted into the fabric of life than natural selection itself" (Bedau 1998).

Steady-state, thermodynamic equilibrium, homeostasis and quasi-state. "Steady state is a characteristic or a condition, such as value, rate, periodicity, or amplitude, exhibiting only negligible change over an arbitrarily long period of time. It may describe a condition in which some characteristics are static, others dynamic" (Considine, 1976). The steady-state deviation of a system may be defined as the system deviation after transients have expired. Another name for a steady state is an attractor-based system (Hageweg

1998), a term often used to emphasize an analogy with a virtual attraction basin of lower energy and higher stability. Steady state must not be confused with thermodynamic equilibrium (Jablonka and Lamb 1995), homeostasy or quasi-state. A steady state is one possible state which may be away from maximum thermodynamic equilibrium and thus can only be maintained at a certain energetic expense. Homeostasy is the process of maintaining the steady state. One system may display one or more steady states. A quasi-state is a collection of steady states and transition areas. In a geographical analogy, the steady states of one system are analogous to valleys and plateaus of a mountain ridge, while the quasi-state is the virtual surface occupied by all steady states of a system plus the paths among them.

Stereo-isomer. A molecular architecture that can be arranged in two geometric ways. Mirror images of each other that cannot be placed one over the other.

Structure. The disposition of the concrete elements or parts of a being (Valenzuela 2002). See Organization.

Substrate-level transduction. The formation of energy currencies such as ATP in solution. As opposed to chemiosmotic transduction (chemiosmotic phosphorylation) where the formation of ATP is membrane-dependent.

Symbiosis. Mutualism. Association based on sharing properties to the mutual advantage of the participating partners.

Terraforming (terraformation). Transforming the local conditions on another planet into conditions suitable for life on Earth.

Thermal death of the Universe. Because of the consequences of the second law of thermodynamics, every process occurs in such a way that part of the internal energy is transformed into heat. On a large time/space scale, the entire Universe is driven toward a maximum entropy state called the thermal death of the Universe.

Thermodynamic. Concerning chemical reactions; the final equilibrium of the reaction; the state with minimum energy. See Kinetic.

Tierran organisms. With one particularly simple phenotype. Digital simulation of an ancestral organism living in the Tierra artificial life system. In Tierra, each distinct organism executes identical code with identical initial conditions (data). Tierran organisms interact with each other, giving rise to an ecology of phenotypes. See also Avidan organisms.

Transcopic interactions. Interactions among entities situated on very different physical scales between microscopic, macroscopic and megascopic. Transcopic interactions are relevant in explaining the de novo origin of primordial handedness.

Transduction of energy. Cellular transformation of one form of energy into another that is easier to manipulate or store.

Transduction of information. Mechanism of gene transfer mediated by a bacteriophage (the virus of a bacteria) (Darnell et al. 1990).

Turgor pressure. Rigidity of cells due to an increase in hydrostatic pressure inside.

Type of life. Term used to differentiate life (or living entities) that have distinct constructive patterns. As an example, humans from the Sun's solar system belong to one type of life (biological life) while the inhabitants of a planet from the Alpha Centauri system (if they exist and if they are fundamentally different) would represent another type of life that is non-biological.

Units of life. Concept promoted by Eörs Szathmáry (Szathmáry 2002) with the intention of identifying a minimal living unit (see Units of evolution). Pointing out the difference between Units of life and Units of evolution is one possible way to address the mule paradox. See Mule paradox.

Units of evolution. Concept promoted by Eörs Szathmáry (Szathmáry 2002) with the intention of identifying a minimal living unit (see Units of life). Units of evolution must (1) multiply, (2) have heredity and (3) have variability (heredity must not be totally accurate) (Szathmáry 2002). Units of life and units of evolution are largely, but not completely overlapping concepts. Viruses and computer programs undergo evolution but are not alive in the biological sense, while mules and worker honey bees are not capable of reproduction yet they are very much alive.

Virtual life. Theoretical representations of life. Virtual life forms are the ultimate objective of artificial life simulations. Significant differences exist between living entities of the real physical world and virtual life forms. Virtual life forms are not sensitive to the second law of thermodynamics and therefore require energy and matter (virtual representations of matter and energy) only for growth and reproduction but not for maintenance. Unlike living entities in the real world, virtual life entities (whether in the form of mathematical constructs or computer representations) do not need a physical boundary to function.

D Abbreviations

A	Adenine
AA	Aamino acid
AAtRNA synthetase	Amino acyl tRNA synthetase
ActS	Activity-related specificity
Ala	Alanine (amino acid present in proteins)
APS	Adenylyl sulfate
ArchS	Architecture-related specificity
Arg	Arginine (amino acid present in proteins)
ARS	Amino acyl tRNA synthetase
Asp	Aspartic acid (amino acid present in proteins)
ATP	Adenosine triphosphate
C	Cytosine
Cat	Catalysis
CO_2	Carbon dioxide
CompS	Composition-related specificity
CondS	Condensation-related specificity
ConfS	Conformation-related specificity
C_{org}	Organic carbon
CPL	Circularly polarized light
CTP	Cytosine triphosphate
Cys	Cysteine (amino acid present in proteins)
dAMP	Deoxyribose adenine monophosphate
dCMP	Deoxyribose cytosine monophosphate
DeNovoS	De novo specificity
dGMP	Deoxyribose guanine monophosphate
DNA	Deoxyribonucleic acid
ΔS_{Θ}	Entropy variation associated with changes in the quasi state of the system
dTMP	Deoxyribose thymine monophosphate
E	The total amount of energy in a system
E_{fd}	Energy flux density
ET	Extraterrestrial
ETIF	Emergence of Template Information and Functionality

R. Popa, *Between Necessity and Probability: Searching for the Definition and Origin of Life*, Adv. Astrobiol. Biogeophys., pp. 225–226 (2004)
http://www.springerlink.com/

FAD	Flavin adenine dinucleotides
FMN	Flavin mononucleotide
G	Guanine
Gln	Glutamine (amino acid present in proteins)
Glu	Glutamic acid (amino acid present in proteins)
Gly	Glycine (amino acid present in proteins)
GTP	Guanosine triphosphate
Gyr	Giga years (109 years)
HandS	Handedness-related specificity
HetS	Spatial-heterogeneity-related specificity
Hi-fi	High fidelity
His	Histidine (amino acid present in proteins)
K	Equilibrium constant
L-CPL	Left-Circularly polarized light
mRNA	Messenger RNA
NAs	Nucleic acids
nm	Nanometer (10^{-9}m)
O_2	Oxygen
PAHs	Poly aromatic hydrocarbons
PCR	Polymerase Chain Reaction
PMF	Proton motive force
PolyAA_s	Chains of amino acids
PP	Pyrophosphate
PPi	Pyrophosphate
PreCondS	Precondensation-related specificity
Pro	Proline (amino acid present in proteins)
R-CPL	Right-Circularly polarized light
Ref	Reflexive activity
RNA	Ribonucleic acid
S	Entropy
SecS	Secondary specificity
SeqS	Sequence-related specificity
SizeS	Size-related specificity
T	Thymine
U	Uracil
UTP	Uracil triphosphate
UV	Ultra violet
Val	Valine (amino acid present in proteins)

References

1. D.L. Abel: Is life reducible to complexity? In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 57–71
2. P. Adawadker, W.D. Wilson, W. Brey, E.J. Gabbay: Stereospecific interaction of dipeptide amides with DNA. Evidence for partial intercalation and bending of the helix. J. Am. Chem. Soc. **87**, 1958–1961 (1975)
3. R.A. Alberty, R.J. Silbey: *Physical Chemistry* (Wiley, New York 1992)
4. J. Alexander: *Life: Its Nature and Origin* (Reinhold Publishing Company, New York 1948)
5. A.D. Altstein: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 5–16
6. M. Anbar: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 16–17
7. M. Anbar: What is life? In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 73–85
8. U. An der Heiden, G. Roth, H. Schwegler: Principles of self-generation and self-maintenance. Acta Biotheoretica **34**, 125–142 (1985)
9. P. Apte: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 17–18
10. D.H. Ardell: On error minimization in a sequential origin of the standard genetic code. J. Mol. Evol. **47**, 1–13 (1998)
11. G. Arrhenius: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 17–18
12. G. Arrhenius, R. De Birhas, H. Alfvén: Origin of the ocean. In: *The Sea*, Vol. 5, ed. by E.D. Goldberg (Wiley 1973) Cap. **23**, 839–861
13. V.A. Avetisov, V.I. Goldanskii: Chirality and the equation of the biological big-bang. Phys. Lett. **172**, 407–410 (1993)
14. V.A. Avetisov, V.I. Goldanskii: Mirror symmetry breaking at the molecular level. Proc. Natl. Acad. Sci. USA **93**, 11435–11442 (1996)
15. V.A. Avetisov, V.I. Goldanskii, V.V. Kuzmin: Handedness, origin of life and evolution. Physics Today **44**, 33–41 (1991)
16. J.L. Bada: Origins of homochirality. Nature **374**, 594–595 (1985)
17. J.L. Bada, G.D. McDonald: Amino acid racemization on Mars: Implications for the preservation of biomolecules from an extinct Martian biota. Icarus **114**, 139–143 (1995)
18. K. Badahur, S. Ranganayaki: The primordial formation of self-sustaining coacervates. J. Brit. Interplanet. Soc. **23**, 813–829 (1970)
19. P.A. Bachman, P.L. Luisi, J. Lang: Autocatalytic self-replicating micelles as models for prebiotic structures. Nature **357**, 57–59 (1992)

20. B.G. Bag, G. von Kiedrowski: Templates, autocatalysis and molecular replication. Pure Appl. Chem. **68**, 2145–2152 (1996)
21. J. Baggott: *Perfect Symmetry. The Accidental Discovery of Buckminsterfullerene* (Oxford University Press, Oxford 1994) p. 310
22. P. Bak: *How Nature Works. The Science of Self-Organized Criticality* (Copernicus, New York 1996)
23. H. Baltscheffsky: Major 'anastrophes' in the origin and early evolution of biological energy conversion. J. Theor. Biol. **187**, 495–501 (1997)
24. H. Baltcheffsky: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 17–18
25. H. Baltscheffsky, J. Jurka: On protocells, preprokaryotes, an early evolution. In: *Molecular Evolution and Protobiology*, ed. by K. Matsuno et al. (Plenum Press, New York 1984) pp. 207–214
26. H. Baltcheffsky, A. Schultz, M. Baltcheffsky: Fundamental characteristics of life and the molecular origin and evolution of biological energy conversion. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 87–94
27. B. Barbier, J. Visscher, A.W. Schwartz: Polypeptide-assisted oligomerization of analogous in dilute aqueous solution. J. Mol. Evol. **37**, 554–558 (1993)
28. A. Bar-Nun, E. Kochavi, S. Bar-Nun: Assemblies of free amino acids as possible prebiotic catalysis. J. Mol. Evol. **39**, 116–122 (1994)
29. N.A. Barricelli: On the origin and evolution of genetic code. J. Theor. Biol. **67**, 85–109 (1977)
30. N.A. Barricelli: On the origin and evolution of the genetic code. II. Origin of the genetic code as a primordial collector language. The pairing release hypothesis. BioSystems **11**, 19–28 (1979)
31. J.D. Barrow, F.J. Tipler: *The Anthropic Cosmological Principle* (Oxford University Press, Oxford 1986)
32. B.L. Bass, T.R. Cech: Specific interaction between the self-splicing RNA of Tetrahymena and its guanosine substrate: implications for biological catalysis by RNA. Nature **301**, 820–826 (1984)
33. H.C. Bastian: cited in H.C. Bastian (1905), *The Nature and Origin of Living Matter* (Fisher Unwin, London 1872) p. 147
34. L.S. Beale: *Disease Germs, their Nature and Origin* (London 1872) p. 9
35. M.A. Bedau: Four puzzles about life. Artificial Life **4**, 125–140 (1998)
36. M.A. Bedau, J.S. McCaskill, N.H. Packard, S. Rasmussen, C. Adami, D.G. Green, T. Ikegami, K. Kaneko, T.S. Ray: Open problems in artificial life. Artificial Life **6**, 363–376 (2000)
37. T. Behmoaras, J.J. Toulmé, C. Hélène: A tryptophan-containing peptide recognizes and cleaves DNA at apurinic sites. Nature **292**, 858–859 (1981)
38. F. Békés: Simulation of kinetics of proliferating chemical systems. BioSystems **7**, 189–195 (1975)
39. B.P. Belusov: (In Russian) Sb. Ref. Radiat. Med. Moscow (1958)
40. C.R. Benn: The Moon and the origin of life. Earth, Moon and Planets **86**, 61–66 (2001)
41. S.A. Benner, M.A. Cohen, G.H. Gonnet, D.B. Berkowitz, K.P. Johnsson: Reading the palimpsest: contemporary biochemical data and the RNA world. In: *The RNA World*, ed. by R.F. Gesteland, J.F. Atkins (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY 1993)

42. J.M. Berg, R.H. Holm: Structures and reactions of iron–sulfur protein clusters and their synthetic analogs. In: *Iron–Sulfur Clusters*, ed. by T.G. Spiro (Wiley, New York 1982) pp. 1–67
43. A.M. Bergareche, K. Ruiz-Mirazo: Metabolism and the problem of its universalization. BioSystems **49**, 45–61 (1999)
44. J.D. Bernal: Molecular structure, biochemical function and evolution. In: *Theoretical and Mathematical Biology*, ed. by T.H. Waterman, H.J. Morowitz (Blaisdel, New York 1965)
45. J.D. Bernal: *The Origins of Life* (Weidenfeld and Nicholson, London 1967)
46. C. Bernard (1878a): *Lectures on the Phenomena of Life*. Translated by H.E. Hoff, R. Guillemin, and L. Guillemin (Charles Thomas, Springfield, Illinois 1974)
47. C. Bernard (1878b): *An Introduction to the Study of Medicine*. Translated by H.C. Greene (Macmillan, New York 1927)
48. T.J. Beveridge: Ultrastructure, chemistry, and function of the bacterial wall. International Review of Cytology **72**, 229–317 (1981)
49. C.K. Biebricher, M. Eigen, R. Luce: Template-free RNA synthesis by Q(beta) replicase. Nature **32**, 89–91 (1986)
50. E.R. Blout and M. Idelson: Poly-L-glutamic acid preparation and helix-coil conversions. J. Am. Chem. Soc. **78**, 497–498 (1956)
51. E.R. Blout et al.: Polypeptides XII, The optical rotation and configuration stability of α-helices. J. Am. Chem. Soc. **79**, 749–750 (1957)
52. P.W. Boden: Introduction. *The Philosophy of Artificial Life* (Oxford University Press, New York 1996) pp. 25–26
53. M. Boden: Is metabolism necessary? Cognitive Science Research Paper 482, University of Sussex (1998)
54. N. Bohr: Light and life. Nature **131**, 457–459 (1933)
55. L. Boiteau: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 20–21
56. L. Boiteau, R. Plasson, H. Collet, O. Lagrille, J.P. Biron, O. Vandenabeele-Trambouze, J. Taillades, A. Commeyras: Molecular origins of life: When chemistry became cyclic. The primary pump, a model for prebiotic emergence and evolution of peptides. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 211–218
57. W.A. Bonner: Chirality, cosmochemistry and life. Chemistry and Industry **17**, 640–644 (1992)
58. W.A. Bonner: Chirality and life. Orig. Life Evol. Biosph. **25**, 175–190 (1995)
59. A. Brack: *The Molecular Origins of Life. Assembling Pieces of the Puzzle* (Cambridge University Press, Cambridge 1998)
60. A. Brack: The exobiology exploration of Mars: a survey of the European approaches. Planetary and Space Science **48**, 1023–1026 (2000)
61. A. Brack: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 20–21
62. C. Bresch, U. Niesert, D. Harnasch: Hypercycles, parasites and packages. J. Theor. Biol. **85**, 399–405 (1980)
63. D. Brin: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 21–22
64. P. Bro: Chemical reaction automata. Precursors of artificial organisms. Complexity **2**, 38–44 (1997)

65. R. Brooks: The relationship between matter and life. Nature **409**, 409–411 (2001)
66. D.A. Brown, T.J. Beveridge, C.W. Keevil, B.L. Sheriff: Evaluation of microscopic techniques to observe iron precipitation in a natural microbial biofilm. FEMS Microbiology Ecology **26**, 297–310 (1998)
67. S. Brown, M. Sarikaya, E. Johnson: A genetic analysis of crystal growth. J. Mol. Biol. **299**, 725–735 (2000)
68. J. Browning: On the correlation of microscopic physiology and microscopic physics. Monthly Micro. J. **2**, 18 (1869)
69. L. Büchner: *Kraft und Stoff*, 5th edn. (Frankfurt 1858)
70. M. Buiatti, M. Buiatti: The living state of matter. Rivista di Biologia – Biology Forum **94**, 9–82 (2001)
71. R. Buick: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 22
72. S. Bunjun, C. Stathopoulos, D. Graham, B. Min, M. Kitabatake, A.L. Wang, C.C. Wang, C.P. Vivares, L.M. Weiss, D. Soll: A dual-specificity aminoacyl-tRNA synthetase in the deep-rooted eukaryote Giardia lamblia. Proc. Natl. Acad. Sci. USA **97**, 12997–13002 (2000)
73. A.W. Burks: *Essays on Cellular Automata* (University of Illinois Press 1970)
74. A.G. Cairns-Smith: *Genetic Takeover and the Mineral Origins of Life* (Cambridge University Press, Cambridge 1982)
75. A.G. Cairns-Smith, H. Hartman: *Clay Minerals and the Origin of Life* (Cambridge University Press, Cambridge 1986) pp. 193–210
76. M. Calvin: *Chemical Evolution* (Oxford University Press, New York 1969)
77. N.A. Campbell: *Biology*, 3rd edn. (Benjamin Commings, New York 1993)
78. T. Cavalier-Smith: Selfish DNA and the origin of introns. Nature **351**, 110 (1985)
79. T. Cavalier-Smith: Membrane heredity, symbiogenesis, and the multiple origins of algae. In: *Biodiversity and Evolution*, ed. by R. Arai, M. Kato, and Y. Doi (The National Science Museum Foundation, Tokyo 1995) pp. 75–114
80. T. Cavalier-Smith: Membrane heredity and early chloroplast evolution. Trends Plant Sci. **5**, 174–182 (2000)
81. T.R. Cech: A model for the RNA-catalyzed replication of RNA. Proc. Natl. Acad. Sci. USA **83**, 4360–4363 (1986)
82. T.R. Cech: The chemistry of self-splicing RNA and RNA enzymes. Science **236**, 1532–1539 (1987)
83. T.R. Cech: The efficiency and versatility of catalytic RNA: Implications for an RNA world. Gene **135**, 33–36 (1993)
84. R. Cervellati: *Le reazioni chimiche oscillantti* (CLUEB, Bologna 1995)
85. A.C. Chakrabarti, R.R. Breaker, G.F. Joyce, D.W. Deamer: Production of RNA by a polymerase protein encapsulated within phospholipid vesicles. J. Mol. Evol. **39**, 555–559 (1994)
86. O.G. Clark, R. Kok: Engineering of highly autonomous biosystems: Review of the relevant literature. International Journal of Intelligent Systems **13** (8), 749–783 (1998)
87. J.S. Clegg, D.N. Wheatley: Intracellular organization: Evolutionary origins and possible consequences to metabolic rate control in vertebrates. Amer. Zool. **31**, 504–513 (1991)

88. J.P. Collman, X. Zhang, V.J. Lee, E.S. Uffelman, J.I. Brauman: Regioselective and enantioselective epoxidation catalysed by metalloporphyrins. Science **261**, 1404–1411 (1993)
89. G.J. Connell, M. Yarus: RNAs with dual specificity and dual RNAs with similar specificity. Science **264**, 1137–1141 (1994)
90. P.R. Cook, R.A. Laskey: Higher order structure in the nucleus. J. Cell Sci. Supplement 1 (1984)
91. M. Conrad: Origin of life and the underlying physics of the universe. Biosystems **42**, 177–190 (1997)
92. P.G. Conrad, K.H. Nealson: A non-Earth centric approach to life detection. Astrobiology **1**, 15–24 (2001)
93. D.E. Considine: Van Nostrand Scientific Encyclopedia, 5th edn. (Van Nostrand Reinhold, New York 1976)
94. L.M. Coyne: A possible energetic role of mineral surfaces in chemical evolution. Orig. Life **15**, 161–206 (1985)
95. L.M. Coyne, N. Lahav, J.G. Lawless: Dehydration-induced luminescence in clay minerals. Nature **292**, 819–821 (1981)
96. F.H.C. Crick: The origin of the genetic code. J. Mol. Biol. **22**, 361–368 (1968)
97. V. Csanyi: *Evolutionary Systems and Society. A General Theory of Life, Mind and Culture* (Duke University Press, Durham 1989)
98. V. Csanyi, G. Kampis: Autogenesis: The evolution of replicative systems. J. Theor. Biol. **114**, 303–321(1985)
99. J. Darnell, H. Lodish, D. Baltimore: *Molecular Cell Biology* (Scientific American Books, Freeman, New York 1990)
100. R. Dawkins: *The Selfish Gene* (Oxford University Press, Oxford 1989)
101. D.W. Deamer: Role of amphiphilic compounds in the evolution of membrane structure on the early Earth. Orig. Life **17**, 3–25 (1986)
102. D.W. Deamer: The first living systems: a bioenergetic perspective. Microbiology and Molecular Biology Reviews **61**, 239–261 (1997)
103. D.W. Deamer: Membrane compartments in prebiotic evolution. In: *The Molecular Origins of Life. Assembling Pieces of the Puzzle*, ed. by A. Brack (Cambridge University Press, Cambridge 1998) pp. 189–205
104. D.J. DeBeer, D.G. Kourie: Artificial life: an overview. South African Journal of Science **96**, 569–576 (2000)
105. P. Decker: Evolution in open systems. Biostability and origin of molecular asymmetry. Nature Biology **241** (107), 72–74 (1973a)
106. P. Decker: Possible resolution of racemic mixtures by bistability in bioids, open systems which can exist in several steady states. J. Mol. Evol. **2**, 137–143 (1973b)
107. P. Decker: Evolution in bioids: hypercompetitivity as a source of biostability and a possible role of metal complexes as prenucleoprotic mediators of molecular asymmetry. Orig. Life **6**, 211–218 (1975)
108. C. DeDuve: Prebiotic syntheses and the mechanism of early chemical evolution. In: *The Roots of Modern Biochemistry. Fritz Lipmann's Squiggle and its Consequences*, ed. by H. Kleinkauf, H. Von Dohren, L. Jaenicke (Walter de Gruyter, Berlin 1988) pp. 881–894
109. C. DeDuve: *Blueprint for a Cell: The Nature and Origin of Life* (Neil Patterson, Carolina Biological Supply Corp, Burlington, NC 1991)
110. C. DeDuve: *Vital Dust: Life as a Cosmic Imperative* (Basic Books, HarperCollins Publishers, New York 1995)

111. A. DeLoof: Schrödinger 50 years ago: "What is life?" "The ability to communicate," a plausible reply? Int. J. Biochem. **25**, 1715–1721 (1993)
112. A. DeLoof, J.V. Broeck: The key to defining life, death and the force driving evolution – organic-chemistry-based life versus artificial life – communication. Belgian Journal of Zoology **125**, 5–28 (1995)
113. A.H. Delsemme: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 25
114. D.H. Deutsch: A mechanism for molecular asymmetry. J. Mol. Evol. **33**, 295–296 (1991)
115. M. DiGiulio, M.R. Capobianco, M. Medugno: On the optimization of the physicochemical distances between amino acids in the evolution of the genetic code. J. Theor. Biol. **168**, 13–51 (1991)
116. M. DiGiulio: The evolution of aminoacyl-tRNA synthetases, the biosynthetic pathways of amino acids and the genetic code. Orig. Life Evol. Biosphere **22**, 309–319 (1992)
117. M. DiGiulio: Origin of glutaminyl-tRNA synthetase: an example of a palimpsest? J. Mol. Evol. **37**, 5–10 (1993)
118. M. DiGiulio: The phylogeny of tRNA molecules and the origin of the genetic code. Orig. Life Evol. Biosphere **24**, 425–434 (1994)
119. D. Dix: Toward a definition of life – semantic and thermodynamic considerations. J. Theor. Biol. **102**, 337–340 (1983)
120. J. Dzik: Early diversification of organisms in the fossil record. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 219–248
121. F. Dyson: *Origins of Life* (Cambridge University Press, Cambridge 1997)
122. F. Dyson: Colloquium at NASA's Goddard Space Flight Center (2000)
123. E.C. Edblom, M. Orban, I.R. Epstein: A new iodate oscillator – the Landolt reaction with ferrocyanide in a CSTR. J. Am. Chem. Soc. **108**, 2826–2830 (1986)
124. L. Edwards, Y. Peng: Computational models for the formation of protocell structures. Artificial Life **4**, 35–42 (1998)
125. L. Edwards, Y. Peng, J. Regia: Computational models for the formation of protocell structures. Artificial Life **4**, 61–67 (1998)
126. M. Eigen: (1971) Self-organization of matter and the evolution of biological macromolecules. Naturwissenschaften **58**, 465–523
127. M. Eigen, W. Gardiner, R. Winkler-Oswatisch: The origin of genetic information. Sci. Amer. **244**, 88–118 (1981)
128. M. Eigen, P. Schuster: The hypercycle, a principle of natural self-organization. Part A. Naturwissenschaften **64**, 541–565 (1977)
129. M. Eigen, P. Schuster: The hypercycle, a principle of natural self-organization. Part B. Naturwissenschaften **65**, 7–41 (1978a)
130. M. Eigen, P. Schuster: The hypercycle, a principle of natural self-organization. Part C. Naturwissenschaften **65**, 341–369 (1978b)
131. M. Eigen, P. Schuster: *The Hypercycle, a Principle of Natural Self-Organization* (Springer-Verlag, Berlin, New York 1979)
132. M. Eigen, R. Winkler-Oswatisch: *Steps Toward Life. A Perspective on Evolution* (Oxford University Press, Oxford 1992) p. 173
133. F.R. Eirich: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 27

134. C. Emmeche: Life as an abstract phenomenon: is artificial life possible? In: *Towards a Practice of Autonomous Systems*, ed. by F.J. Varen, F. Bourgine, Proc. 1st European Conference on Artificial Life (1992) pp. 466–474
135. F. Engels (ca. 1880): *Dialectic of Nature*. The manuscript was lost for about sixty years. Translated and edited by C.D. Dutt (1940)
136. A.S. Erokhin: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 28
137. A. Eschenmoser: Chemistry of potentially prebiological natural products. Orig. Life Evol. Biosphere **24**, 389–423 (1994)
138. J. Farley: *The Spontaneous Generation Controversy* (John Hopkins University Press, Baltimore 1977)
139. J. Farmer: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 29
140. J.D. Farmer, S. Kauffman, N.H. Packard: Autocatalytic replication of polymers. Physica **22**D, 50–67 (1986)
141. J.P. Ferris: Catalysis and prebiotic RNA synthesis. Orig. Life Evol. Biosphere **23**, 307–315 (1993)
142. J.P. Ferris, G. Ertem: Oligomerization of ribonucleotides on Montmorillonite: Reaction of the 5′-phosphorimidazolide of adenosine. Science **257**, 1387–1388 (1992)
143. J.P. Ferris, A.R. Hill, R. Liu, L.E. Orgel: Synthesis of long prebiotic oligomers on mineral surfaces. Nature **381**, 59–61 (1993)
144. J.P. Ferris, P. Kamaluddin, G. Ertem: Oligomerization reactions of deoxyribonucleotides on montmorillonite clay: the effect of mononucleotide structure, phosphate activation and montmorillonite composition on phosphodiester bond formation. Orig. Life Evol. Biosphere **20**, 279–291 (1990)
145. Y. Fishman, N. Citri: L-asparaginase entrapped in liposomes: preparation and properties. FEBS Letters **60**, 17–20 (1975)
146. G. Fleischacker: Autopoiesis: the status of its system logic. BioSystems **22**, 37–49 (1989)
147. R.J. Flettrick, T. Schroer, R.J. Matela: *Molecular Structure: Macromolecules in Three Dimensions* (Blackwell 1985)
148. J.J. Flores, W.A. Bonner: On the asymmetric polymerization of aspartic acid enantiomers by kaolin. J. Mol. Evol. **3**, 49–56 (1974)
149. J.J. Flores, W.A. Bonner, G.A. Massey: Asymmetric photolysis of (RS)-leucine with circularly polarized ultraviolet light. J. Amer. Chem. Soc. **99**, 3622–3625 (1977)
150. C.E. Folsome: *The Origin of Life* (Freeman, San Francisco 1979)
151. P. Fong: Thermodynamic statistical theory of life: an outline. In: *Biogenesis, Evolution, Homeostasis. A Symposium by Correspondence* (Springer-Verlag, Berlin 1973) pp. 93–101
152. W. Fontana: Algorithmic chemistry. In: *Artificial Life II*, ed. by C.G. Langdon, C. Taylor, J.G. Farmer, S. Rasmussen (Addison-Wesley, Redwood City 1992) pp. 159–209
153. W. Fontana, L.W. Buss: What would be conserved if the tape were played twice? Proc. Natl. Acad. Sci. USA **91**, 757–761 (1994a)
154. W. Fontana, L.W. Buss: The arrival of the fittest – toward a theory of biological organization. Bull. Math. Biol. **56**, 1–64 (1994b)
155. W. Fontana, G. Wagner, L.W. Bus: Beyond digital naturalism. Artif. Life **1**, 211–227 (1994)

156. S.W. Fox: Thermal polymerization of amino acids and production of formed microparticles on lava. Nature **201**, 336–337 (1964)
157. S.W. Fox, K. Dose: *Molecular Evolution and the Origin of Life*, 2nd edn. (Dekker, New York 1977)
158. S.W. Fox, K. Harada: Thermal polycondensation of amino acids. In: *A Laboratory Manual of Analytical Methods of Protein Chemistry*, ed. by P. Alexander, H.P. Lundgren (1966) **4**, 129–151 (1964)
159. S.W. Fox, K. Matsuno: Genetic takeover and the mineral origins of life. Trends in Biochemical Sciences **8**, 341–342 (1983)
160. I. Friedman: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 30
161. E.J. Gabbay: Topography of nucleic acid helices in solutions IX. Models for the interactions of optically active diamines, amino acid amides, diamino acids and lysyl dipeptides with nucleic acid systems. J. Am. Chem. Soc. **90**, 5257–5263 (1968)
162. E.J. Gabbay, R. Kleinman, R.R. Shimshak: Topography of nucleic acid helices in solutions VII. Selective interactions of L-amino acids and peptides with nucleic acid helices. J. Am. Chem. Soc. **90**, 1927–1928 (1968a)
163. E.J. Gabbay, R. Kleinman, R.R. Shimshak: Topography of nucleic acid helices in solutions VI. The effect of amino acid derivatives on the RNA-catalyzed hydrolysis of polyadenylic acid. Demonstration of an asymmetric surface. Biopolymers **6**, 993–996 (1968b)
164. E.J. Gabbay, R.W. Kleinman: Topography of nucleic acid helices in solutions. The interaction specificities of optically active amino acid derivatives. Biochem. J. **117**, 247–250 (1970)
165. E.J. Gabbay, K. Samford, C.G. Baxter: Specific interaction of peptides with nucleic acids. Biochemistry **11**, 3428–3435 (1972)
166. E.J. Gabbay, P.D. Adawadker, W.D. Wilson: Stereospecific binding of diastereomeric peptides to salmon sperm DNA. Biochemistry **15**, 146–151 (1976)
167. T. Gánti: *The Principle of Life* (in Hungarian) (Gondolat, Budapest 1971)
168. T. Gánti: A chemoton elmélet alapjai (in Hungarian). Fizikai Szemle **24**, 97–103 (1974)
169. T. Gánti: Organisation of chemical reactions into dividing and metabolising units: the chemotons. BioSystems **7**, 189–195 (1975)
170. T. Gánti: *A Theory of Biochemical Supersystems* (Akadémiai Kladó, Budapest and University Park Press, Baltimore 1979a)
171. T. Gánti: Interpretation of prebiotic evolution on the basis of chemoton theory (in Hungarian). Biológia **27**, 161–175 (1979b)
172. T. Gánti: *The Principle of Life* (OMIKK, Budapest 1987)
173. T. Gánti: Biogenesis itself. J. Theor. Biol. **187**, 583–593 (1997)
174. A.S. Garay: Origin and role of optical isomery in life. Nature **219**, 338–340 (1968)
175. L. Gatlin: *Information Theory and the Living System* (Columbia University Press, New York 1972)
176. D.R. Gerard: Concepts in biology. Behavioral Sciences **3**, 92–215 (1958)
177. G. Gilat: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 31
178. W. Gilbert: The RNA world. Nature **319**, 618 (1986)

179. M.L. Glassman, A. Hochberg: The origin of life: self-replicating asymmetrical frozen probability. Medical Hypotheses **50** (1), 81–83 (1998)
180. V.I. Goldanskii, V.V. Kuzmin: Chirality and cold origin of life. Nature **352**, 114 (1991)
181. N. Goldman: Further results on error minimization in the genetic code. J. Mol. Evol. **37**, 662–664 (1993)
182. G. Gonzalez, D. Brownlee, P. Ward: The galactic habitable zone: Galactic chemical evolution. Icarus **152**, 185–200 (2001)
183. R. Guerrero: Meeting Frontiers of Science. Cited in Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 31–32
184. C. Guerrier-Takada, S. Altman: Catalytic activity of an RNA molecule prepared by transcription in vitro. Science **223**, 285–286 (1984)
185. R.C. Guimarães: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 33
186. R.C. Guimarães: An evolutionary definition of life: from metabolism to the genetic code. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 95–110
187. R.C. Guimarães, C.H.C. Moreira: Genetic code structure and evolution: aminoacyl-tRNA synthetases and principal dinucleotides. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 249–276
188. V.A. Gusev: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 33
189. E. Haeckel (1866): Quoted in (Oparin 1957) pp. 77–78
190. P. Hageweg: On searching generic properties of non-generic phenomena: An approach to bioinformatic theory formation. In: *Artificial Life VI*, ed. by C. Adami, R.W. Belew, H. Kitano, C.E. Taylor (MIT Press, Cambridge 1998)
191. J.B.S. Haldane (1929): The origin of life. Appendix. In: *The Origins of Life*, ed. by J.D. Bernal (Weidenfeld and Nicholson, London 1967) pp. 242–249
192. G.J. Handschuh, R. Lohrmann, L.E. Orgel: The effect of Mg^{2+} and Ca^{2+} on urea-catalyzed phosphorylation reactions. J. Mol. Evol. **2**, 251–262 (1973)
193. K. Harada: Origin and development of optical activity of organic compounds on the primordial earth. Naturwiss. **57**, 114–119 (1970)
194. H. Hartman: Speculations on the evolution of the genetic code. Orig. Life **6**, 423–427 (1975)
195. H. Hartman: Speculations on the evolution of the genetic code. Orig. Life **9**, 133–136 (1978)
196. H. Hartman: Speculations on the evolution of the genetic code III: The evolution of t-RNA. Orig. Life **14**, 643–648 (1984)
197. H. Hartman: Speculations on the origin of the genetic code. J. Mol. Evol. **40**, 541–544 (1995)
198. H. Hartman, A. Fedorov: The origin of the eukaryotic cell: A genomic investigation. Proc. Natl. Acad. Sci. USA **99**, 1420–1425 (2002)
199. M. Hasegawa, T.A. Yano: Entropy of the genetic information and evolution. Orig. Life **6**, 219–227 (1975)
200. E. Haukioja: Are individuals really subordinate to genes? A theory of living entities. J. Theor. Biol. **99**, 357–375 (1982)

201. R.M. Hazen: Selective adsorbtion of L- and D-amino acids on calcite: Implications for biochemical homochirality. Proc. Natl. Acad. Sci. **98**, 5487–5490 (2001)
202. R.A. Hegstrom, D.K. Kondepudi: The handedness of the universe. Sci. Amer. **262**, 108–115 (1990)
203. A. Henderson-Sellers, A.W. Schwartz: Chemical evolution and ammonia in the early Earth's atmosphere. Nature **287**, 117–118 (1980)
204. R.J.C. Hennet: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 35
205. R.J.C. Hennet: Life is simply a particular state of organized instability. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 109–110
206. J.G. Henrotte: From the faults of Darwinism to the deficiencies of finalism. Recherche **294**, 8–9 (1997)
207. F. Herbert: *Dune* (Berkley Books, New York 1965)
208. M. Hermes-Lima: Model for prebiotic pyrophosphate formation: condensation of precipitated orthophosphate at low temperature in the absence of condensing or phosphorylating agents. J. Mol. Evol. **31**, 353–358 (1990)
209. J. Hertz, A. Krogh, R.G. Palmer: Introduction to the theory of neural computation. Santa Fe Institute (Addison Wesley, Reading, MA, USA 1990)
210. R.D. Hill: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 35
211. N.G. Holm, G. Ertem, J.P. Ferris: The binding and reactions of nucleotides and polynucleotides on iron oxide hydroxide polymorphs. Orig. Life Evol. Biosphere **23**, 195–215 (1993)
212. C. Holden: Evolving toy story. Science **285**, 1663–1663 (1999)
213. N.H. Horowitz: On defining life. In: *The Origin of Life on Earth*, ed. by F. Clark, R.L.M. Synge (Pergamon, London 1959) pp. 106–107
214. N.H. Horowitz: *To Utopia and Back: The Search for Life in the Solar System* (H.W. Freeman, New York 1986)
215. N.H. Horowitz: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 36
216. R.D. Hotchkiss: The assimilation of amino acids by respiring washed Staphylococci. Arch. Bioch. **65** (1), 302–318 (1956)
217. F. Hoyle, N.C. Wickramasinghe: *Our Place in the Cosmos* (J.M. Dent, London 1993)
218. F. Hoyle, N.C. Wickramasinghe: The Universe and life: Deductions from the weak anthropic principle. Astrophysics and Space Science **268**, 89–102 (1999)
219. F. Hucho, K. Buchner: Signal transduction and protein kinases: The long way from plasma membrane into the nucleus. Naturwiss. **84**, 281–290 (1997)
220. J. Hulshof, C. Ponnamperuma: Prebiotic condensation reactions in an aqueous medium: A review of condensing agents. Orig. Life **7**, 197–224 (1976)
221. C.S. Hurlbut: *Dana's Manual of Mineralogy*, 18th edn. (John Wiley and Sons 1971)
222. T.H. Huxley: On the physical basis of life. In: *Lay Sermons, Addresses and Reviews* (New York 1868) p. 129
223. M. Ishigami, K. Nagano: The origin of the genetic code. Orig. Life **6**, 551–560 (1975)

224. K. Ishihara, Y. Iwasaki, N. Nakabayashi: Polymeric lipid nanosphere consisting of water-soluble poly(2-methacryloyloxyethyl phosphorylcholine-co-*n*-butyl) methacrylate. Polymer Journal **31**, 1231–1236 (1999)
225. O.C. Ivanov: Some proteins keep 'living fossil' pre-sequence. Orig. Life Evol. Biosphere **23**, 115–124 (1993)
226. E. Jablonka, M.J. Lamb: *Epigenetic Inheritance and Evolution* (Oxford University Press, Oxford 1995)
227. K.D. James, A.D. Ellington: The search for missing links between self-replicating nucleic acids and the RNA world. Orig. Life Evol. Biosphere **25**, 515–530 (1995)
228. D.C. Jeffares, A.M. Poole, D. Penny: Relics from the RNA world. J. Mol. Evol. **46**, 18–36 (1998)
229. M. Jibu, K. Yasue, S. Hagan: Evanescent (tunneling) photon and cellular 'vision'. BioSystems **42**, 65–73 (1997)
230. W.M. Jones, T.S. Soper, H. Ueno, J.M. Manning: D-glutamate-d-amino acid transaminase from bacteria. Methods in Enzymology **113**, 108–113 (1985)
231. C. Joyce: RNA evolution and the origins of life. Nature **338**, 217–224 (1989)
232. G.F. Joyce: Foreword. In: *Origins of Life: The Central Concepts*, ed. by D.W. Deamer, G.R. Fleischaker (Jones and Bartlet Publishers 1994) pp. xi–xii
233. G.F. Joyce: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 37
234. G.F. Joyce, L.E. Orgel: Prospects for understanding the origin for the RNA world. In: *The RNA World II*, ed. by T. Gesteland (Cold Spring Harbor Laboratory Press, Cold Spring 1999) pp. 49–77
235. G.F. Joyce, A.W. Schwartz, S.E. Miller, L.E. Orgel: The case for an ancestral genetic system involving simple analogues of the nucleotides. Proc. Natl. Acad. Sci. USA **84**, 4398–4402 (1987)
236. S.S. Ju, L.L. Lin, W.C. Wang, W.H. Hsu: A conserved aspartate is essential for FAD binding and catalysis in the D-amino acid oxidase from Trigonopsis variabilis. FEBS Letters **436**, 119–122 (1998)
237. H.B. Kagan, G. Balavoine, A. Mordapour: Can circularly polarized light be used to obtain chiral compounds of high optical purity? J. Mol. Evol. **4**, 41–48 (1974)
238. A. Kanavarioti: Template-directed chemistry and the origins of the RNA world. Orig. Life Evol. Biosphere **24**, 479–494 (1994)
239. R.W. Kaplan: *Der Ursprung des Lebens* (Stuttgart, Thieme 1978)
240. J. Kasting: Habitable zones around stars and the search for extraterrestrial life. The 2nd Astrobiology Science Conference. NASA AMES Research Center, April 7–11 (2002)
241. M.J. Katz: *Templates and Explanation of Complex Patterns* (Cambridge University Press, Cambridge 1986)
242. S.A. Kauffman: Autocatalytic sets of proteins. J. Theor. Biol. **119**, 1–24 (1986)
243. S.A. Kauffman: *The Origins of Order* (Oxford University Press, New York 1993)
244. S.A. Kauffman: *At Home in the Universe: The Search for Laws of Self-Organization and Complexity* (Oxford University Press, New York 1995)
245. S.A. Kauffman: Prolegomenon to a general biology, unity of knowledge: the convergence of natural and human science. Annals of the New York Academy of Sciences **935**, 18–36 (2001)

246. K. Kawamura: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 37
247. A.D. Keefe, S.L. Miller, G.D. McDonald, J.L. Bada: Investigations of the prebiotic synthesis of amino acids and RNA bases from CO_2 using FeS/H_2S as a reducing agent. Proc. Natl. Acad. Sci. USA **92**, 1904–11906 (1995)
248. M. Keller, E. Bloohl, G. Wächtershäuser, K.O. Stetter: Formation of amide bonds without a condensation agent and implications for the origin of life. Nature **368**, 836–838 (1991)
249. L. Keszthelyi: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 38
250. M. Kimura: Stochastic processes in population genetics. In: *Mathematical Topics in Population Genetics*, ed. by K.I. Kojima (Springer, Berlin 1970) pp. 178–209
251. M. Kimura: *The Neutral Theory of Molecular Evolution* (Cambridge University Press, New York 1983)
252. G.A.M. King: Recycling, reproduction and life's origin. BioSystems **15**, 89–97 (1982)
253. G.A.M. King: Was there a prebiotic soup? J. Theor. Biol. **123**, 493–498 (1986)
254. E. Klabunovsky: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 38–39
255. P. Knauth: Ancient sea water. Nature **362**, 290–291 (1993)
256. P. Knauth: Salinity history of the Earth's early ocean. Nature **395**, 554–555 (1998)
257. R.D. Knight, L.F. Landweber: The early evolution of the genetic code. Cell **101**, 569–572 (2000)
258. W.S. Knowles, W.C. Christopfel, K.E. Koenig, C.F. Hobbs: Studies of asymmetric homogeneous catalysts. Advances in Chemistry Series **196**, 325–336 (1982)
259. W.S. Knowles: Application of organometallic catalysis to the commercial production of L-DOPA. Abstracts of Papers of the American Chemical Society **189**, 108–124 (1985)
260. W.S. Knowles: Application of organometallic catalysis to the commercial production of L-DOPA. Journal of Chemical Education **63**, 222–225 (1986)
261. A.L. Koch: Primeval cells: Possible energy-generating and cell-division mechanisms. J. Mol. Evol. **21**, 270–277 (1985)
262. A. Koch, T.M. Schmidt: The first cellular bioenergetic process: primitive generation of a proton-motive force. J. Mol. Evol. **33**, 297–304 (1991)
263. R.R. Kocherlakota, N.D. Acland: Ambiguity and the evolution of the genetic code. Orig. Life **12**, 71–80 (1982)
264. V.M. Kolb: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 38
265. V.N. Kompanichenko: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 38
266. D.K. Kondepudi, G.W. Nelson: Weak neutral currents in molecules and the origin of biomolecular chirality. Nature **314**, 438–441 (1985)
267. J. Konecny, M. Eckert, M. Schöniger, G.L. Hofacker: Neutral adaptation of the genetic code to double-strand coding. J. Mol. Evol. **36**, 407–416 (1993)
268. B. Korzeniewski: Cybernetic formulation of the definition of life. J. Theor. Biol. **209**, 275–286 (2001)

269. K.L. Kovacs: On the physical origin of biological handedness. Orig. Life **9**, 219–233 (1979)
270. W.E. Krumbein: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 38
271. H. Kuhn: Self-organization of molecular systems and evolution of the genetic apparatus. Angew Chem. Int. Ed. Engl. **11**, 798–824 (1972)
272. H. Kuhn: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 40
273. H. Kuhn, C. Kuhn: Evolution of a genetic code simulated with the computer. Orig. Life **9**, 137–150 (1978)
274. H. Kuhn, J. Waser: Molekulare Selbstororganisation des Lebens. Angew Chem. **93**, 495–515 (1981)
275. I.S. Kulaev: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 41
276. V. Kunin: A system of two polymerases – A model for the origin of life. Orig. Life Evol. Biosphere **30**, 459–466 (2000)
277. K. Kurin-Csorgei, M. Orban, A.M. Zhabotinsky, I.R. Epstein: On the nature of patterns arising during polymerization of acrylamide in the presence of the methylene blue sulfide–oxygen oscillating reaction. Chem. Phys. Lett. **295**, 70–74 (1998)
278. N.C. Kyrpides, C.A. Ouzonis: Nucleic acid binding metabolic enzymes: Living fossils of stereochemical interactions? J. Mol. Evol. **40**, 564–569 (1995)
279. J.C. Lacey Jr, G.W. Cook, D.W. Mullins Jr: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 398–418
280. J.C. Lacey, K.N. Pruitt: Origin of the genetic code. Nature, **223**, 799–802 (1969)
281. J.C. Lacey, M.P. Staves: Was there a universal tRNA before specialized tRNAs came into existence? Orig. Life Evol. Biosphere **20**, 303–308 (1990)
282. J.C. Lacey Jr, A.L. Weber, W.E. White Jr: A model for the coevolution of the genetic code and the process of protein synthesis: review and assessment. Orig. Life **37**, 273–283 (1975)
283. J.C. Lacey Jr, N.S.M.D. Wickramasinghe, G.W. Cook, G. Anderson: Couplings of character and of chirality in the origin of the genetic system. J. Mol. Evol. **37**, 233–239 (1993)
284. N. Lahav: *Theories of Life's Origin* (Oxford University Press 1999) p. 349
285. N. Lahav: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 41
286. N. Lahav, S. Chang: Synthesis: The Possible role of solid surface area in condensation reactions during chemical evolution: reevaluation. J. Mol. Evol. **8**, 357–380 (1976)
287. N. Lahav, S. Nir: Life's definition: In search of the most fundamental common denominators between all living entities through the entire history of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 131–133
288. N. Lahav, D.H. White: A possible role of fluctuating clay–water systems in the production of ordered prebiotic oligomers. J. Mol. Evol. **16**, 11–21 (1980)
289. N. Lahav, D.H. White, S. Chang: Peptide formation in the prebiotic era: Thermal condensation of glycine in fluctuating clay environments. Science **20** (1), 67–69 (1978)

290. A.I. Lamond, T.J. Gibson: Catalytic RNA and the origin of genetic systems. Trends Genet. **6**, 145–149 (1990)
291. C.H. Langton (Ed.): Artificial life I. *Proceedings of the First Conference on Artificial Life*, Los Alamos, September (Addison-Wesley, Redwood City 1989)
292. T.A. Langworthy, W.R. Mayberry, P.F. Smith: Long-chain glycerol diether and polyol dialkyl glycerol triether lipids of Sulfolobus acidocaldarius. J. Bacteriol. **119** (1), 106–116 (1974)
293. P.C. Lauterbur: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 41
294. A. Lazcano: In: *Early Life on Earth*, ed. by S. Bengton, (Columbia University Press, NY 1994) pp. 60–69
295. A. Lazcano, V. Valverde, G. Hernandez, P. Gariglio, G.E. Fox, J. Oró: On the early emergence of reverse transcription: Theoretical basis and experimental evidence. J. Mol. Evol. **35**, 524–536 (1992)
296. R. Law, R.D. Morton: Alternative permanent states of ecological communities. Ecology **74** (5), 1347–1361 (1993)
297. J.G. Lawless, A. Banin, F.M. Church, J. Mazzurco, R. Huff, J. Kao, A. Cook, T. Lowe, J.B. Orenberg, E. Edelson: pH profile of the adsorbtion of nucleotides onto montmorillonite. Orig. Life. **15**, 77–88 (1985)
298. D.H. Lee, J.R. Granja, J.A. Martinez, K. Severin, M.R. Ghadiri: A self-replicating peptide. Nature **382**, 525–528 (1996)
299. A.L. Lehninger: Supramolecular organization of enzyme and membrane systems. Naturwissenschaften **53**, 57–63 (1966)
300. A.L. Lehninger, D.L. Nelson, M.M. Cox: *Principles of Biochemistry*, 2nd edn. (Worth, New York 1993)
301. P.E. León: Inhibition of ribozymes by deoxyribonucleotides and the origin of DNA. J. Mol. Evol. **47**, 122–126 (1998)
302. L. Levine: Gaia goddess and idea. BioSystems **31** (2–3), 85–92 (1993)
303. D.R. Lide, H.P.R. Frederikse (Eds.): *CRC Handbook of Chemistry and Physics. A Ready-Reference Book of Chemical and Physical Data*, 77th edn. (CRC Press, Boca Raton 1996)
304. V. Liebl, V.J.A. Novak, Z. Masinovsky, B. Pacltova, L. Bejsovcova: The evolution of prebiological self-organization: probable colloid-chemical evolution of first prokaryotic cells. Orig. Life Evol. Biosphere **14** (1–4), 323–334 (1984)
305. S. Lifson: On the crucial stages in the origin of animated matter. J. Mol. Evol. **44**, 1–8 (1997)
306. C.H. Lineweaver: An estimate of the age distribution of terrestrial planets in the universe: Quantifying metallicity as a selection effect. Icarus **151** (2), 307–313 (2001)
307. D. Lippmann: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 41
308. R. Lohrmann, L.E. Orgel: Studies of oligoadenylate formation on a poly(U) template. J. Mol. Evol. **12**, 237–257 (1979)
309. P.L. Luisi: Defining the transition to life: Self-replicating bounded structures and chemical autopoiesis. In: *Thinking about Biology*, ed. by W. Stein, F.J. Varela (Addison-Wesley, New Jersey 1993)
310. P.L. Luisi: About various definitions of life. Orig. Life. Evol. Biosphere **28**, 613–622 (1998)

311. P.L. Luisi: Some open questions about the origin of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 287–301
312. P.L. Luisi, F.J. Varela: Self-replicating micelles – A chemical version of a minimal autopoietic system. Orig. Life Evol. Biosphere **19**, 633–745 (1990)
313. R.D. Lundberg, P. Doty: Polypeptides XVII. A study of the kinetics of the primary amide-initiated polymerization of N-carboxy-anhydrides with special reference to configurational and stereochemical effects. J. Am. Chem. Soc. **79**, 3961–3972 (1957)
314. A.E. Lyubarev, B.I. Kurganov: The concept of biochemical organization and problems of biochemical evolution. In: *Evolutionary Biochemistry and Related Areas of Physicochemical Biology* (Memory of A.I. Oparin), ed. by B.F. Poglazov, M.S. Kritsky, K.L. Gladilin (Bach Institute of Biochemistry and ANKO, Moscow 1995) pp. 127–140
315. A. Macallum: On the origin of life on the globe. Trans. Can. Inst. **8**, 435–436 (1908)
316. J. Maddox: The genetic code by numbers. Nature **367**, 111 (1994)
317. J. Maniloff: The minimal cell genome: On being the right size. Proc. Natl. Acad. Sci. USA **93**, 10004–10006 (1996)
318. S. Mann, D.A. Archibald, J.M. Didymus, T. Douglas, B.R. Heywood, F.C. Meldrum, N.J. Reeves: Crystallization at inorganic–organic interfaces: biominerals and biomimetic synthesis. Science **261**, 1286–1292 (1993)
319. L. Margulis: *Origin of Eukaryotic Cells* (N. Haven 1971)
320. L. Markó: Some characteristics of life and their implications on possible start on Earth. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 303–306
321. E.A. Martell: Radionuclide-induced evolution of DNA and the origin of life. J. Mol. Evol. **35**, 346–355 (1992)
322. S.F. Mason: Biomolecular handedness, origins and significance. Bioch. Pharm. **37** (1), 1–7 (1988)
323. A. Mathews: Nothing like a vacuum. New Scientist 30–33 (1995)
324. H.R. Maturana, F.J. Varela: De máquinas y seres vivos. In: *Una teoría sobre la organizaçion biológica*. (Editorial Universitaria S.A., Santiago, Chile 1973)
325. H.R. Maturana, F.J. Varela: *Autopoiesis and Cognition. The Realization of the Living* (D. Reidel, Dordrecht 1980)
326. B. Mayer, G. Köhler, S. Rasmussen: Simulation and dynamics of entropy-driven molecular self-assembly processes. Phys. Rev. E **55** (4), 4489–4499 (1997)
327. J. Maynard-Smith: *The Theory of Evolution* (Penguin, Harmondsworth 1975)
328. J. Maynard-Smith: *The Problems of Biology* (Oxford University Press, Oxford 1986) Chap. 1
329. J. Maynard-Smith, E. Szathmáry: *The Major Transitions in Evolution* (W.H. Freeman, Oxford 1995)
330. J. Maynard-Smith, E. Szathmáry: *The Origins of Life* (Oxford University Press, Oxford 1999)
331. J.J. McCullough, R.M. Lemmon: The question of the possible asymmetric polymerization of aspartic acid on kaolinite. J. Mol. Evol. **3**, 57–61 (1974)
332. R. McKeon (Ed.): *Introduction to Aristotle* (Random House, New York 1947)
333. B. McMullin: SCL: An artificial chemistry in swarm. (Santa Fe Institute Working Paper, SFI 1997)

334. B. McMullin, F. Varela: Rediscovering computational autopoiesis. In: *Fourth European Conference on Artificial Life*, ed. by P. Husbands, I. Harvery (MIT Press, Cambridge, Mass. 1997) pp. 38–47
335. L.B. Mekler (1980): In: *The Concept of Biochemical Organization and Problems of Biochemical Evolution*, ed. by A.E. Lyubarev, B.I. Kurganov (1995)
336. A.R. Mellersch: A model for the prebiotic synthesis of peptides which throws light on the origin of the genetic code and the observed chirality of life. Orig. Life Evol. Biosphere **23**, 261–274 (1993)
337. E.H. Mercer: *The Foundation of Biological Theory* (Wiley Intersciences, New York 1981)
338. F. Micheel, Z. Krzeminski, W. Himmelmann, A. Kühlkamp: Die condensation von thio-säuren und proteinen. Chem. Ber. **80** (37), 90–105 (1947)
339. S.L. Miller: A production of amino acids under possible primitive Earth conditions. Science **117**, 528–529 (1953)
340. S.L. Miller: Production of some organic compounds under possible primitive earth conditions. J. Amer. Chem. Soc. **77**, 2351–2361 (1955)
341. S.L. Miller: Proc. 4th Congr. on Origin of Life (1973)
342. S.L. Miller: Which organic compounds could have occurred on the prebiotic earth? Cold Spring Harbor Symp. Quant. Biol. (1987)
343. S.L. Miller: The prebiotic synthesis of organic components as a step toward the origin of life. In: *Major Events in the History of Life*, ed. by J.W. Schopf (Jones and Bartlett Publishers, Boston 1992) pp. 1–28
344. S.L. Miller: Communication at the 2nd Astrobiology Science Conference, NASA Ames Research Center, Moffett Field, CA, April 7–11 (2002)
345. S.L. Miller, L. Orgel: *The Origins of Life on the Earth* (Prentice-Hall, Englewood Cliffs, NJ 1974)
346. A. Milosavljevic: Discovering dependencies via algorithmic mutual information: A case study in DNA sequence comparisons. Machine Learning **21** (1–2), 35–50 (1995)
347. E.N. Mirzoyan: A physico-mechanical model of living matter (in honor of the 150th anniversary of N.A. Umov). Izvestiya Akademii Nauk Seriya Biologicheskaya **2**, 243–248 (1997)
348. P. Mitchell: Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biol. Rev. **41**, 445–502 (1966)
349. F. Morán, A. Moreno, E. Minch, F. Montero: Further steps toward a realistic description of the essence of life. In: *Artificial Life V*, ed. by C.G. Langton, K. Shimonara (MIT Press, London 1999) pp. 255–263
350. H. Morii, Y. Koga: Asymmetrical topology of diether- and tetraether-type polar lipids in membranes of Methanobacterium thermoautotrophicum cells. J. Biol. Chem. **269** (14), 10492–10497 (1994)
351. L. Morozov: Mirror symmetry breaking in biochemical evolution. Orig. Life **9**, 187–217 (1979)
352. H.J. Morowitz: *Beginnings of Cellular Life. Metabolism Recapitulates Biogenesis* (Yale University Press 1992)
353. H.J. Morowitz, B. Heinz, D.W. Deamer: The chemical logic of a minimum protocell. Orig. Life. Evol. Biosph. **18**, 281–287 (1988)
354. L. Mörtberg: Nonbiotic origin of optical activity. Nature **232**, 105–108 (1971)
355. H.J. Muller: Life. Science **121**, 1–9 (1955)
356. H.J. Muller: The gene material as the initiator and organizing basis of life. Am. Nat. **100**, 493–517 (1966)

357. M. Muthukumar, C.B. Ober, E.L. Thomas: Competing interactions and levels of ordering in self-organizing polymeric materials. Science **277**, 1225–1232 (1997)
358. C.K.K. Nair: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 45
359. K. Nealson: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 45
360. J. von Neuman: *Theory of Self-Reproducing Automata*, ed. and completed by A.W. Burks (University of Illinois Press 1966)
361. E. Nevo: Evolution of genome–phenome diversity under environmental stress. Proc. Nat. Acad. Sci. USA **98** (11), 6233–6240 (2001)
362. G. Nicolis, I. Prigogine: *Self-Organization in Nonequilibrium Systems* (Wiley, New York 1977)
363. P.E. Nielsen: Peptide nucleic acid (PNA): A model structure for the primordial genetic material? Orig. Life Evol. Biosphere **23**, 323–327 (1993)
364. U. Niesert, D. Harnasch, C. Bresch: Origin of life between Scylla and Charybdis. J. Mol. Evol. **17**, 348–353 (1981)
365. M. Nishihara, T. Kyuragi, N. Sone, Y. Koga: sn-Glycerol-1-phosphate dehydrogenase: a key enzyme in the biosynthesis of ether phospholipids in Archaea. In: *Thermophiles: The Keys to Molecular Evolution and the Origin of Life*, ed. by J. Wiegel, M.W.W. Adams (Taylor & Francis 1998) pp. 281–285
366. H. Noda: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 45
367. B. Norden: Was photoresolution of amino acids the origin of optical activity in life? Nature **266**, 567–68 (1977)
368. G. North: Back to the RNA world – and beyond. Nature **328**, 18–19 (1987)
369. R. Noyori: Asymmetric hydrogenation via architectural and functional molecular engineering. Abstracts of Papers of the American Chemical Society **220**, U77, Part 2 (2000)
370. J.S. Nowick, Q. Feng, T. Tjivikua, P. Ballester, J. Rebek Jr: Kinetic studies and modeling of self-replicating systems. J. Am. Chem. Soc. **113**, 8831 (1991)
371. J.C. Nuño, P. Chaçon, A. Moreno, F. Morán: Compartmentation in replicator models. In: *Advances in Artificial Life*, ed. by F. Morán, A. Moreno, J.J. Merelo, P. Chaçon (Springer-Verlag, Heidelberg 1995) pp. 116–127
372. M.D. Nussinov, V.I. Maron: The universe and the origin of life (origin of organics on clays). J. Brit. Interplanet. Soc. **43**, 3–10 (1980)
373. D.G. Odom, M. Rao, J.G. Lawless, J. Oro: Association of nucleotides with homoionic clays. J. Mol. Evol. **12**, 365–367 (1979)
374. B. Ohler, I. Revenko, C. Husted: Atomic force microscopy of nonhydroxy galactocerebroside nanotubes and their self-assembly at the air–water interface, with applications to myelin. J. Struct. Bio. **133** (1), 1–9 (2001)
375. L. Olendzenski, J.P. Gogarten: Deciphering the molecular record for the early evolution of life: gene duplication and horizontal gene transfer. In: *Thermophiles: The Keys to Molecular Evolution and the Origin of Life*, ed. by J. Wiegel, M.W.W. Adams (Taylor & Francis 1998) pp. 281–285
376. A.E. Oliver, D.W. Deamer: α-helical hydrophobic polypeptides form proton-selective channels in lipid bilayers. Biophys. J. **66**, 1364–1379 (1994)
377. M. Olomucki: *The Chemistry of Life* (McGraw-Hill, New York 1993)

378. A.I. Oparin: *Proishodenie zhizni* (Moscoksky Robotichii, Moscow 1924). Translated in J.D. Bernal: *The Origins of Life*, Appendix (World, Cleveland 1967)
379. A.I. Oparin: *The Origin of Life on the Earth*, 3rd edn. (Academic Press, New York 1957)
380. A.I. Oparin: *Life: Its Nature, Origin and Development* (Academic Press, New York 1961)
381. A.I. Oparin: *Genesis and Evolutionary Development of Life* (Academic Press, New York 1968)
382. M. Orbán: Chemical oscillation during the uncatalyzed reaction of aromatic compounds with bromate 4 stationary and moving structures in uncatalyzed oscillatory chemical reactions. J. Am. Chem. Soc. **102**, 4311–4314 (1980)
383. M. Orbán: Oscillations and bistability in the Cu(II)-catalyzed reaction between H_2O_2 and KSCN. J. Am. Chem. Soc. **108**, 6893–6898 (1986)
384. M. Orbán, K. Kurin-Csörgey, A.M. Zhabotinski, J.R. Epstein: New indicators for visualizing pattern formation in uncatalyzed bromate oscillatory systems. J. Am. Chem. Soc. **120**, 1146–1150 (1998)
385. L.E. Orgel: RNA catalysis and the origins of life. J. Mol. Evol. **38**, 380–393 (1968)
386. L.E. Orgel: *The Origins of Life: Molecules and Natural Selection* (John Wiley, New York 1973) p. 189
387. L.E. Orgel: Darwinism at the very beginning of life. New Scientist pp. 149–151 (1982)
388. L.E. Orgel: RNA catalysis and the origin of life. J. Theor. Biol. **123**, 127–144 (1986)
389. L.E. Orgel: The origin of polynucleotide-directed protein synthesis. J. Mol. Evol. **29**, 465–474 (1989)
390. L.E. Orgel: Molecular replication. Nature **358**, 203–209 (1992)
391. L.E. Orgel: The origin of life on the Earth. In: *Life in the Universe*, Special Issue, Scientific American (W.H. Freeman and Company, New York 1995) pp. 41–52
392. L.E. Orgel: Origin of life – A simpler nucleic acid. Science **290**, 1306–1307 (2000)
393. L.E. Orgel: Current perspectives. Problems and advances in the origin of life. Personal communication at Gordon Conference on Origin of life, Ventura, CA (2001)
394. L.E. Orgel: Communication at the 2nd Astrobiology Science Conference, NASA Ames Research Center, Moffett Field, CA, April 7–11 (2002)
395. J. Oró, A. Lazcano: A holistic precellular organization model. In: *Prebiological Self-Organization of Matter*, ed. by C. Ponnamperuma, F.R. Eirich (DEEPAK, 11, Hampton 1990)
396. J. Oró, E. Stephen-Sherwood: Abiotic origin of biopolymers. Orig. Life **7**, 37–47 (1976)
397. A. Orstan: How to define life – a hierarchical approach. Perspectives in Biology and Medicine **33** (3), 391–401 (1990)
398. C. Ortega, A. Tyrrell: Self-repairing multicellular hardware: A reliability analysis. Advances in Artificial Life, Proceedings Lecture Notes in Artificial Intelligence **1674**, 442–446 (1999)

399. G. Ourisson, Y. Nakatani: The terpenoid theory of the origin of cellular life: the evolution of terpenoids to cholesterol. Chemistry and Biology **1**, 11–23 (1994)
400. T. Owen: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 45
401. N.R. Pace, T.L. Marsch: RNA catalysis and the origin of life. Orig. Life **16**, 97–116 (1985)
402. C. Palache, H. Berman, C. Frondel: *The System of Mineralogy of James Dwight Dana and Edward*, 7th edn., Vol. III (Wiley, New York 1962) p. 16
403. G. Pályi, C. Zucchi, L. Caglioti (Eds.): *Fundamentals of Life* (Elsevier, New York 2002)
404. G. Pályi, C. Zucchi, L. Caglioti: Dimensions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 1–13
405. A.N. Pargellis: The evolution of self-replicating computer organisms. Physica D **98** (1), 111–127 (1996)
406. B.C. Patten, M. Straškraba, S.E. Jørgensen: Ecosystems emerging I. Conservation. Ecological Modeling **96**, 221–284 (1997)
407. S. Paula, D.W. Deamer: Membrane permeability barriers to ionic and polar solutes. Current Topics in Membranes **48**, 77–95 (1990)
408. J. Perrett: Biochemistry and bacteria. New Biology **12**, 68–69 (1952)
409. G.A. Petsko: On the other hand. Science **256**, 1403–1404 (1992)
410. C. Pfeffer (1897): Quoted in L. Von Bertalanffy: *Modern Theories of Development: An Introduction to Theoretical Biology*, English translation (Harper Torchbooks, The Science Library, Harper and Brothers, New York 1933) p. 51
411. N.W. Pirie: The meaning of chiral conventions. Orig. Life **12**, 211–213 (1982)
412. S. Pitsch, A. Eschenmoser, B. Gedulin, S. Hui, G. Arrhenius: Mineral-induced formation of sugar phosphates. Orig. Life. Evol. Biosphere **25**, 294–334 (1995)
413. B.F. Poglazov: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 45
414. R.F. Polishchuck: Life as a negentropy current and problem of infinity. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 141–151
415. C. Ponnamperuma (Ed.): *Exobiology* (North-Holland, Amsterdam 1972)
416. C. Ponnamperuma, A. Shimoyama, E. Friebele: Clay and the origin of life. Orig. Life **12**, 9–40 (1982)
417. R. Popa: *Primitive Metabolism in Archaic Hydrothermal Lava Caves* (NSS Conv. Blacksburg, USA 1995)
418. R. Popa: A sequential scenario for the origin of biological chirality. J. Mol. Evol. **44**, 121–127 (1997)
419. D. Pörschke: Elementary steps of base recognition and helix-coil transitions in nucleic acids. In: I. Pecht, R. Rigler: Mol. Biol. Biochem. Biophys. **24**, 191–218 (1977)
420. N.C. Price, L. Stevens: *Fundamentals of Enzymology*, 3rd. edn. (Oxford University Press, New York 2000)
421. I. Prigogine: *From Being to Becoming* (W.H. Freeman, San Francisco 1980)
422. I. Prigogine, I. Stengers: *Order out of Chaos* (Heinemann, London 1984)
423. S.B. Prusiner: Prion diseases and the BSE crisis. Science **278**, 245–254 (1997)
424. S.B. Prusiner: Prions. Proc. Natl. Acad. Sci. USA **95**, 13363–13383 (1998)
425. D.I. Purich (Ed.): Enzyme rate and inhibitor methods. In: *Methods in Enzymology*, Vol. 63 (Academic Press, New York 1979)

426. D.I. Purich (Ed.): Isotopic probes and complex enzyme systems. In: *Methods in Enzymology*, Vol. 64 (Academic Press, New York 1980)
427. D.I. Purich (Ed.): Intermediates, stereochemistry and rate studies. In: *Methods in Enzymology*, Vol. 87 (Academic Press, New York 1982)
428. D.I. Purich (Ed.): Developments in enzyme dynamics. In: *Methods in Enzymology*, Vol. 249 (Academic Press, New York 1995)
429. W.K. Purves, G.H. Orians, H.C. Heller: Life. *The Science of Biology*, 4th edn. (Sinauer Associates, W.H. Freeman and Co. 1995)
430. A. Putter (1923): Quoted in L. Von Bertalanffy: *Modern Theories of Development: An Introduction to Theoretical Biology*, English translation (Harper Torchbooks, The Science Library, Harper and Brothers, New York 1933) p. 51
431. J. Rebek Jr: Molekulare Erkennung mit konkaven Modellverbindungen. Angew. Chem. **102**, 261–285 (1990)
432. J. Rebek Jr: Molecular recognition with model systems. Angew. Chem. International Edition (in English) **29** (3), 245–255 (1990)
433. J. Rebek Jr: Molecular recognition and the development of self-replicating systems. Experientia **47**, 1096–1118 (1991)
434. T. Reichhardt: Crystal chains invoked as evidence for life on Mars. Nature **410**, 136–136 (2001)
435. H. Rembold, L.E. Orgel: Single-strand regions of poly(G) act as templates for oligo(C) synthesis. J. Mol. Evol. **38**, 205–210 (1994)
436. H. Rembold, R.K. Robins, F. Seela, L.E. Orgel: Polycytidylate and Poly(7-deazaguanylate): A pair of complementary templates. J. Mol. Evol. **38**, 211–214 (1994)
437. J.M. Rivera, T. Martin, J. Rebek Jr: Chiral spaces: dissymetric capsules through self-assembly. Science **279**, 1021–1023 (1998)
438. M. Rizzotti: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 48
439. M. Rizzotti: Living things are far from equilibrium: Which equilibrium? In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 141–151
440. H.D. Robertson: How did replicating and coding RNAs first get together? Science **274**, 66–67 (1996)
441. M.P. Robertson, A.D. Ellington: How to make a nucleotide. Nature **395**, 223–225 (1998)
442. S.N. Rodin, S. Ohno: Two types of aminoacyl-tRNA syntethases could be originally encoded by complementary strands of the same nucleic acid. Orig. Life Evol. Biosphere **25**, 565–589 (1995)
443. E.T. Rolls, S.M. Stringer: On the design of neural networks in the brain by genetic evolution. Prog. Neurobiol. **61**, 557–579 (2000)
444. E.M.A. Ronald, M. Sipper, M.S. Capcarrere: Testing for emergence in artificial life. Advances in Artificial Life, Proceedings Lecture Notes in Artificial Intelligence **1674**, 13–20 (1999)
445. T.A. Ronneberg, L.F. Landweber, S.J. Freeland: Testing a biosynthetic theory of the genetic code: Fact or artifact? Proc. Natl. Acad. Sci. USA **97**, 13690–13695 (2000)
446. R.S. Root-Bernstein, P.F. Dillon: Molecular complementarity I: The complementarity theory of the origin and evolution of life. J. Theor. Biol. **188**, 447–479 (1997)

447. G.D. Rose, L.M. Gierasch, J.A. Smith: Turns in peptides and proteins. Adv. Protein Chem. **37**, 1–109 (1985)
448. R. Rosen: A relational theory of biological systems. Bull. Math. Biophys. **20**, 245–260 (1958)
449. R. Rosen: A relational theory of biological systems II. Bull. Math. Biophys. **21**, 109–128 (1959)
450. R. Rosen: Some results in graph theory and their application to abstract relational biology. Bull. Math. Biophys. **25**, 231–241 (1963)
451. R. Rosen: A note on replication of (M,R)-systems. Bull. Math. Biophys. **28**, 149–151 (1966)
452. R. Rosen: Further comments on replication of (M,R)-systems. Bull. Math. Biophys. **29**, 91–94 (1967)
453. R. Rosen: Some realizations of (M,R)-systems and their interpretation. Bull. Math. Biophys. **33**, 303–319 (1971)
454. R. Rosen: On the dynamical realization of (M,R)-systems. Bull. Math. Biophys. **35**, 1–9 (1973)
455. R. Rosen: Pattern generation in networks. Prog. Theor. Biology. **6**, 161–209 (1981)
456. R. Rosen: *Life Itself. A Comprehensive Inquiry into the Nature, Origin and Fabrication of Life* (Columbia University Press 1991)
457. B. Rosslenbroich: Understanding the organism as a central problem in medicine. Forschende Komplementarmedizin Und Klassische Naturheilkunde **8** (3), 125–136 (2001)
458. O. Rossler: A system-theoretic model of biogenesis. Z. Naturforsch. B **26b**, 741–746 (1971)
459. O. Rossler: Chemical automata in homogeneous and reaction–diffusion kinetics. Notes in Biomath. B **4**, 399–418 (1974)
460. O. Rossler: Deductive prebiology. In: *Molecular Evolution and the Prebiological Paradigm*, ed. by K.L. Rolfing (Plenum, New York 1983)
461. K. Ruiz-Mirazo, A. Moreno, F. Morán: Merging the energetic and the rational-constructive logic of life. In: *Artificial life VI*, ed. by C. Adami, R. Belew, H. Kitano and C. Taylor (MIT Press, Cambridge, Mass. 1998) pp. 448–451
462. K. Ruiz-Mirazo, A. Moreno, F. Morán, J. Pereto, J.J. Merelo: Designing a simulation model of a self-maintaining cellular system. Advances in Artificial Life, Proceedings Lecture Notes in Artificial Intelligence **1674**, 379–388 (1999)
463. M.J. Russell: The generation at hot springs of sedimentary ore deposits, microbialites and life. Ore Geology Reviews **10** (3–6), 199–214 (1996)
464. M.J. Russell: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 48
465. M.J. Russell, R.M. Daniel, A.J. Hall, J.A. Sherringham: A hydrothermally precipitated catalytic iron sulphide membrane as a first step toward life. J. Mol. Evol. **39**, 231–243 (1994)
466. M.J. Russell, A.J. Hall: The emergence of life from iron monosulphide bubbles at submarine hydrothermal redox and pH front. J. Geol. Soc. London **154**, 377–402 (1997)
467. A. Salam: The role of chirality in the origin of life. J. Mol. Evol. **33**, 105–113 (1991)

468. S. Santoli: Life and intelligence in the universe from nanobiological principles: A survey and budget of concepts and perspectives. Acta Astronautica **46**, 641–647 (2000)
469. R. Sattler: *Bio-philosophy* (Springer-Verlag, Berlin 1986)
470. S. Scannerini: No place for man in Gaia. Rivista Di Biologia, Biology Forum **92**, 241–259 (1999)
471. P.K. Schmidli, P. Schurtenberger, P.L. Luisi: Liposome-mediated enzymatic synthesis of phosphatidylcholine as an approach to self-replicating liposomes. J. Am. Chem. Soc. **113**, 8127–8145 (1991)
472. E. Schrödinger: *What is Life? The Physical Aspect of the Living Cell* (Cambridge University Press, Cambridge 1944)
473. K.U. Schöning, P. Scholz, S. Gunta, X. Wu, R. Krishnamurthy, A. Eschenmoser: Chemical ethiology of nucleic acid structure: the -threfruanosyl-(3′-2′) oligonucleotide system. Science **290**, 1347–1349 (2000)
474. G.E. Schultz, R.H. Schirmer: *Principles of Protein Structure* (Springer-Verlag, Berlin 1979)
475. D. Schulze-Makuch, H. Guan, L.N. Irwin, E. Vega: Redefining life: An ecological, thermodynamic, and bioinformatic approach. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 169–179
476. P. Schuster: Evolution between chemistry and biology. Orig. Life **14**, 3–14 (1984)
477. H. Schwegler, K. Tarumi: The protocell: A mathematical model of self-maintenance. BioSystems **19**, 307–315 (1986)
478. R.I. Scorei: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 48
479. A. Scott: *The Creation of Life. Past, Future, Alien* (Basil Blackwell 1986)
480. L. Sertorio, G. Tinetti: Available energy for life on a planet, with or without stellar radiation. (DFTT, Italy, July 1–30, 2001)
481. J.W. Servos: Book review: E. Crawford, Arrhenius. Science **273**, 1512–1513 (1996)
482. C.E. Shannon: *The Mathematical Theory of Communication* (University of Illinois Press, Urbana 1949)
483. C.E. Shannon, W. Weaver: *The Mathematical Theory of Communication* (University of Illinois Press, Urbana 1963)
484. P.A. Sharp: On the origin of RNA splicing and introns. Cell **42**, 397–400 (1985)
485. K.B. Sharpless: Osmium-catalyzed asymmetric aminohydroxylation (AA) of olefins. Abstracts of Papers of the American Chemical Society **211**, 128 ORGN, Part 2 (1996)
486. H.R. Shaw: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 50
487. R.D. Sheardy, E.J. Gabbay: Stereospecific binding of diastereomeric peptides to salmon sperm deoxyribonucleic acid. Further evidence for partial intercalation. Biochem. **22**, 2061–2067 (1983)
488. E.L. Shock: Hydrothermal systems as environments for the emergence of life, Evolution of hydrothermal ecosystems on Earth (and Mars?). Ciba Foundation (John Wiley, New York 1996) pp. 40–52
489. V.P. Skulachev: The laws of cell energetics. Eur. J. Biochem. **208**, 203–209 (1992)

490. K. Soai, T. Shibata, H. Marioka, K. Choji: Asymmetric autocatalysis and amplification of enantiomeric excess of a chiral molecule. Nature **378**, 767–768 (1995)
491. K. Soai, T. Shibata: In: *Advances in Biochirality*, ed. by G. Pályi, C. Zucchi and L. Caglioti (Elsevier, Amsterdam 1999) pp. 125–136
492. D. Soriano: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 50
493. H. Spencer: *The Principles of Biology* (D. Appleton and Co., New York 1884)
494. R.K. Standish: Some techniques for the measurement of complexity in Tierra. Advances in Artificial Life, Proceedings Lecture Notes in Artificial Intelligence **1674**, 104–108 (1999)
495. C. Starr, R. Taggart: *Biology: The Unity and Diversity of Life* (Wadsworth Publications Co. Inc., Belmont CA 1992)
496. L. Stryer: *Biochemistry* (W.H. Freeman, New York 1988) p. 734
497. A. Szabó-Nagy, L. Keszthelyi: Demonstration of the parity-violating energy difference between enantiomers. Proc. Natl. Acad. Sci. USA **96**, 4252–4255 (1999)
498. E. Szathmáry: A classification of replicators and lambda-calculus models of biological organization. Proceedings of the Royal Society of London Series B: Biological Sciences **260**, 279–286 (1995)
499. E. Szathmáry: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002a) p. 50
500. E. Szathmáry: Redefining life: an ecological, thermodynamic, and bioinformatic approach. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002b) pp. 181–195
501. J.W. Szostak, D.P. Bartel, P.L. Luisi: Synthesizing life. Nature **409**, 387–390 (2001)
502. C. Tanford: *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd edn. (John Wiley, New York 1980)
503. K. Tani, T. Yamagata, S. Otsuka, S. Akutagawa, H. Kumobayashi, T. Taketomi, H. Takaya, A. Miyashita, R. Noyori: Cationic rhodium(i) complex-catalyzed asymmetric isomerization of allylamines to optically-active enamines. Journal of the Chemical Society. Chemical Communications **11**, 600–601 (1982)
504. C. Tamponnet, C. Savage: Closed Ecological systems. Journal of Biological Education **28** (3), 167–174 (1994)
505. M. Tegmark, M.J. Rees: Why is the cosmic microwave background fluctuation level 10^{-5}? Astrophysical Journal **499** (2), 526–532 (1998)
506. W. Thiemann: Disproportionation of monomers by precipitation. J. Mol. Evol. **4**, 85–97 (1974)
507. W. Thiemann, H. Teutsch: Possible amplification of enantiomer excesses through structural properties of liquid crystals: A model for the origin of optical activity in the biosphere? Orig. Life Evol. Biosphere **20**, 121–126 (1990)
508. W. Thompson D'Arcy: *On Growth and Form*, 2nd edn. (Cambridge University Press, Cambridge 1942)
509. T. Tjivikua, P. Ballester, J. Rebek: A self-replicating system. J. Am. Chem. Soc. **112**, 1249–1250 (1990)
510. G. Turian: Origin of life II. From prebiotic replicators to protocells. Archives des Sciences **52** (2), 101–109 (1999)

511. S. Tyagi, C. Ponnamperuma: Nonrandomness in prebiotic peptide synthesis. J. Mol. Evol. **30**, 391–399 (1990)
512. T. Ueda, K. Watanabe: The evolutionary change of the genetic code as restricted by the anticodon and identity of transfer RNA. Orig. Life. Evol. Biosphere **23**, 345–364 (1993)
513. G. Unden, A. Kröger: Reconstitution of liposomes of the electron-transport chain catalyzing fumarate reduction to formate. Biochem. Biophys. Acta **682**, 258–263 (1982)
514. P.J. Unrau, D.P. Bartel: RNA-catalyzed nucleotide synthesis. Nature **395**, 260–263 (1998)
515. C.Y. Valenzuela: A biotic Big-Bang. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 197–202
516. C.Y. Valenzuela: Does biotic life exist? In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 331–334
517. E. Vank, L.C. Jain, R.P. Johnston: *Automatic Generation of Neural Network Architecture Using Evolutionary Computation* (World Scientific, Singapore 1997)
518. F. Varela: Life: Identity and cognition. Brain and Cognition (1996)
519. F. Varela, H. Maturana, R. Uribe: Autopoiesis: The organization of living systems, its characterization and a model. BioSystems **5**, 187–196 (1974)
520. C. Viedma: Enantiomeric crystallization from DL-aspartic and DL-glutamic acids: Implications for biomolecular chirality in the origin of life. Orig. Life Evol. Biosph. **31** (6), 501–509 (2001)
521. C.A. Vilee, E.P. Solomon, C.E. Martin, D.W. Martin, L.R. Berg, P.W. Davis: *Biology*, 2nd edn. (Sounders College Publishing 1989) p. 5
522. R. Virchov: Cellular pathology. In: *Disease, Life and Man: Selected Assays* (Stanford University Press, Stanford 1855)
523. S. Vogel: *Life's Devices: The Physical World of Animals and Plants* (Princeton University Press, Princeton 1988)
524. L. Von Bertalanffy: *Modern Theories of Development: An Introduction to Theoretical Biology*, English translation (Harper Torchbooks, The Science Library, Harper and Brothers, New York 1933)
525. L. Von Bertalanffy: *General Systems Theory* (George Brazilier, New York 1968)
526. G. Von Kiedrowski: Ein Selbstreplizierendes Hexadesoxynucleotid. Angew. Chem. **98**, 932 (1986)
527. G. Von Kiedrowski: Molekulare Prinzipen der artifiziellen Selbsreplikation. In: *Gene, Neurone, Qubits & Co.*, Unsere Welten der Information, ed. by D. Ganten and S. Hirzel (Verlag, Stuttgart 1999) pp. 123–145
528. G. Von Kiedrowski: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 38
529. G. Von Kiedrowski, B. Wlotzka, J. Hebling: Sequanxabhängigkeit Matrizengesterurter Synthesen von Hexadesoxynucleotid-Derivaten mit 3′-5′-Pyrophoshatverknüpfung. Angew. Chem. **101**, 1259 (1989)
530. Von Liebig (1868): Quoted in (Engels 1880) p. 190
531. J. Von Neumann (1948): The general and logical theory of automata. Lecture given 1948. In: *Cerebral Mechanisms in Behavior*, The Hixon Symposium, ed. by L.A. Jeffress (John Wiley, New York 1951) pp. 1–41
532. J. Von Neumann: In: *J. Von Neumann: Collected Works*, ed. A.H. Taub (MacMillan, New York 1961–1963) Vol. 5, pp. 288–328

533. G. Wächtershäuser: Before enzymes and templates: Theory of surface metabolism. Microbiol. Rev. **52**, 452–484 (1988)
534. G. Wächtershäuser: The case for the chemoautotrophic origin of life in an iron–sulfur world. Orig. Life Evol. Biosphere **20** (2), 173–176 (1990)
535. G. Wächtershäuser: The cradle chemistry of life: On the origin of natural products in a pyrite-pulled chemoautotrophic origin of life. Pure and Applied Chemistry **65**, 1343–1348 (1993)
536. G. Wächtershäuser: The case for a hyperthermophylic, chemolithoautotrophic origin of life in an iron–sulfur world. In: *Thermophiles: The Keys to Molecular Evolution and the Origin of Life?*, ed. J. Wiegel and M.W.W. Adams (Taylor and Francis 1998) pp. 47–57
537. G. Wald: The origin of optical activity. Ann. NY Acad. Sci. **69**, 352–368 (1957)
538. A.L. Weber: Formation of pyrophosphate, tripolyphospate, and phosphorylimidazole with the thioester, N,S-Diacetylcysteamine, as the condensing agent. J. Mol. Evol. **18**, 24–29 (1981)
539. A.L. Weber: Formation of pyrophosphate on hydroxyapatite with thioesters as condensing agents. BioSystems **15**, 183–189 (1982)
540. A.L. Weber: Prebiotic formation of 'energy-rich' thioesters from glyceraldehyde and N-acetylcysteine. Orig. Life. **15**, 17–27 (1984)
541. A.L. Weber: Oligoglyceric acid synthesis by autocondensation of glyceroyl thioesters. J. Mol. Evol. **25**, 191–196 (1987)
542. A.L. Weber, S.L. Miller: Reasons for the occurrence of the twenty coded protein amino acids. J. Mol. Evol. **17**, 273–284 (1981)
543. A.L. Weber, L.E. Orgel: The formation of peptides from glycine thioesters. J. Mol. Evol. **13**, 193–202 (1979)
544. N. Webster: *Webster's International Dictionary* (Dorset and Baber, NY 1934)
545. N. Webster: *Webster's New Universal Unabridged Dictionary* (Dorset and Baber, NY 1979)
546. S. Weinberger, C. Berman, A. Minskii: Ordered DNA-polypeptide complexes of extreme chirality: Effects of polypeptide handedness on DNA long range asymmetry. J. Am. Chem. Soc. **110**, 8231–8232 (1988)
547. C.J. Welch: Formation of highly enantioenriched microenvironments by stochastic sorting of conglomerate crystals: A plausible mechanism for generation of enantioenrichment on the prebiotic earth. Chirality **13**, 425–427 (2001)
548. N.K. Wessells, J.L. Hopson: *Biology* (Random House, New York 1988)
549. R.D.B. Whalley: State and transition models for rangelands 1. Successional theory and vegetation change. Tropical Grasslands **28**, 195–205 (1994)
550. N. Wiener: *Cybernetics, or Control and Communication in the Animal and in the Machine* (MIT Press, Cambridge, MA 1947)
551. M.J. Wirth, R.W. Peter-Fairbank, H.O. Fatunbi: Mixed self-assembled monolayers in chemical separations. Science **275**, 44–47 (1997)
552. J.T. Wong: A co-evolution theory of the genetic code. Proc. Natl. Acad. Sci. USA **73**, 2336–2340 (1975)
553. J.T.F. Wong: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 53
554. J.T. Wong, P.M. Bronnskill: Inadequacy of prebiotic synthesis as origin of proteinous amino acids. J. Mol. Evol. **13**, 115–125 (1979)

555. J.H. Woodger: *Biological Principles: A Critical Study* (Kegan Paul, French Trubner, London 1929)
556. H. Yanagawa, F. Egami: Formation of organized particles, marigranules and marisomes, from amino acids in a modified sea medium. Biosystems **12**, 147–154 (1980)
557. B.S. Yang, Y.H. Lee, B.K. Choi, H.J. Kim: Optimum design of short journal bearings by artificial life algorithm. Tribology International **34**, 427–435 (2001)
558. M. Yarus: A specific amino acid binding site composed of RNA. Science **240**, 1751–1758 (1988)
559. M. Yarus: Amino acids as RNA ligands: A direct-RNA-template theory for the code's origin. J. Mol. Evol. **47**, 109–117 (1998)
560. H.P. Yockey: Information theory with application to biogenesis and evolution. In: *Biogenesis, Evolution, Homeostasis. A Symposium by Correspondence* (Springer-Verlag, Berlin 1973)
561. H.P. Yockey: *Information Theory and Molecular Biology* (Cambridge University Press, Cambridge 1992)
562. H.P. Yockey: Origin of life on earth and Shannon's theory of communication. Comp. Chem. **24**, 105–123 (2000)
563. H.P. Yockey: Short definitions of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) p. 53
564. H.P. Yockey: Information theory, evolution and the origin of life. In: *Fundamentals of Life*, ed. by G. Pályi, C. Zucchi, L. Caglioti (Elsevier, New York 2002) pp. 335–348
565. S. Yokobori, T. Ueda, K. Watanabe: Codons AGA and AGG are read as glycine in ascidian mitochondria. J. Mol. Evol. **36**, 1–14 (1993)
566. S. Zahn, J.W. Canary: Electron-induced inversion of helical chirality in copper complexes of N,N-dialkylmethionines. Science **288**, 1404–1407 (2000)
567. A.N. Zaikin, A.M. Zhabotinsky: Concentration wave propagation in two-dimensional liquid phase self-oscillating system. Nature **225**, 535–537 (1970)
568. A.P. Zhabotinsky: Biofizika **9**, 306–320 (1964)
569. A.P. Zhabotinsky: Acad. Sc. USSR Moscow (1967)
570. S. Zhang, M. Egli: A hypothesis: Reciprocal information transfer between oligoribonucleotides and oligopeptides in prebiotic molecular evolution. Orig. Life Evol. Biosphere **24**, 495–505 (1994)
571. G. Zieboll, L.E. Orgel: The use of gel electrophoresis to study the reaction of activated amino acids with oligonucleotides. J. Mol. Evol. **38**, 561–565 (1994)
572. G. Zubay: Studies on the lead-catalyzed synthesis of alsopentoses. Orig. Life Evol. Biosphere **28**, 13–26 (1998)