Basic Biological Anticipation

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Jan-Hendrik S. Hofmeyr

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Abstract

Living organisms persist as functional wholes far beyond the individual lifetimes of their functional components. They achieve this by taking antecedent action, continuously fabricating themselves in anticipation of a future nonfunctional and deleterious internal state. This property of self-fabrication is the most basic expression of biological anticipation and of life itself. Self-fabricating systems must be closed to efficient causation, and in this chapter, I identify the classes of efficient biochemical causes in the cell and show how they are organized in a hierarchical cycle, the hallmark of a system closed to efficient causation. Broadly speaking, the three classes of efficient causes are the *enzyme catalysts* of covalent metabolic chemistry, the *intracellular milieu* that drives the supramolecular processes of chaperone-assisted folding and self-assembly of polypeptides and nucleic acids into functional catalysts and transporters, and the *membrane transporters* that maintain the intracellular milieu, in particular its electrolyte composition.

J.-H. S. Hofmeyr (✉)
Centre for Complex Systems in Transition and Department of Biochemistry, University of Stellenbosch, Stellenbosch, South Africa
e-mail: jhsh@sun.ac.za

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Introduction

All living organisms are constructed from fragile materials, yet they persist as functional wholes far beyond the individual lifetimes of their functional components. They must therefore have the ability to autonomously rebuild themselves in anticipation of the fact that they will deteriorate it they don't. Organisms therefore conform to Rosen's (1985) definition of an anticipatory system, in the sense that they take antecedent action by continuously fabricating themselves in anticipation of a future deleterious internal state. This property of self-fabrication underlies all higher properties of life, such as growth, adaptation, and reproduction, and is the most basic expression of biological anticipation, and of life itself.

The purpose of this chapter is to figure out how, at a molecular level, the living cell, the unit of life, accomplishes this remarkable and defining feat. To the best of my knowledge, this has not been done yet, except for my own preliminary attempt in Hofmeyr (2007). The concept of self-fabrication as *the* feature that distinguishes life from nonlife is of course not new and originated with Rosen's (1958a, b, 1959, 1991) metabolism-repair or (M,R)-systems and Maturana and Varela's (1980) related concept of autopoiesis, both of which focus on the self-fabricating nature of living systems. The relationship between (M,R) systems and autopoietic systems has since been explicated by Letelier et al. (2003). Maturana and Varela also emphasized that an autopoietic system forms a concrete unity in space in which the self-fabricating network is encapsulated. However, neither Rosen nor Maturana and Varela, nor, for that matter, anybody who has since published on this topic, has delved deeply enough into the biochemical underbelly of the cell to pinpoint exactly how the cellular processes form a self-fabricating organization.

With his (M,R)-systems and his formalization of the four Aristotelean causes (material, efficient, formal, final), Rosen gave us the theoretical tools with which to model causal entailment and self-fabrication at an abstract level (Rosen 1991; Louie 2009, 2013). The crucial result is that closure to efficient causation is a necessary condition for self-fabrication, and thus for life. Following Rosen, Aloisius Louie, in his handbook ▶ Chap. 10, "Relational Biology" (which is ideal background reading for the present discussion), defines a *cell* as "(at least) a material structure that realizes an (M,R)-system," and shows that an (M,R)-system is closed to efficient causation and hence anticipatory. However, the mappings in Rosen's (M,R)-diagram, especially the so-called replication map, have been notoriously problematic to realize in terms of real biochemical processes. At journey's end of this chapter, I hope to have shone new light on this matter.

From a biochemical point of view, what I therefore set out to do is identify the sets of efficient causes in the cell and show that the functional organization of cellular

processes is closed to these efficient causes. In the jargon of relational biology, I set out to show how all the efficient causes participate in a so-called *hierarchical cycle*, the hallmark of a system closed to efficient causation (Louie 2009, Louie's handbook ► Chap. 10, "Relational Biology").

The Hierarchical Cycle in the Cell

Before attempting to identify the sets of efficient causes that form a hierarchical cycle, I lay out the cell's underlying network of material transformations, its metabolism. The term *metabolism* usually conjures up the image of a huge network of coupled, enzyme-catalyzed reactions depicted on the wall-charts that typically adorn biochemistry lecture halls. Despite the apparent complexity of this network, it can be simplified considerably to show the functional organization of the metabolic processes (Fig. 1). The degradative processes of catabolism break down nutrients (carbohydrates, proteins, lipids) to yield carbon skeletons, chemical energy (high ATP/ADP ratio or high energy charge), and reducing equivalents (high NADPH/$NADP^+$ ratio), which in turn are used by anabolism to synthesize the building blocks for macromolecular synthesis: *amino acids* for proteins, *nucleotides* for DNA and RNA, *fatty acids* for lipids. Catabolism and anabolism form the core of *intermediary metabolism* in all heterotrophic organisms. Both photoheterotrophes and photoautotrophes require the addition of a photochemical block, while photoautotrophes require a further block (the Calvin cycle) which produces sugars that are inputs to the catabolic block. Nevertheless, for the purpose of the present analysis, the whole of intermediary metabolism can be collapsed into the conversion of nutrients to the building blocks for macromolecular synthesis (a single arrow in Fig. 2).

What happens to the building blocks produced by intermediary metabolism? Figure 2 shows the processes relevant to this analysis, namely the maintenance of DNA integrity and the copying of DNA (combined in a single arrow), the production of the three major forms of RNA, namely ribosomal RNA (rRNA), messenger RNA (mRNA), and transfer RNA (tRNA), and the production of membrane lipids. Amino acids, in the form of aminoacyl-tRNAs, serve as substrates for the production of polypeptides. There are of course many other processes that also use products of catabolism and anabolism, but they are peripheral to this analysis and their exclusion does not affect its conclusions.

Efficient Cause 1: Enzymes and Ribosomes Catalyse Covalent Chemistry

The processes in Fig. 2 form the underlying network of *material causation* in the cell. From a chemical viewpoint, this is a *covalent reaction network* in which the bonds that are formed, broken, or rearranged are all covalent bonds. The individual biochemical reactions that comprise this network, while thermodynamically

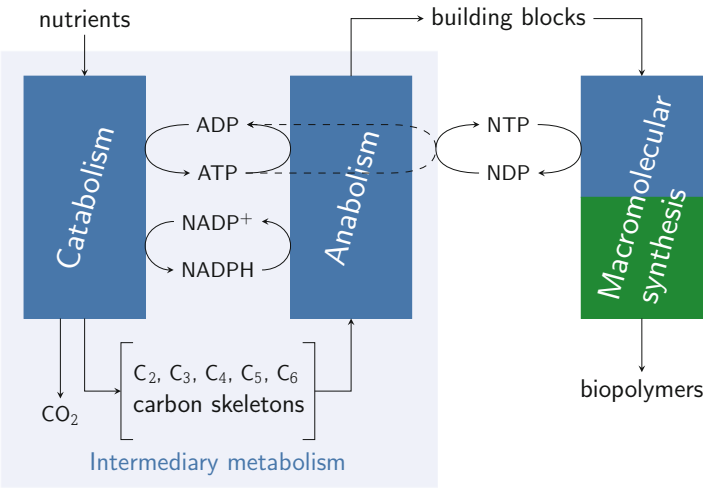

Fig. 1 The functional organization of metabolism. NTP and NDP represent nucleotide triphosphates and diphosphates. The triphosphates ATP, GTP, UTP, and CTP are used in macromolecular synthesis. *Blue* represents catalysis by enzymes, while *green* represents polypeptide synthesis by ribosomes (compare Fig. 2) (Adapted from Atkinson 1977)

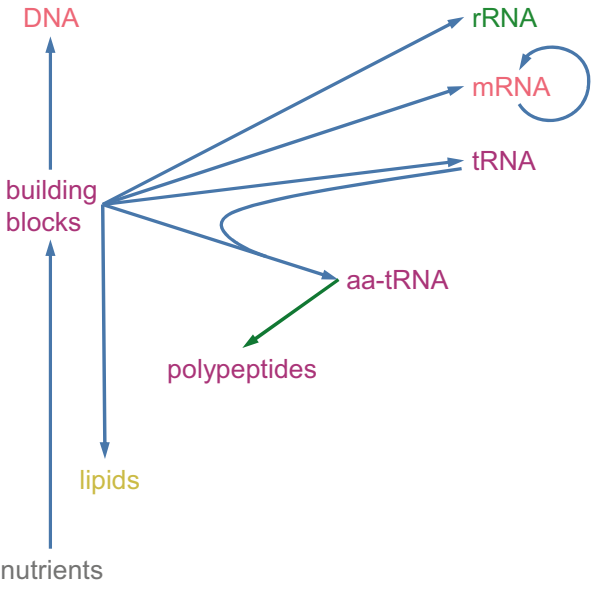

Fig. 2 *Macromolecular synthesis* from monomeric building blocks. The *arrow* from nutrients to building blocks (amino acids, nucleotides, lipids) denotes intermediary metabolism in Fig. 1, while the other *arrows* comprise the macromolecular synthetic block. The *circular arrow* around mRNA represents the formation of mature mRNA through splicing, processing and editing. The substrates for polypeptide synthesis are the aminoacyl-tRNAs

unstable, are kinetically stable and, in the absence of catalysts, proceed at negligible rates. For the network to be kinetically separable and independent from the greater network of spontaneous mass-action chemical transformations in which it is embedded, its reactions must operate on a timescale orders of magnitude faster than the

side-reactions; the greater the time-scale separation, the smaller the effects of these side-reactions and the greater the degree of kinetic autonomy. This can only be achieved by efficient *catalysts* that are highly specific with respect to the substrates they recognize and the reactions they catalyze (Hofmeyr 2007). In the cell these specific catalysts are enzymes, membrane transporters, and supramolecular assemblies such as oligomeric enzymes, ribosomes, proteasomes, spliceosomes, nucleosomes, chaperones, and many others; they are all proteins or, in the case of ribosomes, spliceosomes and nucleosomes, nucleoproteins.

The dashed arrows in Fig. 3 make explicit the catalysis of the covalent chemical processes in Fig. 2 by enzymes and by ribosomes and the transport of nutrients across the cell membrane by transport proteins. Together they comprise the enzyme-catalyzed metabolism and the ribosome-catalyzed repair components of Rosen's (M,R)-system (the detailed biochemistry of the processes depicted in Figs. 1, 2, and 3 can be found in any modern biochemistry textbook and will not referenced here). Whereas the specificity of each enzyme is determined by the architecture of its active site, the specificity of ribosomal synthesis of polypeptides is determined by the ribonucleotide sequences of mature messenger RNAs, each mRNA specifying the amino acid sequence (primary structure) of a unique polypeptide. mRNA in noneukaryotic organisms is mature upon transcription, whereas in eukaryotic organisms its directly transcribed sequence (pre-mRNA) is processed into mature mRNA through a number of processes, including the splicing out of noncoding sequences and rearrangement of the coding sequences (the circular arrow around mRNA in Fig. 3).

Messenger RNA is but one class of RNA that is transcribed from DNA; the other two classes pertinent to this analysis are (i) rRNA, which forms the major structural component of ribosomes and is directly involved in its catalytic function (Steitz and Moore 2003), and (ii) the set of tRNAs. (Besides rRNA and tRNA there are also a host of other noncoding RNAs that fulfill important regulatory, processing, and protective functions, but their fabrication needs nothing more than an extra enzyme-catalyzed arrow from building blocks in Fig. 3.) Each tRNA is covalently coupled with high fidelity to a specific amino acid by an aminoacyl-tRNA synthetase to form an aminoacyl-tRNA (aa-tRNA); the set of aa-tRNAs are the substrates for ribosomal translation of mRNA sequences into polypeptides. The fixed relation between the unique anticodon sequence of three ribonucleotides on a tRNA and its cognate amino acid forms a rule of the genetic code. The set of 20 aminoacyl-tRNA synthetases that most cells possess, one for each amino acid, can therefore be regarded as the efficient cause of the genetic code; the genetic code is inscribed in the active sites of these crucial enzymes (Barbieri 2015).

In Fig. 3 the red dotted arrows emanating from DNA show that DNA acts as a template both for its own copying and error-correcting maintenance and for the synthesis of the different classes of RNA; the red dotted arrow emanating from mRNA shows that it acts as a template for the synthesis of polypeptides. These pathways for the transfer of sequence information from DNA can be regarded as the *formal cause* of polypeptide synthesis.

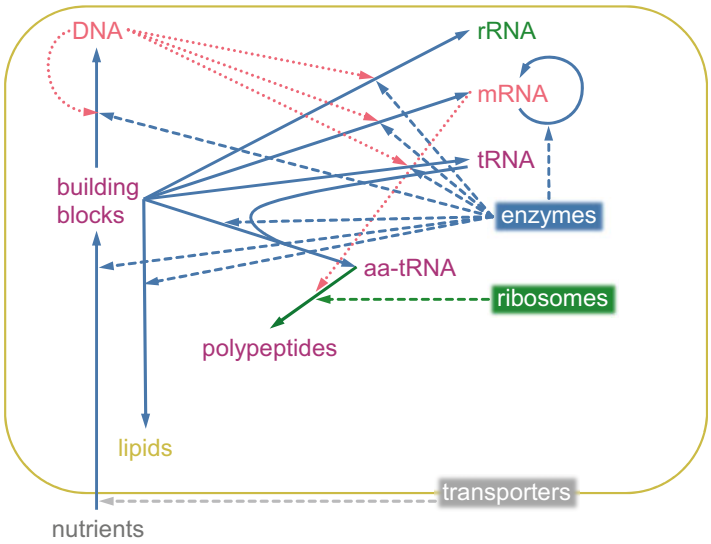

Fig. 3 *Catalysis* as efficient cause of metabolism, macromolecular fabrication, and nutrient transport. The *blue*, *green*, and *grey dashed arrows* indicate the processes that are catalyzed by enzymes, ribosomes, and nutrient transporters, respectively. The *red dotted arrows* show the role of *templates* in the copying and repair of DNA and in the synthesis of the different forms of RNA by transcription and of polypeptides by translation

Figure 3 also makes explicit the fact that nutrients need to be transported across the cell membrane by specific transport proteins.

The polypeptides formed by catalyzed covalent chemistry are as yet *non-functional*. We now turn to the processes that entail their functional state, i.e., the efficient causes that transform nonfunctional polypeptides into the functional catalysts and transporters that comprise efficient cause 1.

Efficient Cause 2: The Intracellular Milieu Enables Supramolecular Chemistry

In order to become functional, say as an enzyme, a newly synthesized polypeptide needs to fold into the correct three-dimensional conformation that forms the active binding and catalytic site. Higher-order structures such as proteasomes and chaperones need to self-assemble from pre-folded proteins, while ribosomes and spliceosomes need to self-assemble from pre-folded proteins and RNA. These processes of *folding* and *self-assembly* are driven by noncovalent interactions and are now called *supramolecular chemical processes* (Lehn 1995) (see Fig. 4).

The pioneering studies on ribonuclease in the 1950s and 1960s by Anfinsen, Sela, and White (Anfinsen 1973) and on the assembly of viruses by Caspar and Klug (1962) established that, given a watery environment with a specific pH, temperature,

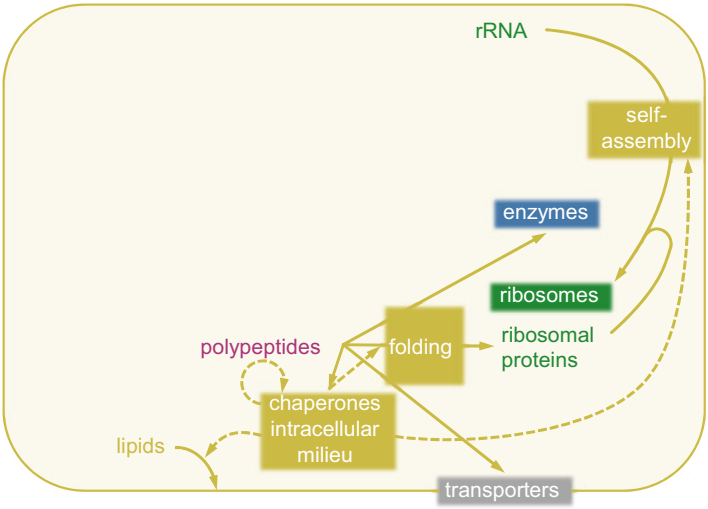

Fig. 4 The *supramolecular processes* of *polypeptide folding* and *macromolecular assembly* that generate *functional catalysts* such as enzymes, transporters, and ribosomes, as well as chaperones that assist in folding and self-assembly (the *yellow solid arrows*). The intracellular space is enclosed by the cell membrane, which is formed from lipids through processes involving self-assembly. Together, the *chaperones* and the membrane-delimited *intracellular milieu* act as efficient causes of folding and self-assembly (the *yellow dashed arrows*)

ionic strength, and electrolyte composition, the folding of polypeptides into their functional state is a spontaneous process that is determined by their amino acid sequences (primary structures). Similarly, the self-assembly of proteins and/or nucleic acids into higher-order structures is spontaneous, the specificity of the process being determined by the properties of the prefolded subunits. The generality of these facts has since been confirmed by the many studies that show that a very large number of pure denatured proteins refold or re-assemble spontaneously to their functional conformations when the denaturant is removed (Ellis et al. 1998). Nevertheless, while the specificity of folding and self-assembly is determined by the hydrogen bonding and electrostatic properties of the polypeptides and subunits themselves, the main driver for folding and assembly is the *hydrophobic force* exerted by the watery environment, which imparts an important agency to the intracellular milieu (intracellular meaning "inside the cell" and milieu the "environmental condition"). Furthermore, for the intracellular milieu to be effective as an efficient cause of supramolecular chemistry, its pH, temperature, ionic strength, and electrolyte composition have to be strictly maintained, since these factors determine the state of dissociation and solvation of functional groups on proteins and nucleic acids.

Since the discovery by Laskey et al. (1978) that the assembly of histones and DNA into nucleosomes is allowed only in the presence of a protein called

nucleoplasmin that prevents aggregate formation, our understanding of protein folding and assembly has been enhanced dramatically. With the introduction of the concept of *molecular chaperone* "to describe a class of cellular proteins whose function is to ensure that the folding of certain other polypeptides and their assembly into oligomeric structures occur correctly" (Ellis 1987, p. 14), we have a new class of efficient causes of supramolecular chemistry. In a recent review, Ellis (2013) suggests "that it is more useful to think of a molecular chaperone as a function, rather than a molecule," a notion that fits perfectly with the idea that, in essence, chaperones can be regarded as catalysts of noncovalent folding and assembly since they assist these processes without becoming permanent components of the structures they help creating. Ellis (2013) suggests that the principle of spontaneous self-assembly should be replaced by the principle of *chaperone-assisted self-assembly*, thus retaining the principle of self-assembly while modifying it to include the need for chaperones that reduce unproductive side reactions, particularly aggregation.

Just like other proteins, chaperones can in principle fold and self-assemble spontaneously, but it is quite probable that it happens with the assistance of other chaperones ("cross-chaperoning"), perhaps even of themselves ("self-chaperoning"); this is suggested by the circular dashed arrow in Fig. 4. Some evidence for this was provided by Lissin et al. (1990) who showed that the chaperone GroEL re-assembles itself in an ATP-dependent process. To my knowledge there are at present no other published studies that explicitly address this issue. The assembly of ribosomes from proteins and rRNA is also assisted by chaperones (Woolford 2002; Karbstein 2010).

It is therefore clear that the efficient causes of the supramolecular processes of folding and self-assembly is an amalgam of, on the one hand, chaperones that prevent mis-folding, mis-assembly, and aggregation and, on the other, a conducive intracellular milieu made up of a pH-buffered solvent with a high dielectric constant (water) and a homeostatically maintained electrolyte composition that differs from that of the external environment. Factors such as macromolecular crowding (Ellis 2001) and the presence of enzyme cofactors (Wittung-Stafshede 2002) also contribute to the effectiveness of the intracellular milieu as efficient cause of supramolecular chemistry.

As depicted in Fig. 4, a controlled intracellular milieu would not exist were it not encapsulated by a barrier that distinguishes and isolates it from the extracellular environment. This barrier is of course the semi-permeable bilayer of lipids called the cell membrane, which contains, among others, proteins that selectively transport nutrients and electrolytes. The cell membrane can be thought of as forming part of the intracellular milieu in that it provides a two-dimensional lipophylic environment that is needed for membrane-bound proteins such as transporters to fold and self-assemble. As with folding, the formation of membranes from amphipathic lipids is a self-assembly process driven by the hydrophobic force of the watery surroundings.

We now have catalysis of covalent biochemistry (efficient cause 1) functionally entailed by the intracellular milieu and chaperones (efficient cause 2) through the supramolecular processes of folding and self-assembly of proteins and nucleic acids, which also account for the self-assembly of membrane lipids. To form a hierarchical

cycle, we need to show that the intracellular milieu itself (efficient cause 2) is functionally entailed by efficient causes that have already been accounted for in Figs. 3 and 4.

Efficient Cause 3: Membrane Transport Maintains the Intracellular Milieu

The main differences between the intracellular and extracellular environments are the marked difference in electrolyte composition and the high intracellular protein concentration, the latter the main contributor to macromolecular crowding. In virtually all microbial, plant, and animal cells the intracellular pH is buffered near 7.2, the most important buffers being inorganic and organic phosphates and proteins, both of which have already been shown to be efficiently caused by metabolism. *Membrane transporters*, which are also already accounted for by protein synthesis and folding, maintain the intracellular electrolyte composition (Fig. 5).

The cytosolic concentration of K^+ is much higher than that of Na^+, while extracellularly the opposite holds. In most organisms, a Na^+/K^+-ATPase maintains the high potassium level inside cells while keeping sodium low. Intracellular Ca^{2+} is also strictly maintained as very low levels. The major intracellular anions are phosphates, sulfate, proteins, and amino acids, while Cl^- is the major extracellular

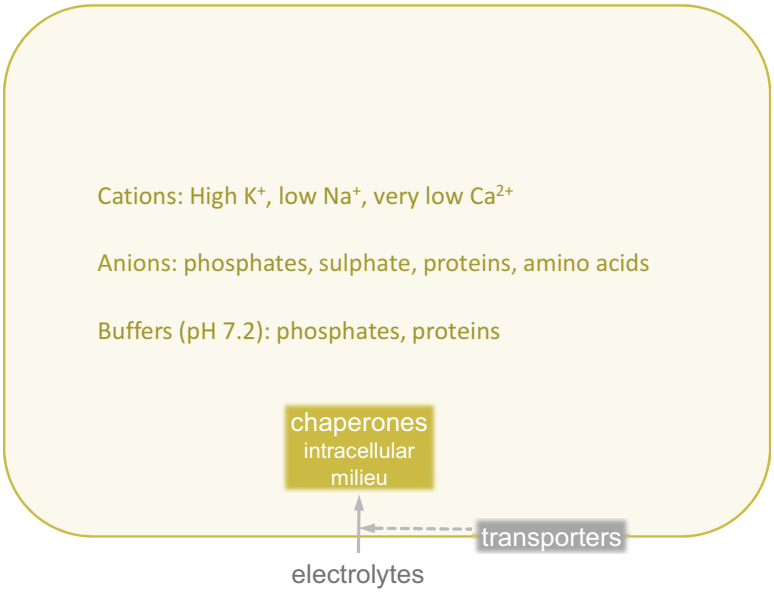

Fig. 5 The homeostatic maintenance of the intracellular milieu by *membrane-bound selective ion transporters* (*grey dashed arrow*), which act as the efficient cause of the electrolyte composition of the intracellular milieu (*grey solid arrow*)

Table 1 Typical ion concentrations in mammalian cytosol and blood (Lodish et al. 2013, p. 485) and in sea water (DOE 1994)

Ion	Cytosol (mM)	Blood (mM)	Sea water (mM)
Potassium	139	4	10
Sodium	12	145	469
Chloride	4	116	546
Bicarbonate	12	29	2
Amino acids in proteins	138	9	
Magnesium	0.8	1.5	53
Calcium	<0.0002	1.8	10

anion; in blood, bicarbonate is also a major anion. Table 1 shows the differences in electrolyte composition of the mammalian cytosol as compared to blood and sea water.

We have already seen that chaperones can be considered part of efficient causation of supramolecular chemistry by the intracellular milieu (efficient cause 2). Like all the other enzymes and transporters they have already been accounted for by polypeptide synthesis, folding and self-assembly.

Closure to Efficient Causation

When stitched together, as in Fig. 6, the three sets of efficient causes depicted in Figs. 3, 4, and 5 form a system in which all efficient causes are produced internally, i.e., a cycle that is closed to efficient causation – a hierarchical cycle.

At first glance this visual representation of the complete network of causation is complicated and difficult to decipher, but it can be simplified, as in Fig. 7, to a form in which the closure to efficient causation is immediately clear. What this representation also shows is the apparent presence in the cell of a constructor in the sense of Von Neumann. In Von Neumann and Burks (1966, Fifth Lecture), he described a theoretical kinematic automaton that could reproduce itself. The automaton consists of a *universal constructor* combined with the description of a machine. The constructor builds the machine from component parts according to its description. If supplied with its own description, the automaton can construct itself. The ribosome that makes polypeptides according to the information in mRNA seems obviously analogous to Von Neumann's constructor, but the analogy is at best partial. The polypeptides constructed by ribosomes are not yet molecular machines; they are still nonfunctional and need to fold, and, in the case of higher order structures, self-assemble in an environment that is conducive to these supramolecular processes. Von Neumann's constructor, given its own description, can make itself, but the ribosome cannot do this: it only makes the protein part of itself, which is not even the functional component: as noted above, ribosomes are ribozymes with rRNA performing the catalytic function. This new understanding of ribosomal function should, I hope, supersede the erroneous view that ribosomes can directly make

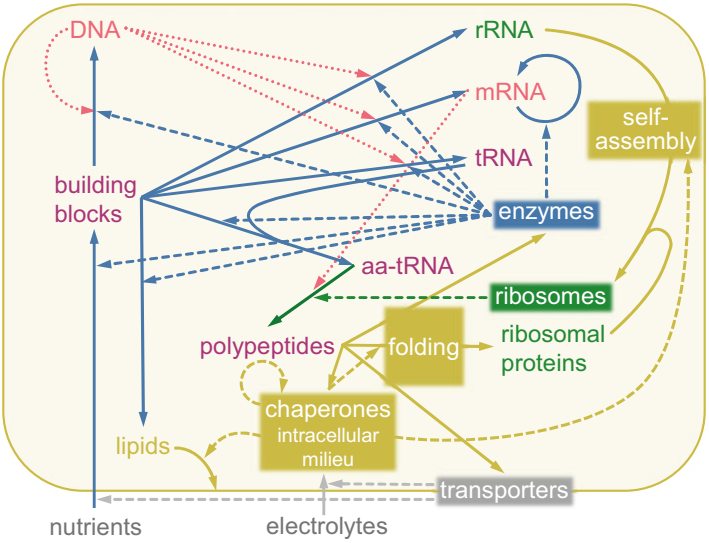

Fig. 6 The superposition of the three sets of efficient causes in Figs. 3, 4, and 5 provides a complete picture of the self-fabricating organization of the cell

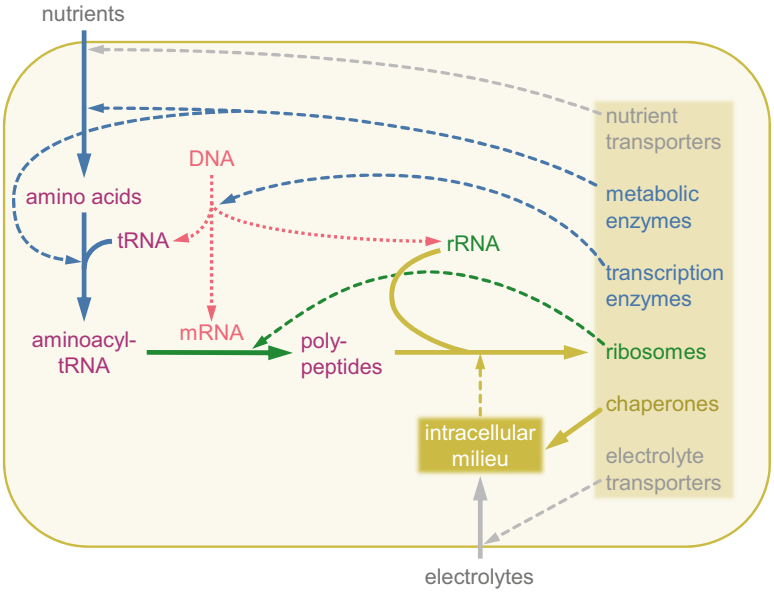

Fig. 7 An alternative view of the self-fabricating organization of the cell depicted in Fig. 6. It shows how all the efficient causes (*dashed arrows*) are produced in the cell, thereby forming an anticipatory hierarchical cycle. The *solid arrows* originate from material causes and the *red dotted arrows* depict formal causation by transfer of sequence information. Lipid and cell membrane synthesis in not included here. The scheme is an expanded version of Fig. 9 in Hofmeyr (2007)

themselves (since some examples of this view were published before the ribozyme nature of ribosomes was known and since the point of this discussion is not polemic I do not provide literature references).

For those readers of a more theoretical bent, the closed-to-efficient causal entailment structure that underlies self-fabrication of the cell is summarized in the diagram of mappings shown in Fig. 8. The catalytic mapping, *e*, includes metabolic and transcription enzymes, ribosomes, and nutrient transporters. As noted above, these macromolecules also contribute as proteins to aspects of the intracellular milieu, *m*, but the main efficient cause of *m* is *t*, the membrane transporters that maintain the intracellular electrolyte composition and ionic strength. As already noted, chaperones, *c*, which assist folding and self-assembly, are considered part of the intracellular milieu, but they are already accounted for by the efficient causes *e* and *m* that fabricate their structure and make them functional, also by cross-chaperoning or self-chaperoning. The intracellular milieu mapping should therefore more correctly be the product of *m* and *c*. It is possible to expand the diagram in Fig. 8a to a version that decomposes the mappings

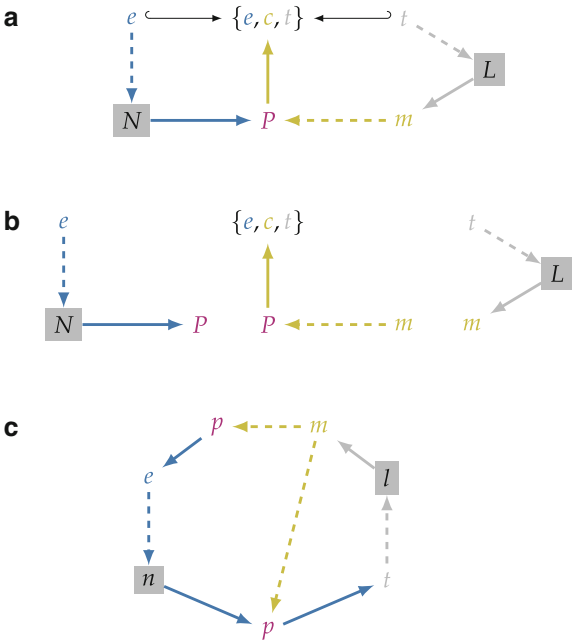

Fig. 8 (**a**) Diagram of the mappings that correspond to Figs. 6 and 7. The *dashed arrows* originate from efficient causes (the mappings *e*, *t* and *m*), the *solid arrows* originate from material causes *N*, *P* and *L*, while the *hooked arrows* to {*e*, *c*, *t*} are inclusion mappings (the inclusion mapping from *c* to {*e*, *c*, *t*} is not shown). *N*, nutrients (extracellular); *L*, electrolytes (extracellular); *P*, polypeptides; *e*, catalysts (enzymes – including nutrient transporters – and ribosomes); *c*, chaperones, *t*, electrolyte transporters; *m*, intracellular milieu, including chaperones. (**b**) Decomposition of the scheme in (**a**) into three mappings that respectively correspond from *left* to *right* to Figs. 3, 4, and 5. (**c**) Chasing elements through the diagram in (**a**) to form a cycle of hierarchical compositions (see Louie (2009) for the theory underlying such diagrams)

into all the details of Figs. 6 and 7, but this will be the subject of a separate publication. Here I just want to provide an abstract representation of how the three major efficient causes described above form the hierarchical cycle that is the most basic expression of biological anticipation and which forms the functional core of all cells, whether prokaryotic or eukaryotic, bacterial, plant, or animal.

While the purpose of this chapter was to analyze closure to efficient causation in terms of cellular biochemistry, we may nevertheless ask where Rosen's (1991) (M,R)-system fits into the present picture. Without going into detail here, the *e* mapping is a composition of the metabolism and repair components of Rosen's diagram and therefore accounts for both metabolism and genetics as in Fig. 3. However, the crucial difference is that the repair is not yet complete because the polypeptides in *P* are still nonfunctional, whereas in the (M,R)-system their functional state is just assumed and not explicitly accounted for as it is in my analysis. The central insight of my analysis is that closure to efficient causation is achieved through an intracellular milieu that serves as efficient cause of the supramolecular chemistry that makes the molecular machinery of the cell functional. In turn these molecular machines create and maintain the intracellular milieu and therefore serve as its efficient cause. What this analysis also makes explicit is that the existence of the intracellular milieu presupposes encapsulation by a self-assembling, semi-permeable isolating barrier that allows selective transport.

The idea that the matrix in which the functional components of a system are embedded can itself be a functional component of the system may be foreign to most readers, but the intracellular milieu actually fulfills Rosen's (1991) criteria for a functional component perfectly. First, it has an input (non-functional polypeptides and polynucleotides) from which it produces an output (functional catalysts and transporters), and second, changing its properties (such as its dielectric constant, pH, ionic strength, or electrolyte composition) affects the effectiveness with which it does this, and so changes the behavior of the system.

The critical reader may protest that the functional organization I describe in Figs. 6 and 7 only pertains to prokaryotic cells (Archaea and Bacteria). While it is true that these diagrams capture the essential details of the functional organization of prokaryotic cells, eukaryotic cells are just elaborations of that functional organization, the essence of which remains unchanged. Of course, the eukaryotic cell is structurally and functionally much more complex than the prokaryotic cell – consider, for example, compartmentation, the cytoskeleton, endomembranes, chromosomes and their remodeling, mRNA splicing and processing, more complex post-translational protein modification, specialized organelles, membrane trafficking, not to mention all the organic codes beyond the genetic code (Barbieri 2015) – but ultimately it remains just a more complicated exemplar of the same three sets of interlinked efficient causes: covalent catalysis by enzymes, supramolecular chemistry driven by the intracellular milieu, and maintenance of the intracellular milieu by membrane transport. Whereas Figs. 6 and 7 do not include these extra eukaryotic features, Fig. 8 applies to all living cell forms. If the Last Universal Common Ancestor (LUCA) was enclosed by a membrane (so creating an intracellular milieu) and had catalysts (whether protein or RNA or both) that needed the correct supramolecular chemistry to become functional

(which depends on the intracellular milieu), then, in principle, Fig. 8 applies to LUCA as well, although some aspects of Figs. 6 and 7 most probably do not. Figure 8 therefore describes the core of the functional organization, if not of life itself, then at least of life as we know it.

Conclusion

In order to fabricate themselves, cells use a single, conceptually straightforward chemical process – polymerization – to create linear macromolecules that fold into functional three-dimensional structures that can self-assemble into higher-order molecular machines. The analysis presented above shows that to make this possible nature had to learn to harness both covalent carbon chemistry and non-covalent supramolecular chemistry. Covalent chemistry is effected by specific catalysts, while supramolecular chemistry is effected by a strictly-maintained intracellular milieu. The functional cellular architecture that ultimately evolved is what I would call a distributed Von Neuman constructor, distributed in the sense that it encompasses three efficient causes: template-directed polymerization to make non-functional polypeptides and polynucleotides (covalent chemistry informed by DNA/RNA sequence information), folding/self-assembly to make these macromolecules functional (supramolecular chemistry), and the maintenance of the intracellular milieu by encapsulation and membrane transport to make supramolecular chemistry possible. To create a link between the world of DNA/RNA sequences and the world of amino-acid sequences in polypeptides a chemical convention (the genetic code) had to be established. Together all of these processes are folded into a hierarchical cycle that forms the basis of basic biological anticipation.

The above analysis of basic biological anticipation also teaches us an important lesson about the functional organization and anticipatory nature of self-sustaining systems in general: in order to qualify for such a system, the relations between *structure, function*, and *context* must form a hierarchical cycle, allowing all three components to be generated from within the system itself. In Hofmeyr (2007) I suggested a mantra for systems biology: "Nothing in an organism makes sense except in the light of functional context." I now want to suggest that one can generalize this by replacing "organism" with "organization." As noted by many before me, we have much to learn from life itself.

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