

Prospects & Overviews

How did bacterial ancestors reproduce? Lessons from L-form cells and giant lipid vesicles

Multiplication similarities between lipid vesicles and L-form bacteria

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In possible scenarios on the origin of life, protocells represent the precursors of the first living cells. To study such hypothetical protocells, giant vesicles are being widely used as a simple model. Lipid vesicles can undergo complex morphological changes enabling self-reproduction such as growth, fission, and extra- and intravesicular budding. These properties of vesicular systems may in some way reflect the mechanism of reproduction used by protocells. Moreover, remarkable similarities exist between the morphological changes observed in giant vesicles and bacterial L-form cells, which represent bacteria that have lost their rigid cell wall, but retain the ability to reproduce. L-forms feature a dismantled cellular structure and are unable to carry out classical binary fission. We propose that the striking similarities in morphological transitions of L-forms and giant lipid vesicles may provide insights into primitive reproductive mechanisms and contribute to a better understanding of the origin and evolution of mechanisms of cell reproduction.

Keywords:

■ evolution; L-form bacteria; protocell; protoplast; spheroplast

Introduction

The emergence of cellular life requires at least two conditions to be fulfilled. At first, compartmentalization is essential to encase a distinct chemical microenvironment and to prevent escape of entrapped replicating genetic components. Secondly, this compartmentalized structure must undergo cycles of growth and division to be capable of self-maintenance. Such a compartment would link phenotype to genotype, assisting in selection of the most successful replicating genetic elements and facilitating Darwinian evolution [1]. The term “protocells” is used for the hypothetical precursing structures of these first cells, which do not yet feature all the attributes of self-sustaining viability, but are at the transition between non-living matter and life. Currently available evidence supports the view in which simple self-assembling lipid vesicles are the type of compartment that most likely represents the earliest protocells [2–5]. Such vesicles cannot only grow [6, 7], they can also undergo competitive evolutionary processes [8, 9] and selectively take up “nutrients” from their environment [10]. Lipid vesicles – preferentially composed of molecules that would presumably be obtainable under prebiotic conditions – are widely used in bottom-up approaches to study the possible mechanisms of protocell growth and division [11]. These vesicles exhibit a diversity in membrane dynamics, depending on their chemical composition and environment. Shape changes, such as intra- and extra-vesicular budding, or formation of protrusions and pearling, followed by fission-like processes indicate that self-reproduction

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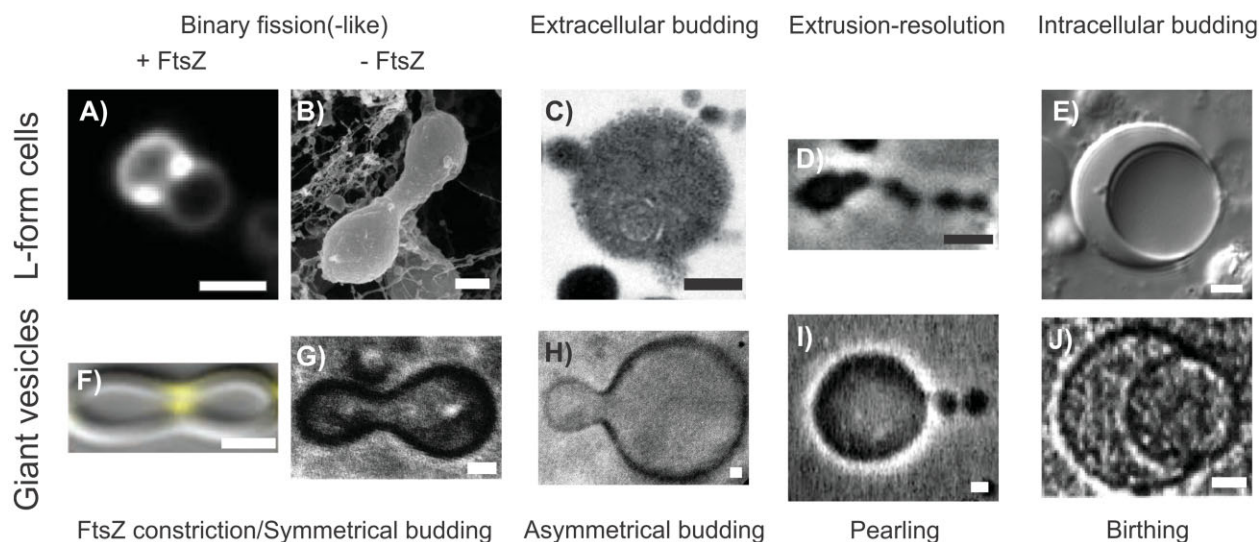


Figure 1. Morphological similarities between giant lipid vesicles, as experimental proto-cells, and L-form bacteria. This overview illustrates the morphological similarities in observations made with L-form bacteria (upper) and experimental protocells (lower). The specific terminology developed in the respective research fields is given for the top-down (L-form bacteria) and bottom-up (lipid vesicles) approaches, respectively. A specific *E. coli* L-form strain with approximately 7% residual peptidoglycan is still able to carry out a binary-fission like division, most likely dependent on Z ring constriction and residual septum formation. A membrane-stained cell is shown (A). FtsZ and enzymes related to septum formation are indispensable for division in this L-form lineage. Many unstable L-form spheroplasts may use a reproductive mode similar to *E. coli* K-12 NC-7 L-forms, which features some residual FtsZ but at a concentration that is probably too low for Z ring formation. Nevertheless, a binary fission-like process takes place (B). It has been demonstrated that FtsZ alone is sufficient for constriction of lipid vesicles. An overlay of light microscopy with fluorescently labeled FtsZ is shown in panel (F). Temperature variations accompanied by surface-to-volume changes induce symmetrical (G) and asymmetrical (H) budding of lipid vesicles, resembling binary fission (B) and extracellular budding (C) in L-forms, respectively. The latter occurs simultaneously with an extrusion-resolution process (D) in some *B. subtilis* L-forms. The resolution of a protrusion from the L-form surface resembles the tubular pearling phenomenon as it occurs in protrusions of lipid vesicles (I). Formation of intracellular progeny vesicles has been observed in both *Listeria* and *Enterococcus* L-forms (E), as well as in giant lipid vesicles featuring auto-catalytic amphiphile synthesis (J). The internal “daughter” vesicles may be released through disintegration of the surrounding L-form membrane (E), or a translocation process termed “birthing” (J). Note that different scale bars have been used: white (2 μm), and black (0.5 μm). Some morphological similarities are present at different size levels, especially in case of (C) versus (H) and (D) versus (I). Modified and reproduced from ref. [29] (panel A), [28] (B), [24] (C, D), [15] (F), [54] (G, H), [65] (I), and [38] (J), with permission from American Society for Microbiology (A, B), Nature Publishing group (C, D), The American Association for the Advancement of Science (F), Elsevier (G, H), Springer (I), and American Chemical Society (J).

of experimental protocells is possible in different ways. Complex morphological changes, driven only by physicochemical forces, may actually reflect the early steps in evolutionary development of cellular reproductive processes [5, 12, 13].

Contemporary (bacterial) life forms have evolved over billions of years. This has resulted in the development of several different very complex, yet tightly orchestrated and strictly regulated division mechanisms. These mechanisms feature highly adapted, interactive multiprotein machineries. This evolutionary process included stepwise acquisition of incremental

levels of complexity, improving the efficiency of previously simple (ancestral) proliferation mechanisms. A prime example is the Z ring, which has been proposed to represent a primordial system for bacterial cytokinesis [14], most likely in a much simpler form than its current version. The ring-like structure consists of filaments of polymerized FtsZ, which is able to generate the force required for constriction of tubular lipid vesicles without the need for other proteins (Fig. 1F) [15]. However, in contemporary bacterial cells, the Z ring functions as a scaffold for recruitment of at least ten other proteins, which then regulate constriction of the ring and fine-tune septum formation [16]. The oscillating MinCDE system positions the ring mid-cell [17]. Active chromosomal segregation processes and DNA translocases assist in the faithful distribution of the genetic material [18]. Together, these incremental improvements promoted development from a primordial, low-efficiency division process that mainly relied on physicochemical membrane dynamics into a highly reliable and actively regulated process, with equal-sized progeny inheriting a single copy of the complete genetic material.

L-forms represent cell wall-deficient yet viable bacteria, featuring a distorted structural organization

L-forms are cell wall-deficient bacteria that are capable of indefinite reproduction [19]. They have been observed and can be isolated from in vivo material [20], but can also be generated by in vitro techniques. Compounds that interfere with cell wall integrity or synthesis (such as certain antibiotics, lytic or

lysosomal enzymes, complement, amino acids, or starvation of certain nutrients required for cell wall biosynthesis) induce L-form transition [21]. The large variability in induction procedures and culture media used, and the fastidious character of L-form cells in culture has resulted in a tremendous heterogeneity in descriptions of their growth requirements, physiology, morphology, growth rate, and stability [22].

L-form cells can either be partially or completely devoid of a cell wall, representing spheroplasts, or protoplasts, respectively. They can exist as both unstable (transient) and stable L-form cell lines, the latter of which are unable to revert to the parental (walled) state. Due to the loss of their shape-determining cell wall, L-forms assume spherical or even pleomorphic shapes, and can vary in size from 0.5 to more than 50 μm . Some L-form cells may accumulate a variable number of intracellular vesicles [19]. L-forms have also been reported to represent multinucleate cells, due to the disruption of the normally existing direct link between genome replication and cellular division processes [23, 24].

Although L-forms feature the full genomic capacity and elaborated biomachinery of a typical walled bacterium, they generally appear to lack the organized structure and cytoskeleton required to carry out classical binary fission. The structural organization normally present in bacterial cells can be defective to various degrees, depending on the L-form state (transient vs. stable) and its parental strain. Whereas certain L-forms may have conserved (part of) the cytoskeletal structure, it is completely absent in others. For instance, the use of fluorescently labeled FtsZ has revealed spots, arcs and complex polymer networks, but no ring structures, in *Bacillus subtilis* L-forms. In fact, FtsZ appears to be dispensable in these cells [24]. Recently, it has been reported that the same L-form strain proliferates in the absence of other cytoskeletal proteins (MreB/MreBH/Mbl, MreC, MreD) and proteins involved in chromosome segregation (Soj, SpoOJ) [25]. Sequence analysis of *Escherichia coli* K-12 LWF+ L-form cells have revealed a mutation in *ftsQ*, which encodes a protein required for divisome assembly [26, 27]. Nevertheless, intact FtsA and FtsZ ring structures are present in these cells [19]. *E. coli* K-12 NC-7 L-forms feature a five-fold reduction of FtsZ per unit of protein; a concentration that appears too low for Z ring formation [28]. *E. coli* K-12 MG1655 L-forms are independent of MreB function, whereas the presence of FtsZ is essential [29]. In conclusion, L-forms represent a morphologically and structurally very heterogeneous group of bacterial derivatives, but generally feature cell wall-deficiency and (at least partially) a defective structural organization.

Do L-forms employ simplified, possibly ancestral cellular reproduction mechanisms?

A large diversity of reproductive mechanisms has been reported to occur in L-form bacteria, ranging from binary fission-like processes, extracellular budding [30], intracellular budding [31], and extrusion-resolution [24] to formation of tiny elementary bodies as reproductive units [32]. We propose that this variability may be best explained if we consider L-forms as contemporary cells that lack one or more of the mechanisms

previously acquired throughout the evolution of bacterial cell division (e.g. Z ring formation and positioning, septum formation, coordinated chromosome segregation, programmed cell elongation, balanced phospholipid production, etc.). According to our hypothesis, L-forms could reflect a regression to a more “primitive” level of cell division, as their proliferative mechanism is typically less efficient, less coordinated, and slower. After cell wall disintegration, individual cells can exit into L-form states, and thereby assume a different functional level that may still be sufficient to support less efficient, but nevertheless sustainable growth and clonal development.

Whereas the structure of some L-form strains seems to be affected only to a minor degree, others seem to be reduced to a more minimal form of cellular life, i.e. simple, phospholipid-enclosed compartments. Multiplication of the latter cellular phenotype may actually reflect the most primitive proliferation pathways; reminiscent of the various and dynamic morphological changes that take place in non-living giant lipid vesicles. Recently, Chen noted the striking similarity between the reproductive mechanism used by a *B. subtilis* L-form strain and some self-reproducing lipid vesicles [33]. Although established L-form model systems are still scarce, and reports on L-form reproductive mechanisms are mostly descriptive, we suggest that morphological similarities between the reproductive mechanisms of giant lipid vesicles and different L-form strains may be more extensive. These similarities may teach us something about primordial cellular reproduction via mechanisms that do not necessarily involve or require complex macromolecules. In addition, we speculate that the different reproductive mechanisms used by adapted, stable L-form strains may provide us with a glimpse of how potential ancestral intermediate cells may have reproduced in the absence of the complex set of structural elements that represents the current bacterial divisome. With this in mind, the diversity of L-form reproductive mechanisms mentioned above presents an exciting view of how most primitive L-forms may be able to thrive simply by using membrane dynamics, and how other L-form strains exploit cellular structures (such as the FtsZ ring) or the peptidoglycan synthesis machinery (Fig. 1).

Formation of internal vesicles can drive multiplication processes of both L-form cells and non-living lipid vesicles

Stable bacterial L-forms obtained from cultures of the Gram-positive genera *Listeria* and *Enterococcus* (and likely other members of the Firmicutes, see below) use a fascinating intracellular budding process for multiplication [34]. Growing L-form cells accumulate an increasing number of intracellular vesicles. These daughter vesicles are eventually released either upon rupture of the mother cell membrane (Fig. 1E) or through non-destructive translocation across the surrounding cell membrane. Viability of daughter vesicles, including their ability to continue propagation processes, has been demonstrated by GFP fluorescence, respiratory activity, and the existence of a membrane potential [31, 34].

Similar self-reproducing behavior through intravesicular budding has been observed in experiments with giant vesicles,

termed “birthing” [35] or “vesicle expulsion” [36]. A common feature of such chemical systems is the autocatalytic production of amphiphiles that constitute the original vesicle’s membrane. This could be achieved through smart selection of catalysts and vesicle components. Catalysis can either take place within the boundary of parent vesicles [37, 38] or in their interior [39]. Although at a low frequency, smaller internal vesicles are apparently released by “translocation” across the surrounding membrane (Fig. 1J).

Pearling instability and membrane fluidity can support offspring formation in lipid vesicles and in L-forms

Cells of a *Bacillus subtilis* L-form strain generated by β -lactam drugs and depletion of peptidoglycan precursors do not require FtsZ for division and proliferate through an “extrusion-resolution” process [24]. In this case, transient pseudopod-like protrusions eventually resolve into several small bodies. Multiple simultaneous budding and eruption of small bodies from the cell surface have also been observed (Fig. 1C, D). Initially, it was proposed that cytoskeletal systems may drive membrane deformation and initiate the protrusions [24]. However, the authors could later show that several known genes that are involved in cytoskeleton, membrane, and chromosome dynamics of the wildtype organism do not play a role in this process [24, 25]. A possible explanation of the phenomenon could be that a small surface area asymmetry between the inner and outer leaflets of the bilayer membrane exists. This might be sufficient to cause local changes in membrane curvature and induce protrusion formation [40, 41]. For instance, absorption of nanoparticles to a vesicle interior leads to formation of protrusions from the vesicular membrane, which grow and shrink [42]; a process which is microscopically similar to the formation of protrusions in *B. subtilis* L-forms [24].

In the context of our new conceptual framework, it is very interesting to note that constriction of *B. subtilis* L-form protrusions into different smaller bodies reveals a striking similarity to pearling instability [33]. Pearling instability is a physical phenomenon that causes tubular vesicles to break down into a string of spherical bodies connected by narrow links (Fig. 1I). Considering the above, pearling of L-form protrusions may be induced by the asymmetry between the bilayer leaflets or the propensity of membranes to reduce their surface area when tension is applied [33, 43, 44].

Complete separation of the daughter cells or fission is the final step in reproduction. The production of membrane lipids with branched fatty acids, and the corresponding higher membrane fluidity, is crucial for this step in the aforementioned *B. subtilis* L-forms [25]. Membrane composition and fluidity therefore play a pivotal role in multiplication. Interestingly, a different *B. subtilis* L-form that grows as a necklace-like structure, consisting of several daughter cells connected by thin links, has also been described [45]. The narrow links could only be broken through mild mechanical forces, i.e. agitation. In this case, the membrane itself may be too rigid to allow for spontaneous fission.

Budding and binary fission-like mechanisms are frequent in both L-forms and lipid vesicles

Budding and budding-like processes originating from a larger “mother” cell and the pinching-off of one or more smaller “daughter” cells have been observed in many L-form bacteria (Fig. 1C) [29, 30, 32, 46–48]. Binary fission-like processes resulting in two progeny cells of almost equal volumes may also occur in L-forms (Fig. 1A, B) [32, 50–52]. Similarly, budding and fission have been reported for giant lipid vesicles under a wide range of different physicochemical forces (Fig. 1G, H) [40, 41, 49, 53–55].

***Escherichia coli* L-forms can adopt different proliferation mechanisms depending on their structural state**

The best example to illustrate the different regressive levels at which L-forms can propagate, depending on the remaining structural elements that normally facilitate controlled binary fission, is the diversity of proliferation mechanisms described for *E. coli* K-12 L-forms [28–30]. Intriguingly, each L-form cell seems to adopt its own specific multiplication mechanism(s). Although the molecular description of different L-form strains is often limited or missing, we propose that reproduction is determined by the presence or absence of residual peptidoglycan synthesis, its remaining ability to form and position a functional Z ring, and the balance between the phospholipid production rate and osmotic state. Unstable *E. coli* K-12 MG1655 L-form-like spheroplasts divide by a budding- or binary fission-like process (Fig. 1A) [29]. Interestingly, they have been shown to retain roughly 7% residual peptidoglycan, the synthesis of which seems to be required for cell division. Whereas inhibition of the actin-like protein MreB has no effect, FtsZ has been found to be essential for division and growth. PBP3, a septum-specific transpeptidase, is also required. So too is PBP1B, a bifunctional transglycosylase-transpeptidase partially located at the septum through interaction with PBP3 [56]. We propose that these are L-form-like cells, where a functional Z ring may still be formed in the context of residual peptidoglycan synthesis at the septal region. However, positioning of the Z ring appears distorted, since both symmetrical and asymmetrical division planes exist. Multiple modes of multiplication exerted by a single unstable *E. coli* K-12 spheroplast have also been described [30]. Interestingly, shortly after conversion from the wildtype to the L-form state, multiplication processes occurred that involved the cytoplasmic membrane as well as constriction of the residual cell wall, often at multiple sites. Both symmetrical and asymmetrical division planes were seen. After several passages, the degree of cell wall-deficiency increased, and the L-form cells divided through budding and segmentation by intracellular membrane formation. These observations seem to reflect an incremental loss of structural organization by bacterial cells as they become L-forms. Whereas cells may initially divide by employing a still functional Z ring and ongoing processes such as residual septum formation, further dismantling may cause the

cell to divide asymmetrically (incorrect Z ring positioning) or without directed septum formation. Eventually, the processes may be further reduced to physicochemical membrane dynamics alone. Another *E. coli* L-form strain (K-12 NC7) generated by exposure to lysozyme and a mutagen, featured a significantly reduced amount of FtsZ that was probably insufficient to form a functional Z ring. No peptidoglycan layer or septum formation was observed in these cells. Nonetheless, these L-form cells adopt a binary fission-like process (Fig. 1B) [28, 57].

Mollicutes show parallels to L-form cells

The Mollicutes are a class of bacteria characterized by a reduced genome size and inherent absence of a murein cell wall. However, they are not considered L-forms because they do not have a walled counterpart. The best-studied genus is *Mycoplasma*. The available evidence indicates that the Mollicutes may have originated by regressive evolution from other low-G + C Gram-positive bacteria, the Firmicutes [58]. It is tempting to speculate that transition to the L-form state observed in this latter group of organisms (e.g. *Listeria* and *Enterococcus*, as described above) could actually reflect a recapitulation of one or more steps in the evolutionary past of *Mycoplasma* [59]. Therefore, mechanisms of cell division in these bacteria may provide an attractive model for comparisons. While the precise molecular mechanisms in *Mycoplasma* cell division are not clear, several reproduction modes appear to exist in parallel. The *ftsZ* gene is present in all species, with the exception of *M. mobile* [60]. Nevertheless, it has been shown that FtsZ may not even be required for cell growth, at least in *M. genitalium*. This species can divide by traction-mediated cytofission, using the force generated by its cytoskeletal motile machinery. This process is proposed to be a redundant mechanism for division in *M. genitalium*, besides FtsZ-based constriction [61]. Symmetrical binary division, budding and segmentation of filamentous cells has been shown to occur in the FtsZ-deficient *M. mobile* [62]. The latter process has also been observed in spoon-like filaments of *M. hominis* [63]. It is especially interesting because of the intriguing similarity to the pearling process in tubular lipid vesicles and the extrusion-resolution mechanism in *B. subtilis* L-forms [24, 43]. In conclusion, the physicochemical membrane dynamics observed in lipid vesicles and L-forms may also be involved in *M. mobile* multiplication. The overall diversity of reproductive modes and the regressive evolution of mycoplasmas show intriguing parallels to observations made for L-forms. Different *Mycoplasma* species seem to exploit one or more division mechanisms dependent on FtsZ, the motility machinery or membrane dynamics, which underlines the plasticity and variability of bacterial division processes.

Morphological coincidence or common ancestry?

The striking morphological similarities between L-forms and experimental protocells (Fig. 1) raise the question as to whether they stem from convergence (different mechanisms

having a similar appearance) or from ancestry, with L-forms as the possible contemporary representatives of bacterial ancestors. Whereas physicochemical forces affecting shape dynamics of chemical lipid vesicles are well documented from both quantitative and theoretical perspectives, studies on L-form multiplication are mainly descriptive; quantitative and molecular studies have only recently been initiated. Nonetheless, it is clear that L-forms are equipped with a full and complex biomachinery, whereas experimental protocells are simple lipid membrane-encased compartments that are liquid-filled or, in some cases, provided with simple catalytic components. These experimental protocells may be too simple a system to study the origin of life, as all known living systems are far more complex; though the evolutionary precursors must have started somehow with a much lower degree of chemical and compositional complexity.

L-forms are able to self-synthesize all components of their membrane, whereas growth in lipid vesicles relies on external addition of amphiphile lipids or directed synthesis of simple amphiphiles in their interior. It is noteworthy that L-form membranes are composed of phospholipids, whereas experimental giant vesicles are mostly constructed from single chain lipids. Generally, physicochemical forces such as minor temperature changes [53, 54], variations in pH [41], spontaneous membrane curvature alterations in response to asymmetry between membrane leaflets [40, 41], and osmotic fluctuations [53, 55] are the factors that drive shape changes in giant vesicles. In contrast, the endogenous and continuous source of phospholipid production has been suggested as the most probable trigger to induce L-form cell shape changes. During rod-to-sphere transitions, the surface-to-volume ratio of L-form cells decreases significantly. As the sphere diameter increases further during growth and cell enlargement, its surface area expands by the $2/3$ power of its volume. Assuming that the cellular phospholipid production rate is linearly dependent and directly correlated to mass or volume [64], an excessive phospholipid pool inherently accompanies L-form growth. It seems reasonable to assume that such a continuous imbalance between available surface area and phospholipid production initiates membrane dynamics that can drive L-form proliferation [14].

As L-forms lack a stabilizing sacculus, their surface-to-volume ratio is also strongly affected by osmolarity. The proportion of vesiculated *L. monocytogenes* L-forms multiplying by an intracellular budding process increases from 35 to 63% when osmolarity is increased by 40%. In addition, the number of vesicles per cell increases drastically (Y. Briers, unpublished results). These findings suggest that excessive membrane leads to additional intracellular vesicle formation; a seemingly simple physicochemical process.

Another fundamental difference between L-forms and giant vesicles is that L-forms encase a protein-dense colloidal milieu, in contrast to the simpler aqueous pool inside giant lipid vesicles. Macromolecular crowding may drastically influence vesicle shape dynamics. Integration of membrane proteins or anchoring of protein complexes to the membrane via lipid anchors can cause local asymmetries in the L-form cell membrane, possibly inducing membrane irregularities and deformations. It has previously been shown for giant lipid vesicles that attachment of hydrophilic polymers [44], contact

with nanoparticles [42], or tension applied by optical tweezers [43] causes local asymmetry and membrane curvature, resulting in shape changes. Moreover, since local addition of lipid material to giant vesicles can lead to drastic shape changes [41, 49], it is not entirely speculative that unevenly distributed phospholipid synthesis by the corresponding (membrane-anchored) enzymes induces membrane deformations in L-forms.

Altogether, currently available observations support the hypothesis that the same phenotypic effects witnessed in L-forms and experimental protocells could, in fact, be produced by different mechanisms in the respective entities. The best explanation may be that the top-down approach starting from L-forms is probing the last universal common ancestor (LUCA), while the bottom-up approach using experimental giant vesicles is probing the first living cellular systems, i.e. the origins of life. The gap between the first protocell and LUCA remains enigmatic. Hence, current knowledge favors coincidence in the origin of the observed morphological similarities between L-forms and experimental protocells.

There is no doubt that L-forms represent an intriguing and highly relevant model system for the study of bacterial evolution and ancestry. The development of a cell wall, the occurrence of FtsZ and all other players in the divisome incrementally improved the efficiency of bacterial division. L-form bacteria lack these upgrades, to varying degrees. Indeed, as L-forms feature only minor [24] or no [34] genetic modifications, they themselves do not represent the proposed bacterial ancestors *sensu stricto*. However, as many of their structural elements dedicated to binary fission are present but not necessarily functional or in use, the wall deficiency and associated changes in life style may resemble a state in bacterial evolution where these components may not have been available. In this context, it has recently been shown that several genes normally involved in cytoskeleton and cell shape determination, membrane dynamics, chromosome segregation, phospholipid production, and membrane stress response actually have no effect on L-form proliferation [24, 25]. Therefore, we believe that L-forms provide a hint as to how bacterial ancestors may have multiplied before the evolution of binary fission involving a genetically determined divisome. Studying the wide diversity of L-form reproductive mechanisms therefore is an attractive top-down approach to evaluate potential (intermediate) primitive proliferative mechanisms. Moreover, the loss of structural elements of the cell wall, cytoskeleton and divisome in the Mollicutes during their intimate association with eukaryote host cells may also help elucidate the origin and functional evolution of these mechanisms. In future studies, it would be useful to obtain more quantitative and molecular data of the mechanisms of L-form proliferation to judge to what degree multiplication rests on simple physicochemical forces and is independent of genetic predisposition, and to clarify the role of divisome components in this process.

Conclusions

The foundation of current theories of the possible multiplication mechanism used by protocells at the dawn of the origin

and evolution of life on earth rests on experiments in which the self-reproducing behavior of lipid vesicles has been investigated. Remarkable advances in the efforts to create a plausible protocell model based on self-reproducing vesicles have been made in recent years, although the origin of the first cell(s) remains a mystery. The growing evidence and understanding of the diverse reproductive processes used by bacterial L-forms simultaneously attests to these findings and provides additional credentials to the existing theories of protocell self-reproduction. Therefore, studies of bacterial L-form cells may contribute to a better understanding of the origin of unicellular life. Their diversity in multiplication strategies also offers a glimpse of the contribution of cellular structure and divisome components to improving the efficiency of primordial cell reproduction. A better understanding of the molecular mechanisms in the various L-form division modes may also contribute to deciphering the evolution of cell division in more general terms.

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