Review Article

Division in synthetic cells

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Cell division is one of the most fundamental processes of life, and so far the only known way of how living systems can come into existence at all. Consequently, its reconstitution in any artificial cell system that will have to be built from the bottom-up is a notoriously complex but an important task. In this short review, I discuss several approaches how to realize division of cell-like compartments, from simply relying on the physical principles of destabilization by growth, or applying external forces, to the design of self-assembling and self-organizing machineries that may autonomously accomplish this task in response to external or internal cues.

Synthetic cells — synthetic life

In the context of synthetic biology, the synthetic cell has become a very attractive goal of research and development. In a fundamental perspective, the cell is the smallest known unit of life by definition, its generation according to cell theory only possible through the division and replication of already living cells. Thus, it is fair to say that nobody has ever witnessed the creation of a living system from non-living building blocks; and whether this process can ever be accomplished in the laboratory is to date a matter of speculation, to be potentially overcome by fundamental research. However, many researchers do not aim at dealing with these rather philosophical implications when they start to design synthetic cells. Many features of living cells, such as their efficient compartmentation of biological function, their generation of biomolecules, and their ability of exchanging matter and/or information with other cells and organisms, are desirable to be reconstituted also in parts in functional biotechnological units. In these entities, division is a dispensable task, prone to create more problems than it solves, with respect to the necessary control over biotechnologically active or bio-functional compounds. However, if one aims at elucidating the origin of life, i.e. the transition of a chemical into a biological system, autonomous compartment division will be one of the central tasks to be accomplished, ideally with the smallest possible number of functional modules. The nature of these modules, and their spatiotemporal orchestration, will crucially depend on the exact realization of the other key elements of such a synthetic life: metabolism, as a prerequisite of spatiotemporal self-organization, and information, establishing a biological identity.

Division as a prerequisite for replication

Besides its central role for the growth of multi-cellular organisms, cell division is an indispensable process for both maintenance and evolution of life. Although a perfect metabolism would potentially be able to entertain cellular functionalities ad infinitum, only the cell’s replication into two or more daughters renders it robust against environmental changes and challenges that could lead to complete annihilation. Moreover, the information transfer that goes along with division establishes a point at which genetic variation and thus, functional evolution, can be most conveniently implemented. Importantly, with respect to the (re-)engineering of biological functionality in synthetic cells, it has to be pointed out that in contrast with metabolism and information transfer, division of compartments has essential physical implications, which makes it an exciting starting point for many biophysical groups working in the field of minimal or bottom-up synthetic biology [1–3].
Division by growth
If one considers a membrane vesicle the most appropriate starting point for the design of a protocell or synthetic cell, division is primarily the task of membrane fission. Lipid membranes as such are very soft objects, the deformation or shape changes of which usually do not require major forces. However, membrane fission, in which the radius in the plane of division is ultimately reduced to zero, is a much more dramatic process than a simple shape change. Locally, the lipids in the division plane are suddenly in the way and have to disappear from the shrinking orifice. Globally, the overall ratio between surface and volume will have to change, most dramatically in the case of spheres, i.e. the equilibrium surfaces of vesicles unless they are forced into different shapes through external forces or scaffolds. To create two spheres out of one, membrane surface needs to be added or volume needs to be disposed of, which is particularly problematic for relatively tight membrane containers made of phospholipids. For the final step of fission, lipids have to be separated laterally, which they usually resist due to their hydrophobic forces. Simple vesicles composed of fatty acid membranes have, in the past, been successfully used [4] for demonstrating the interplay between surface growth and division, as depicted in Figure 1, but phospholipid or other membranes that are much tighter in terms of containment also better withstand the addition and subtraction of material.

Lately, there has been an increasing recognition of non-membrane-bounded compartments with functional roles in cell biology [5]. These originate from liquid–liquid phase separation of certain proteins and nucleic acids in aqueous media, owing to their differential hydrophilicity and other features, such as electrostatic interactions. It has been proposed [6] that such compartments may constitute a more ancient representation of cells, as they are able to separate biochemically distinct spaces without having to establish a certain class of molecules, fatty acids or lipids — entirely as boundaries. Theoretical considerations have proposed that when kept away from thermodynamic equilibrium by the dissipation of energy, such phase-separated droplets can exhibit cycles of growth and division that resemble the proliferation of living cells [7]. In particular, internal flows of matter and energy may mechanically drive shape transformations [8]. Energy dissipation by any sort was suggested to not only reverse Ostwald ripening, i.e. the coalescence of droplets over time, but destabilize them to the point of splitting (Figure 2). Conclusive experiments to quantitatively test these hypotheses are, however, still lacking.

Naturally, biological cells found in nature are much more complex than simple membrane shells or droplet-like liquid phases. Most of them established cell walls or protein coats to maintain certain shapes or at least confer mechanical stability. Thus, they have also developed a wide variety of protein-based machineries to actively regulate shape transformations during division, many of them structural proteins with the ability of self-assembling into filaments or 2D lattices, in order to act over much larger scales than their individual sizes.

Figure 1. Vesicle division by growth.
The surface-to-volume ratio will be increased and the surface destabilized by spontaneous or catalyzed addition of surface molecules, eventually resulting in breakup into two or more daughter vesicles. Lower panel: Experimental realization by synthesis of surface material, reprinted from Castro et al. [18] (CC-BY 4.0 license).
Active division elements

The most iconic biological structures observed in nature that are supposedly responsible for inducing membrane fission, or cell division as a whole, are ring structures formed of self-assembled proteins (Figure 3). The probably most well-known ring structures, because of their large sizes to be directly observed in light microscopes, are actomyosin rings. As already reflected by their names, they are generally composed of actin filaments, as well as motor protein myosin. Since myosin is a directional motor on actin filaments, it has originally been believed that the constriction of these rings may follow along the same lines as known from sarcomeres, with actin and myosin filaments sliding in opposite directions, and thereby constricting the radius of the ring. This has, however, not been confirmed. Instead, myosin appears to induce the contraction of actin filaments by destabilizing them [9]. However, whether this already suffices for ring contraction to the point of membrane fission is, to date, completely unclear, as is the biological trigger to initiate constriction [10].

In prokaryotes, another ring structure of smaller scale is supposedly involved in cell division, the so-called Z ring. It consists of a multitude of proteins, the most central of which, FtsZ, is a bacterial tubulin homolog with remarkable dynamic properties that have only in recent years begun to be elucidated. It polymerizes into bundles of curved polar filaments that were shown to assemble into rings when attached to the inner cell membrane, and to treadmill circumferentially upon GTP hydrolysis [11]. The role of this energy-consuming behavior for the actual division process, accompanied by an apparently constricting ring, is still elusive. Early hypotheses suggested that GTP hydrolysis results in a conformational change of FtsZ monomers, amplified in multimeric filaments into large-scale bending [12]. Recent in vivo studies, in contrast, emphasize a more indirect role of FtsZ treadmillling along the cell periphery, i.e. targeting the synthesis of new cell wall material by recruiting respective enzymes to mid-cell [13]. Due to the small sizes of bacteria and their respective divisomes,
the exact mechanism will be even harder to elucidate by live cell imaging than in eukaryotes. Also, the role of cell wall synthesis in generating sufficient force and energy for division is supposedly hard to investigate by reconstitution studies, because the respective enzymatic machineries are relatively complicated to reconstitute.

Thus, in spite of their apparently intuitive mode of operation, division-associated ring structures have so far largely escaped a detailed mechanistic understanding. This is partly due to the fact that both in eukaryotes and prokaryotes, a multitude of molecular players are thought to be involved in their correct function. But also with respect to convincing theories about how large the required forces and energies for cytokinesis really are, no convergence about a single unifying constriction principle has been obtained that could be used for the rational design of division machineries from the bottom-up. In ‘modern’ cells, membrane- transforming processes are supposedly only of subordinate relevance, and the connection of cell division to cytoskeletal force generation, cell wall recycling, chromosome segregation, and in plant or bacterial cells, turgor pressure, need to be considered. Turgor pressure is an osmotic pressure, induced and controlled by selective exchange of ions through channels and pumps, which likely plays a very important role in contractile force generation [14,15].

Regarding the controlled division of membrane-less compartments in biology, not much is known so far, because, in contrast with membrane-bounded compartments, such as cells and major organelles, they can supposedly form de novo not only by variations in their constituting molecules, but also by changes in the cellular environment, as their existence is linked to phase separation and phase transition.

**Concepts for realizing artificial cell division**

As outlined above, the quest for establishing autonomous division in the context of artificial or synthetic cells has been largely driven by fundamental considerations, in particular in the framework of cellular and membrane biophysics. Thus, most of the experimental work has been targeted towards controllably splitting membrane vesicles. The conceptually simplest way to induce division of any kind of lipid-interfaced compartment is through destabilization and transformation of membranes surface by the addition of ever more surface material, as described above (Figure 1). This has been proposed and experimentally demonstrated mostly using fatty acid vesicles [16,17], and the realization in phospholipid vesicles required the reconstitution of complex biochemical machinery [18]. Here, spontaneous changes in surface area are only possible at high metabolic cost, e.g. catalyzed synthesis of new surface molecules. For more biotechnological or application-oriented endeavors, division of cell-like compartments can also be simply induced by applying direct or indirect external forces, e.g. by extruding them through filters or subjecting them to microfluidic splitters (Figure 4) [19].

**Figure 4. Forced division by mechanical elements, either splitters in microfluidic channels, or pores of extrusion filters.**

Lower panel: Experimental realization of a microfluidic vesicle splitter, reprinted from Deshpande et al. [19] (https://pubs.acs.org/doi/10.1021/acsnano.7b08411; Permissions queries regarding this reprint should be directed to the ACS).
With respect to the functional reconstitution of active elements that could autonomously induce fission or replication of compartments, the biophysical initiatives in the past years have mostly revolved around the eukaryotic actomyosin system, on one hand, and the bacterial divisome from *Escherichia coli*, on the other hand. Owing to the important roles of eukaryotic motor-cytoskeleton systems in cell biology, and the potential physiological impact of their mechanistic understanding, the large majority of studies that are generally concerned with cell mechanics focus on the reconstitution of actin, mostly in connection with cellular effectors, such as anchoring and capping proteins, as well as cross-linkers, and myosin. Many different large-scale transformative processes have so far been observed, of actin meshworks or mimics of cell cortices in general, but also attached to membranes [20], and lately on or within osmotically controlled GUVs, in which many different kinds of large-scale membrane transformation can be observed [21,22]. On the other hand, the reconstitution or purification of actomyosin rings has been accomplished in ghost cells [23] and emulsion droplets [24]. In both bases, constriction of rings could be observed and related to the turnover and homeostasis of actin, or the increase of myosin and the overall shape of the compartment, but a gross deformation in terms of a shrinking waist of the compartment in response to the ring contraction could not be observed, due to the missing attachment and presumably also the high surface tension of droplets. Here, the decisive experiment with actomyosin ring structures tightly anchored to the (inner) surface of a soft membrane vesicle is still missing.

With respect to prokaryotic Z ring reconstitution, the situation is even more complex, although the reconstitution of purified FtsZ, which is at the same time a filament-forming protein and a GTPase, is less complex than for actin and particularly myosin. In contrast with the clearly observable contraction of purified actomyosin rings and cortical networks, no ring-like FtsZ structure has ever been witnessed to actively contract or shrink in real-time in vitro, although radii of curvature of reconstituted FtsZ filament bundles have been observed to range over at least an order of magnitude [25] and membrane necks apparently sculpted by curved FtsZ filaments (Figure 3 inset) have been observed on different spatial scales [26,27]. Instead, FtsZ filaments have shown to directionally treadmill on flat supported membranes along a ring-like periphery of, however, conserved size upon energy dissipation [28,29]. Although a clear connection between the direction of FtsZ treadmilling and the direction of bud-like membrane deformations in osmotically deflated GUVs could be drawn [29], an actual mechanism of how GTP hydrolysis by FtsZ could potentially be coupled to transformative forces on membranes is still elusive. Only very recently, optical tweezer experiments on pulled soft membrane tubes of cell-like diameter have pointed to a fully new model of force generation, by which the circumferential treadmilling of spiral-like filaments in two opposite directions creates membrane tube buckling by shear forces exerted in the plane of the membrane, rather than perpendicular to it as assumed earlier [30]. The observed topology of circumferential spirals shows fascinating parallels to other membrane scission machineries operating on different spatial scales, such as dynamin and the ESCRT-III machinery [31].

Whether the force created by these dynamics could be sufficient to actually divide a large-scale vesicle is, however, still unclear. On the other hand, it has recently been shown that an oscillatory membrane-binding reaction of the MinDE protein system from *E. coli*, with the supposed physiological role of positioning the FtsZ-based divisome to mid-cell, may be able to induce major membrane transformations in GUVs, reminiscent of a symmetric cell division through waist contraction at the geometric middle [32]. This dramatic process is powered by the ATP hydrolysis that rules Min oscillations, but has so far not resulted in a full fission, i.e. separation of the two daughter cells. It is conceivable that when adding FtsZ to such a minimalistic setting, the relatively small shear forces apparently produced by FtsZ torsional treadmilling may be able to provide the small contribution required in a complex energy landscape to tip over to the divided state. At this point, such a mechanism is, however, highly speculative.

With regard to the reconstituted division of droplets or coacervates, it has been demonstrated that active, i.e. energy consuming, systems based on actomyosin on one hand, or FtsZ on the other hand, are able to induce large-scale transformations and symmetric division of phase-separated entities [33,34]. Both active systems are in vivo supposed to bind to membranes in order to unfold their physiological functions, and thus discussed mostly in the context of membrane remodeling. However, it is well conceivable that such active systems, or at least their modes of operation, are more general than originally thought, and already existent in potential predecessors of biological cells without membrane boundaries to entertain a primitive kind of reproduction. Theoretical considerations are at least fully in line with this possibility [7], and to design such self-dividing systems from scratch based on new energy-dissipating molecules is an exciting future challenge of (bio-)supramolecular chemistry.
Lastly, with respect to the bottom-up design of active materials made of functional biomolecules that are not necessarily protein-based, there has been an exciting development on the past years in the field of DNA origami. Originally invented to yield more rationally designed target molecules for x-ray crystallography, the DNA origami technique in combination with modern techniques for DNA synthesis and amplification, as well as computer-aided design, has yielded a wealth of complex DNA-based 3D structures and structure-function relationships [35,36]. Recently, it could be demonstrated that with proper functionalization by cholesterol or peptide groups, DNA origami can be worked into membrane-transforming elements reminiscent of BAR domain, i.e. curved scaffolding proteins involved in endocytosis [37]. Curved origami, particularly when prone to self-assembly into two-dimensional structures, is able to tubulate giant vesicle membranes that it is attached to. Although these tubular transformations are occurring on much smaller scales than the sizes of the vesicles, an extrapolation of this mode of action towards self-assembling and actively transforming ring structures made of DNA origami, rather than protein filaments, is well conceivable. By a smart design and integration of active, i.e., energy dissipating, elements into these origami structures, the bottom-up assembly of a fully artificial contractile biomolecular ring structure may become feasible.

Conclusions
Realizing autonomous, self-regulated division in synthetic cells may still be a rather academic challenge with little practical relevance for more application-oriented synthetic biology. However, the general principle of compartment division constitutes one of the most fundamental requirements for replication and thus, of Darwinian evolution as the precondition of cellular life. It is fair to say that its successful realization in protocells would be a milestone towards a better understanding of the fundamental principles of life. In contrast with metabolism and information storage and processing, division is largely governed by physical, rather than chemical, principles, making it a highly attractive topic for biophysicists and physical biologists concerned with the origin of life research. Although the fundamental principles of cell division have long been mainly considered in the context of membrane vesicles, recent studies on non-membrane-bounded compartments, as formed through liquid–liquid phase separation, hint to an even simpler and more ancient mechanism. Whether it will ever be possible to identify molecular traces of first life forms on earth is highly questionable, because the chemistry on earth may have dramatically changed since then. In contrast, their underlying physical principles and mechanisms may still be observed in today’s living systems. Thus, looking at physical principles, regardless of how they are encoded in functional molecules and modules, appears to be a fascinating view into the history of life on earth, but also under any other possible conditions.

Summary
- Autonomous division is a complex feature to be engineered in synthetic cells.
- Because of its fundamentally mechanical implications, reconstituting cell division is a highly attractive goal for biophysicists.
- The simplest representation of division is through growth of surface and/or volume and destabilization by active processes.
- A contractile ring of self-assembled filaments appears to be the most elegant division module for vesicles, but has so far not been functionally reconstituted in vitro.

Competing Interests
The Author declares that there are no competing interests associated with this manuscript.

References
