

PERSPECTIVES

OPINION

Biology under construction: *in vitro* reconstitution of cellular function

Allen P. Liu and Daniel A. Fletcher

Abstract | We are much better at taking cells apart than putting them together. Reconstitution of biological processes from component molecules has been a powerful but difficult approach to studying functional organization in biology. Recently, the convergence of biochemical and cell biological advances with new experimental and computational tools is providing the opportunity to reconstitute increasingly complex processes. We predict that this bottom-up strategy will uncover basic processes that guide cellular assembly, advancing both basic and applied sciences.

Biology presents many strange and wonderful examples of what is possible in the physical world. We might not fully understand how axons are guided during development or how stem cells divide asymmetrically, but most scientists would accept that there is a physical explanation at the cellular level — given the right molecules and boundary conditions, the known laws of physics govern the interactions that drive biological behaviour. Molecules that are crucial for numerous cellular functions, such as motility and division, have been identified. Furthermore, the importance of boundary conditions, such as membranes, and their fluidity, lateral organization and mechanical compliance, is becoming clear^{1,2}. However, an important question remains: how are the molecules and the boundary conditions linked to generate the remarkably coordinated and robust cellular behaviour that we observe, which often involves the creation of structures with length scales of many micrometres over timescales of minutes to hours?

The concepts of self-organizing systems and cooperative behaviour were first explored in the context of chemical systems in the 1920s, and in the past ten years they have been productively applied to the study of self-assembly in cell biology³. Reconstitutions of cytoskeletal filaments and their interactions with motors and other binding proteins are prime examples of how

dynamic behaviour observed inside a cell can be recreated and studied outside the cell⁴. Advancement towards recreating more complex cell-like behaviour (referred to here as cellular reconstitution), such as cell division and cell motility, will involve confining reactions to small volumes that are bound by a bilayer membrane and incorporating signalling pathways that are controlled by external stimuli. Experiments on living cells, which continue to be crucial for testing theories of how molecules interact to give rise to physiological functions, cannot by themselves satisfy Feynman's idea of understanding: "That which I cannot create I do not understand"⁵. Reconstitution studies using cell-free extracts or purified proteins provide a complementary approach to live-cell studies; taken together, these two approaches can lead to an understanding of biological processes that advances both basic and applied sciences.

This kind of reductionist biology — perhaps more appropriately called constructionist biology — offers three important

“...reconstituting cellular-scale structures and behaviour ... is a crucial step towards answering some of the most fundamental questions of cell biology.”

insights that complement the investigation of living cells. First, conducting *in vitro* reconstitution experiments can confirm and refine molecular models of processes outside the complicating environment of cells. Second, unexpected behaviour can arise in simplified reconstitutions constrained only by known physical laws and specific biochemical interactions, and explaining such behaviour might yield new insight into biological organization. Third, reconstitutions are often amenable to mathematical modelling that considers space, time and a manageable number of other variables to describe the components involved. The level of detailed understanding available from reconstitution studies can help reveal how evolved biological systems work and provide insight into how new biological functions could be engineered.

A growing number of spatially organized processes have now been reconstituted and characterized using purified components and cell-free extracts (see next section; FIG. 1). The recent surge in cellular reconstitutions reported in the literature indicates an increasingly productive convergence of biochemical knowledge, experimental tools and theoretical understanding, which is needed to build cellular structures from scratch. In this Opinion article, we trace the roots of today's cellular reconstitution efforts back to the early days of biochemical reconstitution and discuss technical advances that enable the current work, along with the remaining limitations. We suggest that reconstituting cellular-scale structures and behaviour from component parts, an approach that is poised to become more accessible, informative, and useful than ever before, is a crucial step towards answering some of the most fundamental questions of cell biology.

Early days of reconstitution

When biochemistry emerged as its own field, questions regarding the spatial organization of cellular structures were secondary to more basic questions about their molecular makeup, stoichiometric composition and functional significance (TIMELINE). In the 1940s, actomyosin threads from muscle were reconstituted and biochemically

characterized by Szent-Györgyi, and later by Straub (see REF. 6 for an overview), leading the way for the understanding of how muscles contract. Not long after, the discovery of the structure of DNA by Watson and Crick in 1953 (REF. 7) led to a flurry of investigation on the synthesis of DNA, including Arthur Kornberg's enzymatic study of DNA replication⁸. Simply mixing the four deoxynucleotide components of DNA and *Escherichia coli* extract proved to have little success, as unwanted nuclease activities were present in the extracts. With the purification of what is now known as DNA polymerase I, Kornberg and colleagues successfully reconstituted the synthesis of DNA⁹. The conceptually simple three-component enzymatic reaction, requiring the four deoxynucleotides, single-stranded template DNA and purified DNA polymerase, has paved the way for further studies of DNA replication.

Biochemical reconstitution has been embraced by several fields of modern cell biology beyond the cytoskeleton and DNA replication. The study of membrane trafficking began with the identification and isolation of yeast secretion (Sec) mutants that blocked membrane traffic¹⁰. Later, using a reconstitution approach with purified proteins and semi-intact cells, coat protein I (COPI) and coat protein II (COPII) were identified as proteins involved in protein transport^{11,12}. Biochemical reconstitution has also led to a better understanding of the protein translocation process in the endoplasmic reticulum (ER) and of how other DNA enzymes function^{13,14}. More recently, oscillation of the circadian clock was reconstituted¹⁵.

In these examples, biochemical reconstitution is aimed at identifying specific molecular reactions that operate at molecular-length scales. How cells assemble, organize and use micrometre-scale structures is emerging as a central question in cell biology, one that is now becoming amenable to reconstitution.

Towards cellular reconstitution

Biological activities and reactions are often confined to specific locations in the cell, such as on the plasma membrane, in various organelles and bound to biopolymers, such as the cytoskeleton and chromosomes. Reconstitution of spatially organized processes often requires the specific physical or biochemical boundary conditions that localize reactions or restrict diffusion to be established on the cellular scale, an aspect that was not the focus of early biochemical reconstitutions.

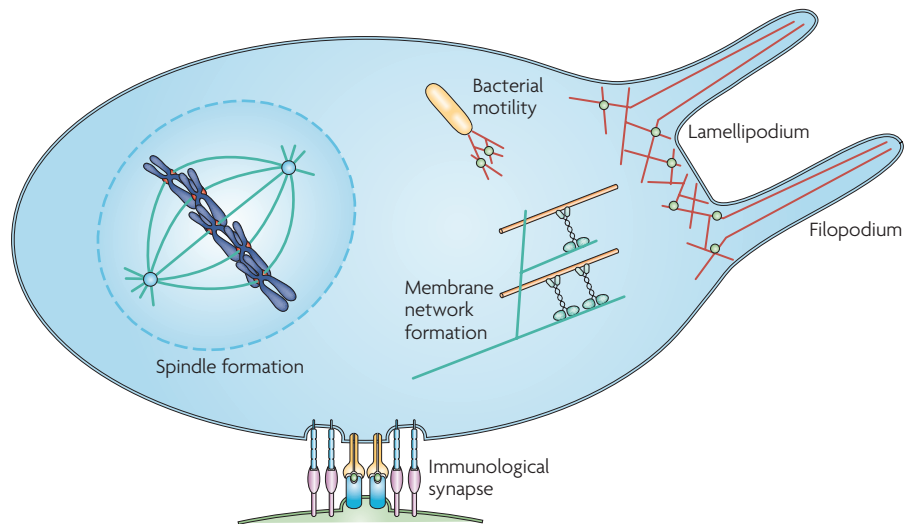


Figure 1 | A cartoon of progress towards reconstitution of cellular processes. Although rebuilding a cell is a long way off, several micrometre-scale structures involved in various cellular processes have been successfully reconstituted using either purified proteins or cell-free extracts. Spindle formation has been reconstituted using *Xenopus laevis* cell extract and DNA-coated microspheres. Actin-based motility of the bacterium *Listeria monocytogenes* has been reconstituted using purified proteins. Growing actin networks involved in lamellipodial protrusions have been shown to displace membranes, and parallel actin filaments growing against membranes have been shown to generate filopodium-like protrusions. An immunological synapse has been reconstituted using a planar lipid bilayer and live T cells. Tubular membrane networks, like those found in cells, have also been reconstituted using purified kinesin molecular motors, microtubules and giant vesicles. In each of these cases, a fairly simple set of components was found to generate a remarkably complex structure that is helping us to understand the assembly, organization and behaviour of cellular processes.

In some cases, the system of biomolecules naturally includes its own boundary conditions. Cytoskeletal polymers reconstituted with binding proteins can spatially organize on the micrometre scale in the absence of physical confinement, with the polymer itself serving as a scaffold for self-assembly. Notable examples include the formation of organized structures, such as asters and vortices that arise from mixtures of microtubules and motor proteins¹⁶, as well as the spontaneous transition from a dendritic actin network to bundled actin filaments¹⁷. The large-scale organization exhibited by the microtubule system is reviewed elsewhere¹⁸.

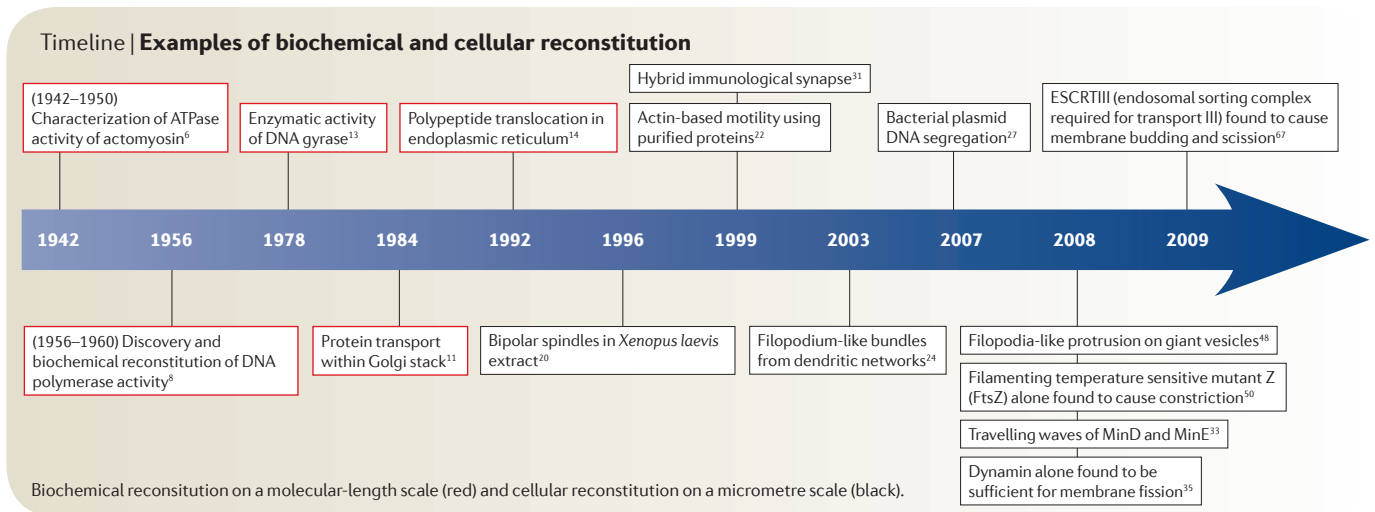
In other cases of reconstitution, the boundary conditions must be added explicitly. Using a new collection of experimental approaches, researchers are 'seeding' the spatial organization of cellular reconstitutions by restricting biochemical activities to surfaces, such as a micrometre-sized polystyrene bead or a planar fluid lipid bilayer (FIG. 2). Confining reactions to two dimensions is not only physiologically appropriate in many cases, but it also enables the use of advanced light microscopy techniques, which result in vivid images of spatially complex processes.

In this Opinion article, we focus on cellular reconstitution in which boundary conditions are added to guide organization. A brief survey of such reconstitutions, summarized in TABLE 1, is organized according to the experimental platform used to reconstitute the cellular-scale process.

Cytoskeleton assembly on microspheres.

Cellular shape change and intracellular motion are driven primarily by the assembly and disassembly of cytoskeletal polymers¹⁹. Polystyrene microspheres are a useful substrate to reconstitute many of the processes that use force generation of cytoskeletal polymers. Polystyrene microspheres come in many different sizes, and proteins or DNA can be physically adsorbed or covalently conjugated onto their surface (FIG. 2a). Furthermore, individual beads can be manipulated using optical traps, magnetic tweezers and micropipettes. However, despite the ease of manipulation, proteins that are directly conjugated to the microspheres may adopt non-physiological conformations and they will have restricted lateral movements.

The first notable use of microspheres in reconstitution was for mitotic spindle formation driven by microtubule dynamics in



a cytoplasmic extract from *Xenopus laevis*. Mitotic spindles were found to assemble around DNA-coated microspheres acting as artificial chromosomes²⁰. The study showed that centrosomes (the microtubule organizing centre) and kinetochores (the region of the chromosome where spindle fibres attach) were not required for spindle formation. More importantly, it showed that bipolarity is an intrinsic property of mitotic spindles because microtubules and motor proteins assemble around DNA in mitotic extracts in the absence of external polarity cues.

Actin filaments produce forces that drive membrane protrusions during processes such as cell motility. Reconstitution of crawling motility began with studies of the pathogenic bacterium *Listeria monocytogenes*. *L. monocytogenes* expresses an actin nucleation-promoting factor (NPF), ActA, which is required for hijacking the cellular machinery of the infected cell to propel its own movement²¹. In an impressive feat, the motility of dead *L. monocytogenes* was reconstituted using NPF-coated microspheres and a minimal set of proteins (actin and three actin filament regulators: the actin-related protein 2/3 (Arp2/3) complex, actin depolymerizing factor and capping protein)²². Using a similar reconstituted system on NPF-coated microspheres, a controversy was recently resolved regarding the role of capping protein, a protein that limits actin filament growth by binding to the filament's barbed end, and yet increases the velocity of actin-based motility. Careful analysis of actin growth on microspheres revealed that actin filament capping leads to an increase in filament nucleation and branching²³. Again using NPF-coated microspheres, bundles of actin filaments that resemble filopodia

(long and thin actin-based protrusions) were reconstituted by introducing the actin-bundling protein fascin²⁴. In addition, these filopodial bundles were shown to be capable of supporting *L. monocytogenes* motility²⁵, which shows that architecturally different actin networks can support cellular motility.

“ In an impressive feat, the motility of dead *L. monocytogenes* was reconstituted using NPF-coated microspheres and a minimal set of proteins... ”

Unlike for eukaryotes, prokaryotic cellular functions were not attributed to cytoskeletal polymers until recent years²⁶, but they are now thought to be important in a large number of processes. Segregation of DNA-coated polystyrene microspheres driven by polymerization of the prokaryotic actin homologue partitioning system M (ParM) has been reconstituted using microspheres and the three-component system of the R1 plasmid, which consists of *parC*, *parR* and *parM* in an operon²⁷. The *parR* gene encodes a protein that binds cooperatively to *parC* to form a complex. In the absence of the ParR-*parC* complex, the ParM polymer is dynamically unstable²⁸, but it is stabilized when it binds to the ParR-*parC* complex. Insertional elongation of ParM and force generation were observed as the ParR-*parC*-conjugated microspheres were pushed apart by the growing polymer. Through these reconstitution experiments it was shown that the R1 plasmid contained all the components necessary for dynamically searching for and then stably separating plasmid copies.

Reconstitution on supported lipid bilayers. To reconstitute membrane-based phenomena, in which diffusion in two dimensions is crucial, a lipid bilayer can be used as a substrate (FIG. 2b). Planar supported lipid bilayers can be made by fusing small liposomes, tiny vesicles made of lipids, onto clean glass coverslips. These bilayers have been used to study lipid diffusion and have been exploited for biomedical applications²⁹. Functional membrane proteins can be incorporated in the supported lipid bilayer using liposomes with inserted proteins³⁰. Planar supported lipid bilayers are particularly suitable for investigating processes that occur at the plasma membrane, such as the formation of the immunological synapse, which occurs at the interface between an antigen presenting cell (APC) and a lymphocyte.

The immunological synapse is a complex supramolecular structure that is involved in the communication between an APC and a T cell. The process of T cell activation was reconstituted by replacing the APC with a planar supported lipid bilayer that contained fluorescently labelled major histocompatibility complex (MHC) peptide and intercellular adhesion molecule 1 (ICAM1)³¹. These molecules, which are expressed by the APC, could diffuse freely on the supported lipid bilayer and associate with cellular receptors on the T cell. The dynamic association and rearrangement of T cell antigen receptor (TCR) and leukocyte function-associated antigen 1 (LFA1) on the T cell with MHC peptide and ICAM1 in the supported lipid bilayer forms the immunological synapse. This consists of a central supramolecular cluster (c-SMAC), in which TCR predominates, and a peripheral supramolecular cluster (p-SMAC), in which adhesion molecules are localized. This reconstitution

indicated that the formation of the immunological synapse provides a mechanism for sustained signalling in engaged T cells. Hybrid neuronal synapses, consisting of neuronal cells and a supported lipid bilayer, were similarly reconstituted³². These studies show that spatially organized and biologically relevant structures can be formed between a living cell and a supported lipid bilayer, providing a controlled environment in which to study cell–cell communication.

Recently, an elegant study reported the self-organization of *MinD* and *MinE* (proteins that are responsible for locating the contractile ring during bacterial cytokinesis) into travelling waves on supported lipid bilayers³³. Supported lipid bilayers can also be formed on glass microspheres instead of on planar glass. Using supported lipid bilayers on glass microspheres and purified proteins involved in actin-based motility, the mechanism behind attachment of the NPF neural Wiskott–Aldrich syndrome protein (*N-WASP*; also known as *WASL*) to actin networks was elucidated³⁴. Similarly, a reconstituted system was recently developed using lipid-coated glass microspheres to study dynamin-mediated membrane fission, and it was found that dynamin and GTP are sufficient for this process³⁵.

Reconstitution using giant lipid vesicles. An alternative to the supported lipid bilayer is the giant lipid vesicle (FIG. 2c). Giant vesicles (10–100 μm in diameter) can be composed of a single lipid bilayer (unilamellar) or multiple bilayers (multilamellar), and they support the diffusion of molecules in-plane, as well as morphological deformations out of plane, such as the formation of protrusions or invaginations. Although small vesicles have been used in many biochemical reconstitutions in which a membrane substrate is required^{36,37}, they are not amenable to investigations of spatial organization by light microscopy. By contrast, giant vesicles are suitable for real-time imaging to directly observe the dynamics and organization of biomolecule–membrane interactions. This approach is limited, however, by the challenge of making large numbers of giant vesicles with a defined size and composition.

Decades ago, thin-section electron microscopy revealed a labyrinth of membrane networks in cells³⁸, and reconstitution studies are now helping us to understand how they might be formed. Tubular membrane networks reminiscent of the Golgi and ER were reconstituted using a truncated kinesin motor from *Drosophila melanogaster* that was bound to giant unilamellar vesicles (GUVs)

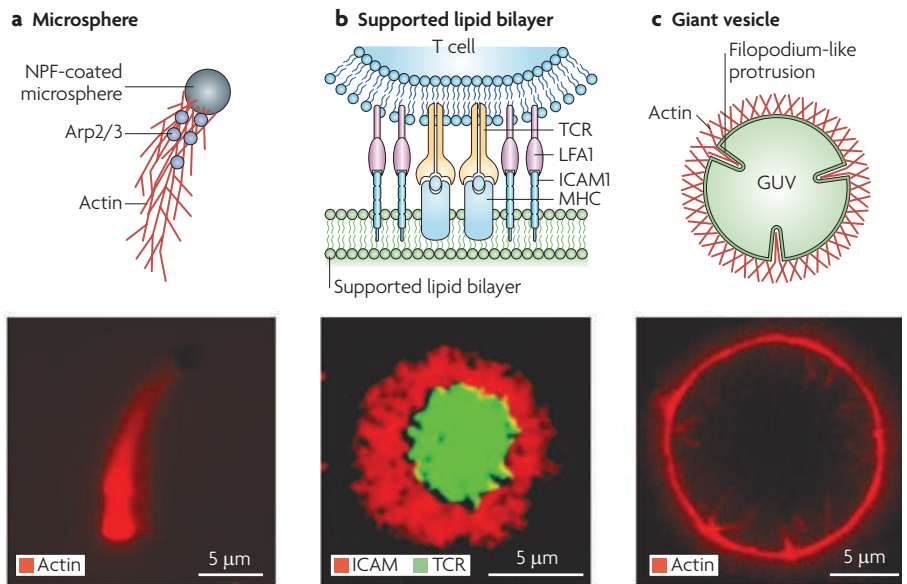


Figure 2 | Strategies for reconstituting cellular processes. Once the specific molecular components that are involved in a cellular process are identified, they must be organized by appropriate boundary conditions to generate functional behaviour. Three primary strategies have been used to define the boundary conditions that initiate spatially organized processes. **a** | The surface of microspheres can be functionalized with proteins such as actin nucleation-promoting factors (NPFs), that spatially organize actin assembly in purified protein solutions or cell extracts by a process that requires the actin-related protein 2/3 (Arp2/3) complex. The lower panel is an epi-fluorescence microscopy image showing an NPF-coated microsphere propelled by a growing actin network. **b** | Supported lipid bilayers ensure that molecular components of the system being studied can freely diffuse. They have been used to reconstitute the immunological synapse. T cell receptor (TCR) and leukocyte function-associated antigen 1 (LFA1) on the T cell bind to intracellular adhesion molecule 1 (ICAM1) and major histocompatibility complex (MHC) peptide reconstituted on the supported lipid bilayer, to form the immunological synapse. The lower panel is an epi-fluorescence microscopy image showing TCR at the centre and ICAM1 at the periphery of the immunological synapse. **c** | Giant unilamellar vesicles (GUVs) represent deformable substrates, and they have been used to reconstitute filopodium-like protrusions. The lower panel is an epi-fluorescence microscopy image showing actin networks on a GUV spontaneously undergoing transitions to form inward protrusions that consist of long unbranched actin filaments. Part **b** is reproduced, with permission, from REF. 62 © (2005) American Association for the Advancement of Science. Part **c** is reproduced, with permission, from REF. 48 © (2008) Macmillan Publishers Ltd. All rights reserved.

on one end and to surface immobilized microtubules on the other end^{39,40}. Processive kinesin molecules were attached to GUVs through a biotin–streptavidin interaction and ‘walked’ along stabilized microtubules to generate tubular networks. Motor-driven membrane tube networks have also been used to dissect the mechanism of dynamin-mediated membrane fission³⁹.

The deformability of lipid bilayer vesicles makes them an ideal tool for elucidating the biophysical effects of force generation by actin^{41,42} and other cytoskeletal polymers. For example, membrane deformations that are dependent on the polymerization of microtubules and actin filaments encapsulated in lipid vesicles have been demonstrated experimentally^{43,44}. Actin shells that are reminiscent of the actin cortex have also been reconstituted on the inner side of GUVs⁴⁵.

The effect of both membrane fluidity and deformability on the assembly of cytoskeletal networks can be studied directly with GUVs. When phase-separated GUVs that contained phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂) were mixed with purified proteins that are involved in dendritic actin network assembly, the actin networks grew external to the GUVs and only on membrane domains that were enriched with PtdIns(4,5)P₂ (REF. 46). It was found that the interaction between actin networks and the membrane acted to stabilize the PtdIns(4,5)P₂-containing membrane domains against temperature-induced mixing with other lipids. The potential influence of an actin network on the spatial organization of membrane components supports the requirement of an intact cytoskeleton in signalling systems⁴⁷.

Table 1 | Summary of recent progress in cellular reconstitution

Reconstituted process or structure	Components used	References
Microspheres		
Cell motility	<i>Listeria monocytogenes</i> and NPF-coated microspheres immersed in <i>Xenopus laevis</i> extracts	63,64
	NPF-coated microspheres, actin, profilin, cofilin, Arp2/3 complex and capping protein	22,23
	NPF-coated microspheres, actin, Arp2/3 complex and fascin	25,24
Mitotic spindle	DNA-coated microspheres and <i>X. laevis</i> cytoplasmic extracts	20,65
Plasmid segregation	<i>ParC</i> coated microspheres, <i>ParR</i> and <i>ParM</i>	27
Supported bilayers		
Immunological synapse	Supported bilayer containing MHC peptide and ICAM1, and T cells	31,62
Neuronal synapse	Supported bilayer containing neuroligin 1, and neuronal cells	32
Bacterial cell division	<i>MinD</i> and <i>MinE</i>	33
Vesicle fission	<i>Dynamin 1</i> and GTP	35
Cell motility	Lipid-coated beads, N-WASP, actin, profilin, cofilin, Arp2/3 complex and capping protein	34
Giant vesicles		
Bacterial cell division	Membrane-targeted <i>FtsZ</i>	50
Membrane tube networks	Kinesin-bound GUV and microtubule tracks	40,66
Actin–membrane interaction	PtdIns(4,5)P ₂ -containing vesicles, actin, N-WASP and Arp2/3 complex	46
Filopodia formation	PtdIns(4,5)P ₂ -containing vesicles, actin, N-WASP and Arp2/3 complex	48
Membrane scission	<i>Vps20</i> , <i>Snf7</i> and <i>Vps24</i>	67

Arp2/3, actin-related protein 2/3; GUV, giant unilamellar vesicle; ICAM1, intercellular adhesion molecule 1; MHC, major histocompatibility complex; NPF, nucleation-promotion factor; N-WASP, neuronal-Wiscott–Aldrich syndrome protein (also known as WASL); PtdIns(4,5)P₂, phosphatidylinositol-4,5-bisphosphate; *Snf7*, sucrose non-fermenting protein 7; *Vps*, vacuole protein sorting protein.

More recently, dynamic filopodia-like membrane deformations were reconstituted on GUVs⁴⁸. It was previously thought that filopodia emerge from branched dendritic actin networks by a process that involves bundling of actin filaments by accessory proteins. Furthermore, the length of cellular filopodia was thought to be limited by membrane resistance force, with filopodia expected to buckle if they grew past the theoretical Euler buckling length — where a structural element can no longer withstand compressive stress. The reconstitution of filopodium-like structures in the absence of both the tip complex (a molecular assembly that promotes actin filament elongation) and the crosslinking protein fascin led to two physical insights: the membrane alone can facilitate the transition of a branched actin network to a parallel array of actin filaments, and the force generated by membrane tension will not buckle filopodia, even

those beyond the Euler buckling length⁴⁹. The reconstitution of complex actin-based structures through interactions with a lipid bilayer suggests that in early cellular life, liposome-encapsulating force-generating polymers could have exhibited rich morphological behaviours that are reminiscent of current living systems.

Last year, the use of multilamellar vesicles to study the bacterial tubulin homologue, filamenting temperature sensitive mutant *Z* (*FtsZ*), revealed that membrane-anchored *FtsZ* alone can self-assemble into ring-like structures known as *Z* rings, which serve as scaffolds for the bacterial division apparatus⁵⁰. The reconstituted *Z* rings generated a constriction force that was dependent on GTP hydrolysis. However, because constriction of the multilamellar vesicles by the *Z* rings was incomplete, it remains possible that additional force-generating activity might be required to complete the process.

Tools for cellular reconstitution

Continued progress in cellular reconstitution will depend on the creation of new tools. Recently, techniques that enable efficient encapsulation of components inside cell-sized vesicles have been developed, making it possible to begin controlling both internal and external biochemical conditions for studies involving transmembrane signalling and transport (BOX 1). Micro-fabrication and patterning tools will also continue to advance, enabling the control of molecular location for activity gradients or three-dimensional confinement^{51,52}.

Central to the study of spatial organization in cells is the widespread use of light microscopy. Traditional biochemical reconstitution techniques typically report the average behaviour of the system and yield little information on spatial organization. By contrast, coordinated cellular processes that are dependent on the collective motion of many molecules often require microscopy to follow their dynamic evolution in real time. Commercial wide-field and confocal light microscopes have become mainstream tools in biology laboratories, and researchers continue to push light microscopy to higher spatial and temporal resolutions. Several techniques that break the diffraction-limited spatial resolution of conventional light microscopes have been demonstrated, and their uses, as well as current developments in fluorescent probe technology, have recently been reviewed⁵³.

Computational tools have been instrumental in guiding experimental approaches to cellular reconstitution as well as in understanding the complex behaviour they create. Mathematical modelling and computer simulations will continue to be crucial for explaining the observations from *in vitro* reconstitution experiments^{16,18,45,54}, and we expect their importance to grow as both experimental and computational techniques improve.

Learning by building

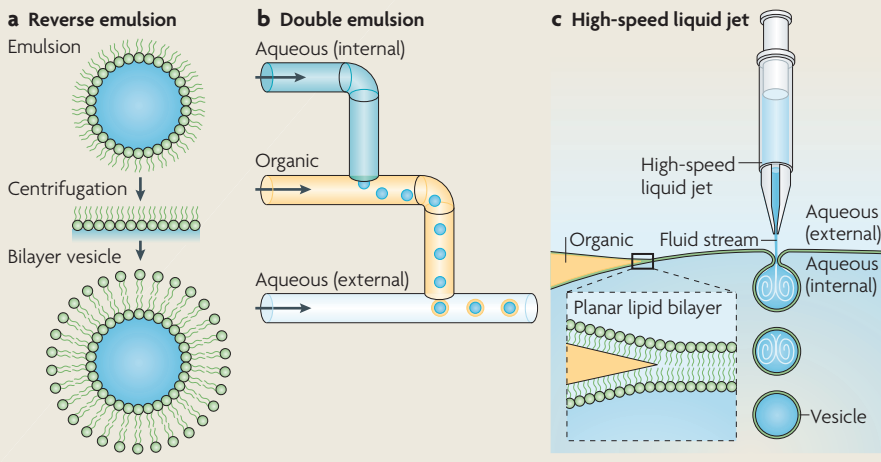
Cell biology has come a long way since Szent-Györgyi and Straub's biochemical reconstitution of muscle actomyosin and Arthur Kornberg's biochemical reconstitution of DNA synthesis more than half a century ago. Although the knowledge of an enzyme's binding affinity and catalytic efficiency gives us sufficient information to predict its turnover capability, we do not yet have the power to predict how a mixture of macromolecules will assemble into spatially complex structures, even with a thorough characterization of the individual parts. Cellular reconstitution offers a way to learn by building.

Box 1 | Advances in vesicle encapsulation

Encapsulation of biomolecules in cell-like vesicles represents an important step in cellular reconstitution, as biological systems naturally operate in confined volumes rather than in test tubes. There have been several reports in recent years that describe new methods for vesicle encapsulation and three are described here. A technique called reverse emulsion was used to make asymmetric lipid vesicles that encapsulate cytoplasmic extract (see the figure, part a)^{55,56}. In this technique, an emulsion of lipid-stabilized aqueous droplets in oil is centrifuged through a planar interface with an aqueous solution, adding a second monolayer of lipids to create bilayer vesicles as they pass into the aqueous phase.

Microfluidic channels have been used to generate monodisperse emulsions and vesicles with high fidelity and high throughput. For example, monodisperse double emulsion (water–oil–water) structures can be made using a fabricated microfluidic device that uses a continuous flow to deposit aqueous droplets into an organic phase and then deposit the emulsion with an oil shell back into an aqueous flow (see the figure, part b)^{57,58}. Double emulsions can become vesicles through solvent evaporation, in which lipid molecules dissolved in the organic phase form a lipid bilayer on removal of the oil⁵⁹.

Recently, a microfluidic jetting technique analogous to blowing soap bubbles was demonstrated. Both double emulsions and unilamellar lipid bilayer vesicles have been formed by shooting a high-speed pulsed liquid jet against a solvent-supported planar lipid bilayer (see the figure, part c)^{60,61}. These techniques hold great promise in cellular reconstitution for encapsulating biomolecules in lipid vesicles.



Advances in imaging technology, combined with clever experimental techniques for controlling boundary conditions, have led to successful reconstitution of several self-organizing biological processes, with more on the way. However, because cellular reconstitution includes only a subset of components used by cells, the approach is not guaranteed to yield the precise mechanistic details of a biological process. So how can we know whether a reconstitution is behaving 'biologically'? Perhaps one day we will need the cellular equivalent of a Turing test — the approach devised by Alan Turing in 1950 to test a computing machine's ability to demonstrate intelligence — to tell the difference, asking the question: does the reconstitution behave like a normal cell in every way? Until then, we will need to use modelling to bridge the gap between small assemblies and cells, and we will need to use cellular reconstitution to complement top-down approaches, all while deliberately peeling away the levels of complexity and regulation that are at work in live cells.

The road map for cellular reconstitution will probably include experiments that are motivated by basic science as well as those that are aimed at creating biological systems that exhibit customized functional behaviour — a kind of bottom-up synthetic biology. It is conceivable that one day our ability to reconstitute basic modules of cellular life will lead us to create a growing and dividing system that can be computationally designed to crawl, swim or fight disease. Now that would be intelligent design.

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doi:10.1038/nrm2746

Published online 12 August 2009

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Acknowledgements

We apologize to those colleagues whose original and important work could not be cited owing to space limitations. We thank D. Richmond, J. Stachowiak and the rest of the Fletcher laboratory, as well as T. Pucadyil, for helpful discussion. A.P.L. is supported by the Natural Sciences and Engineering Research Council of Canada. D.A.F. is funded by National Institutes of Health R01 grants and a Nanomedicine Development Centre grant.

DATABASES

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