Building the cell: design principles of cellular architecture

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Abstract | The astounding structural complexity of a cell arises from the action of a relatively small number of genes, raising the question of how this complexity is achieved. Self-organizing processes combined with simple physical constraints seem to have key roles in controlling organelle size, number, shape and position, and these factors then combine to produce the overall cell architecture. By examining how these parameters are controlled in specific cell biological examples we can identify a handful of simple design principles that seem to underlie cellular architecture and assembly.

Self-organizing process
A process by which a set of components that can, in principle, be connected in various possible patterns will spontaneously associate into a limited subset of patterns, without any external input of information. 'Self-organization' is to be contrasted with 'self-assembly', in which components can only fit together such that only one pattern is possible.

Cilium
A microtubule-based motile and sensory organelle that projects from the surface of many eukaryotic cells.

Flagellum
An alternative term for cilia when applied to eukaryotic cells.

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Cells are highly complex structures[1]. This complexity affects biological functions at many size scales[2]. But how does this complexity arise? What mechanisms pattern the architecture of the cell and where does the information to specify a distinct architecture for different cell types come from?

The amount of information that is needed to direct the action of the cellular assembly pathways could be minimal because cellular morphogenesis is a self-organizing process[3]. Subject to simple inputs that can bias how the system develops, self-organizing processes can generate complex, patterned responses. In principle, therefore, the cell architecture could be specified by a small number of distinct genes, which would serve to modulate the inherent self-organizing processes that lead to the physical shape of cells and organelles. Understanding the origins of cell organization thus requires us to answer the question of how the self-organizing physical and biochemical properties of cellular constituents can convert simple temporal changes in gene-expression levels into complex changes in physical, three-dimensional structures. This review addresses this question at the structural level of the organelle by asking how the geometry (the size, number, position and morphology) of organelles is specified and regulated.

In all cases, we will attempt to identify design principles by which a simple input containing minimal information can produce a complex output in terms of morphological changes. In engineering terminology a design principle is a simple rule that, when followed in the design of a machine, ensures or at least increases the likelihood of proper assembly and function. We note that our use of the term does not imply the existence of a 'designer', but given the current interest in synthetic biology we suggest that the design principles used in evolution could now be used to reprogramme cells in a rational way. For example, we argue that a common strategy used by cells to regulate organelle size is to limit either the assembly or disassembly rate of an organelle by a process that is inherently dependent on organelle size. Other specific principles that we discuss are the control of organelle number by the balance of organelle formation and partitioning; the establishment of cellular polarity by self-organization that is biased by external inputs; the control of organelle position by direct motion on polarized cytoskeletal arrays; and the control of organelle shape by membrane-binding proteins that drive local curvature changes.

How cells measure size and length

Length and size are fundamental parameters for specifying geometry. Organelle size must be appropriately scaled as a function of cell size[4] and physiological demands. Mitochondrion size in yeast, for example, is proportional to cell volume (G. Pesce, The Molecular Sciences Institute, Berkeley, personal communication). Organelle size is tightly controlled: for example, the coefficient of variation in the lengths of eukaryotic cilia and flagella (the two terms will be used interchangeably in this review) is less than 10% (REF. 5). But how are the sizes of subcellular structures determined? Genetic screens have identified genes that are involved in size control for various organelles, including cilia and flagella[6], peroxisomes[7] and lipid droplets[8]. However, in most cases the mechanistic basis for size alterations in these mutants remains unknown.

Measuring length using molecular rulers. One way to control the size of linear structures is for the cell to measure the length of the structure with a molecular ruler protein (FIG. 1a) which has a physical length that

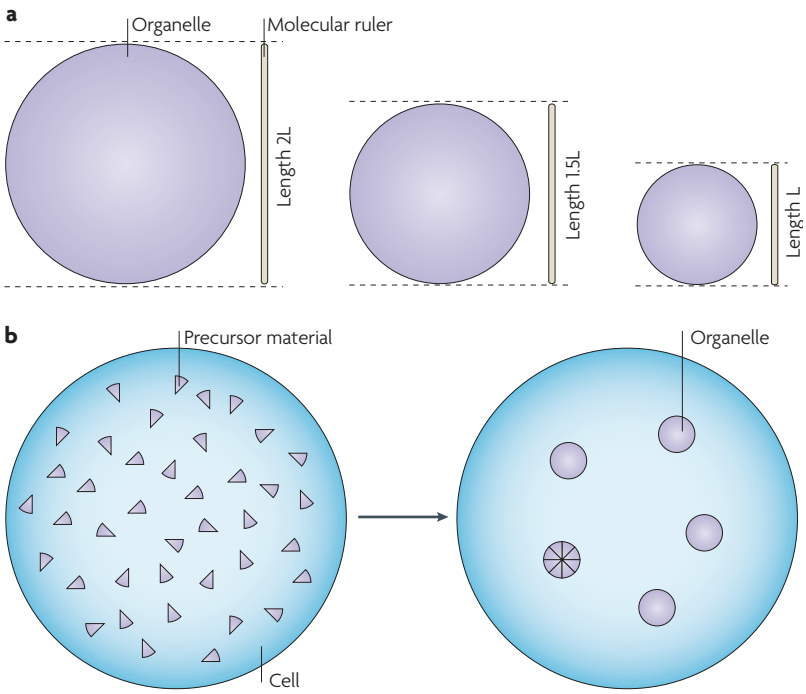

Figure 1 | **Design principles of organelle size control. a** | Organelle size can be controlled by molecular rulers. The physical length of a molecular ruler protein determines the size of a cellular organelle. **b** | Organelle size can be controlled by quantal synthesis whereby the precise amount of precursor material is synthesized by the cell in order to build the correct number of organelles of the correct size.

equals the desired length of the overall structure. For example, the length of the λ-phage tail is dictated by the size of the gene *H* product[9]. Deletions in gene *H* produce shorter tails and insertions produce longer tails. One end of the gene *H* product is attached to the capsid and the other tracks the growing tail, protecting it from a growth-terminating factor until the tail finally exceeds the length of the gene *H* product and growth terminates. Rulers have been proposed for length control of bacterial flagellar hooks[10], bacterial type III secretion needles[11] and sarcomere actin filaments in muscle[12].

Size control by quantal synthesis. Size could be determined by synthesizing precisely enough material to build a structure of the correct size (FIG. 1b), a model that has been termed quantal synthesis[13]. Precursor protein levels affect the length of cilia/flagella in the unicellular green alga *Chlamydomonas reinhardtii*[14] and are under tight regulation during flagellum regeneration[15]. In sea urchin cells, the lengths of cilia correlate with the concentration of the protein tektin, which is produced by the cells[13]. However, new flagella can grow to roughly half of their normal length when protein synthesis is inhibited in *C. reinhardtii*[14], which shows that cells retain a pool of unused precursor protein. Moreover, a simple model in which the amount of precursor directly determines flagellum length would predict a geometric dependence of flagellum length on number. For example, if flagella double in number, then the average length of each flagellum should halve. However, mutants with varying numbers of flagella show a significantly weaker dependence of flagellar length on number[16]. Therefore, it seems that the levels of precursor protein might not be the only length-controlling mechanism at work in flagella.

Another example of an organelle whose size is apparently regulated by the synthesis of components is the endoplasmic reticulum (ER). Forced upregulation of lipid-biosynthesis enzymes leads to an increase in the size of the ER[17]. In this case there seems to be a feedback pathway that controls lipid synthesis as a function of ER size: the unfolded protein response (UPR), a signalling pathway that monitors proper ER function, and controls the transcription of many genes, including those that encode lipid-biosynthesis enzymes[18]. If the amount of lipid controls the size of the ER, and the UPR activates lipid synthesis, then it is predicted that constitutive activation of the UPR pathway should cause an increase in the size of the ER membrane. This prediction has been experimentally verified[19].

One prediction of the quantal synthesis model is that multiple organelles with common limiting components will compete with each other, such that one organelle can only grow if the others shrink. This has been demonstrated experimentally for flagella in *C. reinhardtii*, in which the severing and regeneration of one flagellum in a biflagellate cell induces the other intact flagellum to shorten[20].

Dynamic balance mechanisms. Most cellular structures undergo continuous turnover, and hence size maintenance requires the rates of assembly and disassembly to precisely equal each other only when the correct structure size has been reached. If assembly or disassembly rates are inherently size-dependent then there will only be a limited number of sizes at which the two rates become equal. If there is only one size at which the assembly rate equals the disassembly rate then this will be the steady-state organelle size (FIG. 2a). For example, eukaryotic flagellar microtubules undergo continuous assembly and disassembly at their tips. Of these, the disassembly rate is length-independent and the assembly rate is a decreasing function of length[16,21]. Longer flagella assemble slower than shorter flagella because transport within the flagellum, which is mediated by a kinesin-based system called intraflagellar transport[22], becomes less efficient[16,21]. This limits the availability of precursor protein to support tip assembly, thus reducing the assembly rate with increasing length. As the rate of assembly is a continuously decreasing function of length (whereas the rate of disassembly is length-independent), there is only one length at which the two rates balance, and this sets the steady-state flagellar length.

A similar dynamic balance mechanism might control the length of actin-based structures, such as microvilli or the stereocilia of the mouse inner ear. Actin filaments in these structures constantly treadmill back towards the cell body, with disassembly at the base balanced by continuous assembly at the tip. In turn, this depends on the diffusion of free actin to the tip[23,24]. The requirement for actin to diffuse to the tip provides an inherent

dependence of the assembly rate on the length of the filament. Diffusion-induced length dependence of the assembly rate has also been suggested for the control of bacterial flagella length[25].

Yeast kinesin-8 binds along the length of microtubules, moves processively to their tips and then catalyses disassembly. Longer microtubules recruit more kinesin-8, which results in a length-dependent disassembly process that, when combined with a constant rate of assembly, yields a unique steady-state microtubule length[26]. Unlike flagella and microvilli, it seems to be the disassembly of the microtubules, rather than the assembly, that is regulated as a function of length. In complex cytoskeletal assemblies, such as the mitotic spindle, length control might involve multiple opposing length-dependent processes in a more complicated dynamic balance[27].

The same types of dynamic balance mechanisms that are used to control length could also be used to control the volume or surface area of the organelle. If an organelle such as the Golgi apparatus exchanges vesicles with the ER, vesicle fusion on the organelle occurs at a rate that is dictated by the rate of vesicle budding from the ER, rather than by the organelle size. By contrast, vesicle budding from the organelle could occur at a rate that is proportional to the surface area of the organelle (assuming a constant density of budding sites). The rates of membrane addition and membrane removal would only balance each other for a single surface area value, thereby dictating the surface area of the organelle. If the organelle became larger than this the budding rate would increase, leading to a reduction in surface area: if the organelle became too small the budding rate would decrease relative to the fusion rate and hence it would enlarge to its steady-state surface area.

How cells control organelle number

A typical eukaryotic cell has one nucleus, two centrioles, one ER, multiple mitochondria, scores of endosomes and many ribosomes. How is the number of organelles controlled?

Number control as a dynamic balance. The number of organelles reflects the balance between two competing processes: the increase in number due to *de novo* synthesis or fission of pre-existing organelles; and the decrease in number due to fusion with other organelles, degradation processes such as autophagy, or the partitioning of organelles into daughter cells during cell division. To control the number of organelles, one or more of these processes must be regulated. It has been proposed, for example, that the synthesis of chloroplasts is regulated as a function of the number of chloroplasts in algal cells[28]. Cells that inherit too few chloroplasts will make more, whereas cells that inherit too many will stop chloroplast synthesis. A similar scheme has been experimentally demonstrated for centrioles[29]. Therefore, the control of organelle number shares a conceptually similar strategy to the dynamic balance mechanisms of organelle size control (FIG. 2).

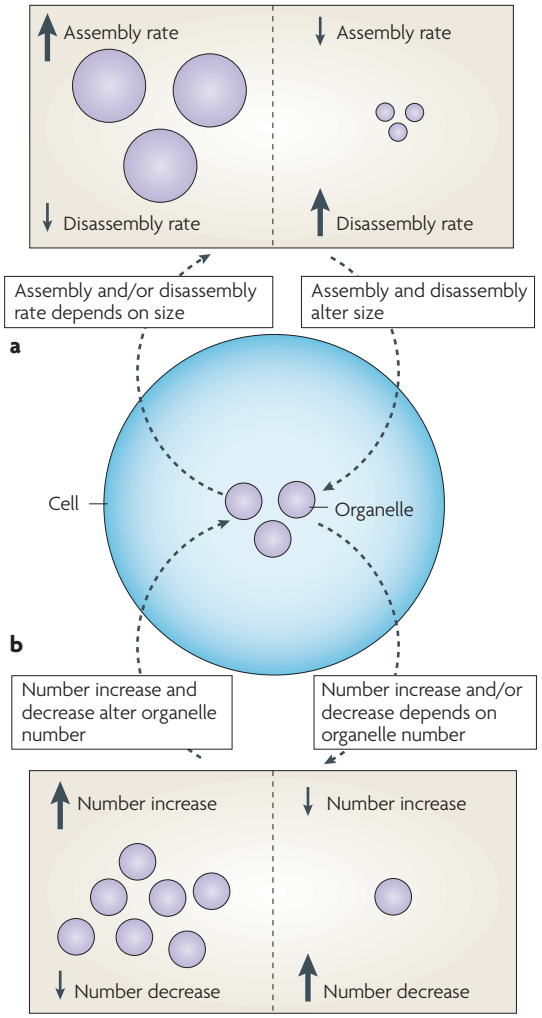

Figure 2 | **The dynamic balance mechanism to control size and number. a** | Dynamic balance to control organelle size. The size of the organelle feeds back onto either the assembly or disassembly rate such that one of these is inherently size-dependent. Thus, the assembly rate and disassembly rate are equal to each other only at the correct size of the organelle. The organelle size changes if the regulation of either the assembly or the disassembly is altered. **b** | Dynamic balance to control organelle number. The mechanisms that regulate either the increase or decrease of organelle number are themselves a function of the organelle such that the correct number of organelles is maintained. The number of organelles changes if the underlying regulation of either the increase (thick arrow) or the decrease (thin arrow) in organelle number is altered.

Controlling the increase in organelle number. The fact that organelle production can be regulated by the existing number of organelles suggests that cells can count. For example, an absence of centrioles activates a *de novo* assembly pathway[30,31], whereas an excess of centrioles (> 2) inactivates further centriole assembly[29]. Peroxisomes undergo highly regulated proliferation, the rate of which is adjusted in response to unknown intracellular cues that control steady-state peroxisome number[7]. Apparently, cells can detect whether the

Mitotic spindle
A highly dynamic array of microtubules that forms during mitosis and serves to move the duplicated chromosomes apart.

Centriole
A short, barrel-like array of microtubules that organizes the centrosome and contributes to cytokinesis and cell-cycle progression.

Autophagy
A pathway for the recycling of cellular contents, in which materials inside the cell are packaged into vesicles and are then targeted to the vacuole or lysosome for bulk turnover.

organelle number has drifted outside of a preset range. One possible mechanism by which cells count organelle number would be for the organelles to generate a diffusible molecular signal at a constant rate per organelle. This signalling molecule would degrade with a constant half-life. As a result, the steady-state concentration of the signal would be proportional to the number of organelles. The concentration could then be used to regulate the assembly of new organelles. One such mechanism seems to function in the control of plasmid replication, in which plasmids encode diffusible *trans*-acting autoinhibitors of plasmid replication at a constant rate per plasmid[32]. This type of mechanism relies on measuring the ratio of organelle number to cell volume and could thus be tested by manipulating cell size[33].

Reducing organelle number. The number (N) of organelles can be reduced by their partitioning between daughter cells. If N organelles distribute randomly between two daughter cells, the number of copies that a given daughter receives will vary randomly according to binomial statistics. For each organelle that segregates at random, it is as though one flips a coin to determine which daughter cell receives that organelle. If one flips a coin a few times, the number of flips that come up heads can vary substantially. Similarly, if organelles partition at random the actual number that a given daughter inherits can fluctuate substantially. One way to dampen such fluctuations is to fragment the organelle into a large number of small pieces so that the effective value of N becomes very large. In this case most daughter cells will inherit a number that is close to N/2. Alternatively, the mitotic spindle can be used to segregate organelles non-randomly between the two daughters, as with centrioles. For many organelles, it is still not entirely clear how they are partitioned and whether this partitioning is truly a random process or not. For example, endosomes and lysosomes associate with spindle poles but still distribute according to binomial statistics[34]. Golgi segregate using the mitotic spindle poles in some organisms[35], whereas in others they fragment and scatter randomly between the daughters[36]. It should be noted that partitioning by the spindle will not necessarily reduce fluctuations in organelle numbers if individual organelles associate with the spindle poles at random[37]. Regardless, partitioning is certain to reduce organelle number because the initial number, N, must somehow be divided among the two daughter cells.

Organelle number might also be reduced by the active destruction of existing organelles when the number exceeds a threshold. Such a process has been documented for peroxisomes and involves an autophagy-like mechanism[38]. In ciliates, the number of cilia in various cortical structures is proportional to the cell size. When the cell volume is experimentally reduced in the absence of cell division, the number of cilia is reduced so as to maintain a cilia number that is in constant proportion to cell volume[39,40]. These experiments suggest that active degradation pathways, such as autophagy, can sense the relative concentration of organelles per unit volume, rather than a strict absolute number. We speculate that active organelle degradation might be triggered by the accumulation of diffusible factors produced by individual organelles, a model that is formally equivalent to the inhibitor-dilution model for control of organelle production that is discussed above[32]. However, the diffusible molecule would trigger degradation rather than inhibit biosynthesis.

Spatial organization and polarity

A key aspect of cell geometry is the fact that both cell shape and intracellular organization are polarized. Cell polarity is an asymmetry of shape or of protein distribution, for example, between the front and the back in motile cells or between the top and the bottom in epithelial cells. A non-polarized cell becomes polarized through the loss of symmetry, occurring as a result of an extrinsic or intrinsic localized cue or occurring spontaneously as a result of underlying self-organizational mechanisms that are inherent in the cell or system. Polarization and directed motility do not require gene transcription or even the nucleus (as demonstrated by experiments using anucleate cytoplasts)[41,42].

Spontaneous polarization has been observed within cells, including the formation of a polarized distribution of the signalling protein Cdc42 at the plasma membrane in *Saccharomyces cerevisiae*[43], the polarization of neutrophils in a uniform bath of chemoattractant[44], and the initiation of motility in stationary keratocytes[45]. In the case of keratocytes, spontaneous myosin-dependent actin contractility at the presumptive rear of the cell leads to the increased inward flow of filamentous (F)-actin and the retraction of the rear. These changes in F-actin organization and dynamics propagate globally and result in the decrease of F-actin flow at the cell rear and the induction of actin-polymerization-based protrusion at the front of the cell, thus leading to persistent keratocyte motility[45]. The common design principle in establishing cell polarity seems to be the existence of intrinsic self-organizing polarity systems, in which the cell exhibits fluctuating asymmetry in the localization of signalling proteins or cytoskeletal dynamics. The cell then responds to a stochastic amplification in these fluctuations by initiating a positive-feedback loop, which involves both signalling networks and cytoskeletal structures that locally amplify the initial symmetry-breaking event and further regulate other mechanochemical polarizing processes throughout the cell (FIG. 3a,b). Positive feedback alone would lead to constant uniform activation of polarizing processes. Therefore, polarization to a discrete site also requires long-range negative interactions to suppress the spread of polarization[43,46]. The combination of both local positive feedback and the long-range inhibition of polarizing processes allows spontaneous symmetry breaking and self-organization. Such systems are then biased by upstream spatial inputs in order to drive polarization in a desired direction.

Organelle positioning in response to polarity. Cell polarity directs the positioning of intracellular organelles. This positioning is often crucial for their function, for example, in determining the orientation of stereocilia

Binomial statistics
A statistical distribution that describes the probability distribution that is obtained by several successive decisions, each of which has two possible outcomes with constant probabilities. For example, the distribution of the numbers of heads or tails after a particular number of coin flips.

Fluctuating asymmetry
Asymmetry resulting from slight stochastic differences in the molecular concentration in two different regions of a cell.

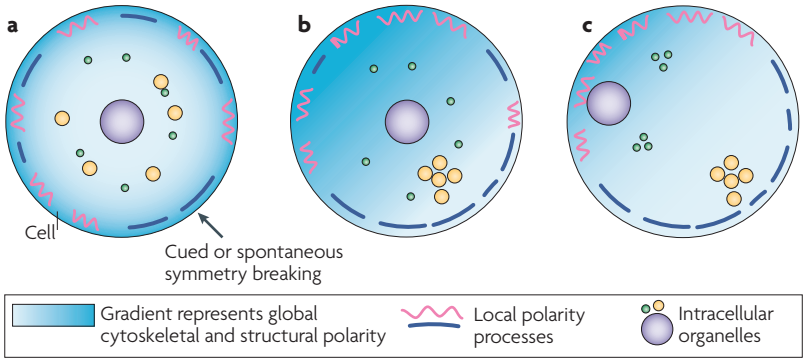

Figure 3 | **Design principles of polarity and organelle positioning. a** | Local polarity processes (including cytoskeletal dynamics and protein localization) undergo stochastic fluctuating asymmetry. The cell responds to either a stochastic or cued amplification in these fluctuations by locally amplifying this symmetry-breaking event. Meanwhile, organelles are randomly positioned within the cell. **b** | Local polarity processes induce global cytoskeletal or structural polarity (shown by the gradient). Long-range suppression of these polarity processes prevents them from being activated uniformly throughout the cell. Together, the local amplification and long-range suppression of polarity processes amplify the overall polarity of the cell. Some organelles (yellow) are positioned by this global cell polarity. **c** | The positioning of some organelles (yellow) further amplifies the global cytoskeletal or structural polarity (the gradient steepens) and controls the positioning of other organelles (green and grey) in the cell.

bundles in response to planar cell polarity cues in the cochlea[47,48] or the orientation of the secretory apparatus towards the targets of natural killer immune cells[49]. In migrating cells, many organelles, including mitochondria and the Golgi apparatus, are positioned relative to the axis of polarization[50,51]. A particularly dramatic example of polarized organelle positioning occurs during the development of neurons, in which the positions of the Golgi apparatus and the centrosomes strongly correlate with sites of neurite outgrowth[52].

Mechanistically, it is not clear how organelles become repositioned in response to polarity cues. Organelles might move by random diffusion until they collide with a target docking site, although it is unclear whether the diffusion of organelles in the crowded cytoplasm is fast enough to allow such a diffuse-to-capture mechanism to operate[53–56]. Perhaps for this reason, organelle positioning often involves the cytoskeleton, which can allow for more rapid, directed transport of organelles by motor proteins or specific subcellular localization through the regulation of cytoskeletal distribution and dynamics. For example, cortical actin flow drives the polarized redistribution of the cortical ER and myoplasm (mitochondrially enriched cytoplasm) in Ascidian oocytes[57] and establishes the nucleus–centrosome axis in migrating cells during wound healing[58].

Feedback from organelle positioning. One common theme in polarized cells is the non-random distribution and dynamics of the microtubule array. Work has recently focused on the plus-end microtubule-tip-binding proteins and motors that track with microtubule plus ends and interact with other signalling or motor proteins that are localized to the cell cortex[59–61]. These proteins allow the selective stabilization of microtubule dynamics and the proper localization of the microtubule cytoskeleton within the cell[62,63]. For example, the microtubule array is polarized and microtubules are selectively stabilized towards the leading edge in motile cells[64].

The position of the microtubule array can in turn affect the position of organelles, such as the Golgi apparatus in mammalian cells. The Golgi is located adjacent to the centrosome and its position is dependent on both the microtubules and the motor protein dynein[65–67]. The cisternal compartments of the Golgi have a polarized organization, and their orientation depends on the cell type and on both dynein and kinesin molecular motors moving on polarized microtubules[65]. For example, in motile cells the *trans*-Golgi network (TGN), from which vesicles that are destined for the plasma membrane bud, is orientated towards the outside of the cell, whereas the *cis*-Golgi is orientated inward. The TGN has been shown to harbour microtubule-nucleation ability and Golgi-attached microtubules exhibit increased stability[68]. It was recently further shown that the TGN protein GCC185 recruits CLASPs (CLIP-associated proteins), which stabilize small microtubule seeds that are nucleated at the TGN[69]. This leads to a population of stabilized microtubules emanating from the TGN, preferentially towards the leading edge[69], which might be required for cell motility[70]. Thus, the positioning of the Golgi in response to cell polarity might itself contribute further to processes that promote cell polarity. This sort of organelle position-dependent positive-feedback loop could potentially lead to the spontaneous formation of a polarized intracellular axis, although to our knowledge this has not been tested.

Organelles can also feedback on the positioning of other organelles. In *C. reinhardtii*, which exhibits stereotyped positioning of its intracellular organelles, the mother centriole directs the positioning of the daughter centriole, the nucleus and possibly the contractile vacuole[71]. This suggests that perhaps the mother centriole acts more generally to coordinate the overall geometry of the many subcellular structures in this organism.

After the initial polarization, some organelles are repositioned in the cell in response to the now orientated cytoskeleton (FIG. 3b). These organelles can in turn redirect the position of the cytoskeleton and further enhance the global cell polarity, and can cause the reposition of other organelles through an organelle position-dependent feedback loop (FIG. 3c). Because the underlying polarity system is self-organizing, it does not require high information content in its inputs, but because it is coupled to the cytoskeleton and ultimately to organelle positioning, the polarity system can lead to complicated responses to simple inputs.

Dynamic shape specification

Organelles take on a striking variety of shapes that range from interconnected tubular networks of the mitochondria and peripheral ER to carefully localized stacks of membranes in the Golgi cisterna, and to the large continuous membrane sheets in the nuclear envelope and regions of the ER. How are these distinct shapes achieved?

Centrosome
An organelle that contains the centrioles and that anchors the 'minus' ends of microtubules.

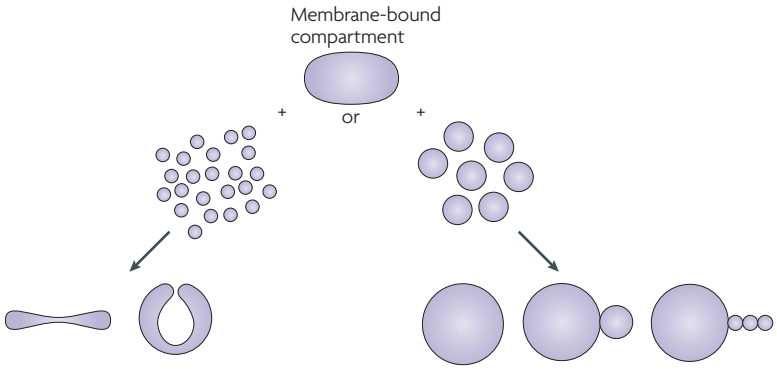

Figure 4 | **The regulation of organelle shape by changing the vesicle size.** Depending on the number and size of the vesicles that fuse with a membrane-bound compartment, the resultant compartment can display significant shape differences. Small vesicles would increase the surface area of the compartment more than the volume, whereas large vesicles would increase the volume over the surface area. While the possible resulting shapes of the compartment are extreme, this sort of control mechanism could also create many more subtle variations in compartment shape and function.

Shaping membrane curvature. The shape of an organelle can be completely defined by specifying the local curvature of the surrounding membrane at each point. The membrane curvature depends on the surface-area-to-volume ratio (FIG. 4) and the preferred intrinsic curvature of the membrane. Indeed, changes in the surface-area-to-volume ratio and the difference in area between the two lipid monolayers can lead to a wide range of dramatic transitions in shape, such as to spheres, flattened discs, cup-shapes and even complex star-like shapes[72] (FIG. 5a). These shapes can be explained by theoretical models that minimize the energetics of membrane deformation[73–75]. Different lipids preferentially stabilize different degrees of membrane curvature and can profoundly affect the organelle shape[76]. Local membrane curvature can drive the segregation of lipids, which can then influence further curvature changes, suggesting a potential for complicated feedback loops between local curvature and lipid composition[77,78] (FIG. 5a). Phase-separated vesicles and tubules can even spontaneously bud owing to the induced local membrane curvature of segregated domains[79–81].

Membrane-associated proteins can also affect membrane curvature[82–84] (FIG. 5b), as is believed to be the case for the BAR-domain-containing proteins[85]. An extreme example is that when purified matrix protein of Newcastle disease virus is added to membranes, they deform and bud off vesicles of similar sizes to virus particles in infected cells[86].

Determining the tubular morphology of ER. The ER consists of a membrane-bound compartment that is continuous with the nuclear envelope. There are two types of ER: the rough ER, which is usually composed of membrane sheets, and the smooth ER, which is usually composed of a tubular network that extends to the cell periphery in mammalian cells or is attached to the periphery in yeast[87]. Membrane tubes can be formed *in vitro* by the activity of motor proteins, which pull the lipid membrane into tubes as they move along microtubules[88–90] (FIG. 5c). These membrane tubes resemble tubular ER networks. However, membrane tubes can also be formed from purified ER-membrane fractions and cytosol, in the absence of microtubules, in a process that is dependent on cytosol-regulated vesicle–membrane fusion[91]. This suggests that a microtubule-independent regulation of ER networks exists. The movement of the ER along and the direct attachment of the ER to microtubules, however, is required for its peripheral localization in mammalian cells[92–93].

Insights into how the tubular morphology compared with the sheet-like morphology of the ER is regulated has come from the identification of the reticulon proteins in yeast and mammalian cells, which localize to the tubular ER and are excluded from the sheets[94,95]. Altering the amounts of these proteins in cells induces transitions between a more tubular ER and a more sheet-like ER[94]. Reticulons contain two long hydrophobic domains that are thought to induce membrane tubulation by constraining local membrane curvature[94,96].

Dynamic morphology of mitochondria. Depending on the cell type, mitochondrial shapes range widely from small individual organelles, as commonly described in textbooks, to large interconnected networks[97]. Mitochondrial morphology involves a combination of the underlying tubular structure, the constant tubule fusion and fission dynamics which regulate the degree of mitochondrial fragmentation, and the tubule movement along or the attachment to the cytoskeleton and cell periphery[98–101]. In mammalian cells mitochondria form tubular networks that spread throughout the cell along the microtubule cytoskeleton[97], whereas in budding yeast they form an interconnected tubular network that is localized to the inner cell surface which forms a cage-like structure around the cell[102]. The cell regulates the overall degree of mitochondrial fragmentation in response to the cell cycle[103,104] or apoptotic signals[105]. The mitochondrial tubule fusion and fission dynamics constantly remodel the connections within the mitochondrial network, and therefore perhaps provide a mechanism for the cell to remodel the mitochondrial network, for example, from a more simplified to a more complex and ramified network, as observed in yeast cells in response to changes in growth condition[106].

The wealth of imaging and genetic tools available to study mitochondrial morphology makes this organelle an ideal model for determining the minimum requirements that are needed to establish a complex organelle morphology from simple algorithmic rules. It is also an ideal model for understanding the basic design principles that underlie the precise regulation of mitochondrial morphology in response to the functional needs of a cell. These sorts of studies, however, require rigorous methods to quantify the dynamic morphology of mitochondria to compare them with modelled structures.

Membrane-bound organelle morphology results from basic physical effects (including surface-to-volume-driven curvature distributions and curvature-induced lipid partitioning; FIG. 5a) combined with protein-induced

Dynamic morphology
A term that considers complex organelles not as static structures, but as encompassing the effects of constant shape-altering dynamics.

curvature changes (FIG. 5b) and the attachment to and motor-driven movement along cellular structures (FIG. 5c). Thermodynamically, energy input into the system by the cytoskeleton, by the production and turnover of membrane-associated proteins and by the generation of specific lipid composition changes allows the membranous systems to attain more complicated structures than a simple lipid-bound vesicle could attain at thermodynamic equilibrium. Further precise control of the shape-altering dynamics of these structures imparts a flexibility to their morphologies that allows them, in a controlled manner, to regulate the cellular functions accomplished by these complex structures.

Propagation of structure

Because cells are dynamic systems, their organization must be continuously regulated. It seems most likely that the genome exerts its control over cellular structure not by explicitly dictating where each part of the cell has to go and how it is to be shaped, but rather by providing small course corrections that steer the evolving organization of the cell in particular directions. The steering of dynamic systems by small perturbations is an important principle of control-system theory and has been applied to control biological systems, such as heart rhythms.

The dynamic nature of the cell structure also means that the influence of a given pattern of gene expression on the cell organization will be a strong function of the current state of the cell — the history of a structure of a cell directly affects its future dynamic organization. It has been observed that cells can re-form their original shape after the removal of chemical inhibitors that disrupt cell shape[107–109]. Cells thus have a persistent state which is gradually modified by dynamic processes.

If cells can restore their geometric state following transient perturbations, one might wonder how much of cellular structure persists following a round of division: cell division is, in essence, a geometrical perturbation. This question can be potentially addressed by comparing the structural similarity between sister cells. In fact, several studies have suggested that sister cells are similar and might even be mirror images of each other[110–113]. However, these studies have been based on visual observation and the statistical strength of the similarity between sisters is yet to be rigorously demonstrated. The degree to which sister cells are mirror images of each other is therefore an open question, whose answer will have strong implications for the importance of pre-existing structure in the dynamic organization of the cell.

In cells with sufficiently complex structures, it has been possible to test for the inheritance of spatial organization by experimentally rearranging cellular components and testing whether the rearranged geometry persists. The famous experiments of Beisson and Sonneborn on inverted ciliary rows in *Paramecium aurelia* clearly show that artificially rearranged structures can be propagated through division[114]. This phenomenon has been extensively investigated with a series of elegant experiments in various types of ciliates, which have shown that certain aspects of a rearranged organization can be inherited and other rearrangements can self-correct. An example is provided by the oral apparatus,

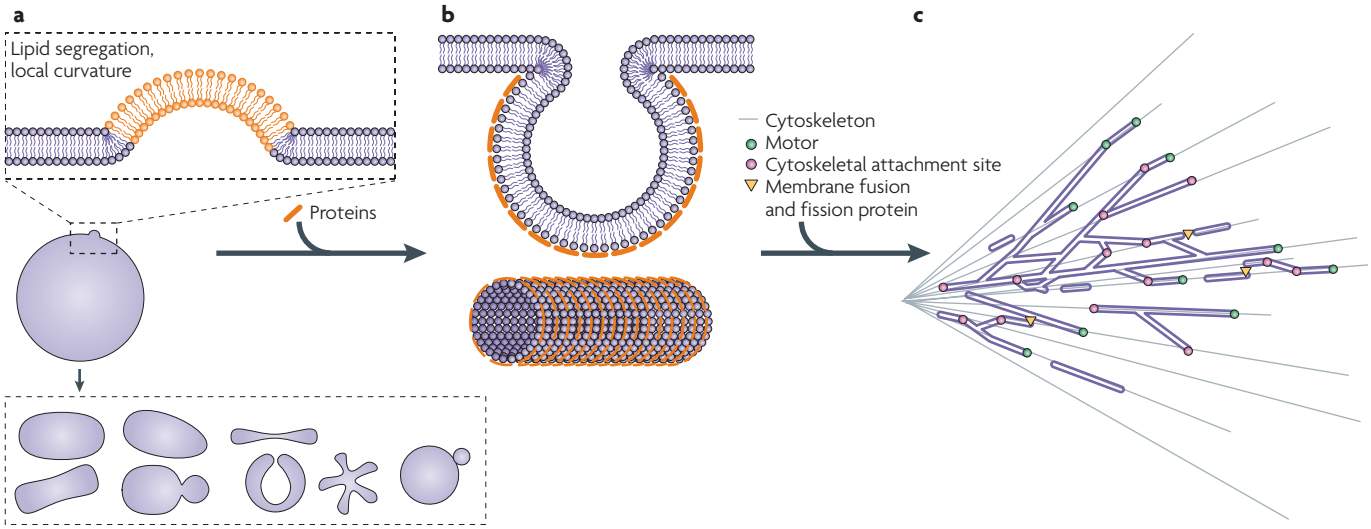

Figure 5 | **Design principles of dynamic shape specification. a** | Possible changes in membrane curvature and vesicle shape based on lipid size and composition. The segregation of lipid domains occurs spontaneously within a lipid bilayer and can affect localized curvature (top). Changing the surface-area-to-volume ratio and the relative surface areas of each monolayer within the bilayer can induce a wide range of vesicle shapes simply by minimizing the energetics of membrane curvature (bottom). **b** | The addition of membrane-binding proteins or integral membrane proteins can induce or stabilize localized membrane curvature, including the extreme curvatures that are seen during the budding of smaller vesicles from the membrane (top) and during the formation of membrane tubes (bottom). **c** | The cytoskeleton provides a framework along which membrane-protein structures can be moved and attached. Regulated fusion and fission dynamics can constantly remodel the resultant complex membrane networks. The overall network morphology depends on the localization of the cytoskeletal attachment sites and the localization of the fusion and fission proteins within the network.

Table 1 | **Design principles for cellular architecture**

Parameter	Design principle	References*
Size	Dynamic balance of assembly and disassembly	21
Number	Balance of organelle formation and partitioning	28
Polarity	Self-organization biased by external inputs	43
Position	Directed motion on polarized cytoskeletal arrays	67
Shape	Energetics of protein binding drive curvature change	94

* We have provided a single reference here as an example, although there are numerous others.

a spiral-shaped array of cilia. The oral apparatus is a polarized structure with a distinct anterior and posterior end, and is also chiral, showing a pronounced left–right asymmetry. When the oral apparatus is repositioned to different places in the cell, it retains its normal left–right asymmetry but switches its anterior–posterior asymmetry in response to the new cellular context. This suggests that the structure relies strictly on local self-propagating cues for left–right position but responds to global cellular cues for anterior–posterior positioning[115]. This has been interpreted as reflecting a varying level of input of local information (which can be rearranged and thus propagated) for some aspects of structure compared with global cellular polarity information (which cannot be rearranged by local manipulations and therefore cannot be propagated). If genomic tools could be used to probe the underlying events of these rearrangements, they might aid the understanding of the origins of cellular geometry and its propagation.

Perspective

As cell biologists accumulate details concerning cellular composition and geometry, new approaches that combine physics, engineering and biology will be needed to understand the systems that build a cell. TABLE 1 outlines some of the general design principles that seem to apply to cellular geometry, based on examples discussed above. One important current goal is to determine whether these simple mechanisms, such as local membrane curvature changes driven by protein binding, are sufficient to explain the full complexity of cellular structures. To achieve this goal, computational modelling combined with quantitative measurements will be needed to ask whether some proposed set of potential mechanisms could be sufficient to generate a particular structure that is observed *in vivo*. For instance, one could simulate the combined effects of membrane-binding proteins by using a mechanical model for the equilibrium of membrane curvature and ask whether this yields the structure of a Golgi stack. Ultimately, such approaches should yield a design-level understanding of cell architecture, allowing us to programme cell organization by the modification of strategically chosen gene-expression levels.

Within the realm of regenerative medicine, the differentiation of stem cells into a desired cell type is just the first step. In order for cells to perform their correct physiological function, they must not only express the correct genes but also attain the correct morphology. The ability to reprogramme and design cell organization might thus have a crucial role by allowing cells to be driven towards the functionally required architecture.

There is currently great interest in 'synthetic biology', whereby circuits and systems can be engineered using biomolecular building blocks such as promoters and DNA-binding proteins. However, the synthetic systems built to date are trivial compared with real physical machines built out of metal or silicon. One reason for this is that current synthetic biology relies on solution-phase reactions with diffusible molecules. Because the molecules that carry information, such as the value of digital bits, are diffusible, it is impossible to use spatial patterning such as that used in integrated circuits to build up complexity. Hence, a synthetic biological logic circuit can only have as many logic gates as there are diffusible signalling molecules. If molecular assemblies with defined three-dimensional structures can be engineered within cells, it might be possible to take synthetic biology into a realm of vastly increased complexity. Understanding how cells assemble and control complex structures might thus provide the basis for a whole new type of engineering.

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DATABASES

Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
gene *H*
UniProtKB: http://ca.expasy.org/sprot
Cdc42 | dynein | tektin

FURTHER INFORMATION

Wallace F. Marshall's homepage:
http://biochemistry.ucsf.edu/labs/marshall

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