Size control in dynamic organelles

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The mechanisms that control organelle size are unknown. Flagellar length regulation is the most accessible of all organelle-size-control problems, and experiments on flagellar assembly have provided important clues to how flagellar length is controlled, as a balance of assembly and disassembly. I propose that the inherent length dependence of intraflagellar transport might be what allows the flagellum to reach a defined length. This model of the flagellum might represent a general scheme for organelle size control that could apply to any organelle whose maintenance involves continuous assembly balanced by disassembly.

Published online: 7 August 2002

A major question in cell biology is how the one-dimensional genome specifies the three-dimensional morphology of a cell. In particular, how are the number, size and position of organelles determined? In this article, I begin to attack these problems by considering organelle size control.

What does it mean for size to be controlled? Intuitively, the question of size control arises when the size distribution for an organelle is extremely tight; that is, the organelle size appears to be somehow ‘defined’. Size control is not an issue for structures whose size is inherently determined by their composition, such as a protein complex of defined subunit stoichiometry. Rather, it becomes an issue when a structure can, in principle, take on a range of sizes (as in a polymeric structure made of repeating subunits) but in fact only takes on a single size in vivo. The action of a size-control mechanism can also be inferred when there are mutants that alter the size of the organelle. Size-altering mutants have been identified for many subcellular structures, including stereocilia [1], eyespots [2] and flagella (see below). Size control also becomes evident when size changes as a function of developmental stage or hormonal activity. Size control is important because of the tight connection between form and function; for example, flagella that are too short are generally less effective for swimming.

If size is controlled, how is such control achieved? To discuss models for size control, we distinguish between two types of structure that require different size control mechanisms: static and dynamic. Static structures are those that, once assembled, remain intact and do not undergo further assembly or disassembly. For example, pulse-labeling experiments indicate that centrioles do not undergo turnover and hence are probably static structures [3]. Dynamic structures, by contrast, are constantly turning over.

Many cellular structures are dynamic, including microvilli [4], actin thin filaments in striated muscle [5], the Golgi apparatus [6], mitochondria [7] and flagella (see below). Size control in static structures takes place only during the period of initial assembly, whereas size control in dynamic structures must operate continuously in order to ensure a balance of assembly and disassembly.

Once we have decided whether a given organelle is static or dynamic, we can consider possible size-control mechanisms. A priori, these mechanisms fall into four broad classes. The simplest mechanism for controlling organelle size is for the cell to produce a limited quantity of one or more crucial precursor molecules, whose abundance directly determines the size of the organelle. A limiting-precursor model like this transforms the problem of organelle size into a simple problem of regulating protein abundance. Size control of the yeast spindle-pole body (SPB) might involve this type of mechanism. Overproduction of SPB structural components results in increased size, either of the entire SPB [8] or of specific SPB substructures [9]. This dependence of size on protein levels is consistent with a limiting-precursor model. In such a model, one would expect that increased absolute gene dosage would cause a proportional increase in organelle size, and this is indeed the case for the SPB: diploid cells have SPBs twice the size of haploid SPBs.

The second size-control method is a ruler mechanism, in which organelle size is fixed by the physical size of a molecule composing the structure. Examples include bacteriophage tails [10,11] and sarcomeres, whose length appears to be determined by the length of the protein titin [12]. Ruler models can, in theory, include more-complex vernier schemes [12,13], in which size is set by an array of subunits that self-assemble into a structure of fixed size, which then acts as the ruler.

A third type of size-control mechanism is sensor-based size control, in which a signal-transduction pathway monitors organelle size and modulates assembly accordingly. Such a mechanism would benefit from the robustness and stability achievable by feedback control systems, but raises the question of how size could actually be sensed. A possible feedback sensor can be proposed for the endoplasmic reticulum (ER), based on the observation that the unfolded protein response (UPR) pathway, which becomes activated in response to misfolding of proteins in the ER, upregulates genes involved in membrane biosynthesis [14]. Because the ability of secreted proteins to be processed properly in the ER depends on the volume of the ER, it has been suggested that this could represent a type of feedback control to regulate the amount of ER in the cell [15]. If the ER becomes too small during cell growth, it would be overwhelmed with protein, trigger the UPR and thereby induce additional ER formation. However, there is also evidence against this idea: UPR pathway
components are not essential except under conditions of stress; mutants in the UPR appear to have normal ER morphology [16]; and only a subset of the membrane-biosynthetic machinery is upregulated by the UPR. Thus, it remains unclear whether the UPR does in fact play a crucial role in ER size control.

A fourth type of size-control mechanism is equilibrium balance, which applies only to dynamic organelles. For a dynamic organelle to maintain a defined size, the rates of assembly and disassembly must balance each other. If either the assembly or the disassembly rate is inherently size dependent in an appropriate way then the two rates will only balance at a unique size, thus accounting for organelle size control. I discuss here the possibility that such an equilibrium mechanism might, in fact, be the predominant mechanism of length control in at least one well-understood system: the eukaryotic flagellum.

**Flagellar length control**

The flagellum is the most convenient system for studying organelle size control. Perhaps the most important reason for this is that flagella are essentially one-dimensional structures whose size can be determined simply by measuring their length. This contrasts with more three-dimensional organelles such as the ER, whose size can only be measured accurately by tedious serial-section reconstructions. Measurements of flagellar length distributions suggest that flagellar length is tightly controlled [17]. Further evidence that length is controlled comes from the existence of mutants that affect flagellar length. Genetic analysis of flagellar length exploits Chlamydomonas reinhardtii, a unicellular biflagellate green alga that has many of the same advantages for genetics as yeast [18] but, unlike yeast, has flagella identical to those of animal cells. Using the power of Chlamydomonas genetics, several mutants have been discovered that affect flagellar length, including *lf* (long flagella) mutations that cause flagella to be too long [19–22] and *shf* (short flagella) mutations that cause flagella to be too short [23,24] relative to the wild-type length of 10 µm. These genetic studies reveal genes whose products are necessary for maintaining flagellar length and provide a possible entry point into dissecting the length-control pathway. Unfortunately, the sequences of the *lf* and *shf* genes have not yet been reported.

The first step in determining the mechanism of flagellar size control is to determine whether the flagellum is static or dynamic. Flagella have sometimes been considered to be static, because they are stable when isolated and do not disassemble in the presence of colchicine. However, measurement of protein turnover within the flagellum using radioactive pulse labeling [25–27] clearly demonstrates that the flagellum is dynamic and undergoes continuous turnover. This turnover was visualized by examining the incorporation of epitope-tagged tubulin into non-growing flagella [28], which localized the site of the turnover to the distal end of the flagellar outer doublet microtubules (Fig. 1a). These studies imply that there must be a mechanism to balance the rates of assembly and disassembly in order to maintain a defined length.

Evidently, either the assembly rate or the disassembly rate must be modulated as a function of length. The key to dissecting these two rates is the
Intraflagellar transport (IFT) is mediated by particles that are assumed to move at a constant velocity $v$ from the cell body to the tip and back. In fact, the anterograde and retrograde rates are different, so $v$ represents the harmonic mean (the product divided by the arithmetic mean) of the two velocities. A single particle moving from the cell body to the tip will travel a distance $L$ equal to the length of the flagellum, and this trip will take time $L/v$. In order for the particle to move to the tip, deliver cargo and return to collect more cargo, the total round-trip time will be $2L/v$. The frequency of which this particle makes a delivery of cargo to the tip is the reciprocal of the round-trip time and is therefore equal to $v/2L$.

Quantitative immunofluorescence indicates that there is a constant number of IFT particles in a flagellum, regardless of length [28]. If there are $N$ particles moving back and forth, each delivering cargo with a frequency $v/2L$, the total frequency with which cargo is delivered to the tip is $Nv/2L$. If we assume that assembly at the tip is limited by precursor availability then the assembly rate is proportional to the cargo-delivery frequency. Therefore, including a constant of proportionality $C$, we conclude that the assembly rate is $CNv/2L$. The key result is that the assembly rate decreases as $L$ increases.

This model is, however, contradicted by an elegant and insightful experiment [24] in which Chlamydomonas shf1 mutants that have flagella half as long as the wild type were mated to wild-type cells. Because each parent cell had two flagella, after cell division, one daughter cell had a long flagellum, and the other had a short flagellum. The two daughter cells were then followed through their first two cell cycles. In the wild-type cells, the flagella became longer, but in the mutants, they became shorter (Fig. 2). This demonstrates that the assembly rate is a direct consequence of the mechanism of IFT and does not depend on the action of a length-sensing pathway. Because assembly decreases monotonically as a function of length and disassembly is length independent, the assembly and disassembly rates will only balance at a single value of length (Fig. 2a). This length represents a stable equilibrium because, if the length becomes longer than this value, disassembly will predominate and cause the flagellum to shorten; conversely, if the flagellum is shorter than the equilibrium length, it will elongate. Thus, the facts that steady-state assembly requires an intrinsically length-dependent transport process (IFT) and that steady-state disassembly is length independent immediately yield a simple mechanism for flagellar length control. This mechanism does not involve sensors, rulers or a precisely limited quantity of precursor but instead falls under the general category of equilibrium models.

Can this simple equilibrium model account for the available data on flagellar length control? Perhaps the most important piece of information is that there are mutants in which flagella are reproducibly too long or too short. As described in Fig. 2b, two ways for the equilibrium mechanism to produce short flagella: by either increasing the efficiency of disassembly or decreasing the efficiency of assembly. The latter case has been experimentally verified by showing that, when IFT is partially blocked using a temperature-sensitive IFT mutant grown at intermediate temperatures, short flagella could be stably obtained [28]. Likewise, there are two ways to obtain long flagella (Fig. 2c): by increasing assembly or by reducing disassembly. Consistent with the latter prediction, at least one mutant of Chlamydomonas shows a decreased rate of tubulin turnover, presumably reflecting a decrease in steady-state disassembly [28].
Fig. 2. Model for flagellar length control. (a) The assembly and disassembly rates of tubulin at the distal end of outer doublet microtubules. The disassembly rate \( D \) is independent of length as determined from flagellar shortening kinetics in \( fla10 \) mutants. Assembly rate is inherently length dependent owing to the properties of intraflagellar transport and is proportional to \( 1/L \) (Box 1), resulting in a hyperbolic curve as shown. The two curves intersect at a single point, which is the only length for which the assembly and disassembly rates are equal, therefore representing a steady-state length. (b) Short-flagella mutants can arise by changes in either the assembly or the disassembly rates. In each case, the dotted line represents the original rate-versus-length curve, whereas the solid line represents the new rate-versus-length curve in a mutant. Either increasing the disassembly rate or impeding the assembly process lead to a new shorter length, as indicated by the new intersection point of the two curves. (c) Long-flagella mutants can also arise by two different means, either by making the assembly process more efficient or by reducing the disassembly rate. Again, the new curves intersect at a new point, which in this case occurs at a length greater than in the wild type.

Fusion a quadriflagellate intermediate was formed with two full-length and two half-length flagella. Because the recessive \( shf1 \) mutation is rescued by the wild-type cytoplasm, the half-length flagella immediately start growing. These half-length flagella grow significantly more quickly than did wild-type flagella that had reached half length during normal regeneration [24]. It was concluded that the flagellar growth rate is not inherently length dependent, contradicting the fundamental basis of the length-control model presented here. Unfortunately, the wild-type flagellar regeneration kinetics reported in this work were much slower than all other published examples, including very careful studies on single cells as well as populations of cells under various conditions [31]. If the \( shf1 \) regeneration kinetics are compared with the wild-type regeneration kinetics observed by others, it appears that wild-type flagella at half length grow at essentially the same rate as \( shf1 \) flagella, implying that growth rate is indeed a function of length as predicted by the equilibrium-balance model. Of course, when there is a discrepancy in a measurement between two different groups, one can never know with certainty which result is actually correct. At any rate, this discrepancy means that we should be cautious in interpreting the results of the \( shf1 \) mating experiment and suggests that this important experiment should be repeated.

Thus, it appears that flagellar length control can be explained by a very simple equilibrium model without invoking any sort of length sensor or ruler. Although this model does not require a signal-transduction pathway as an intrinsic part of the length-control machinery, extrinsic signal-transduction pathways might impinge on the length-control mechanism, allowing the cell to make its flagella longer or shorter when necessary. For instance, the cilia lining the oviduct undergo reproducible changes in length during the menstrual cycle. Pharmacology has begun to identify pathways that modulate flagellar length. Lithium, for example, causes flagella to elongate [32]. The pathway by which lithium affects flagellar length remains unclear, although exciting new work suggests that glycogen synthase kinase 3 (GSK3) might be the primary downstream target of lithium in flagella [33]. The key point to recognize is that, just because the length-control mechanism does not involve signaling as an integral component, this does not preclude signaling pathways from impinging on the underlying length-control machinery in order to modify flagellar length in particular situations.

**General model for organelle size control?**

Can this model be extended to cover size control in other organelles? I first consider organelles whose geometry resembles flagella – structures that protrude from the cell body out into the environment. Obvious examples include microvilli, stereocilia and filopodia. These share with flagella the feature that the underlying cytoskeletal framework (here, actin instead of tubulin) is polarized, such that growth has to occur at the distal tip. Actin must somehow be transported from the cell body to the tip and, provided that this transport is length dependent, length control could occur by the same balance mechanism proposed for flagella.

Actin photobleaching in brush-border epithelia has revealed that actin bundles in microvilli undergo continuous treadmilling with assembly at the distal end balanced by disassembly at the proximal end [4]. To support the ongoing assembly, actin is continuously transported to the tip by diffusion [4]. We can assume that the assembly rate depends on the actin concentration at the tip. However, because assembly at the tip removes free actin from solution, the assembly rate also dictates the slope of the diffusion gradient in the microvillus. Consequently, the assembly rate can only match the retrograde treadmilling rate at a single value of length, assuming a constant concentration of free actin in the cytoplasm. This length represents an equilibrium length for the microvillus. Of course, as with any model, the situation is probably more complex in
reality. For example, myosins play a role in modifying the length of microvilli and stereocilia [1,4], possibly by exerting tension on the membrane at the tip to regulate assembly rates.

But what about most organelles, which are surrounded by cytoplasm and do not undergo polarized growth at a distal site? Does the mechanism proposed for flagellar length control have any bearing on such organelles? The answer might be yes, provided that we generalize the model sufficiently. The key is to realize that flagellar length is, in the model proposed here, maintained because the assembly and disassembly rates have different dependencies on length. As long as the rate-limiting step in either the assembly or the disassembly pathway for an organelle is inherently size dependent, there will be a potential for this size dependence to lead to a defined organelle size.

Figure 3a depicts a membrane-bound organelle, such as the Golgi apparatus, that continuously exchanges vesicles with some large pool of membrane, such as the ER [6]. It is reasonable to assume that the rate of vesicle docking and fusion with the organelle is determined by the rate at which these vesicles are generated at the ER and transported to the organelle, and is therefore independent of the size of the organelle itself. It is also reasonable to propose that the rate of vesicle budding from the organelle membrane is proportional to the surface area of the organelle, because the more surface the organelle has, the more potential budding sites will be present. This situation satisfies the simple criterion outlined above: the assembly rate is independent of size whereas the disassembly rate, being directly proportional to the surface area, is clearly a monotonically increasing function of size. Therefore, the assembly and disassembly rates will only become equal, and hence steady state be achieved, at a unique surface area (Fig. 3b). Thus, in this simple model, size control can be easily achieved by the inherent size dependencies of the assembly and disassembly rates, just as was the case for flagella.

Based on this simple example, I suggest that this general type of size-control mechanism, in which either the assembly or the disassembly processes that maintain a dynamic organelle are inherently size dependent, might apply to a wide range of cellular structures. Clearly, flagella are only the beginning.

Fig. 3. Simple model for membrane-bound organelle size control. (a) The organelle is constantly exchanging vesicles with some pool of membrane such as the endoplasmic reticulum (ER). The rate of vesicle fusion with the organelle is presumed to be limited by the rate of vesicle generation at the ER, which is independent of organelle size. The rate of vesicle budding from the organelle is assumed to be proportional to organelle surface area because a greater surface area implies more potential budding sites. (b) The assembly and disassembly rates are proportional to the vesicle fusion and budding frequencies, respectively. This graph is entirely different from that describing flagellar length control because, in this case, it is the disassembly rate and not the assembly rate that is size dependent. However, the end result is the same: the two curves only intersect at a single value for the surface area, representing the steady-state size of the organelle.

Acknowledgements
I thank J. Rosenbaum and members of his lab for many valuable discussions about flagellar length control, and M. Tyska, M. Mooseker and V. Fowler for discussions about actin-based structures. My work has been supported by a Helen Hay Whitney Foundation postdoctoral fellowship and a Leukemia and Lymphoma Society Special Fellowship.
Centromeres become unstuck without heterochromatin

Pascal Bernard and Robin C. Allshire

In most if not all eukaryotes, sister-chromatid cohesion, which is mediated by the chromosomal complex Cohesin, is destroyed by proteolysis at the transition from metaphase to anaphase. In metazoans, Cohesin is removed from chromosomes in two steps, and the centromere and its associated pericentric heterochromatin constitute the last point of linkage between sister chromatids at metaphase. Mechanistic insights are now emerging on the way in which cells distinguish cohesion at the centromere from cohesion along chromosome arms. We discuss recent advances in our understanding of the role of centromeric heterochromatin in sister-chromatid cohesion and propose a causal relationship between this specialized type of chromatin and the removal by proteolysis of Cohesins that are associated with it.

The proliferation and development of living organisms rely on the accurate transmission of genetic content. Each time a cell divides, the complete chromosome complement, which has been duplicated in the preceding S phase, separates into two equivalent sets, giving rise to daughter cells with an identical genetic content. Precision and coordination are key factors in this process, because errors that lead to the gain or loss of chromosomes (aneuploidy) can have disastrous repercussions for both individual cells and the organism. Such aberrations can drive cancer in somatic tissues and lead to debilitating human conditions such as Down’s syndrome (caused by trisomy of chromosome 21), Edward’s syndrome (chromosome 18), Patau syndrome (chromosome 13), Turner’s syndrome (X chromosome in females) and Klinefelter’s syndrome (X chromosome in males).

From their synthesis in S phase until the onset of anaphase, sister chromatids are linked firmly together. This cohesion is essential because it allows sister centromeres and their associated kinetochores to be captured by microtubules that emanate from opposite spindle poles long after chromosome duplication has been completed. After bilateral attachment, chromosomes align at the spindle equator, resulting in the characteristic metaphase configuration. This state reflects the equilibrium between two antagonist forces: the poleward traction that is exerted by microtubules on kinetochores, and the tethers that hold sister chromatids together. At anaphase onset, cohesion between sister chromatids is released completely, thereby allowing sister chromatids to separate and move towards the opposite poles of the spindle.

Sister-chromatid cohesion is mediated by the chromosomal complex Cohesin, which includes at least four proteins that were initially identified in the budding yeast Saccharomyces cerevisiae as Scc1/Mcd1, Scc3, Smc1 and Smc3. Cohesin is evolutionarily conserved: Scc1 is known as Rad21 in fission yeast (Schizosaccharomyces pombe), Drosophila and several vertebrates (for a recent review, see [1]).

The metaphase to anaphase transition is the crucial step of the cell cycle in which cells take the irreversible decision to release cohesion by removing Cohesins (Fig. 1). This is achieved through the proteolytic cleavage of Scc1/Rad21 by an endopeptidase known as Separase. Until anaphase onset, Separase is inhibited by an associated protein, Securin. When all of the chromosomes are aligned properly on the metaphase spindle, the Anaphase Promoting Complex/Cyclosome (APC/C), a ubiquitin ligase, triggers the destruction of Securin. Separase is then freed, Scc1/Rad21 is cleaved and anaphase ensues [1]. To guard against the disastrous premature release of Cohesin, the spindle checkpoint inhibits the activity of the APC/C until all chromosomes are attached bilaterally and thus ready to segregate to opposite poles [2].

Unexpectedly, it has been recently shown in metazoans that a fraction of Cohesin dissociates from chromosome arms as early as prophase—a stage during which the spindle checkpoint is thought to