Current Biology 22, R330–R339, May 8, 2012 ©2012 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2012.03.046

Organelle Growth Control through Limiting Pools of Cytoplasmic Components

Review

Nathan W. Goehring and Anthony A. Hyman

The critical importance of controlling the size and number of intracellular organelles has led to a variety of mechanisms for regulating the formation and growth of cellular structures. In this review, we explore a class of mechanisms for organelle growth control that rely primarily on the cytoplasm as a 'limiting pool' of available material. These mechanisms are based on the idea that, as organelles grow, they incorporate subunits from the cytoplasm. If this subunit pool is limited, organelle growth will lead to depletion of subunits from the cytoplasm. Free subunit concentration therefore provides a measure of the number of incorporated subunits and thus the current size of the organelle. Because organelle growth rates are typically a function of subunit concentration, cytoplasmic depletion links organelle size, free subunit concentration, and growth rates, ensuring that as the organelle grows, its rate of growth slows. Thus, a limiting cytoplasmic pool provides a powerful mechanism for size-dependent regulation of growth without recourse to active mechanisms to measure size or modulate growth rates. Variations of this general idea allow not only for size control, but also cell-size-dependent scaling of cellular structures, coordination of growth between similar structures within a cell, and the enforcement of singularity in structure formation, when only a single copy of a structure is desired. Here, we review several examples of such mechanisms in cellular processes as diverse as centriole duplication, centrosome and nuclear size control, cell polarity, and growth of flagella.

Introduction

In a series of pioneering experiments, E.G. Conklin [1] provided key insights into how the size of subcellular organelles is set by cytoplasmic determinants. A general correlation was known to exist between the size of a cell and the size of its internal components, including the nucleus, the spindle, and centrosomes (Figure 1A; reviewed in [2]). However, Conklin found that it was not the physical dimensions of the cell, but the amount of associated cytoplasm that was the dominant factor in specifying organelle size. By centrifuging embryos of the sea snail *Crepidula plana*, he was able to induce the separation of yolk from cytoplasm. Because these two phases were often differentially inherited during cleavage, the amount of cytoplasm in the resulting blastomeres was no longer proportional to cell volume (Figure 1B). In these blastomeres, the size of the nucleus, the spindle, and the centrosomes did not scale with the physical dimensions of the cell, but with the amount of cytoplasm in which they were suspended, indicating that the volume of cytoplasm was playing a direct role in setting the size of organelles. But how could cytoplasm regulate the size of cellular structures, let alone provide mechanisms to both measure the size of structures and alter their growth rates accordingly?

Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), 01307 Dresden, Germany.
E-mail: goehring@mpi-cbg.de

Nearly a century after Conklin first posed this question, answers are beginning to emerge. In this review, we explore how these and other examples of organelle growth control can be explained by a consideration of the cytoplasm as a pool of available building blocks. We highlight a general class of mechanisms, which rely on either local or cell-wide depletion of this cytoplasmic pool as a way to regulate the size and number of cellular structures. As long as subunit amounts are limiting, the growth of intracellular structures necessarily leads to depletion of cytoplasmic subunit pools. In turn, reduction in the concentration of free subunits reduces growth rates. Ultimately, this coupling between organelle size, cytoplasmic concentration, and growth rates enables robust mechanisms for size-dependent and number-dependent control of organelle growth in cells. At the same time, because they depend on depletion of diffusible cytoplasmic subunits, these mechanisms require careful consideration of appropriate length and time scales, as well as mechanisms to accurately specify protein concentrations in cells.

How Can the Cytoplasm Provide Dynamic Size Control?

Cytoplasmic Concentrations Govern Assembly Kinetics

The primary role of the cytoplasm in the assembly of cellular structures is to provide a pool of available subunits from which to draw. Typically, the rate of product formation by a chemical reaction is a function of substrate concentration. As a consequence, the growth of a structure such as a microtubule depends on the concentration of its component parts, in this case, free tubulin dimers [3]. Thus, the concentration of subunits in the cytoplasm can directly influence the assembly rate of cellular structures.

Limiting Pools Allow Size-Dependent Growth Rates Without Measuring

Just as knowing only the velocity of an object tells one nothing about how far it will travel, in the absence of other constraints, knowledge of the rate of assembly of a structure tells one nothing about its ultimate size. Assembly must therefore be constrained to achieve a target size. Growth can be limited by simple time or physical constraints (Figure 2A,B) or it may reflect a dynamic balance, in which assembly competes with a size-dependent disassembly process (Figure 2C). Recognition that the cytoplasm is finite raises yet another simple mechanism of exerting size-dependent growth control, a so-called 'limiting pool' mechanism (Figure 2D). If the supply of subunits is limited, assembly of a structure will by its very nature deplete the cytoplasmic subunit pool, reducing the cytoplasmic concentration. Consequently, the rate of subunit incorporation will decrease as the structure grows larger. Thus, a limiting pool provides size-dependent control of growth rates without requiring the cell to either measure the current size of a structure or to actively modify assembly rates, for example, through changes in protein activity, protein synthesis, or degradation. Instead, limiting pool models are inherently self-correcting: deviations away from the characteristic size of a structure result in changes in the assembly–disassembly balance that naturally drive the system back towards steady state. For example, if a fully grown structure is severed, subunits are returned to the pool, stimulating growth.

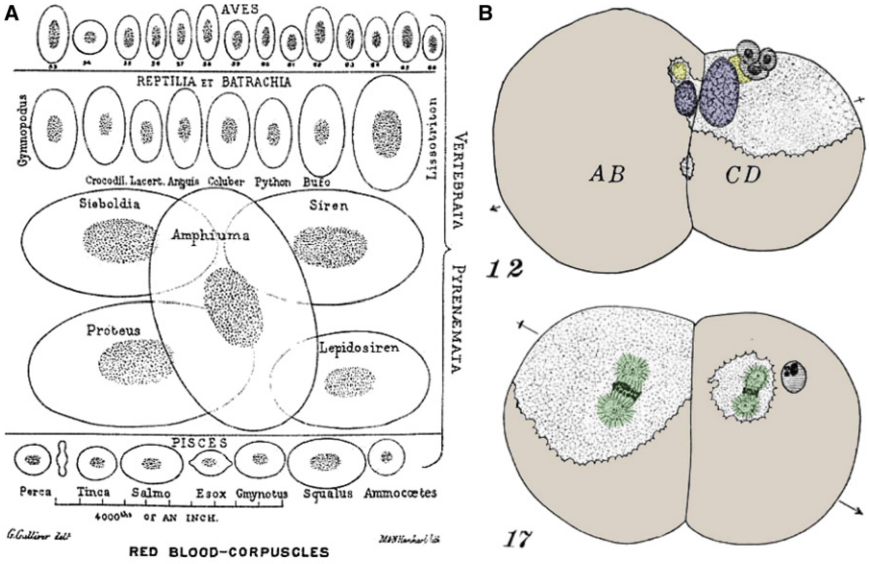

Figure 1. Organelles scale with cell cytoplasm.

(A) Sketches of red blood cells taken from various vertebrate species by George Gulliver (1875) highlighting the scaling of nucleus to cell size. Reproduced with permission from [80]. (B) Sketch of two centrifuged *Crepidula* two-cell embryos by E.G. Conklin (1912). Due to centrifugation along the indicated axis (arrows) during cleavage, cell size and the amount of cytoplasm (dotted) and yolk (brown) are not proportional in the two blastomeres. The size of spindles (green), nuclei (purple), and centrosomes or 'spheres' (yellow) depend on the volume of cytoplasm in which they find themselves rather than the physical dimensions of the cell. Adapted with permission from [1]. Note that the organelles do not scale in strict proportion to cytoplasmic volume, possibly reflecting the possibility that other factors may also come into play, e.g. an upper size limit on the mitotic spindle [29].

Natural Scaling of a Structure to Cell Size

Another advantage of limiting pools is that they can account for the scaling of structures in cells of different sizes. Consider the case of two cells that possess an identical initial concentration of subunits for a given structure, but differ in size. Because the starting subunit concentration is the same, the initial growth rates of the structures in each cell will also be the same. As the individual structures grow and cytoplasmic pools are depleted, growth rates in both cells will slow. However, because the smaller cell possesses a proportionally smaller pool of subunits (*concentration · volume*) than the larger cell, its subunit concentration will decline faster as the structure grows. Therefore, growth of the structure in the smaller cell will slow more rapidly and plateau at

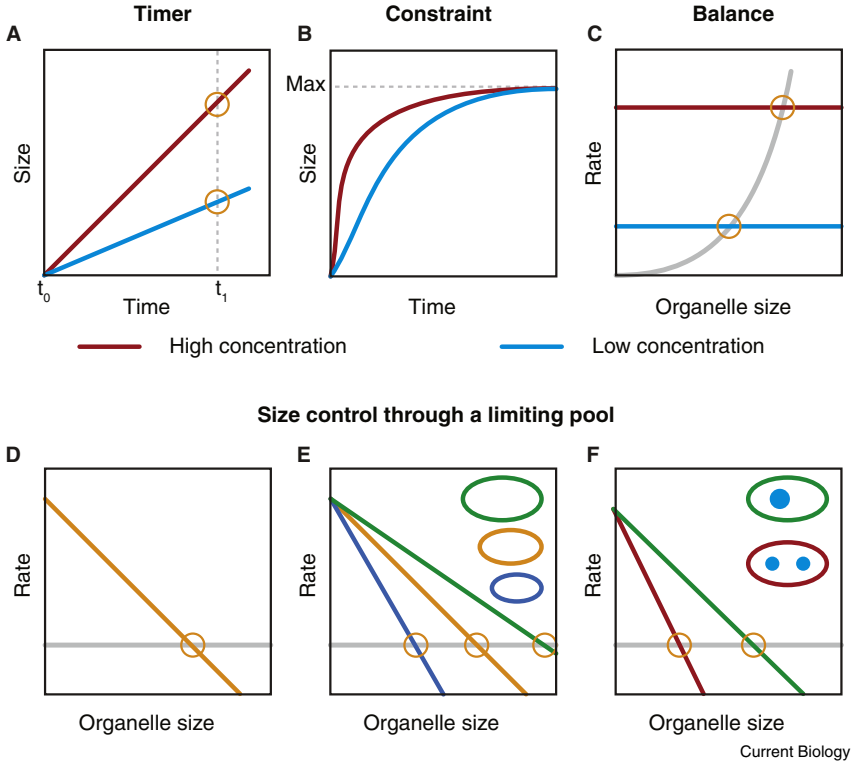

Figure 2. Models for growth control.

(A–C) Mechanisms for size control that do not rely on cytoplasmic depletion. In these models, we assume concentration is fixed, resulting in a constant, concentration-dependent assembly rate. (A) Timer: because higher subunit concentrations allow faster assembly, a cell with a higher subunit concentration (red line) will show faster assembly rates compared to a cell with a lower subunit concentration (blue line). Thus, over a given timespan (t_0–t_1, dashed gray line), the cell with a higher concentration can assemble a larger structure. Orange circles indicate the size of each condition at t_1. (B) Physical constraint: a system with a higher concentration will grow faster, but physical constraints limit the maximum achievable size (Max). (C) Size-dependent disassembly: steady-state size is specified by the point at which a size-dependent disassembly rate (gray line) precisely balances the assembly rate (red/blue lines). If the concentration is increased, thereby increasing the rate of assembly, the balance point (shown as an orange circle) shifts to a higher size. See [81] for an example of such a mechanism. (D–F) Size control through depletion of a limiting cytoplasmic pool. As in (C), orange circles indicate the balance point where assembly and disassembly are balanced. (D) Growth of a structure depletes the cytoplasm, thereby reducing the assembly rate (orange). Steady-state size is given by the point at which the rate of assembly exactly matches the rate of disassembly (gray). (E) As in (D), but for three cells containing identical subunit concentrations but differing in volume. Because total pool scales with cell size, for a given increase in size, a smaller cell suffers a proportionally larger depletion of the pool, resulting in a smaller steady-state size. (F) Competition for a limiting pool: if multiple structures compete for subunit from a common pool (red), the pool will be depleted more rapidly relative to a single, isolated structure given an identical cytoplasmic pool (green). Here, total size, independent of number (N), is constant, with mean size scaling as 1/N [63].

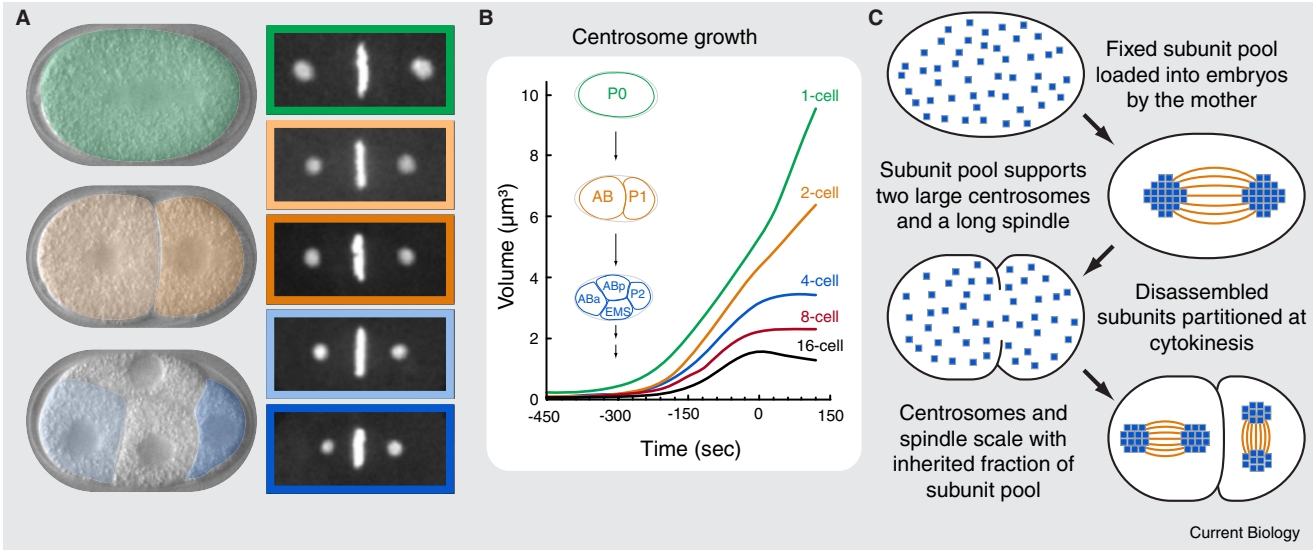

Figure 3. Centrosome scaling via a limiting component.

(A) Spindle size and centrosome size scale with cell size during early development of *C. elegans.* (Assembled from images courtesy of G. Greenan.) (B) Centrosome growth is limited by cell volume. Modified with permission from [6]. (C) Schematic of a limiting pool model for centrosome and spindle scaling during development. A fixed precursor pool is loaded into the embryo, which enables growth of two centrosomes in the one-cell embryo. Centrosome disassembly returns precursor to the cytoplasm. Thus, each daughter receives a precursor pool with a size proportional to volume. As a result, the centrosomes in the daughters will scale with cell size. Because centrosome size governs spindle size, the spindles of the two-cell embryo are smaller than in the one-cell embryo.

a smaller final size (Figure 2E). Thus, when pools are limiting and the initial subunit concentration is equal across cells, a structure scales to the size of the cell in which it is contained.

Coordination of Growth between Multiple Structures

What about multiple structures growing in a single cell, and therefore sharing a common cytoplasmic subunit pool? If the diffusivity of subunits in the cytoplasm is sufficiently high, local depletion around a growing structure will cause a cell-wide reduction in subunit concentration. In such a regime, the concentration of the cytoplasmic pool provides a measure of the total number of subunits incorporated within individual structures across the cell, thereby providing an indirect measure of the combined total number and size of these structures. In the simplest case, if all structures draw from the same cytoplasm, the total combined size of all structures will be limited by the total subunit pool (Figure 2F). Once that pool is depleted, a structure can only grow if another disassembles, ensuring the total combined amount of incorporated subunits remains constant. As we shall see later in other contexts, this notion of competition opens the door to more complex phenomena, such as size equalization between spatially separated structures (flagella) and regulation of structure number through effects on the nucleation of structure assembly (centrioles, cell polarity).

Thus, a simple consideration of the cytoplasm as a finite and potentially limiting pool of subunits for the assembly of cellular structures reveals robust mechanisms for limiting the size of cellular structures, scaling structures to cell size, and coordinating the growth of multiple structures within a single cell. In the following sections, we illustrate the potential impact of limiting pools in several classic examples of size control.

Control of Centrosome Size

One clear example of a limiting pool mechanism controlling the size of a cellular organelle is the regulation of centrosome size. The scaling of the centrosome with cell size was recognized by the first decades of the 20th century [1,2]. One purpose of centrosome scaling, at least in *Caenorhabditis elegans*, is to adjust the length of the mitotic spindle to match the rapid decline in cell size that occurs during early embryonic cell divisions. During these divisions, which result in a 558-cell larva that is no bigger than the one-cell zygote [4], metaphase spindle length declines in a manner that correlates with centrosome size [5,6] (Figure 3A). Although the precise mechanism by which centrosomes specify spindle length remains unclear, a direct link was demonstrated by experimental reduction of centrosome size through partial depletion of the centrosome assembly factor SAS-4, which led to corresponding changes in spindle length [5,7]. This observation, however, begs the question of how centrosomes are able to scale with cell size.

Centrosomes are composed of pericentriolar material (PCM), the assembly of which is templated by a pair of centrioles, which specify both the number of centrosomes and where they will form [8]. Centrosome growth occurs through a process of maturation, in which PCM gradually accumulates around centrioles, reaching a peak size during mitosis, when the centrosomes direct organization of the mitotic spindle [9]. The centrosomes then fragment and disassemble during cytokinesis, returning PCM components to the cytoplasm. Presumably, cells must therefore regulate PCM recruitment to ensure formation of centrosomes of the proper size.

In a modern re-examination of Conklin's experiments, recent work in *C. elegans* development [6] showed that, as cells become smaller, the growth rate of centrosomes slows, resulting in smaller centrosomes (Figure 3B). Importantly,

centrosomes in blastomeres of abnormally small embryos were smaller relative to those in identical blastomeres in normal-sized embryos, consistent with centrosome size being limited not by cell identity but by the volume of cytoplasm. The total volume of all centrosomes in the embryo was also conserved, both through development or in cells manipulated to contain ectopic centrosomes, matching what one would expect if centrosomes are competing for the same pool of PCM components (as in Figure 2F). Thus, a fixed pool of PCM components is loaded into the oocyte, which is then partitioned between cells at each division, with each cell inheriting a pool of PCM components in direct proportion to its volume (Figure 3C). In small cells, the correspondingly smaller PCM pool is depleted more quickly, resulting in smaller centrosomes. Supporting this hypothesis, centrosomes could be induced to grow larger by increasing the pool of available PCM through overexpression of the centriole/centrosome component SPD-2 [6].

Experiments on centrosome growth in syncytial *Drosophila* embryos also found that centrosome size was dependent on the rate of incorporation of the core centrosome component Cnn [10]. Doubling or halving the levels of Cnn yielded corresponding changes in centrosome size. However, centrosome growth did not exhibit any plateau, but rather grew steadily throughout S phase before abruptly ceasing upon mitotic entry. Thus, a cell-cycle timer rather than pool depletion appears to limit centrosome growth. It is tempting to speculate that the extremely large volume of the syncytial embryo in which these nuclear divisions take place provides an effectively unlimited pool, and thus the embryo must rely on other growth control mechanisms.

Centriole Duplication

Intriguingly, cytoplasmic depletion may also play a role in controlling the number of centrioles, which in turn sets the number of centrosomes. Control of centriole number requires that, during each cell cycle, a single new daughter centriole is nucleated in the immediate vicinity of each mother centriole in a process termed centriole duplication (reviewed in [11]). It is known that cells have sufficient material to form extra centrioles because the removal of existing daughter centrioles from around the mother centriole allows formation of additional daughter centrioles [12]. Thus, depletion of the overall cellular concentration does not limit centriole nucleation. Rather, daughter centrioles locally suppress nucleation events.

One mechanism by which growing daughter centrioles could inhibit nucleation of additional centrioles is through local, as opposed to global, depletion of centriole components around the mother centriole. In contrast to growth rate, nucleation of structures often exhibits strong, nonlinear dependence on protein concentrations, leading to effective nucleation thresholds. Thus, one can clearly find concentration regimes where nucleation is effectively suppressed, but growth of existing structures is sustained. One could imagine that the local depletion of centriole components by growing centrioles could be sufficient to push concentrations below the nucleation threshold so that no new centrioles will be nucleated around the mother centriole, while still allowing the daughter centriole to grow. Consistent with this model, overexpression of centriole components, which should overwhelm the effects of local depletion, leads to formation of multiple daughter centrioles [13–15]. Also, as one might expect given the sensitivity of the system to concentration, the cellular concentration of at least one of these components, SAS-6, is tightly regulated [16]. Thus, we speculate that proper spindle morphology relies on a combination of global and local depletion of cytoplasmic protein pools to control, respectively, the size and number of centrosomes.

Nuclear Size Control — Limiting Component or Active Control?

As early as the late 19th century, biologists noted a striking correlation between the size of the nucleus and the size of the cell (Figure 1A; reviewed in [2]). The precise scaling relationship, known as the *kern–plasma* (nuclear–cytoplasm) ratio [17], has been the subject of considerable controversy. The ratio appears nearly constant in some systems and more variable in others. This is in part due to limits in obtaining precise measurements of nuclear and cytoplasmic volumes, complicated by the presence of yolk, vacuoles, and other organelles that displace cytoplasm. Moreover, in many systems, the nucleus often continues to grow right up to mitotic entry, meaning that nuclear size also depends on both the duration of interphase and the time at which it is measured [1]. Nonetheless, a general correlation between cell size and nuclear size appears nearly universal [18].

The mechanisms behind nuclear scaling remain largely unknown. One hypothesis, based on a general correlation between DNA amount and nuclear size, is that nuclear size is a function of DNA content [2,19]. However, in yeast, changes in ploidy do not result in direct changes in cell or nuclear size, and no specific increase in nuclear size was seen during S phase, as predicted by a DNA content model [18,20]. Moreover, a direct causal relationship between nuclear size and DNA content makes it difficult to explain why nuclei in different cells of the same organism are of different size [1].

An alternative hypothesis is that the size of the nucleus is a function of cell size. Supporting this view, if a nucleus is transferred from a small donor cell into a larger host cell, the transferred nucleus expands, responding to the size of the host cell (Figure 4A) [21,22]. In both budding and fission yeast, nuclear size increased proportionally with cell growth, maintaining a remarkably constant ratio of nuclear to cell volume over a broad range of cell sizes and growth conditions [18,20]. In fact, a cell-size model likely also explains many cases of coupling between DNA content and nuclear size. Haploid and polyploid cells are typically smaller and larger, respectively, compared with their diploid counterparts [2,23].

How does this work? Similar to the case of centrosomes, in centrifuged *Crepidula* embryos, nuclear size did not correlate with cell size, but rather with the amount of inherited cytoplasm (Figure 1B). This is consistent with a model in which a finite pool of cytoplasmic factors limits nuclear size [1]. Further support for this idea comes from cells containing multiple nuclei. When HeLa nuclei are injected into the cytoplasm of *Xenopus* oocytes, individual or sparsely spaced HeLa nuclei grow much larger than nuclei contained within large clumps of several hundred nuclei (Figure 4B) [21]. A qualitatively similar result was seen in cytokinesis-defective fission yeast, which harbor numerous irregularly spaced nuclei (Figure 4C) [18]. Thus, nuclei appear to be competing locally for some diffusion-limited component. Normally, as nuclei take up components, the local pool is replenished by the influx of components from elsewhere in the

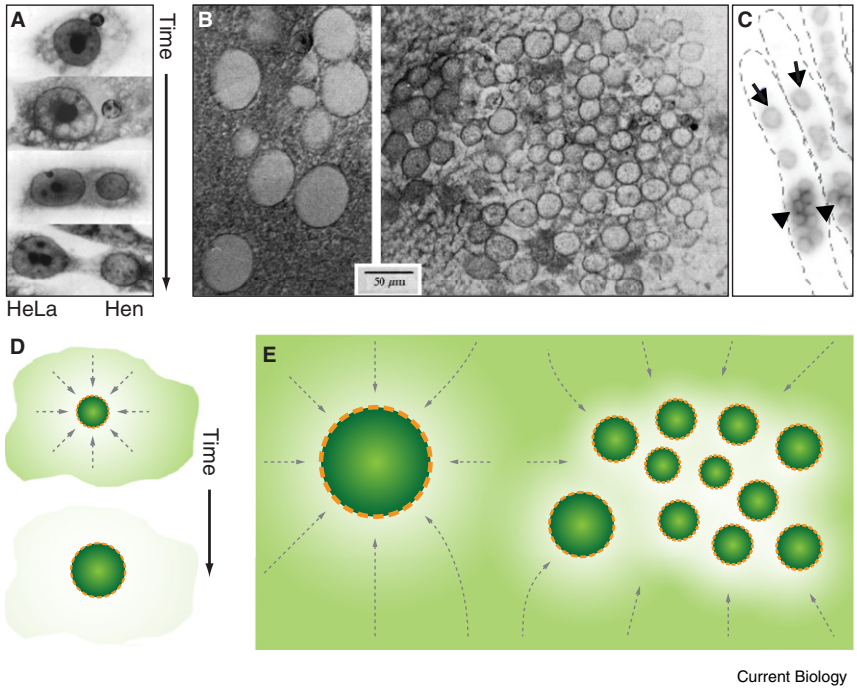

Figure 4. The kern-plasma ratio and scaling of nuclei.

(A) Diminutive hen erythrocyte nuclei injected into HeLa cells and fixed at various times post-injection. The hen erythrocyte (right) gradually increases in size until it almost reaches parity with the HeLa nucleus (left). Adapted with permission from [22]. (B) HeLa nuclei injections into *Xenopus* oocytes reveal that nuclei fail to grow substantially when competing with other nuclei in large clusters (right), compared with when nuclei are relatively isolated (left). Adapted with permission from [21]. (C) The same is true in cytokinesis mutants of *Schizosaccharomyces pombe* where nuclei in clusters (arrowheads) are smaller than more isolated nuclei (arrows) in the same cell. Adapted with permission from [18]. (D,E) Size control through depletion of a diffusion-limited precursor. Growth of a nucleus will tend to deplete precursor (green) from the local cytoplasm, which is replenished due to diffusion of precursor from elsewhere in the cell (arrows). In a single cell (D), this depletion will eventually reduce concentrations throughout the cytoplasm, limiting growth. (E) If total precursor is not limiting, for example, if cell volume greatly exceeds nuclear volume, or if precursor is constantly synthesized, there will be few limitations on the growth of isolated nuclei, allowing them to grow continuously unless limited by some other factor. However, the effects of depletion may still limit the growth of multiple, clustered nuclei if their combined local uptake sufficiently exceeds the rate of precursor diffusion. This effect will be particularly strong for nuclei at the center of clusters, since nuclei at the edges will tend to take up precursor before it can reach the center. Such a scenario would lead to a situation as in (B) where nuclei in the center of the cluster show almost no growth.

cell (Figure 4D). However, in nuclear clusters, the nuclei at the edge will take up components as they diffuse in from outside (Figure 4E). Consequently, there is insufficient flux of components into the center of the cluster, keeping the local concentration low and limiting growth. Strikingly, when clusters of HeLa nuclei were injected directly into the nucleus of unfertilized *Xenopus* oocytes, known as the germinal vesicle (GV), the HeLa nuclei expanded uniformly until constrained by the physical limits of the GV membrane [21]. This is presumably because the GV is enriched in whatever components are normally limiting for nuclear growth. Consistent with this interpretation, when the GV was ruptured, HeLa nuclei in the cytoplasm were able to attain a much larger size [21]. The precise nature of these size-limiting components remains unclear, although nuclear lamins are strong candidates due to their known role in nuclear expansion [24–26].

This limiting-pool mechanism for scaling the nucleus to cell size has obvious advantages during early development when cell size often changes rapidly. However, early animal development also presents several situations where a limiting-pool mechanism breaks down. One example is the early *Xenopus laevis* embryo. From the mid-blastula stage onward, the scaling between nuclear size and cell size is typical of what one would expect for a pool-limited model [27,28]. However, nuclear size control is very different within the initial four embryonic cell divisions, where nuclear size remains constant, despite a roughly 16-fold mean reduction in cell volume [27–29]. Why is the typical coupling between cell and nuclear size absent in these early cells? The answer may be a matter of size. *X. laevis* one-cell embryos exceed a diameter of 1.2 mm, ~30-fold larger than the 40–50 μm diameter nucleus typical of cells in these stages, equating to a 27,000-fold difference in volume. Thus, the pool of nuclear components is in such excess that the formation of a nucleus has little effect on the concentration of components in the cytoplasm. Consistent with the pool of components being effectively unlimited, these early nuclei grow unchecked until the nuclear envelope breaks down at mitosis [28]. Contrast this to the mid-blastula where the ratio of nuclear to cell volume is closer to 50. In these smaller cells, nuclear growth reaches steady state during the cell cycle. Moreover, in these later stages, the total volume of all nuclei in the embryo is constant between stages, consistent with a limiting amount of precursor being partitioned into each cell according to volume [27]. Mitotic spindle length in *X. laevis* shows a similar pattern, with scaling to cell size absent in early cell divisions, but evident in embryos entering the mid-blastula stage and beyond [29].

The large size of cells in the early *Xenopus* embryo appears to have prompted development of alternative mechanisms of nuclear size control. Recently, nuclear size was found to be significantly different in both early stage embryos and in oocyte extract from *X. laevis* compared with its smaller relative *X. tropicalis*. These differences were traced to altered rates of import of lamin B [28], an essential architectural component of the nuclear envelope known to be required for nuclear growth [24,25]. The rates of lamin B import were found, in turn, to be set by the levels of importin-α. *X. tropicalis* embryos have lower levels of importin-α, and thus nuclei in *X. tropicalis* grow more slowly than in *X. laevis*. Consequently, *X. tropicalis* nuclei are smaller in size at the onset of mitosis.

Interestingly, this same work also identified lamin B as a potential limiting cytoplasmic component for controlling nuclear size scaling in later stage *Xenopus* embryos.

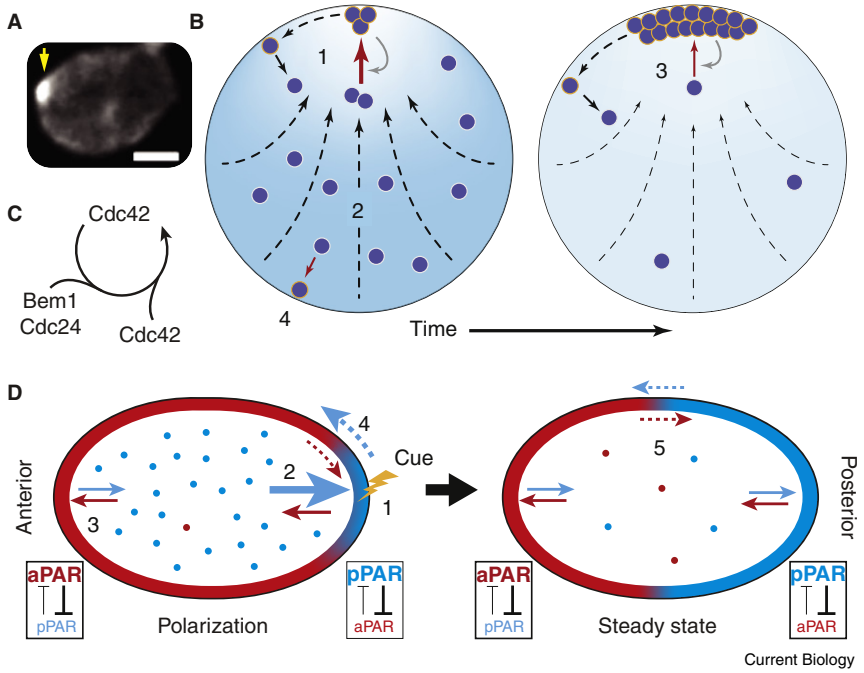

Figure 5. Cell polarity: a question of size and number.

(A) Polar Cdc42 cap in yeast. Modified from [38]. (B) Generic scheme for cell polarization of a single component based on Cdc42 polarization: (1) Active signaling molecules at the membrane recruit additional molecules from the cytoplasm. These molecules will diffuse away from the domain and eventually return to the cytoplasm due to spontaneous dissociation or internalization. (2) Local cytoplasmic depletion by the growing domain induces diffusion of molecules from elsewhere in the cell, leading to global depletion of the pool. (3) As the pool is depleted, recruitment by the existing domain slows, leading to stalling of domain growth. (4) At initial time points, high cytoplasmic concentrations may permit nucleation of additional polarity domains. However, the large domain can outcompete the newly formed domain for subunits in a 'winner takes all' situation. As the cytoplasm is depleted, nucleation events become increasingly difficult. (C) Self-amplifying feedback loop: active Cdc42 recruits Bem1 which in turn stimulates further recruitment and activation of Cdc42. (D) Schematic of PAR polarity establishment in *C. elegans*. Polarization involves the formation of two opposing polarity domains, an anterior PAR (aPAR)-dominant domain (red) characterized by net aPAR association and net dissociation of posterior PARs (pPAR), and the converse pPAR-dominant domain characterized by net pPAR association and net aPAR dissociation (blue). Polarization is triggered through a local cue (1) that induces a small pPAR domain. Because cytoplasmic pPAR concentration is high, further addition of pPAR is strongly favored (2). By contrast, a substantial fraction of aPAR is already on the membrane, reducing the pool of available aPAR in the cytoplasm. Thus, accumulation of additional aPAR is less favored (3). Thus, at the onset of polarization the system strongly favors recruitment of pPAR and expansion of the pPAR domain (4). As the pPAR domain grows, the pool of cytoplasmic pPAR is depleted. Simultaneously, the aPAR domain shrinks and aPARs are returned to the cytoplasm. This process eventually reduces the rate of pPAR association and increases the rates of aPAR association until the tendency of the two domains to expand is equalized (5).

Specifically, increasing lamin B or its import by importin-α in mid-blastula cells allowed nuclei to reach larger sizes before plateauing [28]. Thus, in *Xenopus* there appear to be two size-control regimes, each involving a limitation on the ability of the nucleus to take up lamin B. In large cells, where nuclear growth is not limited by cytoplasmic depletion and nuclei do not scale with cell size, nuclear size is set through a timer mechanism (Figure 2A). Maximum nuclear size attained is a function of the rate of lamin B import and the duration of the interphase period prior to nuclear envelope breakdown. As cell size decreases, cells enter a pool-limiting regime in which depletion of the cytoplasmic pool of lamin B and possibly other components begins to limit growth in a size-dependent fashion, resulting in scaling of nuclei with cell size.

It is tempting to speculate about what would happen to nuclei in the early *Xenopus* embryo if the cell-cycle timer were relaxed and nuclei were allowed to grow over much longer timescales. Intriguingly, the GV (the nucleus of the unfertilized oocyte) reaches a size of nearly 400–500 μm, yielding a nuclear:cytoplasmic ratio much closer to that seen in smaller mid-blastula cells [21,27]. The key difference between the GV and the nucleus of the one-cell embryo may be that GV growth is not time limited. Rather, the GV expands over weeks to months, allowing it to reach sizes where cytoplasmic depletion could begin to limit its growth. Thus, the basic premise of pool limitation may still apply in large cells, so long as there is sufficient time for nuclear growth. However, in the early embryo, the demands for rapid divisions ensure that the duration of interphase is too short for nuclei to reach a size at which cytoplasmic pool limitation comes into play.

Cell Polarity — Global Control of Size and Number

Cell polarity typically involves the formation of a membrane domain — or two opposing membrane domains — that orient the cell along a unique geometric axis. Once a polarity axis is established, the formation of additional polarity domains must be suppressed and the size of existing domains limited to prevent a domain from expanding to occupy the entire cell membrane, thus rendering the cell unpolarized. Although not exactly an assembly process, it raises similar issues of size control: the cell must somehow sense that a domain already exists, determine how big it is, and then adjust the polarization machinery accordingly to prevent both the expansion of existing polarity sites as well as the formation of new sites, all while allowing the existing structure to persist. Although such feedback control of domain size would appear complicated to engineer, a limiting cytoplasmic pool of a critical component provides an elegant and simple solution. Here we focus on the role of a limiting pool in two model systems, Cdc42 polarity in the yeast *Saccharomyces cerevisiae*, and PAR polarity in *C. elegans* (Figure 5), although the general themes we discuss apply to a variety of systems [30].

Cdc42 polarity and PAR polarity share several key features that combine to generate pattern-forming systems. First, the enrichment of a given polarity protein within a domain relies on auto-catalytic feedback that drives local enrichment within domains. Second, the local enrichment involves local

conversion of signaling molecules from rapidly diffusing, inactive, cytoplasmic states to more slowly diffusing, active, membrane-associated states. This slow membrane diffusion allows membrane-associated species to be concentrated in space, while rapid cytoplasmic diffusion ensures that local autocatalysis has access to the total cytoplasmic pool of inactive molecules. Finally, there is at least one critical component for which the pool of available protein is limiting. In other words, the total pool is small enough that its recruitment to a polarity domain results in a decline in its cytoplasmic concentration, reducing its ability to be added to the membrane. The importance of feedback and diffusion in pattern-forming systems was first noted by Turing [31] (for an accessible discussion for the general reader see [32]).

Polarization of budding yeast in the absence of pre-existing landmarks depends on a positive feedback loop involving Cdc42 [33,34]. Currently, it is thought that active Cdc42 recruits its own activator, the GTP exchange factor Cdc24, via the scaffold Bem1, resulting in further local recruitment of active Cdc42 [35] (Figure 5B,C). This feedback allows small, local fluctuations of Cdc42 to be rapidly amplified, resulting in the accumulation of active Cdc42 to high levels within a local patch [36] (Figure 5A,B). However, because Cdc42 fluctuations can occur at any time and place within the cell, in the absence of a limiting factor the number of patches would simply increase over time, violating the requirement for a single polarity axis. The limiting factor appears to be the scaffold protein Bem1, which is recruited to the Cdc42 patch as it forms [37]. The eventual depletion of cytoplasmic Bem1 reduces the strength of the positive feedback as the patch expands, limiting further accumulation of Cdc42 to the patch [36]. In addition, because depletion reduces cytoplasmic Bem1 concentrations throughout the cell, the probability of stochastic Cdc42 fluctuations being sufficiently large to form a stable second patch drops dramatically. If a second domain is able to form, competition between the two patches for available Bem1 leads to a 'winner takes all' scenario in which only one domain can persist [36,37]. Consistent with this model, overexpression of Bem1, which would reduce the ability of a single focus to deplete the cytoplasmic pool, increased the frequency of multiple Cdc42 patches. (For a recent review of these basic features of the Cdc42 polarity network, see [38].)

In PAR polarity, domain formation is driven by reciprocal negative feedback between two antagonistic groups of PAR proteins (anterior PARs and posterior PARs). Each is enriched on the membrane in the absence of the other, and is capable of displacing the opposing group from the membrane [39,40]. Consequently, a local advantage of one PAR species over the other will tend to be amplified: by displacing its antagonist, a given PAR species enhances its own enrichment at the membrane. Thus, reciprocal negative feedback functions similarly to the single positive feedback loop described for Cdc42. Because of this feedback, the membrane will tend towards one of two states — an anterior-like, high-anterior PAR/low-posterior PAR state, or the reciprocal posterior-like, low-anterior PAR/high-posterior PAR state (Figure 5D). Such a model of reciprocal negative feedback permits segregation of the membrane into domains [41–44], but there is no *a priori* reason why one domain would be favored over the other, let alone ensure that the cell is divided into two, roughly equal-sized domains. Domain size control appears to rely on limiting pools of PAR protein. Cytoplasmic depletion of the posterior PAR protein PAR-2 was found to be coupled to domain expansion, and overexpression or underexpression of PAR-2 led to corresponding changes in the relative size of the two polarity domains [43]. Thus, a limiting pool of PAR protein ensures that, as a PAR domain expands, it depletes the supply of components required for its further growth. It is currently unclear whether this pool is also necessary to enforce singularity in the system, which may be less of a problem in the *C. elegans* embryo, given that polarization relies on a polarity cue acting at a single site [45,46].

A survey of current cell polarity models reveals that depletion of a cytoplasmic pool is frequently invoked to limit expansion of polarity domains [30,36,42,47–49]. However, there are relatively few cases beyond Bem1-mediated polarity in yeast and PAR polarity in *C. elegans* where the role for cytoplasmic depletion has been demonstrated experimentally. Both the robustness and flexibility of limiting pool mechanisms in regulating the size and number of polarity domains suggest a broad role for limiting cytoplasmic pools in polarizing systems.

Flagellar Length Control — Competition for Limiting Components

The function of eukaryotic flagella depends on their length [50]. In *Chlamydomonas*, efficient swimming requires not only that the flagella be of proper length to enable an optimal swim stroke but also that the two flagella be of equal length to promote forward-directed (rather than circular) motion [51]. Thus, length control mechanisms must exist both to specify an optimal length for an individual flagellum and to coordinate length between flagella.

The mechanisms governing flagellar length control have been the subject of several excellent reviews but are still the subject of some controversy [50,52]. Assembly and disassembly of subunits into the flagella occur continuously at the tip [53], with the net balance of assembly and disassembly setting the flagellar growth rate [54]. Measurements suggest that, while the rate of subunit dissociation is independent of length [54,55], the rate of incorporation is reduced as flagella lengthen [55,56]. As a consequence, assembly and disassembly will balance at precisely one length. This is the so-called balance point at which flagellar length is stable [55]. But how are assembly rates controlled in a length-dependent fashion? The answer does not appear to be depletion of the cellular pool of flagellar components during flagellar growth. Rather, the rate of subunit transport to the growing tip appears to slow as the flagellum lengthens [50,55,56]. How this change in transport rates occurs remains unclear. Some form of length-dependent feedback signal could be involved [52,57] and the recent discovery of length-dependent phosphorylation of an aurora-like kinase is tantalizing [58]. However, clear support for signaling-based feedback control of flagellar length remains lacking.

One factor that has not been sufficiently explored is the control of assembly rates by a limiting pool, because this does not appear to be the mechanism for specifying length [59]. However, the limiting nature of flagellar components can be revealed in flagella severing experiments. Normally, if both flagella are severed, they regrow in unison to their original lengths [60] (Figure 6A). However, if protein synthesis is blocked, flagella regrow to only half their original length [60,61] (Figure 6B). The flagella will even regrow after a second round of severing, but now to only a quarter of their original length. Thus, synthesis of flagella represents

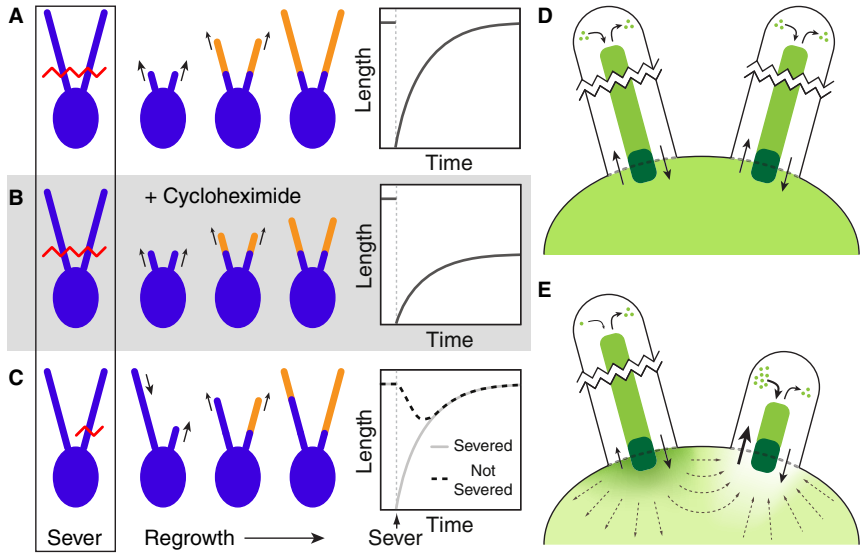

Figure 6. Flagellar length control in *Chlamydomonas*.

(A–C) Schematic summary of flagellar-severing experiments. Orange indicates new growth following severing. Graphs show flagellar length over time in each severing experiment. Time of severing is indicated by the dashed vertical line. (A) Normal regrowth of flagella following loss of both flagella. (B) If protein synthesis is blocked, flagella can regrow using the remaining cytoplasmic precursor pool from the cell body. Due to lack of new synthesis, this pool will be depleted, leading to stalling of growth at a shorter length. (C) If only one flagellum is severed, the uncut flagellum (dashed black line) initially shrinks, while the severed flagellum regrows (gray line). Once the two flagella reach equal length, their growth curves converge and they grow out together. (D) At steady state, assembly and disassembly are balanced, the length of the two flagella remains constant, and there are no concentration gradients of precursor since uptake and loss of components presumably remain balanced (black arrows). (E) After severing of one flagellum, the rapid regrowth of the shorter flagellum results in a dramatic increase in precursor uptake (large arrow). This increased uptake locally depletes subunits from the cytoplasm, creating a concentration gradient, which will cause precursor to diffuse toward the site of the regrowing flagellum (dashed arrows), eventually leading to global reductions in precursor concentration. As reduced cytoplasmic concentration begins to limit assembly rates, the balance between assembly and disassembly in the longer flagellum tips to favor disassembly. Consequently, the longer flagellum will shrink, resulting in a net donation of precursor to the cytoplasmic pool, where it can help fuel growth of the shorter flagellum.

a significant drain on the cytoplasmic precursor pool. Without new synthesis, depletion of this pool eventually reduces assembly rates sufficiently to limit growth. It is important to note that flagella do not stop growing because they run out of precursor. In each case, a significant pool remains in the cell to support regrowth [60,62,63]. Rather, as previously suggested [54], in the absence of new synthesis, regrowth reduces the precursor concentration of the cytoplasmic pool, thus lowering assembly rates, leading to a lower steady-state size. Consistent with a general role for limiting pools, the length of the primary cilium in mammalian cells was strongly affected by changes in the availability of free tubulin [64]. However, at least in *Chlamydomonas*, the size-limiting effect of depletion is prevented due to tight regulatory coupling between flagellar growth and precursor synthesis, which ensures sufficiently large pools of precursor [65–67].

Evidence does suggest, however, that competition for a limiting pool underlies equalization of flagellar length. The equalization process can be observed if only one of the two flagella is sheared off at its base. The severed flagellum does not simply regrow to match the remaining one. Rather, as the severed flagellum begins to regrow, the longer flagellum shrinks until the two flagella are equal in size, after which both grow out in unison [54,60] (Figure 6C). How can we explain this rather striking result? From the above experiments, we know that the severing of a flagellum results in the loss of a significant fraction of the total pool of flagellar components, which, at least in the period before flagellar component synthesis is upregulated, will be limiting for flagellar growth. Also, because assembly rates decline with length, the newly shortened flagellum will take up precursor at a higher rate than the longer flagellum. As a consequence, if pools are limiting, the shorter flagellum can outcompete the longer flagellum, ensuring that the short flagellum will grow at the expense of the longer until size is equalized or the pool is sufficiently replenished such that precursor is no longer limiting [54] (Figure 6D,E). If the cytoplasmic pool of flagellar components were simply maintained at high levels such that precursor concentrations were not limiting, such a model would not be possible. Perhaps the selective advantage of rapid flagellar equalization has led to maintenance of flagellar component concentrations within a narrow window.

Concluding Remarks

The notion of a finite, limiting pool seems intuitive. With more building blocks available, more and larger structures can be assembled. This provides a simple mechanism for both competition between growing structures in the same cell, as well as cell-size-dependent scaling: a larger cell will typically contain more building blocks than a smaller cell. Importantly, subunits need not literally ‘run out’. Rather, the simple reduction in cytoplasmic concentration that accompanies structure growth provides size-dependent regulation of assembly rates, enabling the size control, scaling, and competition mechanisms discussed here. As we continue to analyze the assembly of cellular structures, it is worth keeping such mechanisms in mind as the simplicity and adaptability of such mechanisms suggests that they will be ubiquitous.

Such mechanisms are very attractive in early embryonic development, which is typically characterized by rapid, abbreviated cell cycles in which cell divisions are not accompanied by cell growth. This allows for rapid increase in cell numbers, at the expense of an equally rapid decrease in cell size. In each of these cells, the size of organelles must be adjusted accordingly. Typically, the rapid pace of development necessitates that the embryo begins its life with

a fixed pool of protein, loaded into the oocyte by the mother. New protein is often not synthesized until the embryonic development is well underway. Under the simplest scenario, cells simply receive a protein pool at cytokinesis that is proportional to cell size. For a limiting pool mechanism, the size of the assembled organelles is then simply a function of this pool. Obviously more elaborate mechanisms may, and likely do, exist. For example, we have seen how the extreme size of cells in the early *Xenopus* embryo appear to have necessitated the evolution of alternative size control strategies. Nonetheless, the simplicity of a limiting cytoplasmic pool as a mechanism for scaling and size control suggest that such mechanisms will be widespread in embryonic systems. Whether such mechanisms are dominant in somatic systems remains to be seen. Here, longer cell cycles would in theory allow more freedom to actively adjust cytoplasmic protein concentration, thus allowing greater regulatory control. Testing such ideas will require a greater understanding of how cells modulate protein concentrations.

Limiting pool models require that cells regulate protein synthesis and/or degradation to achieve precise protein levels. In embryonic systems, there is evidence that protein concentration is tightly controlled for key proteins. For instance, regulation of the centrosome-size determinant SPD-2 and the polarity protein PAR-2 appear to exhibit dosage regulation in *C. elegans* ([6] and our unpublished results). Quantitative analysis in yeast indicates that some level of gene dosage compensation exists for up to ~15% of genes, although <5% showed complete compensation [68]. Thus, the number of pathways involving active regulation of protein amounts through some form of feedback control is not negligible.

Indeed, autoregulatory feedback control of protein amounts is ubiquitous, particularly in the biogenesis of multicomponent complexes, where subunits are required in stoichiometric amounts. The coordination of ribosomal protein expression provided the paradigm [69–71], which has subsequently been implicated in regulating the amounts of proteins as diverse as splicing components [72], tubulin [73], and even an E2 ubiquitin ligase [74,75]. A key feature of all these regulatory systems is that the accumulation of excess, unincorporated molecules feeds back to either inhibit the synthesis of additional molecules and/or promote their own degradation, typically through post-transcriptional mechanisms.

While these examples provide mechanisms to coordinate the level of a protein with cellular demands, how cells precisely regulate protein concentrations remains unclear. For example, we know almost nothing about how the protein production machinery in the *C. elegans* gonad ensures that embryos are loaded with the correct concentrations of proteins, although techniques such as varying codon usage, which has strong effects on expression levels, may provide ways to distinguish among mechanisms [6,43,76]. Combining the tools of control theory and dynamical systems with the analysis of synthetic gene networks has provided substantial insight into the properties of regulatory networks that give rise to stability and gene dosage invariance of network output [77–79]. Such approaches will undoubtedly help provide a way forward. Yet, bridging the gap between idealized, simplified model systems to the mechanisms that underlie dosage control in developmental systems will be an important and non-trivial process.

Acknowledgements

We thank S. Reber, C. Brangwynne, O. Wüseke, S. Grill, and several anonymous reviewers for constructive comments on the manuscript. N.W.G. was supported by the Alexander von Humboldt Foundation and a Marie Curie Grant (219286) from the European Commission.

References

1. Conklin, E. (1912). Cell size and nuclear size. J. Exp. Embryol. *12*, 1–98.
2. Wilson, E.B. (1925). The Cell in Development and Heredity, Third Edition (New York: Macmillan).
3. Borisy, G.G., and Olmsted, J.B. (1972). Nucleated assembly of microtubules in porcine brain extracts. Science *177*, 1196–1197.
4. Wood, W.B. (1988). Chapter 8: Embryology. In The Nematode *Caenorhabditis Elegans*, W.B. Wood, ed. (Cold Spring Harbor: Cold Spring Harbor Laboratory).
5. Greenan, G., Brangwynne, C.P., Jaensch, S., Gharakhani, J., Jülicher, F., and Hyman, A.A. (2010). Centrosome size sets mitotic spindle length in Caenorhabditis elegans embryos. Curr. Biol. *20*, 353–358.
6. Decker, M., Jaensch, S., Pozniakovsky, A., Zinke, A., O'Connell, K.F., Zachariae, W., Myers, E., and Hyman, A.A. (2011). Limiting amounts of centrosome material set centrosome size in C. elegans embryos. Curr. Biol. *21*, 1259–1267.
7. Kirkham, M., Müller-Reichert, T., Oegema, K., Grill, S.W., and Hyman, A.A. (2003). SAS-4 is a C. elegans centriolar protein that controls centrosome size. Cell *112*, 575–587.
8. Oegema, K. and Hyman, A.A. Cell division (January 19, 2006), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.72.1, http://www.wormbook.org.
9. Hannak, E., Kirkham, M., Hyman, A.A., and Oegema, K. (2001). Aurora-A kinase is required for centrosome maturation in Caenorhabditis elegans. J. Cell Biol. *155*, 1109–1116.
10. Conduit, P.T., Brunk, K., Dobbelaere, J., Dix, C.I., Lucas, E.P., and Raff, J.W. (2010). Centrioles regulate centrosome size by controlling the rate of Cnn incorporation into the PCM. Curr. Biol. *20*, 2178–2186.
11. Hatch, E., and Stearns, T. (2011). The life cycle of centrioles. Cold Spring Harb. Symp. Quant. Biol. *75*, 425–431.
12. Loncarek, J., Hergert, P., Magidson, V., and Khodjakov, A. (2008). Control of daughter centriole formation by the pericentriolar material. Nat. Cell Biol. *10*, 322–328.
13. Duensing, A., Liu, Y., Perdreau, S.A., Kleylein-Sohn, J., Nigg, E.A., and Duensing, S. (2007). Centriole overduplication through the concurrent formation of multiple daughter centrioles at single maternal templates. Oncogene *26*, 6280–6288.
14. Peel, N., Stevens, N.R., Basto, R., and Raff, J.W. (2007). Overexpressing centriole-replication proteins in vivo induces centriole overduplication and de novo formation. Curr. Biol. *17*, 834–843.
15. Strnad, P., Leidel, S., Vinogradova, T., Euteneuer, U., Khodjakov, A., and Gönczy, P. (2007). Regulated HsSAS-6 levels ensure formation of a single procentriole per centriole during the centrosome duplication cycle. Dev. Cell *13*, 203–213.
16. Puklowski, A., Homsi, Y., Keller, D., May, M., Chauhan, S., Kossatz, U., Grünwald, V., Kubicka, S., Pich, A., Manns, M.P., *et al*. (2011). The SCF-FBXW5 E3-ubiquitin ligase is regulated by PLK4 and targets HsSAS-6 to control centrosome duplication. Nat. Cell Biol. *13*, 1004–1009.
17. Hertwig, R. (1908). Ueber neue Probleme der Zellenlehre. Arch. Zellf. *1*, 1–32.
18. Neumann, F.R., and Nurse, P. (2007). Nuclear size control in fission yeast. J. Cell Biol. *179*, 593–600.
19. Cavalier-Smith, T. (1982). Skeletal DNA and the evolution of genome size. Annu. Rev. Biophys. Bioeng. *11*, 273–302.
20. Jorgensen, P., Edgington, N.P., Schneider, B.L., Rupes, I., Tyers, M., and Futcher, B. (2007). The size of the nucleus increases as yeast cells grow. Mol. Biol. Cell *18*, 3523–3532.
21. Gurdon, J.B. (1976). Injected nuclei in frog oocytes: fate, enlargement, and chromatin dispersal. J. Embryol. Exp. Morphol. *36*, 523–540.
22. Harris, H. (1967). The reactivation of the red cell nucleus. J. Cell Sci. *2*, 23–32.
23. Henery, C.C., and Kaufman, M.H. (1992). Relationship between cell size and nuclear volume in nucleated red blood cells of developmentally matched diploid and tetraploid mouse embryos. J. Exp. Zool. *261*, 472–478.
24. Benavente, R., and Krohne, G. (1986). Involvement of nuclear lamins in post-mitotic reorganization of chromatin as demonstrated by microinjection of lamin antibodies. J. Cell Biol. *103*, 1847–1854.
25. Newport, J.W., Wilson, K.L., and Dunphy, W.G. (1990). A lamin-independent pathway for nuclear envelope assembly. J. Cell Biol. *111*, 2247–2259.
26. Dechat, T., Adam, S.A., Taimen, P., Shimi, T., and Goldman, R.D. (2010). Nuclear lamins. Cold Spring Harb. Perspect. Biol. *2*, a000547.
27. Gerhart, J.C. (1980). Mechanisms regulating pattern formation in the amphibian egg and early embryo. In Biological Regulation and Development: Molecular Organization and Cell Function, R.F. Goldberger, ed. (New York: Plenum Press), pp. 133–316.

28. Levy, D.L., and Heald, R. (2010). Nuclear size is regulated by importin α and Ntf2 in Xenopus. Cell *143*, 288–298.
29. Wühr, M., Chen, Y., Dumont, S., Groen, A.C., Needleman, D.J., Salic, A., and Mitchison, T.J. (2008). Evidence for an upper limit to mitotic spindle length. Curr. Biol. *18*, 1256–1261.
30. Jilkine, A., and Edelstein-Keshet, L. (2011). A comparison of mathematical models for polarization of single eukaryotic cells in response to guided cues. PLoS Comput. Biol. *7*, e1001121.
31. Turing, A. (1952). The chemical basis of morphogenesis. Philos. Trans. R. Soc. Lond. B. Biol. Sci. *237*, 37–72.
32. Meinhardt, H. (1982). Models of Biological Pattern Formation (London: Academic Press).
33. Wedlich-Soldner, R., Altschuler, S., Wu, L., and Li, R. (2003). Spontaneous cell polarization through actomyosin-based delivery of the Cdc42 GTPase. Science *299*, 1231–1235.
34. Irazoqui, J.E., Gladfelter, A.S., and Lew, D.J. (2003). Scaffold-mediated symmetry breaking by Cdc42p. Nat. Cell Biol. *5*, 1062–1070.
35. Kozubowski, L., Saito, K., Johnson, J.M., Howell, A.S., Zyla, T.R., and Lew, D.J. (2008). Symmetry-breaking polarization driven by a Cdc42p GEF-PAK complex. Curr. Biol. *18*, 1719–1726.
36. Goryachev, A.B., and Pokhilko, A.V. (2008). Dynamics of Cdc42 network embodies a Turing-type mechanism of yeast cell polarity. FEBS Lett. *582*, 1437–1443.
37. Howell, A.S., Savage, N.S., Johnson, S.A., Bose, I., Wagner, A.W., Zyla, T.R., Nijhout, H.F., Reed, M.C., Goryachev, A.B., and Lew, D.J. (2009). Singularity in polarization: rewiring yeast cells to make two buds. Cell *139*, 731–743.
38. Johnson, J.M., Jin, M., and Lew, D.J. (2011). Symmetry breaking and the establishment of cell polarity in budding yeast. Curr. Opin. Genet. Dev. *21*, 740–746.
39. Goldstein, B., and Macara, I.G. (2007). The PAR proteins: fundamental players in animal cell polarization. Dev. Cell *13*, 609–622.
40. Bastock, R., and St Johnston, D. (2011). Going with the flow: an elegant model for symmetry breaking. Dev. Cell *21*, 981–982.
41. Jilkine, A., Marée, A.F.M., and Edelstein-Keshet, L. (2007). Mathematical model for spatial segregation of the Rho-family GTPases based on inhibitory crosstalk. Bull. Math. Biol. *69*, 1943–1978.
42. Mori, Y., Jilkine, A., and Edelstein-Keshet, L. (2008). Wave-pinning and cell polarity from a bistable reaction-diffusion system. Biophys. J. *94*, 3684–3697.
43. Goehring, N.W., Trong, P.K., Bois, J.S., Chowdhury, D., Nicola, E.M., Hyman, A.A., and Grill, S.W. (2011). Polarization of PAR proteins by advective triggering of a pattern-forming system. Science *334*, 1137–1141.
44. Dawes, A.T., and Munro, E.M. (2011). PAR-3 oligomerization may provide an actin-independent mechanism to maintain distinct Par protein domains in the early Caenorhabditis elegans embryo. Biophys. J. *101*, 1412–1422.
45. Cowan, C.R., and Hyman, A.A. (2004). Centrosomes direct cell polarity independently of microtubule assembly in C. elegans embryos. Nature *431*, 92–96.
46. Wallenfang, M.R., and Seydoux, G. (2000). Polarization of the anterior-posterior axis of C. elegans is a microtubule-directed process. Nature *408*, 89–92.
47. Gierer, A., and Meinhardt, H. (1972). A theory of biological pattern formation. Kybernetik *12*, 30–39.
48. Gamba, A., de Candia, A., Di Talia, S., Coniglio, A., Bussolino, F., and Serini, G. (2005). Diffusion-limited phase separation in eukaryotic chemotaxis. Proc. Natl. Acad. Sci. USA *102*, 16927–16932.
49. Arai, Y., Shibata, T., Matsuoka, S., Sato, M.J., Yanagida, T., and Ueda, M. (2010). Self-organization of the phosphatidylinositol lipids signaling system for random cell migration. Proc. Natl. Acad. Sci. USA *107*, 12399–12404.
50. Ishikawa, H., and Marshall, W.F. (2011). Ciliogenesis: building the cell's antenna. Nat. Rev. Mol. Cell Biol. *12*, 222–234.
51. Tam, L.W., and Lefebvre, P.A. (1993). Cloning of flagellar genes in Chlamydomonas reinhardtii by DNA insertional mutagenesis. Genetics *135*, 375–384.
52. Wilson, N.F., Iyer, J.K., Buchheim, J.A., and Meek, W. (2008). Regulation of flagellar length in Chlamydomonas. Semin. Cell Dev. Biol. *19*, 494–501.
53. Johnson, K.A., and Rosenbaum, J.L. (1992). Polarity of flagellar assembly in Chlamydomonas. J. Cell Biol. *119*, 1605–1611.
54. Marshall, W.F., and Rosenbaum, J.L. (2001). Intraflagellar transport balances continuous turnover of outer doublet microtubules: implications for flagellar length control. J. Cell Biol. *155*, 405–414.
55. Marshall, W.F., Qin, H., Rodrigo Brenni, M., and Rosenbaum, J.L. (2005). Flagellar length control system: testing a simple model based on intraflagellar transport and turnover. Mol. Biol. Cell *16*, 270–278.
56. Engel, B.D., Ludington, W.B., and Marshall, W.F. (2009). Intraflagellar transport particle size scales inversely with flagellar length: revisiting the balance-point length control model. J. Cell Biol. *187*, 81–89.
57. Howard, J., Grill, S.W., and Bois, J.S. (2011). Turing's next steps: the mechanochemical basis of morphogenesis. Nat. Rev. Mol. Cell Biol. *12*, 392–398.
58. Luo, M., Cao, M., Kan, Y., Li, G., Snell, W., and Pan, J. (2011). The phosphorylation state of an aurora-like kinase marks the length of growing flagella in Chlamydomonas. Curr. Biol. *21*, 586–591.
59. Sloboda, R.D. (2009). Flagella and cilia: the long and the short of it. Curr. Biol. *19*, R1084–R1087.
60. Rosenbaum, J.L. (1969). Flagellar elongation and shortening in Chlamydomonas: The use of cycloheximide and colchicine to study the synthesis and assembly of flagellar proteins. J. Cell Biol. *41*, 600–619.
61. Coyne, B., and Rosenbaum, J.L. (1970). Flagellar elongation and shortening in chlamydomonas. II. Re-utilization of flagellar proteins. J. Cell Biol. *47*, 777–781.
62. Kuchka, M.R., and Jarvik, J.W. (1982). Analysis of flagellar size control using a mutant of Chlamydomonas reinhardtii with a variable number of flagella. J. Cell Biol. *92*, 170–175.
63. Marshall, W.F. (2011). Centrosome size: scaling without measuring. Curr. Biol. *21*, R594–R596.
64. Sharma, N., Kosan, Z.A., Stallworth, J.E., Berbari, N.F., and Yoder, B.K. (2011). Soluble levels of cytosolic tubulin regulate ciliary length control. Mol. Biol. Cell *22*, 806–816.
65. Weeks, D.P., and Collis, P.S. (1976). Induction of microtubule protein synthesis in Chlamydomonas reinhardi during flagellar regeneration. Cell *9*, 15–27.
66. Lefebvre, P.A., Nordstrom, S.A., Moulder, J.E., and Rosenbaum, J.L. (1978). Flagellar elongation and shortening in Chlamydomonas. IV. Effects of flagellar detachment, regeneration, and resorption on the induction of flagellar protein synthesis. J. Cell Biol. *78*, 8–27.
67. Lefebvre, P.A., and Rosenbaum, J.L. (1986). Regulation of the synthesis and assembly of ciliary and flagellar proteins during regeneration. Annu. Rev. Cell. Biol. *2*, 517–546.
68. Springer, M., Weissman, J.S., and Kirschner, M.W. (2010). A general lack of compensation for gene dosage in yeast. Mol. Syst. Biol. *6*, 368.
69. Dennis, P.P. (1974). In vivo stability, maturation and relative differential synthesis rates of individual ribosomal proteins in Escherichia coli B/r. J. Mol. Biol. *88*, 25–41.
70. Fallon, A.M., Jinks, C.S., Strycharz, G.D., and Nomura, M. (1979). Regulation of ribosomal protein synthesis in Escherichia coli by selective mRNA inactivation. Proc. Natl. Acad. Sci. USA *76*, 3411–3415.
71. Nomura, M., Gourse, R., and Baughman, G. (1984). Regulation of the synthesis of ribosomes and ribosomal components. Annu. Rev. Biochem. *53*, 75–117.
72. Preker, P.J., Kim, K.S., and Guthrie, C. (2002). Expression of the essential mRNA export factor Yra1p is autoregulated by a splicing-dependent mechanism. RNA *8*, 969–980.
73. Yen, T.J., Machlin, P.S., and Cleveland, D.W. (1988). Autoregulated instability of beta-tubulin mRNAs by recognition of the nascent amino terminus of beta-tubulin. Nature *334*, 580–585.
74. Biederer, T., Volkwein, C., and Sommer, T. (1997). Role of Cue1p in ubiquitination and degradation at the ER surface. Science *278*, 1806–1809.
75. Ravid, T., and Hochstrasser, M. (2007). Autoregulation of an E2 enzyme by ubiquitin-chain assembly on its catalytic residue. Nat. Cell Biol. *9*, 422–427.
76. Redemann, S., Schloissnig, S., Ernst, S., Pozniakowsky, A., Ayloo, S., Hyman, A.A., and Bringmann, H. (2011). Codon adaptation-based control of protein expression in C. elegans. Nat. Methods *8*, 250–252.
77. Becskei, A., and Serrano, L. (2000). Engineering stability in gene networks by autoregulation. Nature *405*, 590–593.
78. Acar, M., Pando, B.F., Arnold, F.H., Elowitz, M.B., and van Oudenaarden, A. (2010). A general mechanism for network-dosage compensation in gene circuits. Science *329*, 1656–1660.
79. Bleris, L., Xie, Z., Glass, D., Adadey, A., Sontag, E., and Benenson, Y. (2011). Synthetic incoherent feedforward circuits show adaptation to the amount of their genetic template. Mol. Syst. Biol. *7*, 519.
80. Gulliver, G. (1875). On the size and shape of red corpuscles of the blood of vertebrates. Proc. Zool. Soc. Lond. *1875*, 474–495.
81. Varga, V., Leduc, C., Bormuth, V., Diez, S., and Howard, J. (2009). Kinesin-8 motors act cooperatively to mediate length-dependent microtubule depolymerization. Cell *138*, 1174–1183.