Title
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Permalink
https://escholarship.org/uc/item/3g37d8sc

Journal
Trends in Cell Biology, 25(12)

ISSN
0962-8924

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Publication Date
2015-12-01

DOI
10.1016/j.tcb.2015.08.008

Peer reviewed

How cells measure length on subcellular scales

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Abstract

Cells are not just amorphous bags of enzymes, but precise and complex machines. With any machine, it is important that the parts be of the right size, yet our understanding of the mechanisms that control size of cellular structures remains at a rudimentary level in most cases. One problem with studying size control is that many cellular organelles have complex three-dimensional structures that make their size hard to measure. Here we focus on linear structures within cells, for which the problem of size control reduces to the problem of length control. We compare and contrast potential mechanisms for length control to understand how cells solve simple geometry problems.

Keywords

organelle size; molecular rulers; limiting precursor; organelle dynamics; cytoskeleton; gradients

Length control of cellular structures: a paradigm for organelle size regulation

> Tous les problèmes de géométrie se peuvent facilement réduire à tels termes, qu'il n'est besoin par après que de connaître la longueur de quelques lignes droites, pour les construire. [All problems of geometry can be reduced to terms such that it is only necessary to know the length of several straight lines to construct them]
>
> - René Descartes. *La Géométrie*. 1637.

Cells have complex geometries that are directly linked to their function. Where does the information come from that specifies the three dimensional architecture of cells, and how is this information translated into actual structures? Important geometrical properties for cellular components include length of linear structures, and surface area and volume of membrane-bound organelles. Cells need a way to ensure that their components are of the appropriate sizes, but how exactly does this happen? Many biological regulatory systems,

such as pathways that regulate membrane potential or lactose synthesis rates, involve sensors that allow cells to measure the quantity that is being regulated. Here we will consider how cells might sense and measure length and distance.

Using length to determine geometry

The most obvious biological role of length measurement is to fix the size of those cellular structures that happen to be essentially linear (Figure 1). Cells carefully control the length of actin filaments in stereocilia (Manor et al., 2011; Narayanan et al., 2015) and sarcomeres (Littlefield and Fowler, 2008) to tune mechanical sensitivity and force-generation, respectively. In prokaryotes and viruses, the lengths of bacterial injectisomes (Wagner et al., 2009), bacterial flagellar hooks (Kawagishi et al., 1996), and bacteriophage tails (Katsura, 1990), are tightly controlled to a narrow range of lengths, thereby allowing proper biological function. For the injectisome, the optimal length is likely to reflect a tradeoff between mechanical stiffness and the ability to reach out to a target cell over some distance. Microtubules in eukaryotic cilia and flagella are set to pre-defined lengths presumably to help optimize their mechanical properties for swimming (Wemmer and Marshall, 2007), and microtubule bundles in spindle midzones (Hu et al., 2011) also are regulated to within a narrow length distribution. In each case, the cell is able to control the length of an essentially polymeric structure, which amounts to controlling the number of monomers that assemble into the polymer. Linear dimensions of more complex structures such as the mitotic spindle (Dumont and Mitchison, 2009; Goshima and Scholey, 2010) are also regulated by the cell and these can be considered as cases of length determination even though the structures in question may also have to control other geometric features such as cross sectional area or total volume.

In addition to directly controlling the size of a structure, linear elements of defined size can be used to set distances between other structures. Simple molecular spacers, in the form of coiled coil domains, are used to set the spacing between layers of the yeast spindle pole body (Kilmartin et al., 1993) and between the axial elements of the synaptonemal complex of meiotic chromosomes (Tung and Roeder, 1998). Within centrioles, the coiled coil domain of the centriole cartwheel protein Bld10/Cep135 appears to contribute to stabilizing the ninefold symmetry of the cartwheel by setting the length of the cartwheel spokes, thus regulating the diameter of the centriole and hence the number of triplets that can be accommodated around the circumference of the centriole (Hiraki et al., 2007; Rodrigues-Martins et al., 2007). This mechanism appears to act in parallel with the direct self-assembly of ninefold symmetry by the cartwheel protein SAS6 (reviewed in Gonczy, 2012).

At larger spatial scales, such use of linear elements to dictate spacing between structures seems to be relatively uncommon. One clear case occurs in the phototactic unicellular green alga *Chlamydomonas reinhardtii*, in which the length of the rootlet microtubule bundles seems to set the distance between the flagella and the eyespot, a geometrical parameter that is critical for proper phototaxis (Boyd et al., 2011). In the ciliate *Nassula,* a transient microtubule bundle determines the distance of the contractile vacuole pore relative to a specific set of basal bodies, with the bundle arranged like a ruler running from the basal bodies to the pore (Tucker et al., 1971). In fission yeast, the position of the nucleus at the

center of the cell is established by means of microtubule bundles that probe the length of the cell (Piel and Tran, 2009), and if the microtubule bundles are too long or too short the nucleus positioning is defective. While this is not a case where the absolute length of a linear structure dictates position of some other structure, it does represent a situation in which proper positioning of an important structure (the nucleus) relies on proper length of a linear element (microtubule bundles).

To summarize the discussion thus far, the length of linear structures is used in cell biology to regulate the length of the structure themselves, the length of directly associated structures, and in at least some cases the distance between discrete structures.

Determining length inside a cell

Given the multiple ways in which length can be used to control other aspects of cell geometry, the next question is how length itself may be determined. In the case of single protein molecules, such as coiled-coil based spacer molecules, their length is determined directly by their protein sequence. This approach has the advantage of not requiring any additional molecular components, but has the disadvantage that in order to change the length of the spacer, it would be necessary to change the length of the amino acid sequence. Such changes in amino acid sequence length could of course be accomplished by alternative splicing, in which case a given genome could encode more than one size spacer for a given task. However, these mechanisms are not capable of changing the size of more complex, repeating structures such as cytoskeletal filaments composed of multiple identical subunits, which could, in theory, have almost any length. What mechanisms control the length of such polymeric structures?

In considering how to control the length of biological multimeric structures, it is important to first consider what would happen if there was no length control imposed. It is intuitively clear that in the absence of additional regulatory mechanisms, the length distribution of multimeric structures will depend on the number of separate structures in the cell (which in turn is dictated by the number of nucleation events), the dissociation constant of monomers from the ends of the multimers, and the quantity of monomer contained in the cell. For polymers assembling in solution, the equilibrium length distribution is predicted to be exponential (Oosawa and Kasai, 1962) and this is generally confirmed when the equilibrium size distribution of in vitro assembled filaments of actin and other polymers are analyzed (Kawamura and Maruyama, 1970). Both theory and experiment have shown that it is possible to transiently obtain length distributions of actin that have a distinct peak, for example via the combined actions of polymerization and fragmentation, even though the population will eventually become exponential at equilibrium (Ermentrout and Edelstein-Keshet, 1998; Kuhlman, 2005). This result suggests that length could in principle be controlled by quenching an assembly process before the assembling structures can reach an equilibrium length distribution. It is unclear if this type of mechanism is actually used in vivo, but it does show that a narrower-than expected length distribution can be achieved by very simple means that do not require the cell to sense how long the structures actually are. Since the rates of assembly, disassembly, fragmentation, and annealing all contribute to the final length distribution (Fass et al., 2008), it is possible to change the average length by

modifying any of these rates. For example spindle microtubule length distributions can be reprogrammed by changing the activity of the microtubule severing protein katanin (Loughlin et al., 2011).

Even simple influences such as the finite volume of a cell can constrain length distributions. Conceptually this effect arises because a fixed volume constrains the maximum length that a structure can attain. The lack of long structures means there is more precursor available to allow the shorter structures that would otherwise be present to grow, thus both ends of the distribution are truncated, producing a tighter range than exponential. The volume of the cell has been shown to be capable of playing a role in setting the length distribution of microtubules, such that microtubules confined to a finite cellular volume switch from exponential length distributions to a much narrower distribution (Gregoretti et al., 2006; Janulevicius et al., 2006). However the resulting length distributions, while narrower than an exponential, are still broad enough to result in a range of lengths comparable to the entire diameter of the cell. For many cellular engineering problems, such a size distribution is still too broad and the cell needs a way to further restrict length variation.

A number of different length control mechanisms have been proposed for various cellular structures, but in general such mechanisms seem to fall into four different categories (Figure 2). We consider each of these in turn.

Molecular Rulers

The simplest length control method is the molecular ruler (Figure 2A). In this case one protein, usually an extended coiled coil protein, has a physical length that corresponds to the desired final size of an assembling polymeric structure. Molecular rulers are often considered an innovation of prokaryotes and viruses, regulating the length of such structures as bacteriophage tails.

A central question concerning molecular ruler models is how, mechanistically, the length of the ruler is actually compared to the length of the growing structure. In one hypothetical type of ruler mechanism, the ruler could have a domain at one end that changes the binding affinity of monomer for the growing structure, so that as the polymer grows, the ruler would have no effect until the polymer reaches the length of the ruler, at which point the last monomer would bind more tightly. Such a scheme has been shown capable, in theory, of giving precise length control based on calculations predicting a narrow length distribution for a population of polymers under this type of control (Wagenknecht and Bloomfield, 1975). Alternatively, one end of the ruler protein attaches to the base of the growing structure, and the other end associates with the growing end, protecting it from a capping or terminator protein which, once bound, arrests further growth. Once the polymer grows longer than the ruler, its end can no longer be protected and so further growth stops, fixing the length to be the same as the ruler molecule. In either case the tertiary structure of one protein (the ruler) determines the quaternary structure of a set of other proteins that assemble together into a linear polymer. The standard test for a ruler is to make the ruler molecule longer or shorter and ask if this produces corresponding changes in the final assembled structure. This test has proven the identity of molecular rulers for bacteriophage tail,

bacterial injectisomes, and bacterial flagellar hooks (Katsura, 1987; Shibata et al., 2007; Wagner et al., 2009; Wee and Hughes, 2015).

Are rulers also found in eukaryotic cells? Nebulin was originally hypothesized to act as a ruler to set actin thin filament length in muscle sarcomeres. However, while the length of nebulin between cell types correlates with the length of the thin filaments (Labeit and Kolmerer, 1995), the length of the actin thin filament extends beyond the length of nebulin itself, implying other mechanisms must exist to dictate the final thin filament length (Castillo et al., 2009). Recent work has identified a molecular ruler that sets the 96 nm spacing of motility-related structures within cilia and flagella. It has long been known that dynein arms and radial spokes form a characteristic longitudinal pattern that repeats every 96 nm. This spacing appears to arise from a 96 nm long protein complex composed of the proteins FAP59 and FAP172 (Oda et al., 2014). This complex passes the key test of a ruler – experimental elongation of the complex by domain duplication increased the spacing of axonemal structures from 96 nm to 128 nm.

Although rulers are a simple and elegant way to regulate length, they lack flexibility. Indeed, length can only be changed by expressing a different isoform of the ruler protein, which makes quick and dynamic size adjustments extremely difficult. Another limitation of molecular rulers is that they only work on length scales that are small enough to be spanned by a single molecule. More complicated "vernier" schemes have been proposed to understand bacteriophage length (Paulson and Laemmli, 1977). Here two rulers of different lengths span an object and only align perfectly at intervals given by the least common multiple of both lengths. However, it is unclear if other structures follow a similar model.

Limiting Precursor

Length can also be controlled by limiting the production of a key precursor molecule for the object in question (Figure 2B). If the resulting structure is an equilibrium structure, we would expect exponential length distributions, but if the precursor molecules are completely consumed in an irreversible assembly reaction, then the size would be directly determined by the quantity of precursor made. However, the cell has to carefully control how many copies of the structure it is building. Indeed, as a fixed quantity of precursor is distributed among multiple copies of the structure as they assemble, the size of each structure will scale as the reciprocal of the number of copies. This relation has been used to test limiting precursor models, and has supported such a model for centrosome size (Decker et al., 2011) and rejected it for eukaryotic flagellar length (Kuchka and Jarvik, 1982). While the effect of copy number on average size solves one problem (length or size control), it creates two new problems (control of precursor synthesis and the number of structures being assembled). A recent review discusses this class of size control models in more detail (Goehring and Hyman, 2012). Even in cases where limiting precursor pools are not the sole mechanism regulating size, precursor synthesis is expected to have an effect on the final size of the structure, such that when more precursor is available, the structure grows larger. For example the length of microvilli appears to correlate with the availability of free actin in the cytoplasm (Stidwell and Burgess, 1986), while depletion of precursor protein causes cells to grow shorter than normal flagella (Rosenbaum et al., 1969), and competition between two

flagella in one cell for a common pool of precursor is sufficient for cells to equalize flagellar lengths (Ludington et al., 2012). This influence of precursor quantity on assembly may help to explain the tendency for cellular structures to scale in size proportional to the overall size of the cell (Chan and Marshall, 2010; Levy and Heald, 2012). Elegant experiments with cell extracts encapsulated in droplets have provided evidence that spindle length is determined by the volume of cytoplasm and potentially by the quantity of tubulin (Good et al., 2013; Hazel et al., 2013).

While the limiting precursor mechanism is in some sense a "null hypothesis" if one assumes that the quantity of precursor is fixed, cells are still able to continually make more precursor suggesting that the mechanism is not as simple to implement. Exactly how a cell controls how much precursor it has made, especially if a substantial portion of the precursor is sequestered in assembled structures, is a fundamental problem for this type of control mechanism. Most biological regulatory mechanisms that control protein production levels do so by sensing the soluble protein concentration, for example by having the protein act as a repressor for the gene that encodes it, but this type of feedback becomes problematic when a large fraction of the protein is incorporated into an insoluble structure.

Balanced assembly/disassembly

A third general class of length control methods exploits the fact that cellular structures are dynamic, undergoing constant assembly and disassembly, so that if either the assembly or the disassembly rate is rendered length dependent, then it is possible to have a single unique steady state length (Figure 2C). For example the microtubule depolymerizing kinesin-8 binds along the length of microtubules, moves to the plus ends, and mediates disassembly, such that the longer the microtubule, the faster the recruitment of kinesin-8 (Varga et al., 2009). In this way, short microtubules are allowed to grow, while long microtubules are caused to shorten, resulting in a narrower length distribution at steady state. For bacterial flagella, the growth rate is independent of length while the breakage rate is predicted to increase as length increases, so that the steady state length distribution is a function of the constant growth rate and the length-dependence of breakage (Turner et al., 2012).

A similar mechanism appears to work in eukaryotic flagellar microtubules, which disassemble at a constant rate (Marshall and Rosenbaum, 2001), but assemble at a rate inversely proportional to their length (Marshall et al., 2005). It has been hypothesized that the length dependence of assembly might be due to the length-dependence of kinesin-based intraflagellar transport, which is required to bring tubulin to the growing tip (Marshall and Rosenbaum, 2001; Marshall et al., 2005; Engel et al., 2009). The original idea was that if a fixed number of these transport complexes are present inside the flagellum, then as the flagellum becomes longer, each complex should need to travel a longer distance, and transport efficiency should drop. However photobleaching analyses have shown that intraflagellar transport complexes move in and out of the flagellum (Ludington et al., 2015), indicating that the entry of these complexes is somehow regulated. Quantitative measurements show that the rate of intraflagellar protein entry into flagella is proportional to $1/L$ where L is the length of the flagellum (Ludington et al., 2013). The mechanism that creates the length dependence is presently not known. It has also been reported that the

loading of cargo onto transport complexes is also length dependent (Wren et al., 2013). Thus far this loading regulation has only been reported for a flagellar motility-related cargo that is not involved in length determination, and so it still remains an interesting question to determine whether length-dependent cargo loading will play a central role in length determination. In addition to regulation of transport either at the transporter injection or cargo loading levels, additional regulatory inputs could modify length by controlling parameters such as assembly and disassembly rates (Avasthi et al., 2012).

Molecular Gradients

Finally, it is worth considering if the cell ever actually "knows" the length of any of its structures. By "know" what we mean is that some internal signaling pathway would have its activity modulated as a function of length. For example, if a kinase existed whose activity was proportional to the size of a cellular structure, it could be used in a feedback loop to control the assembly rate. One can imagine different mechanisms by which a signaling pathway could respond to the size of a structure, but the simplest and most easily generalizable would be a molecular gradient. For example, a diffusible ligand could be produced at one end of the structure and sensed at the other, such that the shape of the resulting ligand gradient would provide information about the distance between the two ends (Figure 2D). This type of scheme was initially proposed for flagella based on theory (Levy, 1973) and more recently has been implicated in the control of mitotic spindle length, for which a RanGTP gradient originating at chromatin and read out by spindle microtubule regulatory proteins is seen to extend over a length range comparable to the length of the spindle (Kalab and Heald, 2008). Furthermore, theoretical models based on RanGTP diffusing out of the cilium have been used to describe the entry of intraflagellar complexes as a function of length (Ludington et al., 2013). However, diffusion is only one way to construct a length-measurement device. Length can also be measured by the time it takes a small molecule to move along or through the elongating structure. This type of scheme has been proposed for bacterial flagellar hooks in which the dwell time of a growth-terminating factor within the hook is predicted to increase as the hook becomes longer, thus giving the factor more time to arrest growth (Keener, 2010).

Probing length control with quantitative methods

As the field of cell biology moves from the study of individual molecules to the study of their assembly and function as large systems, it will become increasingly important to learn how cells solve geometry problems. The measurement and control of length within cells is an important part of the overall puzzle of cellular geometry, and it is clear that we have only scratched the surface of the total space of possible mechanisms. Quantitative approaches have played important roles in revealing important aspects of size control, for example by showing scaling relations between transporter complex injection and length in flagella (Engel et al., 2009; Ludington et al, 2013; Wren et al., 2013). Given the complexity of organelles and their assembly pathways, theoretical modeling combined with quantitative measurement is going to be necessary for further progress. For example, length distributions can potentially be a rich source of information about length control mechanisms. The population balance method described by Aizawa and co-workers for studying length

regulation of the bacterial flagellar hook (Koroyasu et al., 1998) allows growth rate to be determined as a function of length just from a steady-state length distribution, even in cases where live cell imaging is difficult. This approach was adopted to distinguish short-flagella mutants in Chlamydomonas that affect length via different mechanisms (Kannegaard et al., 2014).

Static distributions are just one way to analyze length quantitatively. Analysis of dynamic length fluctuations using time series analysis methods should yield important insights into the underlying control mechanisms. Conceptual tools from control systems theory should provide new ways to understand and predict potential length control mechanisms. In both of these cases, the fact that length is a single scalar quantity makes it much easier to relate to the standard engineering signals and systems literature, which historically has focused mostly on single-valued signals as a function of time.

Because length is an easily measured feature of cells, and yet it results from complex regulatory pathways, understanding length control is a classic example of trying to understand the workings of a black box from a series of measurements of the final outcome. As such it poses an interesting and tractable challenge for quantitative cell biology.

Concluding Remarks

As the molecular composition of cells becomes increasingly well understood, there is an ever greater urgency in understanding how those components are assembled into larger scale structures within cells. The control of size within cells is a key element of cell geometry. As discussed in Outstanding Questions box, there are many open questions about the size of even simple linear structures, but it is also worth considering how lessons learned from studying linear structures might apply more generally within the cell. Although we focus here on one-dimensional structures within the cell, most cellular structures exist as two or three dimensional shapes. Will the lessons that we learn about length control be in any way applicable to understanding size regulation of two or three dimensional cellular structures? In at least some cases such as the actin network at the leading edge of the cell, although the structure is two-dimensional, its width can be viewed as a one-dimensional size scale, and hence might be regulated by some of the length-controlling mechanisms discussed here. For membrane bound organelles, it seems likely that their size will be set by trafficking pathways that regulate total membrane surface area, and this would not involve a length measurement. However, a balance between vesicle fission and fusion to determine organelle steady state size would be similar to the dynamic balance mechanism discussed for length control. Thus, the potential relations between length control of 1D structures, and size control of 2D and 3D structures, remains very much an open question.

Outstanding questions box

- **How important are molecular rulers in eukaryotic cells?** While rulers have been extensively reported in bacterial and bacteriophage structures, the role of rulers in determining the size of eukaryotic structures has been less emphasized in the literature. Is this a real difference between eukaryotes and prokaryotes, or does it represent some sort of observer bias?

- **How does organelle size or length affect function?** This has been an impossible question to ask because in the absence of mechanistic understanding of size control, there is no way to predictably alter size and then test function. Recent progress on identifying size control systems now makes this question accessible to experimental testing.

- **Can mechanisms for controlling length be applied to controlling surface area of volume?** Length is a particularly easy parameter to measure and study, but it remains unclear whether the control mechanisms that cells use to control length will serve as paradigms for how cells control other aspects of organelle geometry.

Perhaps the most important open question is how size relates to function. Intuitively it seems clear that the size of a structure will have some bearing on its physiological function, yet experimental evidence for such a link is still mostly absent. One important reason for trying to understand the mechanisms that control size is that such an understanding should reveal the molecular control knobs that can be used to alter size, which is a pre-requisite for testing the effect of altered size on biological function.

Acknowledgments

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Trends box

- The mechanisms that control organelle size are not known. Length control of linear structures provides a simplified version of the more general organelle size control problem.

- A number of distinct mechanisms have been proposed for regulating length of different structures, including molecular rules, limiting precursor production, balanced assembly/disassembly, and molecular gradients.

- While molecular rulers play a prominent role in regulating length in prokaryotes, it is less obvious whether rulers are similarly important in eukaryotic cells.

- Organelle size control, as a problem that spans molecular and cellular scales of organization, is emerging as a key challenge for quantitative cell biology.

Trends in Cell Biology
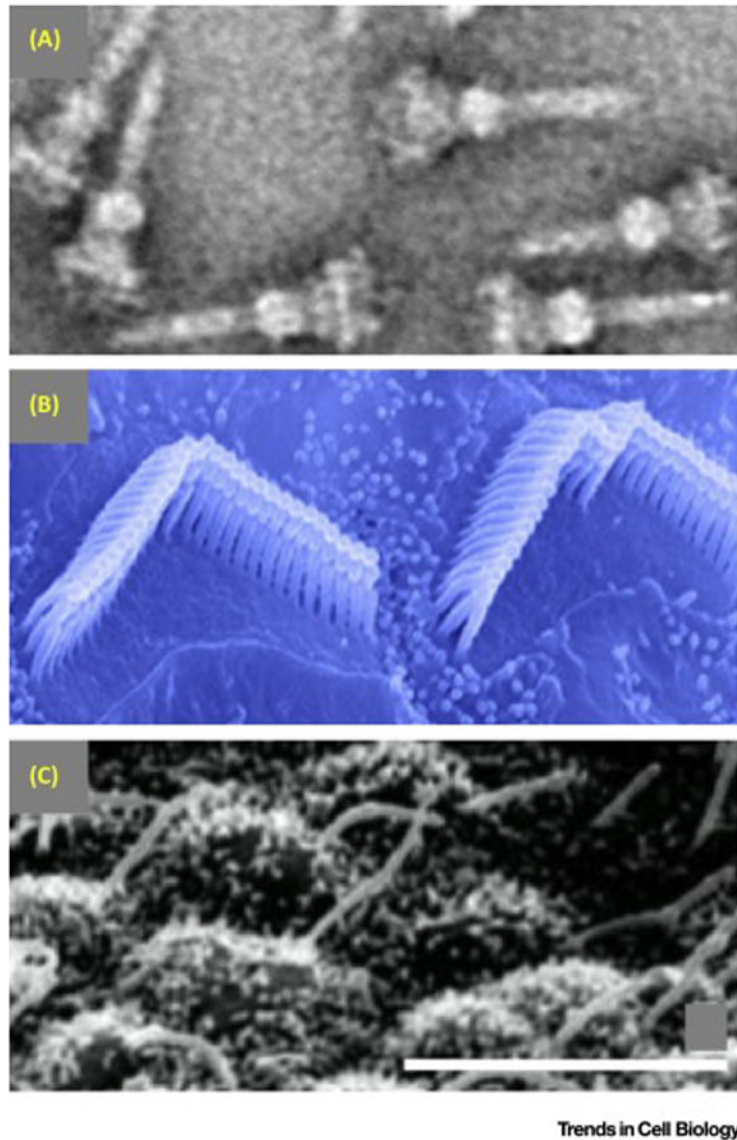
Figure 1.
Examples of linear structures with defined lengths. (A) Bacterial injection needle (Schraidt et al., 2010). Each needle is approximately 50 nm long. (B) Stereocilia (source: https://commons.wikimedia.org/wiki/File:Stereocilia.jpg). Image shows two adjacent hair cells from the ear, each possessing an array of actin-based stereocilia. (C) Cilia from the node of a developing mouse embryo (Nonaka et al., 2005). Scale bar 5 μm. These different structures on different length scales, composed of different molecular building blocks, all show precise length control but employ radically different mechanisms to achieve it.
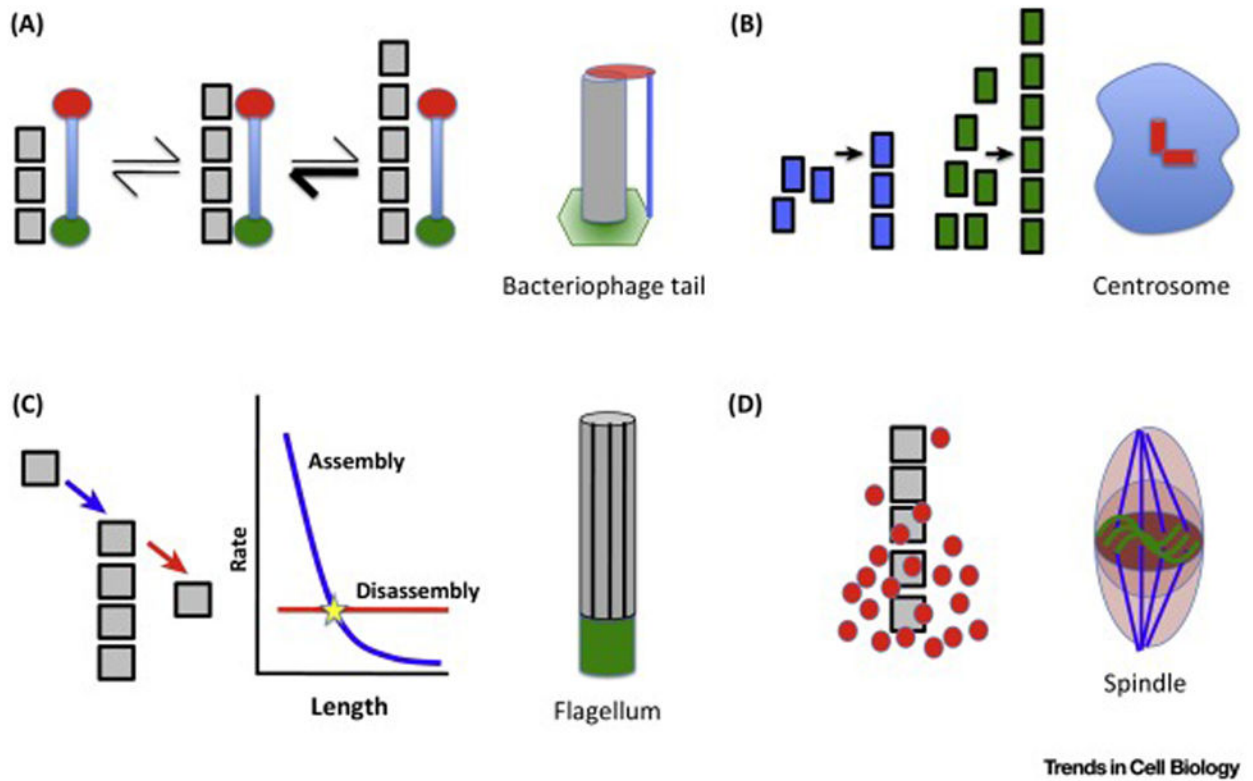
Figure 2.
Schematic of length control mechanisms. (A) Molecular ruler. Multimeric structure (monomers indicated by gray squares) assembles at one end while attached to the base (green) of a ruler molecule. When the end of the assembly passes the tip (red) of the ruler molecule, further growth is inhibited. Biological example: bacteriophage tail (Katsura, 1990). In the bacteriophage tail example, the tail assembles onto an initiator complex (green) and continues growing as long as its end is protected by the ruler molecule, which is physically as long as the tail (blue line). Growth is inhibited when the tail becomes too long because the end of the ruler (red) protects the tail from a depolymerizing factor, so that if the tail elongates past the ruler, disassembly increases as indicated by the bold arrow. (B) Limiting Precursor. Size of structure is determined by complete incorporation of a fixed quantity of precursor. If the cell makes more precursor (green), the final structure can be longer. Biological example: centrosome diameter (Decker et al., 2011). (C) Dynamic balance. Length of structure is determined by balance of assembly (blue arrow) and disassembly (red arrow). If the assembly rate is a decreasing function of length, while disassembly rate is length independent (inset graph) then there is a unique steady-state length (star). Biological example: eukaryotic flagella (Ludington et al., 2015). In the flagellum, microtubules (grey) grow from the basal body (green) by adding new tubulin at their distal ends. At steady state, tubulin is being continually added to and removed from the distal end, far from the basal body. As indicated in the graph, the assembly rate is length-dependent, in this case because the transport of tubulin to the tip is length dependent. (D) Diffusion-based measurement system. A diffusible ligand (red) produced near the base of a linear structure diffuses away from its source to set up a spatial gradient, which can be read

by other molecules to determine distance from the source. This information about distance can then be used to determine where to stop assembly of the structure. Biological example: the mitotic RanGTP gradient (Kalab and Heald, 2008). In the mitotic spindle, a gradient of RanGTP (pink) is generated with a source at the chromatin (green). This activated Ran diffuses and forms a gradient whose length matches the length of the spindle and is thought to influence formation of spindle microtubules (blue).