The Minus End in Sight

Review

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Microtubules are intrinsically polar structures. A consequence of this polarity is that the two ends of the microtubule polymer exhibit different properties. The more dynamic plus ends and the mechanisms that regulate their behavior have been the focus of much recent attention. Here, we concentrate on the dynamics and regulation of minus ends, which play distinct but equally critical roles in microtubule function. In the first part of this review, we compare the *in vitro* and *in vivo* behavior of microtubules from a minus end perspective. This comparison suggests that cells possess conserved mechanisms to specifically inhibit minus end polymerization, and perhaps also to actively promote depolymerization. In the second part, we focus on the spatial positioning of minus ends, which is achieved by localized microtubule nucleation, minus end capping and minus end anchoring as well as by motor-dependent sorting. These mechanisms are used in different biological contexts to generate the diversity of organized microtubule arrays in cells.

Introduction

Microtubule arrays direct intracellular organization and help to define cellular morphology. The cellular functions of microtubules critically depend on their intrinsic polarity, which results from the head-to-tail association of the α/β tubulin subunits (for a primer on microtubule structure see Box 1). This polarity is central to the ability of motor proteins to move unidirectionally on the polymer lattice and execute their diverse functions [1]. Microtubule polarity is also reflected in the distinct dynamic properties of the two polymer ends. Based on the analysis of microtubules assembled from purified tubulin, the faster polymerizing end was termed the 'plus' end and the more slowly polymerizing end the 'minus' end. Microtubule plus ends and proteins that affect their behavior have been the subject of much recent attention [2,3]. In this review, we focus on the dynamics and organization of minus ends, which play an equally important role in the functions of the microtubule cytoskeleton.

A Modern View of Microtubule Minus Ends in Cells

Microtubule arrays in cells are generally portrayed with dynamic plus ends exploring the cytoplasm and

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inert minus ends anchored at microtubule organizing centers. However, direct observation of microtubules in a variety of cell types has shown that the fraction of anchored minus ends varies extensively, from essentially all to practically none (Figure 1A). Within organized arrays, the minus ends of microtubules can also be dynamic, as is the case for spindle microtubules in metazoans (Figure 1A; reviewed in

Box 1

Microtubules are intrinsically polar polymers.

Microtubules are polymers of tubulin, which is itself a heterodimer composed of α- and β-tubulin. During microtubule polymerization, the head-to-tail association of α/β-tubulin heterodimers results in linear protofilaments. Within the microtubule, thirteen protofilaments associate laterally to form a hollow cylindrical structure that measures 25 nm in diameter. Heterodimers are oriented within the polymer lattice with β-tubulin pointing towards the faster polymerizing 'plus' end, and α-tubulin pointing towards the slower polymerizing 'minus' end. α- and β-tubulin are highly related proteins, ~50% identical at the amino acid level, and both bind GTP. When tubulin dimers polymerize, the GTP bound to β-tubulin is hydrolyzed, and the resulting GDP does not exchange as long as the heterodimer remains in the polymer lattice. In contrast, α-tubulin binds GTP in a non-exchangeable manner and does not hydrolyze its bound nucleotide during polymerization. The uniform orientation of tubulin heterodimers in the microtubule lattice confers a polarity that plays a crucial role in the functions of the microtubule cytoskeleton. For a detailed review of microtubule structure, see [99].

25 nm

Key:
- α-tubulin-GTP
- β-tubulin-GTP
- β-tubulin-GDP
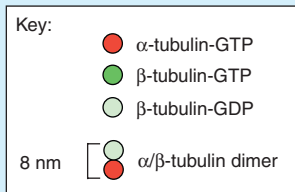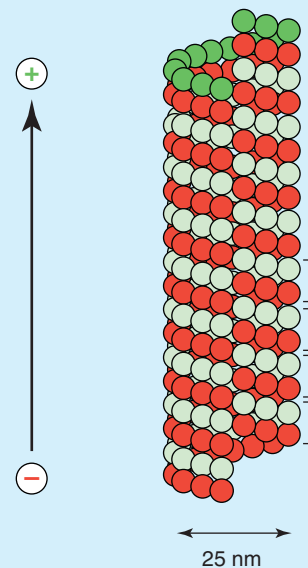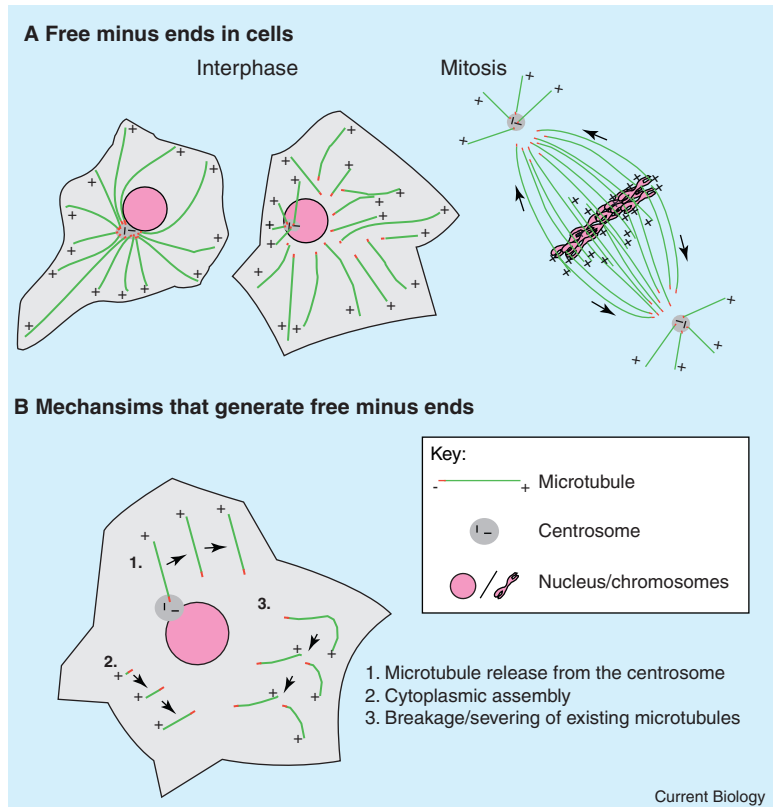- 8 nm α/β-tubulin dimer

Figure 1. Free microtubule minus ends in cells.

(A) The number of free minus ends in cellular microtubule arrays varies widely, from essentially all to practically none. The familiar text book view of a radial interphase array in which the majority of minus ends are embedded in a microtubule organizing center such as the centrosome (left interphase cell) is actually quite rare in cultured vertebrate cells. Cell types with such arrays include the human osteosarcoma cell line U2OS, the African green monkey kidney cell line CV-1 and its derivative COS-7, and the rat kangaroo epithelial cell line PtK-2. More commonly observed is a loosely organized interphase microtubule array with a large number of free microtubule minus ends (right interphase cell), as found in the widely used human cervical carcinoma cell line HeLa and mouse 3T3 fibroblasts. During mitosis, the minus ends of the 'astral' microtubules associated with each spindle pole are firmly anchored at the centrosomes and do not depolymerize. In contrast, the minus ends of spindle microtubules depolymerize, concomitant with the translocation of the microtubule lattice towards the spindle poles (arrows). (B) Free microtubule minus ends may be generated by: (1) release from microtubule organizing centers such as the centrosome, (2) cytoplasmic assembly, or (3) breakage or severing of existing microtubules. Figure 1B adapted from [97].

A Free minus ends in cells

Interphase   Mitosis

B Mechansims that generate free minus ends

Key:

— Microtubule

Centrosome

Nucleus/chromosomes

1. Microtubule release from the centrosome
2. Cytoplasmic assembly
3. Breakage/severing of existing microtubules

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[4]). Microtubules with free minus ends may be generated by release from a microtubule organizing center [5–7], cytoplasmic assembly [8,9], or breakage/severing of existing microtubules [10–12] (Figure 1B). In different cellular contexts, each of these mechanisms is thought to make an important contribution to the steady state nature of the cellular microtubule array.

An interesting example of an array with a majority of unanchored microtubules is found in the lamellae of migrating epithelial cells, where 80–90% of microtubules have free minus ends. In this subcellular domain, spontaneous nucleation and centrosomal release contribute only negligibly to microtubule number. Instead, the majority of new microtubules arise via breakage of existing microtubules in a 'convergence' zone where the retrograde flow of filamentous actin and microtubules from the lamellum collides with the slower anterograde flow of filamentous actin from the cell body [12,13]. This effectively couples generation and turnover of microtubules to the dynamics of the actin cytoskeleton. A strikingly similar behavior has been observed in the migrating growth cones of neurons [11], suggesting that this mechanism may have general relevance.

These and other studies have led to the current view that cellular microtubule arrays are composed of microtubule populations with both anchored and free minus ends. The existence of large populations of free microtubule minus ends suggests that, as with the plus ends, there are likely to be conserved mechanisms that regulate their behavior.

Minus End Dynamics *in vitro*

For microtubule plus ends, a quantitative comparison of the dynamic properties of microtubules polymerized from purified tubulin *in vitro* to those of microtubules in living cells stimulated the discovery of several conserved regulators (reviewed in [14,15]). Here we present a similar comparision for minus ends, beginning with a description of the dynamics of minus ends *in vitro*.

Both the plus and minus ends of microtubules polymerized from pure tubulin *in vitro* exhibit persistent phases of polymerization and depolymerization with infrequent transitions between these two states, a behavior termed dynamic instability (reviewed in [15]). This specialized non-equilibrium behavior is powered by the polymerization-coupled hydrolysis of GTP bound to β-tubulin. The free energy of GTP hydrolysis is stored in the microtubule lattice as mechanical strain, which, upon release, drives rapid depolymerization of the microtubule. Polymerizing microtubules are stabilized by a 'cap', whose exact nature is unclear. Transitions to rapid depolymerization, called 'catastrophes', result from loss of this stabilizing cap, either in a stochastic manner or due to the action of catastrophe promoting factors. Depolymerizing microtubule ends may recover ('rescue'), and reinitiate growth. Microtubule ends can also 'pause' exhibiting neither polymerization nor depolymerization, a behavior often observed in living cells.

Measurement of dynamic instability for both ends of microtubules polymerized from pure tubulin *in vitro* revealed that the behavior of the plus and minus ends

Box 2.

The γ-tubulin ring complex (γTuRC).

γTuRC
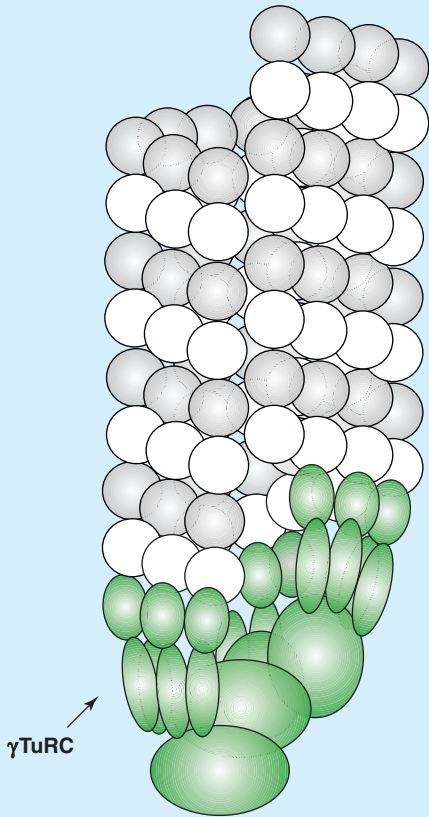
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γ-tubulin is a member of the tubulin superfamily thought to be important for microtubule nucleation and minus end capping. In animal cells, γ-tubulin is present in a large multiprotein complex, ~2 million Daltons in size, with the shape of an open ring approximately the diameter of a microtubule (25nm). Because of its characteristic shape, this complex has been named the γTuRC (for γ-tubulin ring complex; [31]). γ-tubulin-containing complexes have been studied primarily in the context of microtubule nucleation. Microtubule nucleation, or the formation of new polymer ends, is a kinetically limiting process. Nucleation is highly dependent on tubulin concentration because it requires the coalescence of multiple tubulin heterodimers to generate a seed or 'nucleus' that can subsequently elongate (reviewed in [43]). γ-tubulin-containing complexes accelerate the rate of microtubule nucleation *in vitro*, but since the γTuRC does not eliminate the steep tubulin concentration dependence of nucleation [31], the mechanistic basis of this acceleration remains unclear.

The γTuRC could also have a role in capping minus ends to inhibit their dynamics. Electron microscopy suggests that the γTuRC binds to microtubule minus ends in a cap-like configuration (see Figure) with one molecule of γ-tubulin at the end of each protofilament [30,100,101]. *In vitro* studies indicate that the γTuRC can inhibit the growth of microtubule minus ends [30,31] and may slow their depolymerization [30]. Whether the γTuRC plays an active role in capping microtubule minus ends *in vivo* is not known. Figure adapted from [101].

two-fold lower rate, exhibit slightly fewer catastrophes and are more likely to be rescued than plus ends [16]. The relative stability of minus ends is also reflected in their behavior after cutting of single microtubules. While newly created plus ends rapidly shorten, most minus ends resume polymerization [17,18]. Despite these differences, both plus and minus ends depolymerize at similar rates and polymerize over an essentially identical range of tubulin concentrations [16].

Minus End Dynamics *in vivo*
Elegant observations in various systems have documented the dynamics of free microtubule minus ends in cells. Below, we focus on some of the key conclusions from these studies and speculate on the origin of the differences between the behavior of minus ends *in vivo* and minus ends of microtubules polymerized from pure tubulin *in vitro*.

Free Minus Ends Never Polymerize
One strikingly consistent feature of free minus ends in cells is that they never polymerize. This is true for all of the diverse cell types that have been studied to date, as well as for microtubules assembled in crude cytoplasmic extracts (for examples see [8,12,19–26]). This lack of polymerization is independent of the mechanism by which the minus ends were generated. As the minus ends of microtubules assembled from purified tubulin polymerize readily at tubulin concentrations similar to those in cells, this striking difference suggests that specific cellular mechanisms prevent minus end growth.

In principle, minus end polymerization in cells could be inhibited by a protein that binds to tubulin dimers and specifically blocks their assembly onto minus ends, a post-translational modification of tubulin that prevents assembly onto minus ends, or a minus end capping factor. There is some experimental support for each of these ideas. A factor associated with tubulin dimers that prevents minus end polymerization was postulated based on work with sea urchin egg extracts [25]. However, such a factor has not yet been purified. The idea that a post-translational modification may be involved also appears plausible, because tubulin dimers chemically modified with N-ethylmaleimide (NEM) can specifically block elongation of purified tubulin at the minus end [27]. However, although tubulin is extensively modified *in vivo* (reviewed in [28]), a modification that would result in similar properties as NEM treatment has not been identified. Prevention of minus end polymerization by capping factor(s) is currently the most favored idea. However, such factors would have to be able to associate rapidly with minus ends, as minus ends generated by breakage of existing microtubules do not even exhibit transient growth. The best candidate for a minus end capping factor is the γTuRC, a ring shaped protein complex containing, among other proteins, the specialized tubulin isoform, γ-tubulin (see Box 2). γ-tubulin-containing complexes can cap microtubules *in vitro* [29–31], and soluble cytoplasmic pools of these complexes are present in cells [32,33]. Surprisingly, however, the key experiment of depleting γ-tubulin from cytoplasmic extracts and

is overall very similar but exhibits some quantitative differences. Minus ends polymerize at an approximately

Figure 2. An overview of centrosome structure.

Schematic representation of centrosome structure in a vertebrate somatic cell. A pair of centrioles is shown, each composed of 9 triplet microtubules ~400 nm in length [98]. Surrounding the centrioles is an electron-dense matrix termed the pericentriolar material. This matrix contains γ-tubulin ring complexes (γTuRCs) that nucleate microtubules. During duplication, a new ('daughter') centriole forms near the proximal end of the existing ('mother') centriole. The mother centriole has additional appendages near its distal end. These appendages are acquired by centrioles at the onset of mitosis, after centriole formation (reviewed in [37]). They appear to be the main site of microtubule anchorage at the centrosome and specifically contain a number of proteins, including ninein. Centriolar satellites, electron-dense spherical granules ~70–100 nm in diameter, are enriched in the vicinity of the centrosome. These granules play an important role in the microtubule-dependent recruitment of pericentriolar proteins [60].
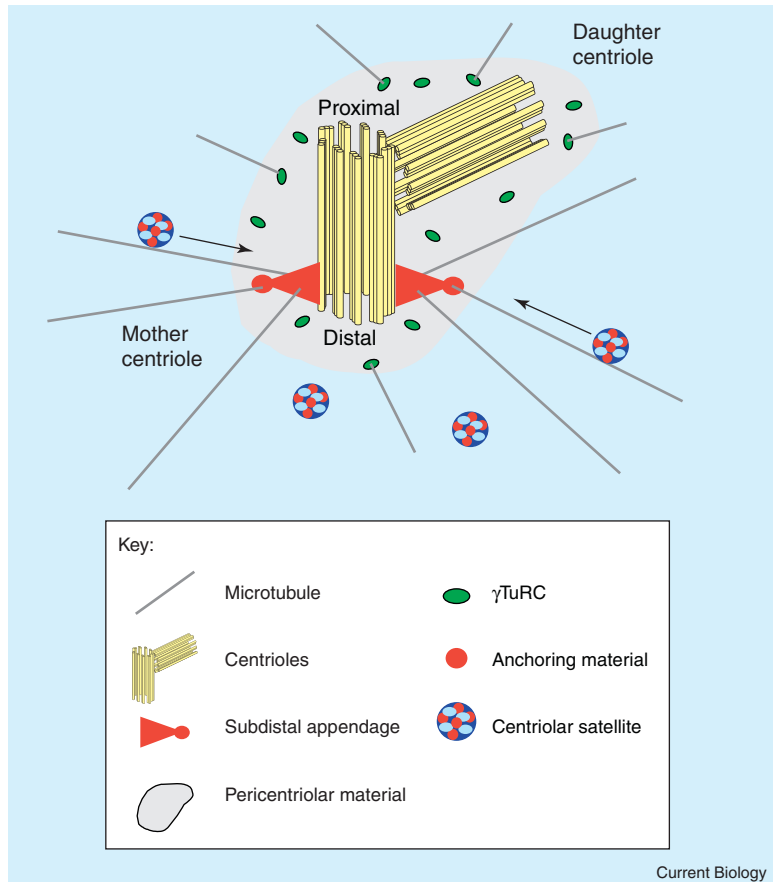
assessing the effect on minus end polymerization has not yet been done.

In summary, minus ends consistently fail to polymerize *in vivo*, indicating that robust and widely conserved mechanisms prevent their polymerization within cells. However, a clear picture of the mechanisms that underlie this dramatic difference in behavior has yet to emerge. Defining these mechanisms may help address the more fundamental question of why minus end polymerization has been so strongly selected against *in vivo*.

Free Minus Ends Are Stable or Persistently Depolymerize

In cells, free minus ends are either stable or depolymerize persistently. The stability of free microtubule minus ends typically correlates inversely with the proportion of microtubules that are anchored at microtubule organizing centers. In cells with tightly focused microtubule arrays, free minus ends generated by breakage rapidly transit to depolymerization (for examples see [7,22,23,34]). In contrast, in cells with a high fraction of unanchored microtubules, free minus ends are more stable [8,12,22]. However, even in these cell types, minus ends frequently depolymerize without rescuing, suggesting that persistent depolymerization from minus ends is a general mechanism for microtubule turnover. Minus end depolymerization is also a characteristic feature of spindle microtubules (reviewed in [4]).

The persistent depolymerization of minus ends observed in cells could be an active process that requires extrinsic factors. Such an active mechanism has been suggested primarily in the context of minus end depolymerization at spindle poles, but could also apply to free minus ends in the cytoplasm. To date, only the Kin I family of microtubule-destabilizing kinesins is known to depolymerize minus ends of artificially stabilized microtubules [35]. However, as is the case with γ-TuRCs and their role in minus end capping, it is not known whether Kin I kinesins depolymerize free minus ends *in vivo*.

In summary, the mechanisms preventing minus end polymerization and potentially promoting persistent minus end depolymerization in cells have not been defined. Deciphering these mechanisms should provide insight into the biological roles of minus end dynamics.

Radial Microtubule Arrays Organized by Centrosomes

In vivo, microtubules are often organized into radial arrays with their dynamic plus ends exploring the cell periphery and their less dynamic minus ends in the center. Radial microtubule arrays direct intracellular traffic, position organelles and orient the axis of cell division. In animal cells, radial arrays are generally organized by a small, specialized organelle, the centrosome.

Centrosomes consist of a pair of centrioles surrounded by an electron-dense matrix called the
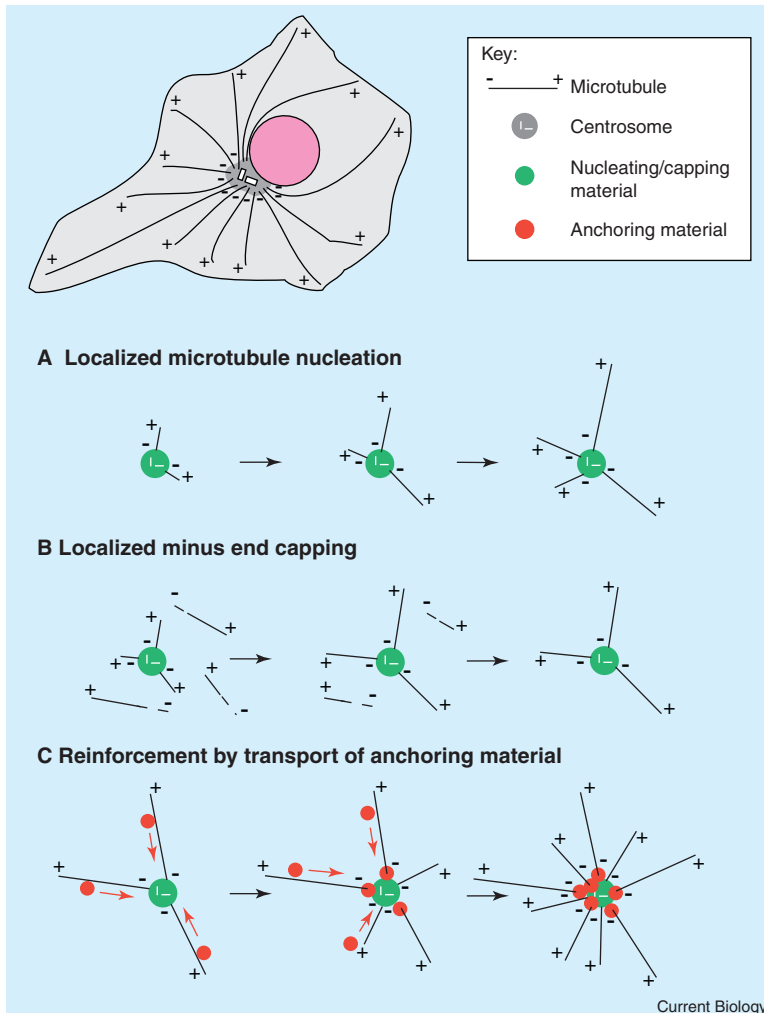
Figure 3. Mechanisms contributing to the formation of centrosomal microtubule arrays.

In cell types containing centrosomes, microtubules are frequently organized into radial arrays. Centrosomes contribute to this radial organization in three distinct ways: (A) Localized nucleation results in microtubules polymerizing outward in all directions from a focal point. (B) Localized minus end capping in combination with destabilization of free minus ends gives centrosomal microtubules a selective advantage. (C) Microtubule-dependent transport of anchoring material reinforces the nascent array established using the above two mechanisms.

pericentriolar material (Figure 2; reviewed in [36,37]). Centrioles are required for centrosomal integrity. When centrioles are disassembled by injection of antibodies to polyglutamylated tubulin, the surrounding cloud of pericentriolar material disperses [38]. Recent experiments suggest that centrioles specify not only the location but also the extent of the pericentriolar material accumulation. Progressive loss of a centriolar component, SAS-4, results in structurally defective centrioles that have proportionally less pericentriolar material associated with them [39]. Self-assembly of pericentriolar material may also help to create the discrete structural entity that is the centrosome. Support for this idea comes from laser ablation of centrosomes in vertebrate cells. If centrosomes are destroyed, a focus of pericentriolar material is reformed. This focus lacks centrioles but is still capable of organizing microtubules [40].

Organization of radial arrays by centrosomes has been primarily discussed in terms of the ability of the pericentriolar material to promote microtubule assembly. However, as discussed below, it is becoming increasingly clear that formation of a centrosomal microtubule array in cells involves the integration of multiple mechanisms that act on minus ends.

Localized Microtubule Nucleation

An important mechanism for the assembly of radial centrosomal arrays is the concentration of microtubule nucleation activity in the pericentriolar material (Figure 3A). This is evident from the observation that, upon reversal of microtubule depolymerization, microtubules regrow primarily from the centrosome [33,41,42]. Studies in many organisms support a conserved role for γ-tubulin complexes in centrosomal microtubule nucleation (Box 2; reviewed in [43,44]). γTuRCs are present within the pericentriolar material and at the minus ends of microtubules nucleated from isolated centrosomes [45]. Treatment of isolated centrosomes with chaotropic agents extracts the γTuRCs, leaving behind an insoluble fibrous matrix that is unable to nucleate microtubules. This matrix can recover nucleating activity after incubation with cytoplasmic extract, but fails do do so when incubated with extract that was depleted of γ-tubulin [46,47]. However, incubation of the matrix with purified γTuRCs fails to restore nucleating activity, suggesting that additional factor(s) are required [46]. Although a number of candidates for such a factor have been proposed [48–51], none of these has yet been shown to restore the nucleating capacity of extracted centrosomes when combined with purified γTuRCs.

The association of γ-tubulin with centrosomes is dynamic and changes during the cell cycle. In vertebrate cells, a large fraction of centrosome-associated γ-tubulin is slowly turning over with a half-life of about 40–60 minutes [52]. As cells enter mitosis, the level of centrosomal γ-tubulin and the capacity of centrosomes to nucleate microtubules increase 3–5 fold [52–54]. Neither the continuous turnover nor the mitosis-specific increase in centrosomal γ-tubulin requires intact microtubules [52,54]. Thus, transport by microtubule motors does not appear to play a role in the targeting of γ-tubulin to centrosomes.

The increase in centrosomal γ-tubulin during entry into mitosis is controlled by the protein kinases Aurora A and Polo, both of which localize to centrosomes ([54,55]; reviewed in [56]). The analysis of Polo function is complicated by its involvement in cell cycle progression. However, the idea is appealing that co-ordinated regulation by Polo and Aurora A ensures the accurate timing of γ-tubulin increase at centrosomes during mitotic entry.

Localized Minus End Capping

The concentration of minus end capping at centrosomes also contributes to the formation of radial arrays (Figure 3B). The minus ends of centrosomal microtubules do not depolymerize, suggesting that they are capped [7,57]. In contrast, the minus ends of free cytoplasmic microtubules often transit to depolymerization (as discussed above). The increased stability of their minus ends confers a selective advantage to centrosomal microtubules, reinforcing the radial array that is established by localized nucleation. The γTuRC may be responsible for capping minus ends at centrosomes, although direct evidence for this is difficult to obtain.

A role for minus end capping in the formation of radial microtubule arrays is an old idea [58] whose importance is becoming increasingly clear. An elegant experiment performed in centrosome-free cell fragments highlights the importance of localized capping [22]. In fragments derived from cells with focused microtubule arrays, free minus ends were continuously depolymerizing, whereas in fragments of cells with a large population of non-centrosomal microtubules the free minus ends were relatively stable. These results suggest that removal, or 'selective editing', of non-centrosomal microtubules by minus end depolymerization contributes to the radial organization of centrosomal arrays. In cell types with prominent radially organized arrays, free microtubules are more likely to depolymerize from their minus ends [7,34,59], suggesting that this mechanism may be widely relevant.

Reinforcement by Transport of Microtubule Anchoring Material

In addition to localized nucleation and capping, generation of a robust radial array requires anchoring of microtubules at centrosomes. In theory, a single protein complex such as the γTuRC could carry out all three of these functions. However, it is becoming increasingly clear that the ability to anchor microtubules at centrosomes is separate from their ability to nucleate microtubules. For instance, depletion or overexpression of the putative microtubule-anchoring protein ninein affects the ability of centrosomes to organize microtubules without perturbing nucleation [5,60]. Conversely, depletion of γ-tubulin in *C. elegans* embryos severely reduces the ability of the mitotic centrosome to nucleate microtubules but does not prevent radial organization of the remaining microtubules [61].

Unlike γ-tubulin, factors involved in microtubule anchoring at centrosomes require intact microtubules and dynein/dynactin motor function for their recruitment [62]. Moreover, this process requires PCM-1, a protein that localizes to the centriolar satellites, which shuttle between centrosomes and the surrounding cytoplasm in a microtubule and dynein-dependent manner (Figure 2; [60,63]). Disrupting transport by targeting PCM-1 or dynein/dynactin motor function results in a steady state distribution in which few microtubules are associated with centrosomes, although the centrosomes retain the ability to nucleate microtubules in regrowth experiments. Thus, the recruitment of anchoring factors by minus end-directed transport reinforces radial microtubule organization established by localized nucleation and is essential to maintain robust centrosomal arrays (Figure 3C).

While the capacity for nucleation is distributed throughout the pericentriolar material, the anchoring of centrosomal microtubules in vertebrate cells is localized to the subdistal appendages of the mother centriole, which appear to lack γ-tubulin (Figure 2; [32,64,65]). A number of centrosomal proteins, including cenexin/ODF2 [66], centriolin/CEP110 [67,68], ε-tubulin [69], and ninein [70], localize almost exclusively to these subdistal appendages. Of these, only ninein has so far been shown to be required for microtubule anchoring at centrosomes.

An interesting variation on the microtubule nucleation and anchoring theme is based on observations of centriole behavior in undifferentiated epithelial cells [65]. While both mother and daughter centrioles nucleate microtubules, only the mother centriole, via its subdistal appendages, is able to anchor them. Hence, microtubules nucleated by the daughter centriole are released into the cytoplasm, unless they are close enough to be captured by the anchoring material associated with the mother centriole. This predicts that the ability of the centrosome to release microtubules depends on the spatial separation between the centrioles, which has been found to vary much more extensively than previously thought [65].

In summary, the radial microtubule arrays organized by centrosomes arise via a combination of mechanisms. Centrioles direct the assembly of a small focus of pericentriolar material. γTuRCs, localized to this material, promote microtubule nucleation. Minus end capping by γTuRCs or other, as yet unidentified, factors confers a selective advantage to centrosomal microtubules. Radial arrays initiated by these mechanisms are reinforced by minus end directed transport of microtubule anchoring factors, such as ninein.

Non-Centrosomal Radial Microtubule Arrays

In addition to centrosomal organization, focused microtubule arrays can also form by other mechanisms. In
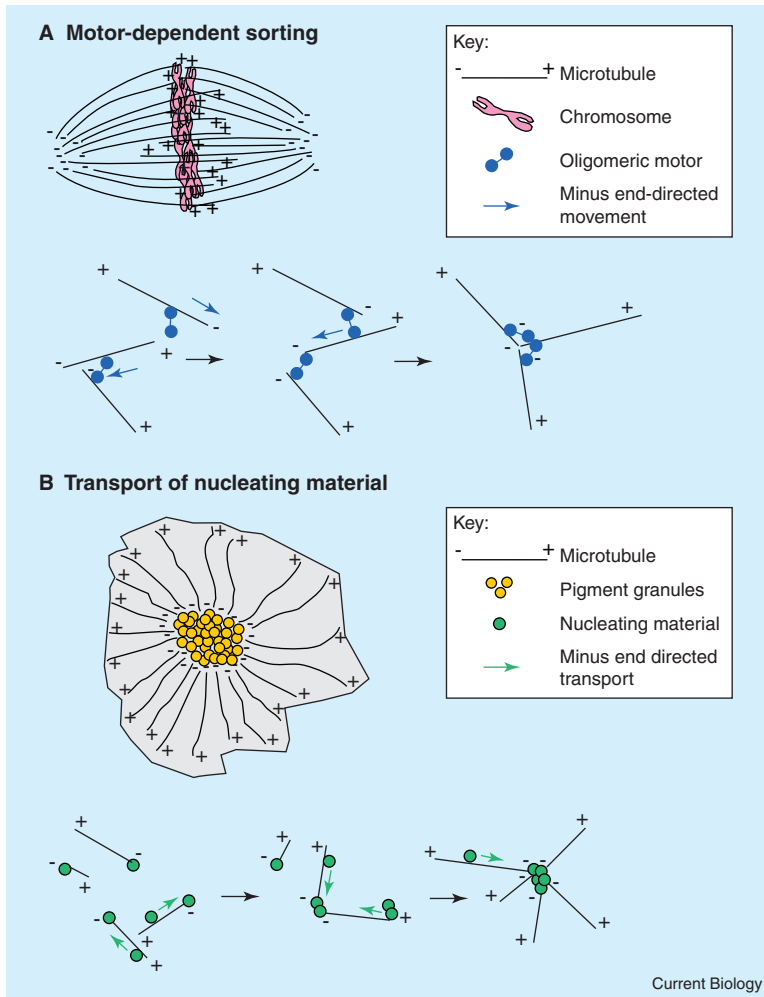
Figure 4. Centrosome-independent mechanisms for generating radial microtubule arrays.

(A) In acentrosomal spindles, such as those found during female meiosis in many organisms, minus ends are concentrated at spindle poles by directed movement of microtubules relative to each other. This movement is driven by oligomeric minus end-directed motor proteins. Such a motor-dependent sorting mechanism may also contribute to spindle assembly in cells containing centrosomes. (B) An unusual type of radial microtubule array is formed in acentrosomal cytoplasmic fragments of fish melanophores upon induction of pigment aggregation. Remarkably, formation of this array occurs without movement of microtubules relative to each other. Instead, radial microtubule organization arises through a combination of the minus end-directed transport of pigment granules bearing microtubule nucleation sites and continuous microtubule turnover [82].

this section, we describe the role of microtubule motor proteins in focusing minus ends during spindle pole formation. We then discuss an alternative mechanism that can generate radial organization by combining minus end-directed transport of nucleating material with microtubule turnover.

Organization of Microtubules by Oligomeric Motors

During cell division, the minus ends of spindle microtubules are tightly focused at the spindle poles. This ensures that segregating chromosomes move to a single location, where the nucleus of the daughter cell will eventually form. In the absence of centrosomes, spindle poles can form by self-organization of microtubules (reviewed in [4]). This self-organization occurs as a consequence of motor protein dependent movement of microtubules relative to each other (Figure 4A). In cells containing centrosomes, motor-dependent focusing has also been shown to contribute to pole formation [71,72].

In vitro, mixtures of oligomeric motor proteins and free microtubules self-organize into radial arrays [73,74]. Oligomerization plays a key role in this process, as it allows the motors to interact simultaneously with multiple microtubules and move them relative to each other (Figure 4A). At the spindle poles, self-organization

requires the minus end-directed motor cytoplasmic dynein, its activator dynactin, and the large coiled coil protein NuMA [75–77]. These proteins function as a complex, within which NuMA plays the critical role of oligomerizing the motor [76]. Minus end-directed movement of microtubules has never been observed during interphase when NuMA is sequestered in the nucleus, potentially because dynein cannot oligomerize.
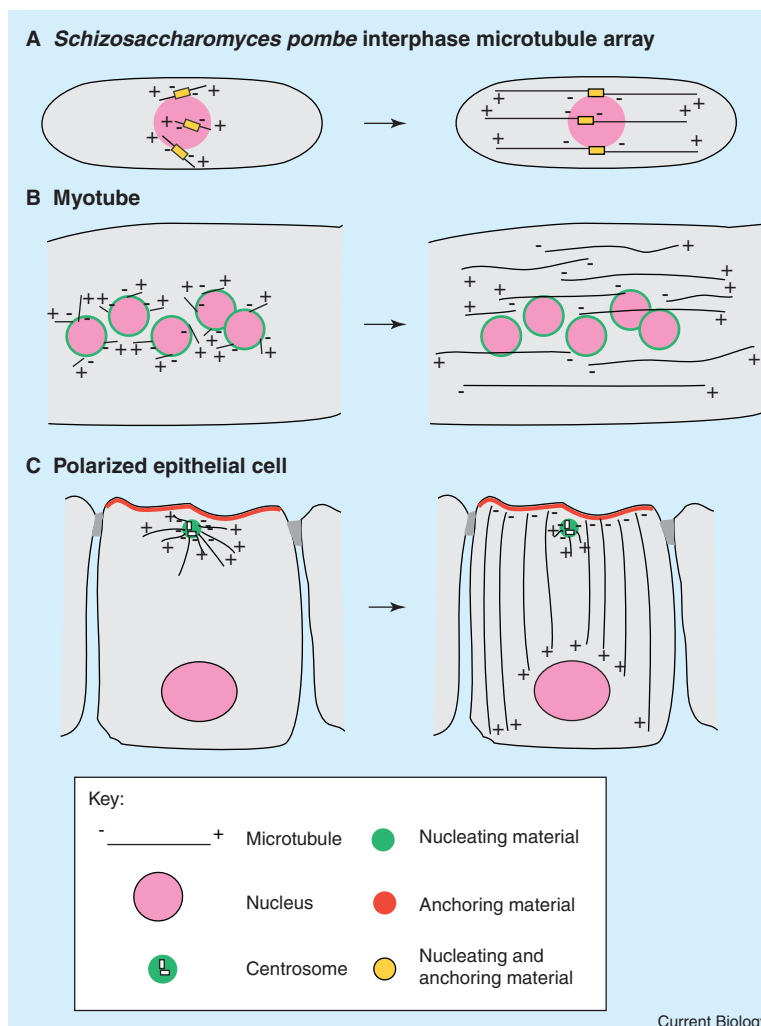
In all cases in which self-organization plays an important role in spindle pole formation, spindle microtubules continuously translocate towards the poles, where their minus ends are depolymerizing (reviewed in [4]). This is in contrast to centrosomally anchored, astral microtubules, which remain capped at their minus ends during mitosis [57]. Coupled poleward movement and minus end depolymerization of spindle microtubules contributes to the movement of chromosomes during anaphase [78–81]. Motor dependent self-organization may contribute to spindle function by allowing poles to remain focused in the face of continuous minus end depolymerization.

Minus End Directed Transport of Microtubule Nucleating Material

A new mechanism for organizing microtubule minus ends into radial arrays has emerged from work on

Figure 5. Specialized non-radial microtubule arrays.

The specialized functions of many cell types are often best served by non-radial microtubule arrays. The three examples discussed in the text are illustrated here. The left panels depict cells in the early stages of recovery from microtubule depolymerization, indicating the sites where microtubules are preferentially nucleated. The right panels indicate the microtubule distribution at steady state. (A) During interphase in the fission yeast *S. pombe*, cytoplasmic microtubule bundles originate from distinct sites on the nuclear envelope and span the length of the cell. Microtubules are firmly anchored at these sites, as they can withstand the pushing forces exerted on them during nuclear positioning. Microtubules regrow from these sites during recovery from depolymerization, indicating that they can nucleate as well as anchor microtubules. (B) During myogenesis, both pericentriolar material proteins and microtubule nucleation potential redistribute from the centrosome to the nuclear periphery. Thus, as shown on the left, microtubules regrow from the nuclear surface following recovery from depolymerization. At steady state, microtubules are still concentrated in the vicinity of nuclei but predominantly run parallel to the long axis of the myotube, suggesting an absence of anchoring factors on the nuclear periphery. (C) In polarized epithelial cells, centrosomes remain the kinetically dominant site of microtubule nucleation. However, at steady state, microtubules do not remain associated with the centrosome but are oriented along the apico-basal axis with their minus ends anchored at the apical surface.

A *Schizosaccharomyces pombe* interphase microtubule array

B Myotube

C Polarized epithelial cell

Key:

− ——— + Microtubule

Nucleus

Centrosome

Nucleating material

Anchoring material

Nucleating and anchoring material

Current Biology

centrosome-free fragments of fish melanophores. In response to adrenaline stimulation, radial microtubule arrays assemble in these cell fragments and pigment granules that were originally dispersed throughout the cytoplasm now aggregate at the center (Figure 4B). Surprisingly, assembly of these radial arrays does not occur by the movement of microtubules relative to each other [82]. Instead, minus end directed movement of nucleating activity in the presence of continuous microtubule turnover is postulated to generate radial organization in this system (Figure 4E; [82]). It will be interesting to determine whether this conceptually appealing mechanism also operates in other cell types. Since γTuRCs have not been reported to be transported towards minus ends, further investigation of this mechanism may also help identify alternative microtubule nucleating factors.

Non-radial Microtubule Arrays
Non-radial arrays are frequently generated to expand the functional repertoire of the microtubule cytoskeleton. This is particularly true during the differentiation of specialized cell types in multicellular organisms, but also applies to unicellular eukaryotes. The formation of non-radial arrays involves many of the mechanisms

discussed above in the context of radial arrays. In this section, we focus on three specific examples that illustrate how nucleation and minus end anchoring are manipulated to help generate non-radial microtubule arrays. Motor-dependent self organization may also contribute to formation of non-radial arrays, as is suggested by localization of the kinesin Eg5 [83] and the dynein-oligomerizing protein NuMA [84] in neurons. However, as there has been no further exploration of such a mechanism outside of cell division, it will not be discussed here.

The Interphase Microtubule Array in Fission Yeast
During interphase in the fission yeast *Schizosaccharomyces pombe*, between 3 and 8 microtubule bundles originate from sites on the nuclear envelope and extend along the long axis of the cell (Figure 5A; reviewed in [85]). This non-radial microtubule array plays a key role in targeting growth to the cell ends and positioning the nucleus within the cell [86,87]. One of the microtubule organizing sites associated with the nuclear envelope is the spindle pole body, the fungal equivalent of the centrosome. However, the other sites are present only during interphase and have been termed interphase microtubule organizing

centers (iMTOCs); [86]). During recovery from depolymerization, microtubules regrow from iMTOCs with their plus ends facing the periphery. Microtubule attachments to iMTOCs can withstand the considerable pushing forces that are exerted on them during nuclear positioning, indicating that the minus ends are firmly anchored [86]. Thus, the interphase microtubule array of *S. pombe* is generated by localizing both nucleation and anchoring to discrete transient structures. Similar observations have been made in the fungal plant pathogen *Ustilago maydis* [88], suggesting that this may be a common mechanism for the assembly of non-radial arrays in fungi.

Microtubule Reorganization During Myogenesis

Conceptually, the simplest method to generate a non-radial microtubule array would be to redistribute microtubule nucleating material to other sites within the cell. This is what appears to occur during myogenesis, as muscle progenitor myoblasts elongate and fuse to form myotubes (Figure 5B). While myoblasts contain a radial microtubule array, organized by the centrosome, such arrays are not found in myotubes. Instead, microtubules run parallel along the contractile axis of the cell with some enrichment near the nuclear periphery. This specialized microtubule array is involved in myofibril assembly [89,90]. In myoblasts, microtubule regrowth is first observed at centrosomes during recovery from depolymerization. By contrast, in myotubes, microtubules first appear near the surface of the nucleus (Figure 5B; [91]), suggesting a redistribution of nucleating material. Consistent with this idea, pericentriolar proteins, including pericentrin, are redistributed from the centrosome to the nuclear periphery during myogenesis [92]. Whether γ-tubulin is concentrated at the nuclear periphery is an interesting question that has not yet been addressed. The fate of the centrioles themselves is uncertain. While they are readily detected in surrounding satellite cells, centrioles are rarely observed in skeletal muscle fibers and may be discarded at some stage during the differentiation process [93].

Microtubule Arrays in Polarized Epithelial Cells

In polarized epithelial cells, microtubules form an apico-basal array with their minus ends concentrated near the apical surface (Figure 5C). This array directs vesicle trafficking that is central to the function of epithelia (reviewed in [94]). Centrosomes are still present but have only a few microtubules associated with them. However, in contrast to myotubes, microtubule regrowth following depolymerization occurs from centrosomes [33]. This suggests that redistribution of microtubule anchoring activity, but not nucleating activity, occurs during generation of the non-radial array in epithelial cells. Consistent with this, the putative anchoring protein ninein is concentrated at the apical surface [70].

The origin of the microtubules that are anchored at the apical surface remains unclear. One possible mechanism would be release of centrosomal microtubules followed by transport to the cell periphery [95]. However, microtubule release from the centrosome is a rare event in the cell types in which it has been examined [7,8,12,23]. Release of microtubules may be enhanced by microtubule severing proteins such as katanin [6,96], or simply reflect the absence of anchoring factors, such as ninein [5]. While microtubule transit to anchoring sites is likely to involve active transport, motors that mediate this process have not been identified. A better understanding of the mechanism by which microtubule arrays are established in polarized epithelial cells awaits analysis by live imaging.

Perspective

The minus ends of microtubules have long remained in the shadow of their more dynamic plus end counterparts. In this review, we have attempted to redress the balance. The observed lack of minus end polymerization in cells is not an inevitable consequence of microtubule structure. Instead, it appears to be specifically inhibited in cells through currently unknown mechanisms. This inhibition of polymerization may allow stable anchorage of one end and, thus, aid the organization of microtubule arrays. Radial as well as non-radial microtubule arrays depend on the localization of microtubule minus ends to particular sites within the cell. This may be achieved by localizing nucleation, minus end capping and/or minus end anchoring activities, as well as by transporting pre-formed microtubules. With a few notable exceptions, the formation of organized microtubule arrays has not been studied using live microscopy. Similarly, the molecular basis of many of the activities that are centered around minus ends—nucleation, capping, anchoring—remains unclear. In conclusion, given the many gaps that remain in our knowledge of how minus end behavior is regulated in cells, the end is clearly not in sight.

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