

Pathways of Spindle Pole Formation: Different Mechanisms; Conserved Components

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A central event during cell division is the transformation of an interphase network of microtubules into a bipolar spindle. For most animal cells the centrosomes, a pair of centrioles surrounded by electron-dense pericentriolar material, represent the microtubule organizing centers from which interphase microtubules are nucleated, with the microtubule minus ends at the pole and the rapidly growing, free plus ends extending away. At, or just before, the time of nuclear envelope fragmentation, the duplicated centrosomes separate from each other using microtubule-dependent motors that push against the astral microtubules nucleated by each centrosome. Microtubules penetrate the nucleus, and in a mechanism called “search and capture” (Kirschner and Mitchison, 1986) some attach to kinetochores, specialized regions that assemble onto the surface of centromeres (Fig. 1 A). As a result, most mitotic animal cells have spindles with two clearly defined spindle poles at which the microtubules (kinetochore attached, pole-to-pole, or astral) converge in a focal area around each centrosome (Fig. 1 B). This, and the proven ability of centrosomes to nucleate microtubules efficiently *in vitro* (Mitchison and Kirschner, 1984), have fueled the general view that centrosomal microtubule organizing centers are essential features of spindle assembly and organization.

Attractive as this paradigm is, several examples of meiotic spindles, as well as early embryonic mitotic spindles in animals, have been found without centrosomes, displaying a spindle morphology that is more reminiscent of a barrel shape and lacking astral microtubules. Also, many plant cells are devoid of morphologically recognizable centrosomes. Several proposals to explain this discrepancy have been offered, perhaps the most prominent of which was that of Mazia (1984), who suggested the existence of flexible centrosomal material, aligned on a ribbon-like structure that can fold or extend in cell-type specific ways

and act in microtubule organization in all cells. Two candidate proteins for such microtubule organizing material are γ tubulin and pericentrin, both centrosomal components in “conventional” spindles. γ Tubulin has been found in ring-like structures (Zheng et al., 1995) that may be aligned on a cage-like lattice, most likely provided by pericentrin (Dichtenberg, J., W. Carrington, F.S. Fay, and S.F. Doxsey. 1995. *Mol. Biol. Cell.* 6:40a), and both proteins are found at poles in mouse oocytes and early embryos, although no centrosomes can be detected (Gueth-Hallonet et al., 1993). Surprisingly, however, there are mitotic and meiotic cells (e.g., in *Drosophila*; Matthies et al., 1996; Wilson et al., 1997) that appear to have neither centrosomes nor detectable amounts of γ tubulin; furthermore, pericentrin is dispensable for centrosome-independent formation of microtubule asters and half spindles *in vitro* (Kallajoki et al., 1992; Gaglio et al., 1996).

Spindle Poles without Centrosomes

How do spindles form in the absence of preexisting microtubule organizing sites? Live observations of meiotic spindle formation in *Drosophila* oocytes (Matthies et al., 1996) have revealed that the spindles form by an “inside-out” mechanism in which microtubules reorganize around the mass of chromatin (Fig. 2 A). This process may involve the action of chromatin-bound, plus end-directed microtubule motors, including such candidates as the chromatin-associated, kinesin-like proteins chromokinesin (Wang and Adler, 1995), its frog homologue Xklp1 (Vernos et al., 1995), and *Drosophila* Nod (Afshar et al., 1995). With the microtubule minus ends oriented away from the chromatin in these developing spindles, the organization of the microtubules into bipolar spindles may then be achieved by the action of multivalent, minus end-directed microtubule motor complexes that can tether parallel-oriented microtubules into bundles and stabilize converging microtubules into poles (Fig. 2 B). As Matthies et al. (1996) showed, in *Drosophila* oocytes this process is clearly dependent on the presence of the minus end-directed motor Ncd, although there seem to be other motor proteins with redundant functions involved.

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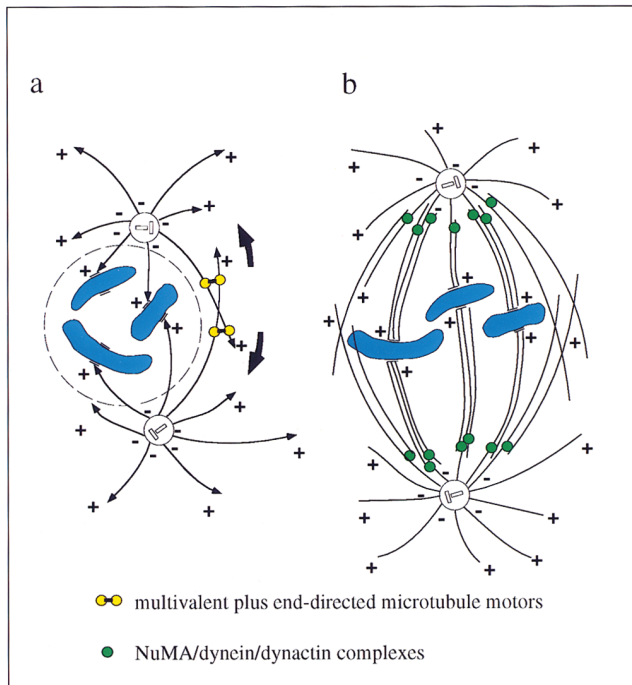


Figure 1. Spindle formation in centrosome-containing cells. (a) Microtubules are nucleated from the duplicated centrosomes with their growing plus ends pointing away from the centrosomes. Microtubules that penetrate the perforated nuclear envelope in prometaphase are captured by the kinetochores of the chromosomes. Multivalent plus end-directed motors of the bimC family may be involved in the separation of the two centrosomes and the establishment of a symmetric spindle axis (*big arrows*). (b) In the mature spindle, microtubule minus ends disconnect from the centrosomes and are anchored to the body of the spindle by complexes of NuMA/dynein/dynactin. (The chromosomes are indicated in blue.)

Dynein-dependent Spindle Pole Assembly with or without Centrosomes

The concept of centrosome-free spindle pole formation depending on the action of microtubule motors was directly demonstrated during spindle assembly in extracts from metaphase-arrested frog eggs (Heald et al., 1996). Using DNA-coated beads as chromosomal substitutes, microtubules were nucleated and organized into a bipolar spindle apparatus without specialized centromere sequences on the DNA and without centrosomes at the poles. Addition of an antibody to the intermediate chain subunit of the microtubule motor cytoplasmic dynein blocked the organization of microtubule arrays into focused poles without affecting the assembly of a bi-oriented array of microtubules emerging from the centrally localized, bead-bound DNA. The establishment of bipolarity without centrosomes thus involves two independent mechanisms. The first is sorting of microtubules into a bipolar axial array, which may be achieved by plus end-directed, multimeric motors that can promote anti-parallel microtubule sliding and axial alignment. Candidates for such an activity are the tetrameric motors of the BimC family, such as Eg5 and KRP130 (Kashina et al., 1996). The second is bundling of these oriented microtubules into

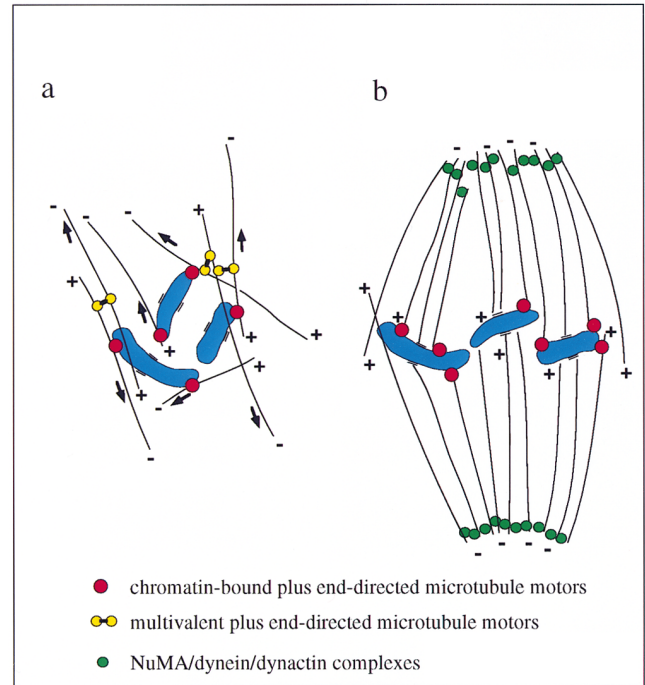


Figure 2. Spindle formation in centrosome-free cells. (a) Spindle formation is driven by chromatin-associated, plus end-directed microtubule motors, orienting chromatin-attached microtubules with their minus ends outward (*arrows*). Multivalent plus end-directed microtubule motors of the bimC family can interconnect antiparallel microtubules and establish a bipolar organization of the spindle by moving the microtubule ends apart. (b) During spindle pole formation, complexes composed of NuMA, dynein, and dynactin induce convergent arrays of microtubules at the spindle poles and provide stability to the spindle by tethering the microtubule minus ends.

poles, involving the minus end-directed, microtubule motor cytoplasmic dynein. Consistent with this, dynein has been implicated by a variety of in vitro studies in frog and mammalian mitotic extracts in which dynein-dependent, centrosome-free spindle pole formation was mimicked by the induction of microtubule asters in the presence of the drug taxol (Verde et al., 1991; Gaglio et al., 1996), as well as by immunolocalization of dynein on spindle poles in dividing cells (Pfarr et al., 1990; Steuer et al., 1990).

A Complex of Cytoplasmic Dynein, Dynactin, and NuMA Tethers Centrosomes to Spindle Microtubules

Through what mechanism can dynein provide stability to spindle poles with or without centrosomes? In work using *Xenopus* egg extracts, Heald et al. (1997) showed that addition of an antibody against cytoplasmic dynein intermediate chain blocks the translocation of fluorescently tagged spindle microtubules along each other. Since organization of microtubules into poles is also blocked by this same dynein antibody, the evidence suggests that minus end-directed microtubule gliding is a prerequisite to organize microtubules into convergent polar arrays. Furthermore, Heald et al. (1997) demonstrated that dynein acts as a microtubule

tethering factor at the spindle poles, irrespective of the presence or absence of centrosomes.

Cytoplasmic dynein-dependent microtubule tethering at poles requires at least two microtubule binding sites that in principle could be achieved by the dimeric dynein heavy chain head domains. However, other efforts have clearly demonstrated that dynein does not act by itself. Rather, dynein-associated proteins are needed, including its motility-activating complex dynactin (Gaglio et al., 1996) and NuMA, a 240-kD protein with a ~1,500-amino acid-long helical domain, separating globular head and tail regions. NuMA is nuclear during interphase but localizes to the spindle poles in mitosis as well as to centrosome-free spindles in meiosis (Tang, T.K., C.J.C. Tang, and H.M. Hu. 1995. *Mol. Biol. Cell.* 6:422a; Navara, C.S., C. Simerly, D.A. Compton, and G. Shatten. 1996. *Mol. Biol. Cell.* 7: 208a). The NuMA tail binds to microtubules *in vitro*, and NuMA in frog egg extracts is associated in a nearly stoichiometric complex with cytoplasmic dynein and dynactin (Merdes et al., 1996).

Several lines of evidence demonstrate that NuMA plays a critical role in microtubule tethering at poles. Immunodepletion of the NuMA/cytoplasmic dynein/dynactin complex from frog egg extracts does not affect assembly of a bi-oriented array of microtubules with centrally oriented chromosomes but does completely block the aggregation of the microtubule minus ends into focused spindle poles, despite the presence of centrosomes (Merdes et al., 1996). As there is a many-fold excess of cytoplasmic dynein and dynactin over NuMA, disruption of pole formation must reflect a necessity for NuMA not diminution of cytoplasmic dynein or dynactin. This phenotype is almost indistinguishable from the effect of inhibitory anti-dynein antibodies added to a similar spindle formation assay (Heald et al., 1996, 1997). Similarly, immunodepletion of NuMA from mammalian mitotic extracts completely blocks taxol-induced microtubule aster formation (Gaglio et al., 1995, 1996), as does addition of a monoclonal antibody to the dynein intermediate chain. The latter, reported on pages 1055–1066 of this issue, apparently leads to the disconnection of dynein from dynactin (Gaglio et al., 1997), even though this antibody does not affect the motility of purified dynein itself (Heald et al., 1997). Further, depletion of either cytoplasmic dynein or dynactin in the taxol-induced aster formation assay yielded only randomly oriented microtubules with NuMA scattered all over the microtubule length. Direct support for an involvement of dynactin in spindle formation came from overexpression of p50/dynamitin, one of the nine known components of the dynactin complex. This caused disruption of the complex and resulted in aberrant spindle morphology with irregular poles (Echeverri et al., 1996).

The simplest view is that microtubule tethering into poles is mediated by a large complex containing NuMA, dynein, and dynactin, using the motor activity of dynein to power the complex toward the microtubule minus ends and the distinct microtubule binding sites on NuMA (Merdes et al., 1996) and the associated p150 dynactin component (Karki and Holzbaur, 1995; Vaughan and Vallee, 1995) to provide the needed crosslinking. The displacement of NuMA upon dynein or dynactin depletion or upon microinjection of anti-dynein antibody into cells (Gaglio et al.,

1997) supports the idea that NuMA is one of the specific cargos of the dynein motor during cell division.

Why Is Microtubule Tethering to Spindle Poles Needed?

From the viewpoint of the centrosome as the nucleator of spindle microtubules, why is a NuMA/dynactin/cytoplasmic dynein complex necessary for pole assembly? The most obvious and direct answer emerged initially from serial sectioning of a mammalian mitotic spindle. Unlike many simplified text book views, this revealed that up to 75% of the interpolar microtubules do not connect directly to the centrosome but end within a distance of ≥ 1 μm thereof (Mastronarde et al., 1993). Hence, most spindle microtubules cannot be directly attached to the pole. Moreover, removal of the centrosome by micromanipulation does not grossly affect the integrity of the spindle (Nicklas, 1989; Nicklas et al., 1989). A plausible model for what keeps these microtubules in place invokes the NuMA complex, which is distributed in a broad, crescent-shaped area between the centrosome and the spindle microtubule bundles, rather than focused directly at the centrosome. NuMA thus is likely to be one of the connecting molecules that anchor the large number of free microtubule minus ends to the microtubules still directly nucleated by the centrosome. Furthermore, as Heald et al. (1997) and Gaglio et al. (1997) now demonstrate, cytoplasmic dynein plays an essential role in linking centrosomes to spindles. Addition of one dynein intermediate chain antibody to spindles formed *in vitro* (Heald et al., 1997), as well as microinjection of that same antibody into cultured cells (Gaglio et al., 1997), leads to the disconnection of the centrosome from the rest of the spindle. Similar effects were previously observed upon microinjection of anti-NuMA antibodies (Gaglio et al., 1995) or overexpression of p50/dynamitin of the dynactin complex (Echeverri et al., 1996). Thus, in both centrosome-free and centrosome-containing spindles, NuMA, dynein, and dynactin are involved in stabilizing the spindle poles.

While Heald et al. (1997) do demonstrate that there is dynein-dependent poleward flow of labeled microtubules added *in vitro* and provide a plausible explanation for pole formation by dynein-driven poleward microtubule movement in noncentrosomal meiotic spindles, it seems less likely that this reflects the *in vivo* mechanism of pole formation in centrosome-containing cells, because the dominant microtubule nucleation site is located at the centrosome and not at the kinetochore (Mitchison et al., 1986; Geuens et al., 1989). Thus, early in mitosis, the majority of the microtubules should be centrosome bound, but as the mitotic cycle proceeds, some of these disconnect from their nucleation centers. The newly freed (possibly uncapped) minus ends may be essential for a mechanism called “poleward microtubule flux,” seen in metaphase and anaphase of mitosis. Poleward flux involves translocation of microtubules towards the spindle poles, while the microtubule plus ends at the spindle equator elongate and the minus ends at the poles shorten simultaneously. This flux is likely to be powered by microtubule motors such as dynein bound to the moving microtubules or alternatively, plus end-directed microtubule motor proteins such as Eg5 or Xklp2 (Sawin et al., 1992; Boleti et al., 1996) bound to

an immobilized spindle pole matrix, comprised, at least in part, of NuMA. Further evidence consistent with this is the ability of NuMA to form a filamentous meshwork when overexpressed in the cytoplasm (Saredi et al., 1996), as well as unusually long spindle-like structures assembled in the absence of NuMA (Merdes et al., 1996), suggesting the loss of part of the "flux" machinery necessary for the shortening of microtubule minus ends.

Centrosomes Versus No Centrosomes

With many conserved components between centrosome-containing and centrosome-free spindles, a key difference may simply be the high abundance of these components in eggs and early embryos that may enable these systems to form spindles by self organization of microtubules through recruitment of any necessary factors from the large cytoplasmic pool. In somatic cells, such supplies are more limited, and spatial constraints within the cell, as well as the requirement of a specific orientation of the cell in a tissue, may favor spindle formation from preexisting, centrosomal microtubule organizing centers. As demonstrated in the paper by Heald et al. (1997), the potential of centrosomes to organize microtubules provides a kinetic advantage to the cell, and when present, centrosomes are dominant over self assembly of microtubules around chromatin. In both cases of spindle assembly, however, the underlying principles of pole organization relying on NuMA/cytoplasmic dynein/dynactin-dependent microtubule tethering remain largely similar.

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