Spindle assembly: asters part their separate ways

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Cells have developed diverse ways to separate two microtubule asters to form a mitotic spindle. Here, I focus on two mechanisms used to position asters around chromosomes during mitosis: first, aster migration around the nuclear envelope and, second, aster attachment to a contractile cortex at the plasma membrane after the nuclear envelope has broken down. Although certain cell types use one mechanism predominantly, most rely on both to ensure proper spindle assembly.

To guarantee that a cell's entire genome is faithfully transmitted, chromosomes duplicate, condense, and segregate on a mitotic spindle. The mitotic spindle consists of two microtubule asters that span either side of the condensed chromosomes. During prophase, when the spindle starts to assemble, the centrosomes that nucleate asters separate around the nuclear envelope (Fig. 1a). After the nuclear envelope has broken down (NEBD) during prometaphase, the asters continue to separate until they become aligned on either side of the condensed chromosomes (Fig. 1b). Once the asters have completed migration, some of the aster microtubules will bind to the chromosomes and align them at the metaphase plate.

For a spindle to assemble correctly, the centrosomes must migrate in the correct direction and stop migrating once they reach their appropriate location on either side of the chromosomes. Moreover, in most cases the spindle must position itself centrally within a cell so that the two daughter cells produced are of the same size and composition. Even in cases in which a cell is programmed to divide asymmetrically, it first forms a spindle that fits symmetrically in a cell and then rotates or shifts the spindle to make the asymmetric division (for an example, see ref. 1). Therefore, spindle assembly must be considered in conjunction with spindle positioning within the cell.

Although the spindle was discovered over 120 years ago (see ref. 2 for a review), we still know surprisingly little about the processes that drive its assembly. Although genetics and cytological analyses of fixed and live cells have greatly increased our knowledge of how a spindle assembles, much recent progress has come from *in vitro* spindle self-assembly assays. However, it is important to note that the spindles that were assembled in these *in vitro* assays use an alternative pathway that is typical to cells that do not contain centrosomes. Cells without centrosomes do not nucleate asters that separate around chromosomes, but instead build their spindles 'inside-out' by nucleating and organizing microtubules around chromosomes after NEBD[3,4]. Although these self-assembly mechanisms can assist chromosome alignment and spindle maintenance (reviewed in ref. 5 and discussed later), they are not the primary mechanism that drives aster separation in somatic cells. Because there are several excellent reviews on spindle self-assembly mechanisms[6–8], I will not discuss them here. Instead, I will focus on the mechanisms used to position two asters on either side of condensed chromosomes in mitotic somatic cells. Furthermore, I hope to highlight the gaps in our knowledge so that future studies will give a better understanding of these mechanisms, and perhaps unveil new mechanisms by which spindle assembly occurs.

Aster separation around the nuclear envelope

All eukaryotes contain a nucleus, and so it is not surprising that most of these cells use the nuclear envelope to help assemble the mitotic spindle. The nuclear envelope provides a direct path for the two halves of a spindle to separate and position themselves on either side of condensed chromosomes (Fig. 1a, c). The extent to which a cell uses the nuclear envelope to separate the two asters depends on when NEBD occurs. For instance, in most somatic cells NEBD can occur at any time during aster separation[9] (Fig. 1d), whereas in early *Caenorhabditis elegans* and *Drosophila* embryonic cells it occurs very late[10] (Fig. 1c). In either case, the primary path of the centrosomes before NEBD is around the nuclear envelope. In eukaryotes such as yeast and *Dictyostelium,* in which the nuclear envelope does not break down, the spindle pole bodies or centrosomes attach to the nuclear envelope at prophase, as in other cells[11]. However, spindle assembly is likely to differ in these cell types because it takes place inside the nucleus. For clarity, I omit discussion of spindle assembly within the nucleus and focus here on mechanisms for aster migration around the nucleus.

Although nuclear-envelope-dependent separation of asters is seen in most cells, surprisingly little is known about the mechanism involved. So far only one microtubule motor, cytoplasmic dynein, has been shown to be important for this movement. Cytoplasmic dynein is a minus-end-directed microtubule motor, which is located at the nuclear envelope and at the cell cortex[12]. It is required for the asters to attach and move along the nuclear envelope in both *Drosophila* embryos and mammalian cells in culture[13,14]. However, how dynein contributes to centrosome separation around the nuclear envelope is not clear. One possibility is shown in Fig. 2a. In this model, cytoplasmic dynein attaches the centrosomes to the nuclear envelope, presumably by the ZYG-12 and SUN-1 proteins, which mediate this attachment during interphase[15]. The minus-end motor activity of dynein would continuously pull the

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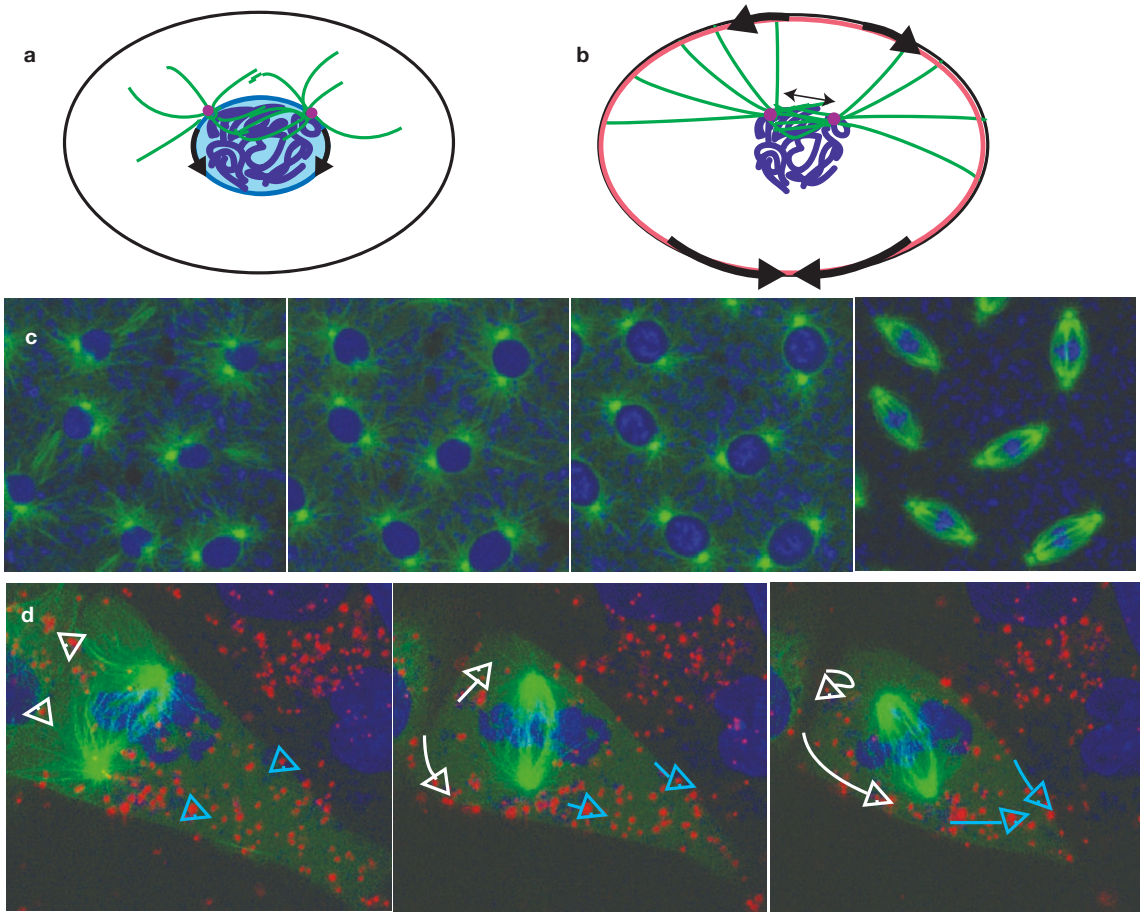

Figure 1 Two mechanisms by which asters separate during mitotic spindle assembly. Microtubules are in green and chromosomes are in blue. (**a**) Each of the two centrosomes (magenta) nucleates an aster, which separate on the cytosolic surface of the nuclear envelope. (**b**) Once the nuclear envelope breaks down (NEBD), further aster separation becomes dependent on attachment to, and movement on, the actin- and myosin-based cell cortex (red). (**c**) Early *Drosophila* embryos contain many nuclei in a syncytium in which the nuclear envelope persists until just before metaphase, allowing the asters to complete their separation by moving on the nuclear envelope, without the need to interact with the cortex. (The still micrographs from a movie of spindle assembly in *Drosophila* were kindly provided by B. Fasulo and B. Sullivan.) (**d**) In most somatic cells, NEBD occurs at various times during aster separation. Once NEBD occurs, further aster separation depends upon astral contacts to a moving cortex, as in this PtK2 epithelial cell in culture. Red beads mark the circumferential movement of the cortex (cortical flow); white arrows (with a tracked bead in the arrowhead) mark movement of the cortex in the direction of the separating asters; and blue arrows mark the cortical contraction on the opposing side of the cell. In panels **c** and **d**, the spindle is shown in green and DNA is shown in blue.

centrosomes towards the nucleus so that they are held firmly against the nuclear envelope. Finally, dynein, localized to the cortex by the dynactin complex[12], may pull asters apart by reeling them in towards the cortex[16].

Although aspects of the model may be correct, it does not provide a complete picture for how centrosomes migrate around a nuclear envelope at prophase. One problem is that asters can separate to some extent in dynein mutants[13], suggesting that other forces contribute to aster separation. In principle, these forces could come from plus-end motors binding to either the nuclear envelope or to anti-parallel microtubules. Another problem with this model is that the motor activity of dynein at the cortex would be expected to pull the asters straight to the cortex instead of around the nucleus. To translate this pulling force into one that moves the asters around the nuclear envelope, the dynein activity would, in theory, also need to move around the cortex. Furthermore, a careful study of dynein localization throughout mitosis in MDCK cells shows that dynein does not localize to the cortex until after NEBD[12]. Thus, if dynein does contribute to pulling asters apart from the cortex during prophase, it does not use this force in all cells. Alternatively, because dynein does localize to the nuclear envelope during prophase[12], it could separate the centrosomes by simply moving along the nuclear envelope. Although there is no evidence for this, the integral nuclear membrane proteins, ZYG-12 and SUN-1, which attach dynein to the nuclear envelope, may interact with the recently found cortical actin network at the inner nuclear membrane[17]. Contraction or flux within this network could cause net movement of the nuclear envelope and the attached proteins and asters (similar to cortical flow, described below). Such a mechanism, albeit highly speculative, could provide a simple means for centrosomes to migrate directly around the nuclear envelope. Finally, the model in Fig. 2a does not address how aster separation ceases once the asters have completed their migration to opposite sides of the nucleus. Therefore, this model needs either to be augmented with additional forces, or entirely new mechanisms must be explored.

Aster separation by attachment to a moving cortex

Once NEBD occurs, asters can no longer separate along the nuclear envelope and further separation becomes dependent on another pathway[9,18]. Genetic studies show that Eg5-type

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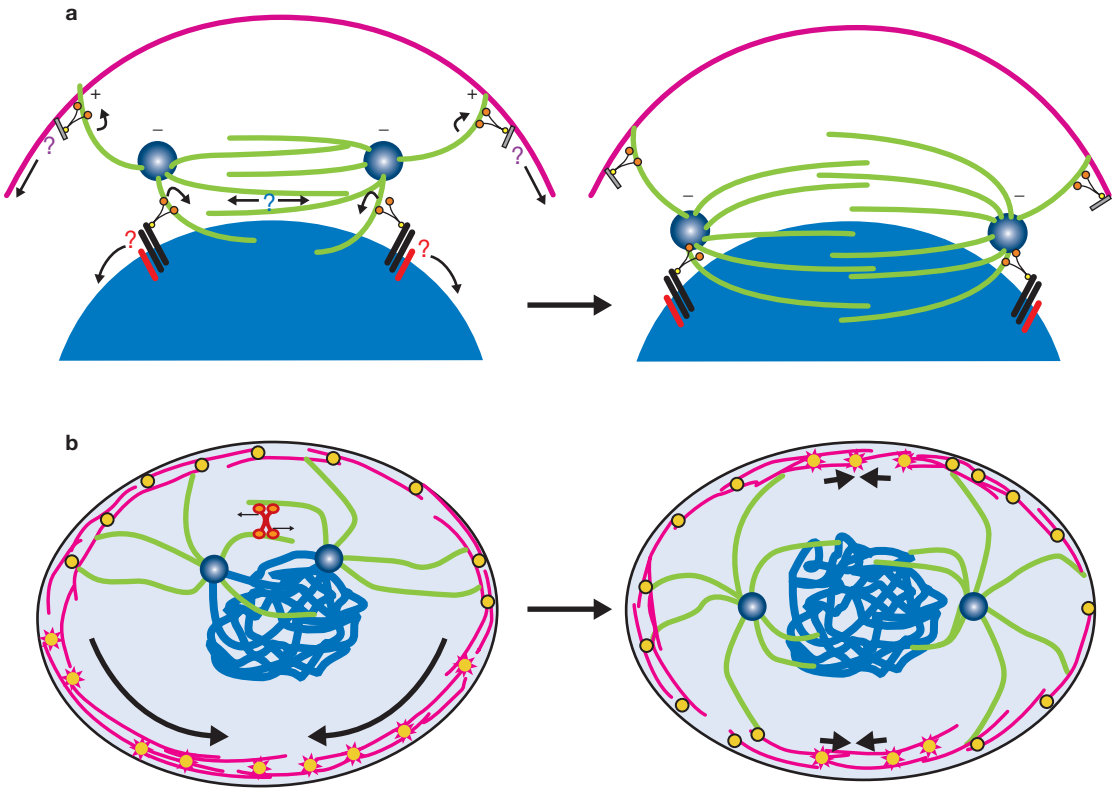

Figure 2 Models for different mechanisms of aster separation. (**a**) One possible model for how asters might separate on the nuclear envelope. The minus-end-directed microtubule motor protein cytoplasmic dynein (orange) attaches the two centrosomes (dark blue) to the nuclear envelope and may also pull the centrosomes apart by reeling in astral microtubules from the cell cortex (pink). Other unidentified kinesin-like motors (blue question mark) on anti-parallel microtubules, or on the nuclear envelope, may also contribute to pushing the asters apart. Additionally, movement of dynein at the cortex (purple question mark) or on the nuclear envelope (red question mark) may also assist centrosome migration. (**b**) A model for how the direction of cortical flow may depend on microtubules locally inhibiting cortical contraction. Actin (pink) and myosin II (yellow) in the cortex actively contract (magenta/yellow stars) everywhere except where the astral microtubules contact the cortex (yellow circles). Thus, when microtubules contact the cortex on one side of the cell in early prometaphase (left panel), cortical contraction is suppressed there; consequently, unsuppressed cortical contraction on the opposite side of the cell pulls apart the asters. When the asters reach opposite poles of the cell, there is no net cortical flow, and the asters stop moving; cortical contraction is now confined to the equator of the cell, where cytokinesis later occurs (right panel). Additionally, the homo-tetrameric kinesin Eg5 (red motors) may push apart centrosomes by sliding apart anti-parallel microtubules. For all models, arrows represent resultant force, not motor direction.

motors are essential for centrosome separation, but function only after NEBD[19–23]. The Eg5 kinesin is a plus-end-directed homo-tetrameric motor that is thought to crosslink and slide anti-parallel microtubules emanating from each centrosome to push centrosomes apart[16,24] (Fig. 2b). However, Eg5 activity alone will only push asters apart; it does not position them around the chromosomes to make a complete spindle.

To complete aster separation and correctly position the asters around the chromosomes, the asters attach to a moving walkway at the cell periphery or cortex[18] (Fig. 1b, d). Although it is not clear which proteins attach the astral microtubules to the cortex, likely candidates include the microtubule-binding proteins EB1 and dynein. This cortical moving walkway, consisting of and dependent on actin and myosin II, is termed cortical flow, and acts in other cell processes apart from mitosis[25]. Blocking cortical flow, by crosslinking the cortex or inhibiting myosin II, blocks asters from separating as soon as NEBD occurs. By tracking cortical movement with extracellular fluorescent latex beads, we have found that the cortex moves in the same direction, simultaneously with aster separation (see arrows highlighting movement in Fig. 1d). This work suggests a model in which contraction of the cortex on one side of the cell pulls the cortex and attached asters apart on the opposite side[18] (Fig. 1b).

Although it is not known what regulates the direction of cortical flow, microtubule-dependent regulation of cortical contraction is likely to be important. Microtubule interactions with the cortex suppress cortical flow in *Xenopus* oocytes[26] and may regulate the location of cortical contraction during cytokinesis. As suggested in the 'astral relaxation' model for cytokinesis, astral microtubule contacts at the cortex suppress contraction at the poles, while allowing contraction in the middle of the cell where astral microtubule contacts are absent[27,28]. In a similar way, during spindle assembly in early prometaphase, polarized astral microtubules on one side of the cell could lead to polarized contraction on the opposing side of the cell (Fig. 2b). Because polarized cortical contraction drives the separation of the asters, eventually the asters become symmetrically distributed on two opposite poles of the cell, thereby suppressing the polarized contraction and, thus, the directed cortical flow. At this point, unsuppressed myosin II contractility would be restricted to the centre of the cell, ready for cytokinesis, as proposed in the astral relaxation model. This hypothetical model is attractive because it simultaneously addresses how asters might separate and how they might stop once asters are aligned symmetrically on either side of the chromosomes.

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Putting it all together

It seems likely that both the architecture of a cell and the timing of NEBD will dictate the primary mechanism that a cell will use for spindle assembly. For example, the spindles that form in the early *Drosophila* embryo syncytium (as seen in Fig. 1b) have limited access to the cortex; because NEBD occurs late, the asters can complete separation by migrating around the nuclear envelope without a requirement for the cortex (Fig. 1a). By contrast, in many somatic cells (including the epithelial cell in Fig. 1d), NEBD often occurs before aster separation is complete and so further aster separation relies on the cortex-dependent mechanism (Fig. 1b); in addition, the spindles in these cells are relatively larger and have easy access to the cell cortex, which also helps to centre the spindle within the cell. Although these examples illustrate the mechanisms used to separate asters during prophase and prometaphase, it is important to note that most somatic cells are likely to use both mechanisms in addition to self-assembly mechanisms to ensure that spindles assemble and position correctly within the cell.

In this way, each mechanism can be considered as a back-up to rescue spindle assembly if other mechanisms fail. For instance, in cases where NEBD is delayed, nuclear-envelope-dependent aster separation is sufficient for spindle assembly when cortical flow is blocked[18]. On the other hand, if NEBD occurs before centrosomes have separated at all, prometaphase forces alone can separate asters around chromatin. Unseparated, monopolar asters with no nuclear envelope can be made artificially by addition of the Eg5 inhibitor monastrol. These monopolar asters can be rescued to form a normal bipolar spindle after monastrol is washed out[22,29]. Presumably, both Eg5 motors and cortical flow collaborate in this rescue, because removing monastrol in the presence of myosin inhibitors prevents complete separation of the asters to opposite sides of the chromosomes (my unpublished observations). Here, self assembly mechanisms may also contribute to monastrol rescue, but are not sufficient without myosin II-dependent cortical flow because centrosomes still separate (as seen in ref. 29) but do not complete migration around chromosomes, with astral microtubules attached but frozen at the cortex.

Furthermore, spindle self-assembly mechanisms around chromatin that operate chiefly in cells that lack centrosomes may also come into play during spindle assembly in other cell types. Ran is a small GTPase protein that is essential for nuclear transport and also acts as an activator of spindle assembly. In extracts, Ran concentrates in a gradient around mitotic chromosomes where it helps catalyse microtubule self-assembly and reorganization into a spindle[30,31]. Although Ran has a central role in spindle self-assembly mechanisms, it also acts in spindle assembly in somatic cells, even though asters can readily separate by nuclear-envelope-dependent and cortex-dependent mechanisms[32]. One way that Ran may promote spindle assembly is by enhancing the assembly of asters near chromosomes, thereby effectively suppressing aster assembly elsewhere. Another possibility is that Ran may help unaligned chromosomes get captured by microtubules. Given that isolated microtubule bundles or clusters get reeled in towards centrosomes after NEBD[33], if a chromosome fails to get captured into the spindle during aster separation, it can induce assembly of an aster that would then get incorporated into the spindle (also see examples in refs 29, 34). Moreover, Ran at chromosomes may help to ensure that spindles maintain their structure once formed[35].

Studying spindle assembly in different cell types has revealed different mechanisms for assembling two asters symmetrically around chromosomes. Whereas some cells favour a particular mechanism, it is becoming clear that many cell types retain the ability to use several mechanisms to ensure that spindles form and position correctly within cells. The functional redundancy of these mechanisms, however, has made it difficult to tease out the different roles that a specific motor might have in each mechanism through classic loss-of-function studies. The use of small molecule inhibitors that block specific motor proteins rapidly and dominantly, together with better techniques to image live cells, should make it easier to dissect these mechanisms and determine how they interact to construct and position the spindle in specific cell types.

ACKNOWLEGEMENTS

I would like to thank L. Cramer, R. Heald, A. Jaffe, A. Lloyd, M. Raff and M. Redd for critically reviewing this manuscript and for helpful discussions about mechanisms. I also thank B. Fasulo and B. Sullivan for contributing pictures of mitosis. Finally, I thank the scientists that have contributed to our understanding of spindle assembly and apologize to those whose work I have had to omit owing to space limitations. My work is supported by Cancer Research UK and BBSRC project grants.

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