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SPOTLIGHT

Separating chromosomes, one way or another

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Chromosome separation during anaphase is driven by two distinct processes, one of which was thought to be absent in early *Caenorhabditis elegans* embryos. In this issue, Henriques et al. (https://doi.org/10.1083/jcb.202505038) show that both processes occur in these cells and that mechanical coupling between the two processes allows one to compensate when the other is compromised.

Anaphase is the dramatic finale of mitosis, when duplicated chromosomes suddenly move apart from one another. It consists of two distinct processes: In "anaphase A," the chromosomes move closer to the spindle's microtubule-organizing centers, also known as its poles (1), while in "anaphase B," the poles move farther apart from one another (2). The overall separation of chromosomes in different organisms depends to different degrees on these two processes, which sometimes occur concurrently but other times occur with distinct timing. The two processes are driven by different mechanisms. During anaphase A, the kinetochore-attached (k-fiber) microtubules that link each sister chromatid to a pole shorten, pulling chromatids closer to the poles. During anaphase B, chromatids are separated indirectly, either by midzone microtubules that lengthen and push the poles outward or by astral microtubules that emanate outward and shorten to pull the poles outward toward the cell cortex. Some of the molecular underpinnings of both processes are known (3, 4), but very little is known about why different organisms might favor anaphase A over anaphase B or how the two processes are differentially regulated.

In their *JCB* paper, Henriques and colleagues used fluorescence microscopy to record the movements of chromosomes and spindle poles in early *Caenorhabditis elegans* embryos (5). The outstanding quality of their live-cell imaging allowed them to track spindle pole separation, chromosome separation, and chromosome movement toward the poles (which they call "chromosome displacement") with high accuracy. It has generally been thought that chromosome separation in early worm embryos is driven entirely by anaphase B, without much, if any, contribution from anaphase A (6). However, by careful tracking, Henriques et al. show clearly that anaphase A also occurs in these cells. Interestingly, it is slightly faster on the anterior side of the cell than on the posterior side, and this asymmetry depends on polarity cues, PAR-2 and PAR-3, and on the cortical dynein adapter, GPR-1/2. RNAi-mediated depletion of any of these molecules individually abolishes the asymmetry but still allows chromosome displacement toward the poles to occur, in some cases faster than in wild-type cells. RNAi depletions also showed that anaphase A in these cells requires the depolymerizing kinesin KLP-7 (MCAK) and is antagonized by another kinesin, KLP-18.

The contributions of anaphase A and B to chromosome separation were examined as embryos developed from the 1-cell stage through 2-, 4-, 8-, 16-, 32-, and 64-cell stages. The magnitude of anaphase A increased, while the magnitude of anaphase B decreased, such that chromosome segregation became more dependent on anaphase A as development proceeded (5). Dissociation of individual cells from 2- and 4-cell embryos showed that these trends are mostly unaffected by the loss of cell–cell contact, suggesting that the balance between anaphase A and B is set by cell-intrinsic regulatory mechanisms.

The most striking new finding is that anaphase A is inhibited by tension in the spindle (Fig. 1). Support for this conclusion comes partly from knockdown of the cortical dynein adapter GPR-1/2, a perturbation that should reduce overall tension on the spindle by reducing cortical pulling forces on astral microtubules (particularly on the posterior side). In the simplest view of spindle mechanics, cortically generated tension is borne entirely by astral and midzone microtubules, bypassing the k-fibers. However, under the low-tension conditions created by knockdown of GPR-1/2, anaphase A chromosome displacement was faster than normal, implying that spindle tension somehow inhibits k-fiber shortening during anaphase (5). Further support for this idea comes from RNAi depletion of the midzone cross-linker, SPD-1, and from laser ablation of the spindle midzone. Both of these perturbations should reduce overall spindle tension by disrupting the mechanical integrity of the midzone. Both resulted in faster-than-normal anaphase A chromosome displacement. Thus, overall spindle tension, which drives anaphase B in these cells, simultaneously puts the brakes on anaphase A. Consequently, if spindle tension is compromised, anaphase A accelerates and sometimes (as in the case of GPR-1/2 knockdown) can compensate for anaphase B slowing. This compensatory effect might explain why anaphase A becomes more important with successive embryonic cell divisions: As cells become smaller, they might generate less cortical

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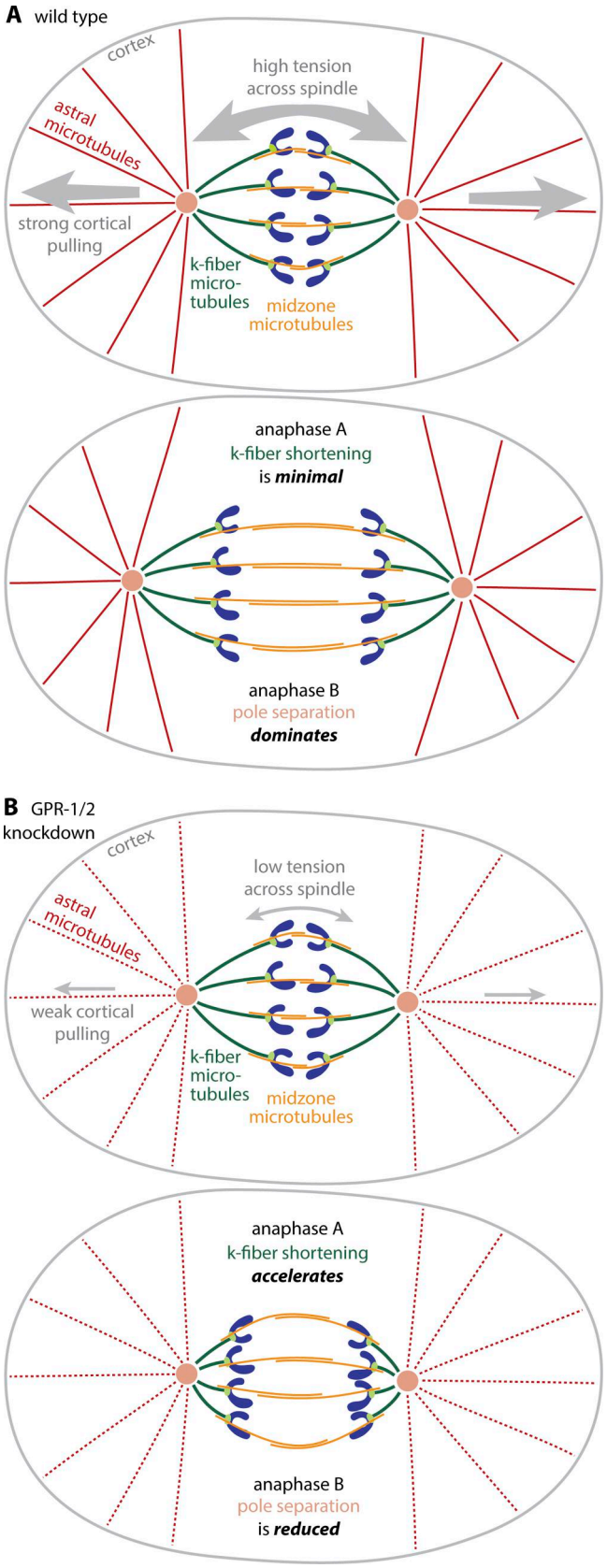

Figure 1. **Anaphase in early *C. elegans* embryos. (A)** In wild-type embryos, interactions between astral microtubules (*red*) and dynein motors anchored at the cell cortex (*gray*) generate strong pulling forces that place the mitotic spindle under high tension. Separation of the chromosomes (*blue*) in these cells is

tension on their spindles, reducing the contribution of anaphase B while also releasing the brakes on anaphase A.

How tension borne by astral and midzone microtubules influences k-fiber microtubule shortening (and thus anaphase A) remains mysterious. The authors speculate that tension transmitted to spindle poles might regulate Aurora kinase, which in turn could modulate KLP-7 (MCAK) activity and anaphase A speed. This would be an interesting parallel to tension-dependent processes proposed to occur at the other end of k-fiber microtubules, where they attach to kinetochores. Alternatively, it seems possible that a simpler and more direct effect of tension on k-fiber dynamics might be at play.

K-fiber and midzone microtubules are distinct in the small spindles of microorganisms like yeast (7), but the midzone microtubules in larger, metazoan spindles interdigitate with the k-fiber microtubules and may also interact directly with the chromosomes (6, 8, 9). The distinction between k-fiber and midzone microtubule subpopulations is therefore blurred in larger spindles, where k-fiber microtubules probably bear some of the overall tension across the spindle. "External" tension (i.e., generated outside the k-fibers themselves) could directly inhibit shortening of the k-fibers, similar to how external tension applied with a microneedle can cause k-fibers to elongate (10). Tension also strongly inhibits the shortening of dynamic microtubules in minimal reconstituted systems that lack depolymerase or Aurora kinase activity (11, 12). Likewise, if enough cortically generated tension is transmitted to k-fiber microtubules in early *C. elegans* embryos, it could directly inhibit k-fiber shortening, slowing anaphase A without necessarily requiring any tension-dependent regulation of Aurora kinase or MCAK. In this view, MCAK might be important to promote microtubule depolymerization globally rather than to respond to local tension-based cues.

Henriques and co-workers saw a rapid recoil of the spindle poles away from each other after severing the central spindle (5), an effect reported earlier (3). This observation shows that midzone tension in these early *C. elegans* embryos normally restricts the anaphase B pole separation driven by cortical force generators. Conversely, other studies have demonstrated that midzones in

driven mainly by movement of spindle poles (*pink*) away from one another (i.e., mainly by anaphase "B"). The k-fibers (*green*) that connect chromosomes to poles shorten only slightly (i.e., anaphase "A" is minimal), perhaps because their shortening is directly inhibited by spindle tension that they carry from the poles to the midzone microtubules (*yellow*). **(B)** Knockdown of the dynein adapter GPR-1/2 weakens cortical pulling forces and reduces separation of the poles, presumably because overall spindle tension is reduced. However, k-fiber shortening is accelerated, thereby increasing the contribution of anaphase A and partially compensating for the loss of anaphase B. The acceleration of k-fiber shortening might arise directly from a reduction in k-fiber tension.

meiotic and mitotic *C. elegans* spindles can actively generate outward pushing forces, thereby promoting rather than restricting separation of the chromosomes or poles (6, 13). It is not surprising or contradictory that when midzone motors bear assistive external loads (i.e., loads in the same direction as their ATP-powered motility), they can act as governors to slow rather than accelerate movement (14). Single motor proteins exhibit this same governor-like behavior (15). When the assistive loads are lost or reversed, these motors can actively drive movement (4). This fundamental mechanical responsiveness of midzone force generators probably explains how midzones can either restrict or drive anaphase B pole separation and suggests that midzones can switch autonomously from restrictive to active mode without complex regulatory feedback.

The coupling between anaphase A and B, demonstrated beautifully by Henriques and co-workers (5), might be another example of direct mechanical responsiveness. Regardless of the underlying mechanisms, it is a striking example of the spindle's ability to adapt itself to changing cellular conditions or large external perturbations. One way or another, by anaphase A or B, by cortical pulling, midzone pushing, or k-fiber shortening, the spindle finds a way to separate chromosomes.

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