Embryonic polarity: **A role for microtubules**

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Researchers have suspected that initial polarization of the *Caenorhabditis elegans* embryo might be directed by microtubules, but demonstrating this has faced obstacles. A new study has cleverly bypassed these obstacles.

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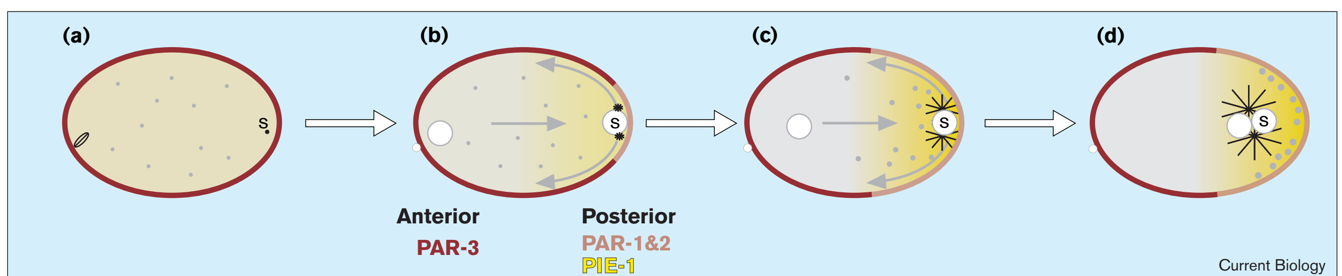
When developing embryos establish internal asymmetries, they often do so by exploiting external spatial cues, such as the sperm entry point, the positions of surrounding tissues, or gravity. Such cues have been identified for many organisms, but how these cues are initially read by cells and subsequently translated into internal asymmetries is not thoroughly understood in any organism [1]. The exploration of these issues touches on a number of important issues in cell and developmental biology, including how cells become polarized, how cell diversity is generated during development, and how the cytoskeleton functions in early embryonic cells.

In the nematode *Caenorhabditis elegans*, the cue for initial polarization of the embryo is the sperm: the sperm normally enters the side of the egg opposite the oocyte nucleus, but when it is experimentally caused to enter on the other side, the orientation of the embryo's anterior–posterior axis is reversed [2]. We do not yet know how a sperm-derived component polarizes the embryo. What little we do know is that the sperm-entry point somehow dictates the axis along which movements of cortical and central cytoplasm occur, and along which cytoplasmic ribonucleoprotein complexes called P granules move. At the same time as these movements occur, certain proteins critical for development, including some of the PAR proteins and the PIE-1 protein, become localized to one side of the embryo or the other (Figure 1).

These movements and the appearance of asymmetries are dependent on an intact actin cytoskeleton, suggesting that movement of the actin-rich cortex may be involved in generating asymmetry [3,4]. For the past few years, the identity of the sperm component that is responsible for initiating asymmetry has been one of the mysteries of early *C. elegans* development. Identifying this component may open a way to explore how critical developmental factors become asymmetrically localized.

One unique gift the *C. elegans* sperm brings to the egg is a centrosome. The egg lacks a centrosome, and after fertilization the egg completes meiosis using acentrosomal meiotic spindles. The sperm-supplied centrosome is a good candidate for the mystery sperm component, because after this centrosome duplicates, the resulting centrosomes begin to nucleate growth of astral microtubules at one end of the cell, and it is at this time that the asymmetries discussed above begin to appear [3–5]. There

Figure 1



Development of asymmetry before the first cell division in *C. elegans*. **(a)** A fertilized egg in meiotic metaphase. The barrel-shaped meiotic spindle is at one end of the embryo, and the sperm-derived components are at other end. **(b)** After completion of meiosis, which produces the polar bodies (small circle at anterior), sperm-derived centrosomes nucleate astral microtubules, cytoplasmic movements begin, PAR protein asymmetry appears as a small patch of membrane-associated PAR-1 and PAR-2 (pink) near the sperm pronucleus [5], and PIE-1 distribution (yellow) begins to become asymmetric [5,11]. **(c)** As astral microtubules continue to grow, PAR-2 distribution expands [5]. **(d)** By pronuclear meeting, all asymmetries are established [3]. Note that P granule size (exaggerated here) normally increases during the first cell cycle. Black dots, centrosomes; black lines, microtubules; gray dots, P granules; gray arrows, cytoplasmic movements; s, sperm pronucleus. Scale: embryo length is about 50 µm.

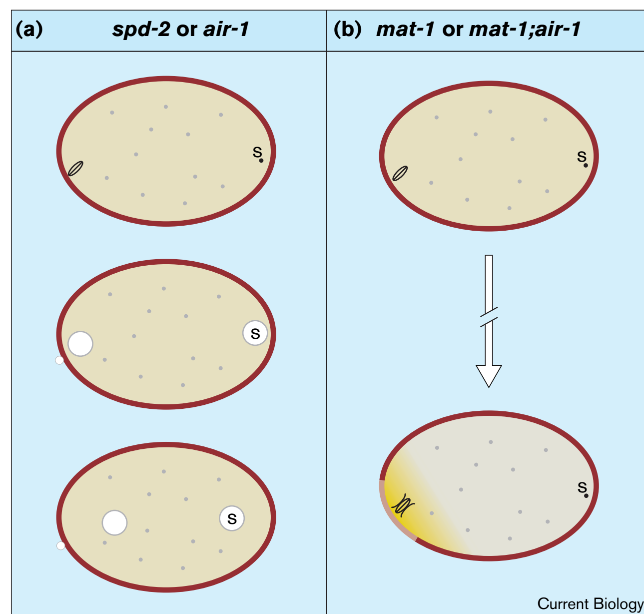
are indications from studies of other cells that astral microtubules can cause movements of an actin-rich cortex in a manner that could potentially cause the polarized redistribution of cellular contents [6].

Testing whether microtubules drive the polarization of the *C. elegans* zygote seems simple enough: treat embryos with drugs that prevent microtubules from polymerizing, and see whether asymmetries appear. Unfortunately, under these conditions, many short microtubules remain associated with the centrosomes and the centrosomes remain positioned very close to the cortex, making it difficult to know whether microtubules have been prevented from interacting with the cortex [7,8]. Instead of preventing microtubule–cortex interactions, it might be possible to test whether ectopic astral microtubules are sufficient to generate asymmetries by injecting a centrosome into an unfertilized *C. elegans* egg, but attempts to do this have encountered technical snags (B.G., unpublished data; S. Strome, personal communication).

Two new papers [5,9] now report the effect of eliminating or ectopically positioning asters by genetic methods. O'Connell *et al.* [9] have characterized *spd-2* mutants, in which the assembly of zygotic centrosomes is defective, and sperm-associated asters do not form at the normal time [8]. They find that none of the normal signs of embryonic polarity appear in the mutants (Figure 2), consistent with the hypothesis that astral microtubules might be required to generate embryonic polarity. There is an important caveat, however, when using mutations in a gene whose function is not fully known as an experimental substitute for microtubule-depolymerizing drugs: the loss of microtubules might be the source of any further phenotypic defects, but it is also possible that the failure of an unknown function of the gene is the cause. Along these lines, O'Connell *et al.* [9] point out that the failure to develop asymmetries may be a consequence of either a defect in aster formation or of a distinct role for *spd-2*. It will be interesting in the future to learn the identity of the *spd-2* gene product and how it functions, not only to understand how embryonic polarity is generated but also to understand how centrosomes assemble.

Wallenfang and Seydoux [5] have examined a class of mutants that arrest in meiotic metaphase — *mat* mutants, named for their defective metaphase-to-anaphase transition. In *mat* mutants, the sperm-pronucleus-associated centrosomes fail to nucleate astral microtubules. The mutants develop partial embryonic polarity and, shockingly, the asymmetries develop with reversed polarity: several proteins that normally localize near the sperm pronucleus instead localize on the opposite end of the embryo, near the oocyte chromatin and meiotic spindle. This reversed polarity might be driven by meiotic spindle microtubules, as it appears only after a prolonged period, during which the meiotic spindle microtubules become less and less confined to the spindle. Curiously, only some of the usual asymmetries appear (Figure 2), indicating that certain aspects of asymmetry might be driven by distinct mechanisms.

Figure 2



Phenotype of embryos either **(a)** lacking sperm-derived astral microtubules (*spd-2* or *air-1* loss-of function), or **(b)** with ectopically organized microtubules (*mat-1* or *mat-1*; *air-1* loss-of-function) [5,9,10]. Some *spd-2* mutant embryos have PAR-2 localized at both poles, rather than as drawn [9]. The stages in (a) are the same as the first three stages in Figure 1; the broken arrow in (b) indicates that asymmetries first appear after 50–60 minutes (M. Wallenfang and G. Seydoux, personal communication), as opposed to about 30 minutes in wild-type embryos. Wild-type asymmetries that do not appear in *mat-1* mutants are P granule localization and polarized cytoplasmic movements [5]. Color code is the same as in Figure 1.

Are microtubules of the meiotic spindle responsible for the reversed polarity of *mat* mutants? To test this, *mat* mutant embryos were treated with the microtubule-destabilizing drug nocodazole, which gave a consistent but still somewhat ambiguous result, cutting in half the percent of embryos that developed polarity. The *mat* mutants were also placed in an *ncc-1* (*cdc2* homolog) mutant background to prevent meiotic spindle formation by preventing entry into meiotic metaphase. This always prevented asymmetries from appearing. Using an *ncc-1* mutant background to test whether oocyte meiotic spindle microtubules play a role is open to the same caveat as discussed for *spd-2* above, but all the results are still consistent with the hypothesis that the sperm-derived centrosome is the mystery sperm component.

How then can the role of microtubules be pinned down? Wallenfang and Seydoux [9] have had a stab at this by ingenius exploitation of the *air-1* gene, which encodes an Aurora/Ipl1-related kinase: *air-1* is known to be required both for the formation of sperm-pronucleus-associated asters and for the development of embryonic polarity. Wallenfang and Seydoux [9] examined the requirement for *air-1* in reversed embryos, in which polarity is established independently of sperm asters. They found that, in this case, *air-1* is no longer required for polarity to develop — indicating that *air-1* itself is not essential for polarity formation. Rather, what is doing the job must be something that is both normally downstream of *air-1* and ectopically positioned in a mutant with reversed polarity. Microtubules fit the description: they are normally downstream of *air-1*, as AIR-1 is a centrosomal kinase required for astral microtubules to form [10], and microtubules are indeed ectopically positioned in the reversed embryos, as *air-1* is not required for the acentrosomal meiotic spindles to form. All of the data presented in these two papers [5,9] are consistent with the hypothesis that microtubules drive embryonic polarity, but this one experiment is the closest thing we have to a smoking gun. Determining exactly how microtubules generate polarity now poses a challenge for the future.

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