521

Spindle positioning during the asymmetric first cell division of *Caenorhabditis elegans* embryos

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Abstract. Cell division during development in many cases generates daughter cells that differ not only in fate, but also in size. We investigate the mechanisms that ensure proper spindle positioning during such asymmetric divisions using the one-cell stage *Caenorhabditis elegans* embryo as a model system. We utilized a UV laser microbeam as an *in vivo* microtubule-severing device to probe the forces driving spindle positioning. Our results indicate that extra-spindle pulling forces acting on the spindle poles dictate spindle position along the anterior–posterior embryonic axis. Importantly, forces acting on the posterior spindle pole appear more extensive than those acting on the anterior one, thus explaining the overall posterior spindle displacement that leads to the asymmetric division of the wild-type one-cell stage embryo. In separate work, we analysed a locus called *zyg-8*, which plays a key role in ensuring proper spindle positioning. Our data show that *zyg-8* is required to promote microtubule growth and/or stability during anaphase. We identified the molecular nature of the *zyg-8* locus in the course of a large-scale RNAi-based functional genomics screen. ZYG-8 harbours two notable protein domains: a Ca^{2+}/calmodulin-dependent kinase domain, and a domain related to doublecortin, a human microtubule-associated protein involved in neuronal migration.

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Asymmetric divisions are central to the generation of cell fate diversity (for review see Horvitz & Herskowitz 1992). During development, asymmetric divisions often give rise to daughter cells that differ not only in fate, but also in size. For instance, in the developing *Drosophila* nervous system, neuroblasts divide asymmetrically to generate a large neuroblast and a small ganglion mother cell (for review see Doe 1996). Similarly, at the fourth cleavage division of sea urchin embryos, vegetal tier cells undergo an asymmetric division that gives rise to a macromere and a

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micromere, which differ markedly in size (for review see Hörstadius 1973). In animal cells, for such divisions to take place, the mitotic spindle must be asymmetrically localized by the end of anaphase, when the cleavage furrow is specified so as to bisect the mitotic spindle (for review see Rappaport 1971). The mechanisms which allow the anaphase spindle to be asymmetrically positioned within the three dimensional space of the cell remain poorly understood.

In this paper, we discuss how we have begun to investigate this question in the one-cell stage *Caenorhabditis elegans* embryo. First, we review how cell polarity is established along the anterior–posterior (AP) embryonic axis, and how anaphase spindle positioning is achieved in wild-type embryos. Second, we discuss experiments in which we have used a UV laser microbeam to probe the forces that act on spindle poles to dictate anaphase spindle positioning. Third, we report our cell biological and molecular analysis of *zyg-8*, a locus that plays a crucial role in ensuring proper anaphase spindle positioning. In conclusion, we summarize our findings and mention some future directions.

Cell polarity and anaphase spindle positioning in the wild-type one-cell stage *C. elegans* embryo

In *C. elegans*, polarity along the AP embryonic axis is established shortly after fertilization (Goldstein & Hird 1996). A sperm component, which remains to be identified, provides an initial polarity cue that determines the future posterior of the embryo. This initial cue is then translated by the concerted action of six maternally required *par* genes (for *par*titioning-defective) to establish polarity along the AP axis (for review see Kemphues & Strome 1997). All six *par* genes have been cloned, and antibodies have been raised to most of the corresponding proteins (Boyd et al 1996, Etemad-Moghadam et al 1995, Guo & Kemphues 1995, Hung & Kemphues 1999, Levitan et al 1994, Watts et al 2000, K. Kemphues, personal communication). Strikingly, several of the PAR proteins have a polarized distribution along the AP axis in the one-cell stage embryo. Thus the PDZ-containing proteins PAR-3 and PAR-6 both localize to the anterior cortex (Etemad-Moghadam et al 1995, Hung & Kemphues 1999). Conversely, PAR-2, a ring-finger containing protein, and PAR-1, a Ser/Thr protein kinase, both localize to the posterior cortex (Boyd et al 1996, Guo & Kemphues 1995). These observations indicate that the polarized distribution of PAR proteins at the cell cortex is essential for establishing proper polarity along the AP embryonic axis.

Interestingly, homologues of some of the PAR proteins have been found in other metazoans where they also have a polarized distribution. For instance, in *Drosophila*, *bazooka* encodes a PAR-3 homologue that localizes to the apical surface of epithelia cells and neuroblasts; moreover, *bazooka* is required for proper polarity in these cells (Kuchinke et al 1998). In mammalian epithelial cells,

the PAR-3 homologue ASIP is present in apically located tight junctions, while a PAR-1 homologue localizes to the basolateral domain (Bohm et al 1997, Izumi et al 1998). These observations suggest that the function of PAR proteins in establishing cell polarity may have been significantly conserved across metazoan evolution.

In the one-cell stage *C. elegans* embryo, establishment of polarity by the PAR proteins leads in turn, among other things, to an asymmetric position of the anaphase spindle along the AP axis. Spindle positioning can be followed with great spatial and temporal resolution in living embryos using time-lapse differential interference contrast (DIC) microscopy (Fig. 1). The spindle is initially set up roughly in the cell centre (Fig. 1, top panel). Then, as the spindle elongates during anaphase B, the anterior spindle pole stays relatively put with respect to overall AP polarity, while the posterior spindle pole is displaced slightly towards the posterior. This results in an asymmetric spindle position along the AP axis by the end of anaphase (Fig. 1, middle panel). As a result, the one-cell stage embryo divides asymmetrically, into a larger anterior blastomere and a smaller posterior one (Fig. 1, bottom panel). In embryos derived from *par* mutant hermaphrodites (hereafter referred to as *par* mutant embryos), posterior displacement does not take place and the first division is symmetric, most likely as a consequence of the earlier defects in establishing polarity along the AP axis (Kemphues et al 1988).

Pulling forces acting on the spindle poles dictate spindle position along the AP axis

The mechanisms by which overall cell polarity, as established by the PAR proteins, is communicated to the cytoskeleton to mediate proper spindle positioning are not understood. Below we discuss two experimental approaches that we have taken to address this question. In the first set of experiments, we sought to identify the forces that act on spindle poles to drive spindle positioning during anaphase. Experiments in other systems have revealed that two types of microtubule-dependent forces can contribute to spindle elongation during anaphase B (Aist & Berns 1981, Aist et al 1993, Leslie & Pickett 1983). First, forces that act on overlapping spindle microtubules can 'push' spindle poles apart. Second, forces that act on astral microtubules can 'pull' spindle poles apart. In some cases, both types of forces may contribute to spindle elongation. Spindle-severing experiments are instrumental for testing whether pulling forces acting along astral microtubules play a role in driving anaphase B. Indeed, if solely intra-spindle pushing forces drive anaphase B, then the two spindle poles should not separate or even collapse onto one another after severing of the spindle, as was observed in diatoms (Leslie & Pickett 1983). In contrast, if extra-spindle pulling

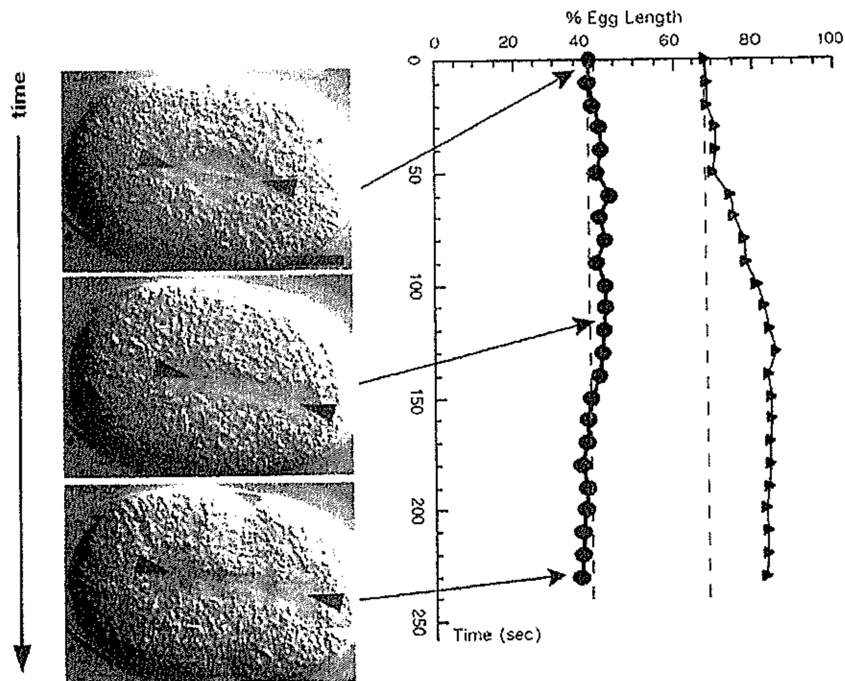

FIG. 1. Anaphase spindle positioning in wild-type embryo. Left: three images taken from a time-lapse DIC recording; arrowheads point to centrosomes and spindle poles. Anterior (0% egg length) is to the left, posterior (100% egg length) to the right in this and all other figures. Time elapsed since the beginning of the sequence is indicated in minutes and seconds. Right: corresponding tracings of aster positions over time. During anaphase, the anterior spindle pole hardly moves with respect to overall AP polarity. In contrast, the posterior spindle pole is displaced slightly towards the posterior. This results in the asymmetric division of the one-cell stage embryo into a larger anterior blastomere and a smaller posterior one. Bar = 10 μm. A Quicktime movie of this sequence can be viewed at *http://www.embl-heidelberg.de/ExternalInfo/hyman/Data.htm*.

forces participate in driving anaphase B, then the two spindle poles should keep moving or even accelerate after severing of the spindle, as was observed in vertebrate Ptk2 cells (Aist et al 1993).

Spindle-severing experiments in wild-type embryos

We conducted spindle-severing experiments in the wild-type one-cell stage *C. elegans* embryo to investigate whether extra-spindle pulling forces participate in driving anaphase B. A 337 nm laser microbeam was used as a local *in vivo*

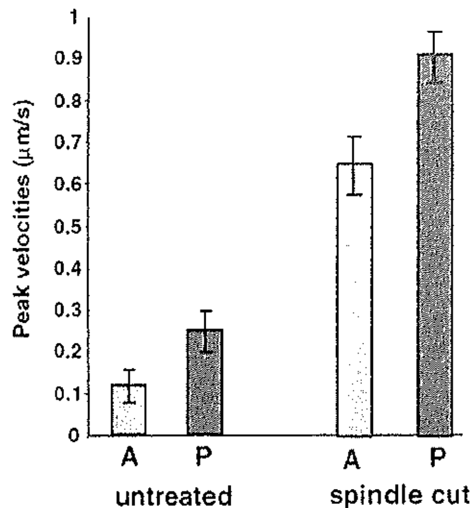

FIG. 2. Peak velocities achieved by the anterior (A) and posterior (P) spindle poles during anaphase. Left: wild-type untreated embryos; $n=5$. Right: wild-type embryos whose spindle has been severed during early anaphase by the UV laser microbeam; $n=34$. The peak velocities of both spindle poles are higher in embryos whose spindle has been severed than in untreated embryos, indicating that extra-spindle pulling forces play an important role in driving anaphase B. Moreover, after severing, the peak velocity of the posterior spindle pole exceeds that of the anterior one, suggesting that a larger pulling force is acting on the posterior spindle pole.

microtubule-severing device. By using a moderately well focused laser microbeam (focal diameter $\sim 3\,\mu m$), spindle microtubules could be destroyed selectively, without affecting astral microtubules. The spindle was severed with the UV laser microbeam during early anaphase, and the position of the spindle poles was monitored using time-lapse DIC microscopy. Strikingly, both spindle poles kept moving, and the peak velocity of each spindle pole was significantly higher than that observed in untreated embryos (Fig. 2). This demonstrates that extra-spindle forces pulling on the spindle poles, presumably along astral microtubules, play an important role in driving anaphase B in the one-cell stage *C. elegans* embryo. It should be noted, however, that these experiments do not rule out the possibility that intra-spindle pushing forces may also contribute to spindle elongation.

Importantly, we observed that the two spindle poles behaved differently following spindle severing: the posterior spindle pole had a higher peak velocity than the anterior one (Fig. 2). This suggests that a more extensive vector sum is pulling on the posterior spindle pole than on the anterior one. This leads us to propose a model in which the asymmetric elongation of the spindle during

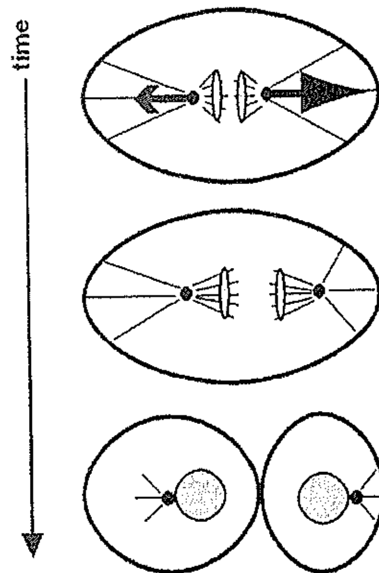

FIG. 3. Model of anaphase spindle positioning in the one-cell stage *C. elegans* embryo. Filled circles, spindle poles and centrosomes; black lines, microtubules. Segregating sets of chromosomes are also indicated, as are nuclei in daughter cells. Spindle-severing experiments indicate that the extent of extra-spindle pulling forces acting on the spindle poles determines spindle position along the AP axis. Forces acting on the posterior spindle pole are more extensive than those acting on the anterior one, thus generating a slight posterior spindle displacement during anaphase. This results in the asymmetric division of the one-cell stage embryo into a larger anterior blastomere and a smaller posterior one.

anaphase results from differential extra-spindle pulling forces acting on the spindle poles (Fig. 3). Because forces acting on the posterior spindle pole are more extensive than those acting on the anterior one, a slight posterior displacement of the spindle is achieved during anaphase in wild-type embryos.

Spindle-severing experiments in par mutant embryos

We conducted spindle-severing experiments in *par-2* and *par-3* mutant embryos to test the validity of this model. In wild-type, PAR-3 protein is restricted to the anterior cortex and PAR-2 protein to the posterior cortex (Boyd et al 1996, Etemad-Moghadam et al 1995). In *par-2* mutants, PAR-3 expands to fill the entire circumference of the embryo, which can be thought of as having anterior character throughout (Etemad-Moghadam et al 1995, Kemphues & Strome

1997). Conversely, in *par-3* mutants, PAR-2 expands to fill the entire circumference of the embryo, which can be thought of as having posterior character throughout (Boyd et al 1996). The first division is symmetric in both *par-2* and *par-3* mutant embryos, presumably as a consequence of the absence of polarity along the AP axis. According to our model, the first division should be symmetric in *par-2* and *par-3* mutant embryos due to distinct alterations in forces pulling on the spindle poles, both of which result in equal forces being exerted on either side. In a *par-2* mutant embryo, forces acting on both spindle poles should be weak, since the embryo has anterior character throughout. Conversely, in a *par-3* mutant embryo, forces acting on both spindle poles should be strong, since the embryo has posterior character throughout.

We tested these predictions by conducting spindle-severing experiments in *par-2* and *par-3* mutant embryos (data not shown). In severed *par-2* mutant embryos, both spindle poles had a velocity that resembled that of the anterior spindle pole of irradiated wild-type embryos. Conversely, in severed *par-3* mutant embryos, both spindle poles had a velocity that resembled that of the posterior spindle pole of irradiated wild-type embryos. These results lend strong support to the model presented in Fig. 3, in which the extent of pulling forces acting on the spindle poles dictates spindle position along the AP axis during the first cleavage division of *C. elegans* embryos.

zyg-8 is required for proper anaphase spindle positioning and encodes a protein kinase related to Doublecortin

In separate work, we screened large collections of maternal-effect embryonic lethal mutations by time-lapse DIC microscopy to identify novel components required for proper anaphase spindle positioning and other cell division processes in the one-cell stage *C. elegans* embryo. In our initial study with a collection of mutations on chromosome III, we identified mutations in a locus called *zyg-8* which gave rise to a spectacular defect in anaphase spindle positioning (Gönczy et al 1999). In most *zyg-8* mutant embryos, the spindle sets up roughly in the cell centre as in wild-type (Fig. 4, top panel). However, during anaphase, both anterior and posterior spindle poles are displaced in an exaggerated manner towards the posterior (Fig. 4, middle panel). As a result, the spindle is located too far towards the posterior by the end of anaphase, and the cleavage furrow is aberrantly placed (Fig. 4, bottom panel). The anaphase spindle positioning phenotype is likely due to a reduction in *zyg-8* function, because it was observed in five out of eight *zyg-8* alleles examined and in the progeny of animals transheterozygous for a *zyg-8* mutation and a deficiency uncovering the region. These observations indicate that *zyg-8* must normally act to somehow restrict the extent of posterior spindle displacement during anaphase.

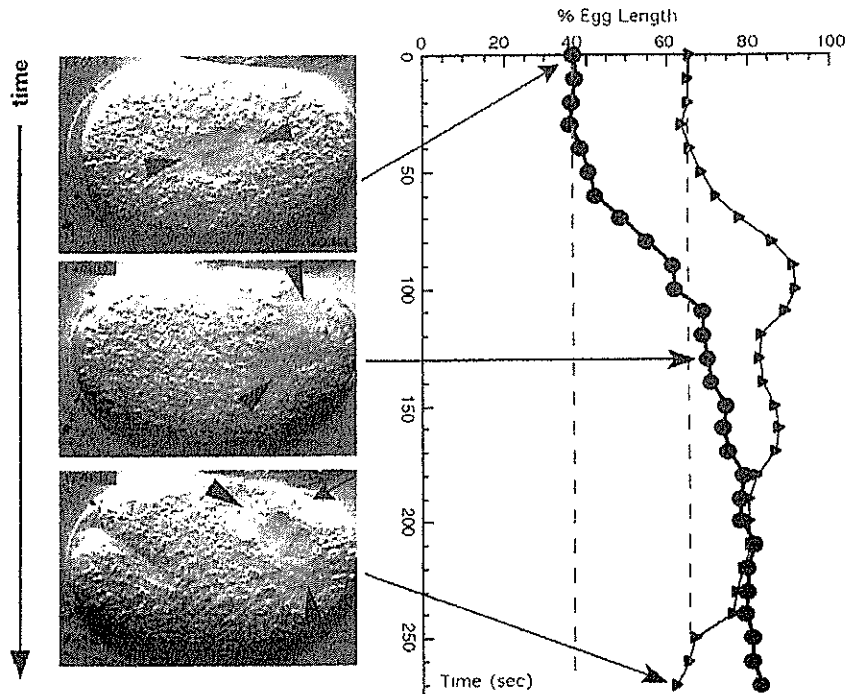

FIG. 4. Anaphase spindle positioning in *zyg-8* (*t1650*) mutant embryo. Left: three images taken from a time-lapse DIC recording; arrowheads point to centrosomes and spindle poles. Time elapsed since the beginning of the sequence is indicated in minutes and seconds. Right: corresponding tracings of aster positions over time. Both anterior and posterior spindle poles move in an exaggerated manner towards the posterior during anaphase. As a result, the cleavage furrow (arrow) is specified too much to the posterior along the AP axis, and has an aberrant orientation. Bar = 10 μm. A Quicktime movie of this sequence can be viewed at *http://www.embl-heidelberg.de/ExternalInfo/hyman/Data.htm*.

zyg-8 regulates microtubule behaviour during anaphase

How could *zyg-8* achieve this function? One possibility is that *zyg-8* could be required to set up proper AP polarity. In this scenario, a polarity defect in *zyg-8* mutant embryos would result, as a consequence, in the spindle going to the wrong position along the longitudinal axis. To address this possibility, we examined the distribution of PAR-1, PAR-2 and PAR-3, as well as P granules, which are cell fate determinants normally segregated to the posterior of the embryo (Strome & Wood 1983). We found that all these markers of polarity were correctly localized in *zyg-8* mutant embryos (data not shown). Thus, *zyg-8* does not appear to play a role in setting up overall embryonic polarity.

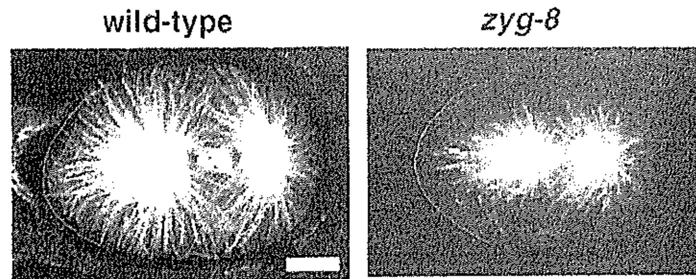

FIG. 5. Distribution of microtubules during anaphase in wild-type and *zyg-8(t1650)* mutant embryos as revealed by staining with anti-tubulin antibodies. White contour marks the anterior cortex. In wild-type, astral microtubules are long, extending all the way to the cell cortex. Astral microtubules in *zyg-8* mutant embryos are shorter, and do not reach the vicinity of the cell cortex. Bar = 10 μm.

Another possibility is that *zyg-8* could be required in some manner for the integrity of the microtubule cytoskeleton, in particular during anaphase. To test this, we examined the distribution of microtubules in fixed wild-type and *zyg-8* mutant embryos (Fig. 5). Strikingly, we found that astral microtubules during anaphase were shorter in *zyg-8* mutant embryos than in wild-type; as a result, a few microns separated the tip of the longest astral microtubule from the cortex in *zyg-8* mutant embryos. Moreover, spindle microtubules appeared altered as well. Taken together, these observations suggest that *zyg-8* normally acts to somehow, directly or indirectly, promote anaphase microtubule growth and/or stability.

We next addressed whether shorter microtubules during anaphase were actually causing exaggerated posterior spindle displacement in *zyg-8* mutant embryos. To this end, we asked whether exaggerated posterior displacement could be generated by shortening microtubules in wild-type embryos during anaphase using low doses of the microtubule-destabilizing agent nocodazole. We found that embryos treated in this manner displayed a *zyg-8*-like anaphase spindle positioning phenotype (data not shown). This leads us to conclude that shorter microtubules during anaphase indeed cause excess posterior displacement and, therefore, likely explain the *zyg-8* mutant phenotype.

zyg-8 encodes an evolutionarily conserved kinase related to Doublecortin

The molecular nature of *zyg-8* was identified in the course of a genome-wide functional genomics screen for cell division genes that was initiated in the Hyman laboratory in 1999. This screen makes use of RNA mediated interference (RNAi). With RNAi, the expression of a given gene in the early embryo can be abolished in a sequence-specific manner via microinjection of corresponding

double-stranded RNA into the gonad of the mother (Fire et al 1998). RNAi is very efficient and phenocopies the null phenotype of the vast majority of genes acting in the early *C. elegans* embryo (C. Mello, personal communication; P. Gönczy, unpublished observations). In our screen, dsRNAs were generated, injected, and the resulting one-cell stage embryos analysed by time-lapse DIC microscopy for potential defects in cell division processes. We initially investigated ~2200 genes located on chromosome III using this screening paradigm. We found a single gene whose RNAi phenotype resembled that of *zyg-8* mutant embryos, and this gene was located in the chromosomal interval to which *zyg-8* had been mapped genetically (C. Mello, personal communication). Therefore, we sequenced this gene in three *zyg-8* mutant alleles, and found three distinct point mutations which all result in premature STOP codons. This indicates that the gene identified during the RNAi-based screen corresponds to the *zyg-8* locus.

The ZYG-8 protein is predicted to be 802 amino acids long and has homologues in *Drosophila* and mammals. ZYG-8 harbours two notable protein domains: a Ca^{2+}/calmodulin-dependent kinase domain, as well as an approximately 220 amino acid domain similar (47% amino acid identity) to human Doublecortin. The *doublecortin* gene is mutated in patients with X-linked lissencephaly and double cortex syndrome (des Portes et al 1998, Gleeson et al 1998). These diseases result from defects in neuronal migration, a process which is normally accompanied by translocation of the centrosome and the associated nucleus through an elongating neuronal process, and which may require proper modulation of the microtubule cytoskeleton (for review see Hatten 1999). Compatible with this view, Doublecortin is a microtubule-associated protein that stimulates polymerization of microtubules *in vitro* (Francis et al 1999, Gleeson et al 1999). This fits well with the phenotype of *zyg-8* mutant embryos, in which anaphase microtubules are short. These observations raise the possibility that the function of protein domains that modulate microtubule dynamics to ensure proper centrosome and spindle positioning may have been significantly conserved across metazoan evolution.

Conclusion and prospects

Asymmetric divisions that give rise to daughter cells of different sizes contribute to the generation of cell fate diversity during development. In this paper, we have discussed what we are in the process of learning about the mechanisms that position the mitotic spindle during the asymmetric division of the one-cell stage *C. elegans* embryo.

We have demonstrated that one of the consequences of establishing polarity along the AP embryonic axis is the generation of differential pulling forces that act on the spindle poles. Forces acting on the posterior spindle pole are more

extensive than those acting on the anterior one, thus explaining the slight overall posterior spindle displacement observed in wild-type embryos. The mechanisms by which the action of PAR proteins results in such differential pulling forces remain to be elucidated. This may include local depolymerization of microtubules to generate spatially restricted depolymerization-coupled movements, or differential action of motor proteins at the anterior versus the posterior cortex.

Our work with *zyg-8* has revealed a requirement for long and/or stable microtubules to prevent exaggerated posterior spindle displacement during anaphase. Why this is the case remains to be determined. The simplest interpretation in light of the model presented in Fig. 3 is that, in wild-type, astral microtubules may need to contact components located at the anterior cortex to generate pulling force on the anterior spindle pole. If these microtubules are too short, as in *zyg-8* mutant embryos, pulling force would not be exerted on the anterior spindle pole. This would result in exaggerated posterior spindle displacement, provided some pulling force is still acting on the posterior spindle pole. Whether this or other scenarios are at play can be tested by conducting spindle-cutting experiments in *zyg-8* mutant embryos.

In conclusion, these studies have shed light on the mechanisms by which animal cells manage to position their spindle in an asymmetric manner, thus contributing to a better understanding of how cell diversity is generated during development.

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DISCUSSION

Simon: What determines the initial cell polarity?

Gönczy: Elegant experiments by Goldstein & Hird (1996) have shown that a sperm component, whose nature remains to be shown, is what determines the posterior of the embryo. This sperm component then somehow leads to polarized distribution of PAR proteins along the anterior–posterior (AP) embryonic axis.

Raff: Does the location of sperm entry determine the polarity?

Gönczy: No. If this were the case, you would expect the side of the embryo to assume posterior character in the rare cases where sperm entry is lateral. Goldstein & Hird (1996) showed this not to be the case. Instead, what they observed is that the male pronucleus and associated centrosomes move along the cell cortex towards the nearest pole, which then becomes the posterior of the embryo. This movement does not require cytoplasmic dynein function (P. Gönczy, unpublished observations) and may also be microtubule-independent.

Raff: So the AP axis is already set up, in the sense that there are poles.

Gönczy: Poles are present indeed.

Nurse: And does it always move to the nearest one?

Gönczy: That is correct.

Raff: How are the poles set up?

Gönczy: That is not known. In a way, this results from the topology of the ooctye, which already has an oval shape.

Reik: Can you parthenogenetically activate the oocyte?

Gönczy: Not that I know of, although I am not sure how much people have tried. One interesting observation related to the origin of polariy in *C. elegans* embryos is that sperm lacking DNA is still able to fertilize the oocyte and set up proper AP polarity in the embryo (Sadler & Shakes 2000). This rules out the male genetic material being required for setting up polarity. However, this leaves open the possibility that the centrosome plays an essential role in this process.

Vande Woude: How did they exclude microtubules from being involved in this activity?

Gönczy: I was referring earlier to the movement of the male pronucleus and associated centrosome towards the nearest pole. The data concerning a potential role for microtubules in setting up embryonic polarity are as follows. If you subject early embryos to the microtubule-destabilizing agent nocodazole, AP polarity does not appear to be affected (Strome & Wood 1983, Hird & White 1993). But we know that nocodazole does not get rid of all microtubules. However, we have identified a number of β-tubulin genes required for microtubule-based processes in the early embryo during the course of our large-scale RNAi-based screen, and it will be interesting to examine AP polarity in β-tubulin RNAi-embryos, which may lack microtubules entirely.

Nurse: Are there mutants which alter either the shape of the ovary or the relationship of the ovary to the AP axis of the worm? If so, are there any effects of this on the polarity of the eggs?

Gönczy: Varying ovary morphology does not seem to have an effect. In fact, Goldstein & Hird (1996) utilized mutants with altered ovary morphology in which oocytes enter the spermatheca in unusual ways, to ascribe the role of a sperm component in determining the posterior of the embryo.

Nurse: What I was really trying to get at were the external developmental cues that might be important for establishing the poles. Presumably polarity is imposed by this organ on the overall animal: is there something you could do to manipulate this to see where the axis comes from?

Ambros: Before the oocyte finally gets cellular it has cytoplasmic bridges to the rest of the gonad. These bridges are at right angles to the AP axis, and could provide a source of polarity.

Chia: How do you rationalize the generation of the posterior movement in the *zyg-8* mutants or nocodazole treated embryos?

Gönczy: There are three scenarios that I can envisage, which are not mutually exclusive. One possibility is that forces acting on the anterior aster are diminished compared to wild-type, but that forces acting on the posterior aster are unchanged. The second possibility is that forces acting on the posterior aster are increased compared to wild-type, but that those acting on the anterior aster are unchanged. The third possibility it that forces acting on both spindle poles are just like in wild-type, but that there is a problem in spindle elongation, which as a secondary consequence leads to the spindle zooming to the posterior. We will be able to distinguish between these three possibilities by severing the spindle in *zyg-8* mutant embryos, as the three scenarios have different predictions in terms of the velocity of each spindle pole after spindle severing.

Leevers: Won't the treatment with nocodazole have cut the spindle?

Gönczy: No, because we are using doses of nocodazole that don't completely abolish the spindle. We are hitting the embryo during anaphase, and the spindle shrinks a bit but not too much during the course of the experiment. We exclude from our analysis those embryos in which the spindle is completely gone. One point that I would like to make is that we don't know anything about the mechanisms of force generation at this point. Force generation could be coupled to depolymerization of microtubules, or result from the work of minus-end directed motor proteins anchored at the cell cortex, which would pull on astral microtubules.

Schaar: Is the pronucleus in the initial spindle set up in the centre?

Gönczy: Yes, the spindle assembles in the cell centre.

Schaar: How does that come about? Are there any astral interactions that this process depends on?

Gönczy: After pronuclear migration and meeting of the pronuclei in the posterior half of the embryo, the pronuclei and associated centrosomes undergo a 90° rotation while going towards the centre of the embryo. This centration/rotation process requires intact microtubules, as well as the function of cytoplasmic dynein and dynactin components (Hyman & White 1987, Gönczy et al 1999a).

Schaar: Do you need *zyg-8* for this process?

Gönczy: To a large extent you don't. However, in about 20% of *zyg-8* mutant embryos, rotation is incomplete, suggesting a partial requirement for *zyg-8* function.

Schaar: But the zipping to other poles occurs in 100%.

Gönczy: That is true in embryos that manage rotation as in wild-type. But in those 20% of embryos that don't, the spindle usually drifts slowly towards the anterior pole. We think this is because astral microtubules are not long enough to reach the posterior cortex.

Kozma: Did your screen uncover other genes with a similar phenotype?

Gönczy: In our earlier mutational analysis of chromosome III, we identified another locus, called *apo-1*, which mutates to a *zyg-8* like phenotype (Gönczy et al 1999b).

Newport: One way to explain your results would be to say that there is a protein which stabilizes microtubules on one side, resulting in more on one side than the other.

Gönczy: This is very difficult to quantitate. As you have seen from the immunofluorescence images, there are many microtubules on either side in wild-type; moreover, anaphase B takes places within a couple of minutes. Therefore, it will be difficult to uncover potential transient changes in microtubule numbers using fixed specimens. However, we have generated a green fluorescent protein (GFP)–tubulin fusion construct in the laboratory which should be appropriate to address these kinds of questions.

Newport: In the absence of PAR-2, PAR-3 can spread uniformly and vice versa. Do you think they bind competitively to the same sites?

Gönczy: Yes, that is a possibility, but we don't know which sites they may bind to. The Kemphues laboratory has done a thorough analysis of the distribution of PAR proteins in various *par* mutant backgrounds, and this may shed light on this question (Kemphues & Strome 1997).

Nurse: Does PAR-2 or PAR-3 start from one pole and spread, or does it just invade?

Gönczy: Initially, PAR-3 has a wider domain of expression, which becomes restricted to the anterior cortex over time, presumably by the action of PAR-2 (Etemad-Moghadam et al 1995).

Vande Woude: Is this correlated with regulated expression, with *par-3* expressed first and then *par-2*? For this to occur, are they simultaneously expressed and you get free assortment, or is there some time-dependent regulation of translation?

Gönczy: The regulation of PAR-2 and PAR-3 translation is an underexplored topic of investigation. I should add that everything that happens in the one-cell-stage embryo is thought to be driven by the maternal and parental genomes, as there appears to be no zygotic transcription at that stage (Edgar et al 1994, Seydoux & Fire 1994).

Nurse: With respect to the astral microtubules emanating towards the cortex, which are shorter in the *zyg-8* mutant, are the pulling forces a consequence of the microtubules in wild-type being attached to the cortex, or are they due to attachment sites in the cytoplasm and associated motors, which means that if you extend longer into the cortex you would have a bigger pulling force than if you are shorter?

Gönczy: We don't know. Indeed, there could be either length-dependent forces which do not require astral microtubules touching the cortex, or forces that require such contact.

Lehner: Have you looked at *par-2/zyg-8* double mutants?

Gönczy: Yes. Because AP polarity appears normal in *zyg-8* mutant embryos, we expected that *zyg-8* mutant embryos would be able to respond to changes in AP polarity. In other words, if a *zyg-8* mutant embryo is also lacking *par-2* or *par-3* function, the spindle should no longer shoot to the posterior, but instead go to random locations around the circumference of the embryo. This is exactly what we observed, at least to a first approximation.

Edgar: What happens to the spindle?

Gönczy: The spindle is not stable, contrary to the situation in a *par-2* or *par-3* single mutant.

Ambros: Do you have any evidence for the *par*s or *zyg-8* acting later in development, for example in postembryonic asymmetric cell divisions?

Gönczy: par genes are required strictly maternally.

Ambros: In your RNAi experiments with *zyg-8*, do you see animals that survive embryogenesis, and then show nervous system defects?

Gönczy: We haven't looked.

Vande Woude: What is the similarity in the structure between PAR-2 and PAR-3 proteins?

Gönczy: Their molecular nature is completely different. PAR-3 is a PDZ-containing protein that has *Bazooka* and ASIP as *Drosophila* and mammalian cousins, respectively (Izumi et al 1998, Kuchinke et al 1998). PAR-2 is a ring-finger containing protein that does not have a characterized *Drosophila* or mammalian orthologue.

Nurse: Are the attempts to furrow failing because the spindle is moving all the time? Could it be that it sends a signal, furrowing starts and then the spindle moves, so that the signal is lost?

Gönczy: Indeed, these observations suggest that cleavage furrow specification does not occur at a single time point, but instead that the signal might be needed for a while.

Raff: In relation to Bill Chia's results, can you summarize in a few sentences what is known about the 90° turn in the spindle?

Gönczy: What Bill showed is that Bazooka, Inscuteable and Pins are required for a 90° spindle rotation in *Drosophila*. In the one cell stage *C. elegans* embryo, it appears that PAR-3, the Bazooka homologue, is not required for the 90° rotation of centrosomes that precedes spindle assembly (Cheng et al 1995).

Raff: What is required?

Gönczy: We know of a requirement for astral microtubules, cytoplasmic dynein, the dynactin components p50 and p150, as well as a protein called LET-99 (Hyman & White 1987, Gönczy et al 1999b, Rose & Kemphues 1998).

Raff: Is the original spindle vertical to the AP axis?

Gönczy: No. There is a 90° rotation of the centrosome pair and associated pronuclei that precedes spindle assembly.

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