However, current inferences about the evolution of introns are at best tentative because virtually no information exists about intron content for a vast majority of animal phyla and major clades. The sampling of species from each group is meager, and our ability to reliably map the intron gain and loss on ancestral evolutionary lineages is highly limited. A case in point is the observation that the tunicate *Ciona*, a deuterostome, contains far fewer introns per gene than its closest relatives (fish and human). If its genome were the only one available for deuterostomes, we would have erroneously inferred that deuterostomes lost introns early in their evolutionary history.

Finally, the observation of intron loss in several independent lineages of animals may be an indication that the increased number of alternatively spliced gene products in the cell, afforded by an increased intron content, was not the prelude to a higher phenotypic complexity of animals. Perhaps, as suggested by Lynch and Conery (2003), the evolution of introns is attributable to smaller population sizes of bigger (more complex) organisms. This allows introns to escape natural selection and to become fixed in the genome without initially having an adaptive role. In this case, the complexity and diversity of advanced animal body plans arose independently of the intronic enrichment of their genomes.

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Playing Ping Pong with Pins: Cortical and Microtubule-Induced Polarity

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Cortical cell polarity controls mitotic spindle orientation in many cell types. In this issue of *Cell*, Siegrist and Doe (2005) turn this around and show that the transfer of polarity information between the cortex and the spindle is not just one way. In *Drosophila* neuroblasts, the spindle also has polarizing activity on the cortex.

Many animal cells have polarized functions. They can separate inside from outside, undergo directed migration, grow in a defined direction, or divide to give daughters of different fates. So it is important to understand how cells become polarized and how this polarity is communicated and coordinated with cellular functions.

A popular model system for addressing these questions is the study of neuroblasts in the fruit fly *Drosophila* (reviewed in Betschinger and Knoblich, 2004). In *Drosophila* embryos, neuroblasts delaminate basally from a polarized epithelium, the neuroectoderm (Figure 1). These neuroblasts become polarized along their apical/basal axes and undergo asymmetric cell divisions to generate two daughter cells of different sizes and fates, a larger apical neuroblast and a smaller basal ganglion mother cell. Before division, the spindle rotates to orient along the apical/basal polarity axis. Understanding how cortical polarity information controls the orientation of the mitotic spindle is a major focus of research. Siegrist and Doe (2005), in this issue of *Cell*, show that information does not just flow from the cortex to the inside of the cell but that the spindle also communicates to the cortex to ensure the robust coordination of spindle orientation with cortical polarity.

The cortical polarity of neuroblasts is controlled by a set of apically localized proteins: the conserved Par complex (consisting of Bazooka, Par-6, and atypical protein kinase C) and the Inscuteable protein (reviewed in Betschinger and Knoblich [2004]). Disruption of the Par/Insc pathway leads to defects in spindle orientation and mislocalization of basal proteins

such as Miranda. Par/Insc activity controls spindle orientation through induction of apical crescents of Gαi and Pins. Pins is a receptor-independent Gα regulator that binds directly to Gαi through GoLoco domains. Pins-like proteins and Gα subunits are central to the regulation of spindle position in species as diverse as worms and humans (Willard et al., 2004).

The starting point for the work of Siegrist and Doe (2005) was the finding that although *insc* mutant neuroblasts fail to form Pins crescents by the normal time (early prophase), crescents are present by metaphase (Cai et al., 2003; Figure 1). These Insc-independent crescents further differ from the wild-type in that they can be found at any position on the cortex rather than at the normal apical position. An important observation is that the crescents are always found at a spindle pole.

In a series of experiments, the authors show that in the absence of Insc, the spindle both induces and positions the Pins crescent. They identified three components that are critical for this function: astral microtubules, Discs large (Dlg, a membrane-associated guanylate kinase [MAGUK] protein) and Khc-73, a Kinesin 3 family member similar to human GAKIN. Interfering with the function of any of these in *insc* mutants prevents formation of Pins crescents (Figure 1). Dlg binds to Pins and normally forms apical crescents during the transition from prometaphase to metaphase (Albertson and Doe, 2003; Bellaiche et al., 2001). As was found for Pins, Dlg crescent formation also requires microtubules in the absence of Insc. Therefore, Dlg and Pins crescents can be induced by two different mechanisms: via Par/Insc apical polarity or by a spindle pole. How are these activities coordinated when both are active?

A clue comes from the phenotype that is induced by inhibiting the spindle pole pathway (by loss of Dlg or Khc-73) in the presence of Par/Insc activity. Here, Pins crescents form in the normal location at the apical cortex. However, the mitotic spindle is not always perfectly aligned with the center of the crescent (Figure 1). This could mean one of two things. The spindle might require Dlg and Khc-73 to respond to the location of Pins or some other aspect of apical information. Alternatively, it is possible that the activity of the spindle pole pathway causes a slight alteration of crescent position with respect to the spindle. This latter possibility is consistent with the observation that in the absence of Insc the Pins crescent is aligned with the spindle pole.

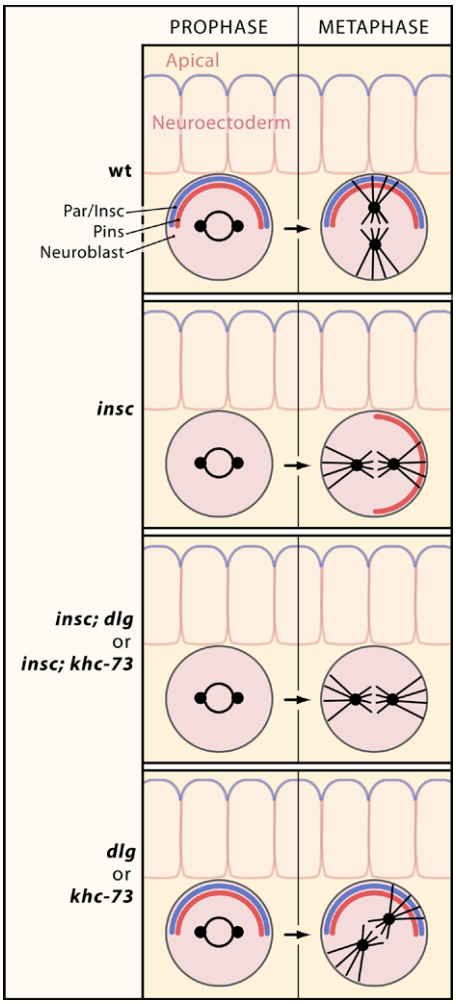

Figure 1. Cortical Pins Polarity Induced by Two Mechanisms

Each set of two panels shows a neuroblast that has delaminated from the overlying neuroectoderm, progressing from prophase (left column) to metaphase (right column) In prophase, centrosomes are represented as black dots outside of the intact nuclear envelope. In metaphase, the fully formed mitotic spindle is depicted. Genotypes are given on the left. (Top row) In the wild-type (wt), Par proteins and Insc (Par/Insc, together represented as a blue crescent), and Pins (red crescent) are localized to the apical cortex by prophase and remain there in metaphase and later. (Second row) In *insc* mutant neuroblasts, Par proteins are not apically enriched and there is no Pins crescent at prophase; however, by metaphase, a Pins crescent overlies a spindle pole. (Third row) The *insc*-independent Pins crescent requires Dlg, Khc-73, and astral microtubles: it does not form in *dlg* mutants (*insc*; *dlg*) after RNAi of *khc-73* (*insc*; *khc-73*), or when astral microtubules are removed (not shown) in an *insc* background. (Bottom row) Inhibition of *dlg* or *khc-73* in a wt background causes spindle misalignment.

Do the Par proteins and the spindle independently control asymmetric localization of Pins-like proteins in other systems? In the one-celled embryo of the nematode *C. elegans*, the Pins-like proteins GPR-1/2 are enriched at the posterior cortex and control asymmetric spindle position together with Gα subunits (reviewed in Betschinger and Knoblich [2004]). Unlike in *Drosophila* neuroblasts, asymmetric localization of GPR-1/2 is completely dependent on Par polarity (Colombo et al., 2003; Gotta et al., 2003). Dlg and Khc-73 homologs exist in the worm, but thus far neither appears to have a role in the first division. However, there is evidence that the mitotic spindle in *C. elegans* does have polarizing activity. In *spn-4* mutants, spindle orientation of the P1 cell at the two cell stage is incorrect and not aligned with the polarity axis (Gomes et al., 2001). However, during prometaphase, polarized molecules are redistributed to overlie a spindle pole, analogous to the metaphase induction of the Pins crescent in neuroblasts lacking Insc. Interestingly, redistribution appears to depend on G proteins (see discussion in Gomes et al., 2001). The oblong shape of the *C. elegans* eggshell constrains the orientation of the first mitotic spindle to lie along the long axis of the embryo,

which is the polarity axis, and this may also help in coordinating spindle orientation with cell polarity.

What is the relationship between Dlg and Khc-73? Siegrist and Doe (2005) found that Dlg and Khc-73 physically interact, as was previously found for vertebrate homologs of these proteins (Asaba et al., 2003; Hanada et al., 2000). They also detected Khc-73 at the plus ends of microtubules but failed to see Dlg on microtubules. A study of Dlg and the Khc-73 homolog GAKIN in MDCK cells provides a possible functional parallel for the neuroblast study (Asaba et al., 2003). Expression of GAKIN induces long projections that contain Dlg and GAKIN at their tips. Significantly, these projections are dependent on microtubules for their formation. Therefore, Dlg/Khc-73 might have a general role in regulating polarity via microtubules. How could this work?

Siegrist and Doe (2005) put forth a model whereby Khc-73 at microtubule plus ends contacts and clusters Dlg that is already at the cortex of the cell. Dlg clustering in turn would lead to Pins/Gαi crescent formation, via binding of Dlg to Pins. They suggest that Khc-73 could induce clustering by blocking Dlg intramolecular interactions to promote Dlg intermolecular interactions. Intermolecular Dlg interactions do appear to occur, because they found that endogenous Dlg binds Dlg-eGFP in vivo. If Dlg crescent formation is a consequence of blocking intramolecular interactions, then a protein that is enriched at the cortex would be expected to provide this function in the absence of Khc-73.

An alternative model is that the Khc-73 kinesin transports Dlg along microtubules and delivers it to the cortex leading to Dlg enrichment at a pole. Although Siegrist and Doe (2005) could not detect Dlg on microtubules, it may be that Dlg levels are below their detection limit. Human homologs of Dlg physically interact with microtubule binding proteins (Brenman et al., 1998; Niethammer et al., 1998), supporting the idea that Dlg might be associated with the spindle. In this view, Dlg intermolecular interactions could be promoted by a higher local concentration of cortical Dlg, induced either by transport on microtubules or recruitment by apically enriched polarity proteins.

A previous study from the Doe lab made additional links between Dlg and the spindle (Albertson and Doe, 2003). First, the Miranda protein, which is normally localized to the basal cortex, decorates the spindle in *dlg* mutants. This suggests that Miranda might be trafficked on the spindle and that trafficking depends on Dlg. Second, the sizes of the spindle poles in neuroblasts are abnormal in *dlg* mutants. Thus, if Dlg is transported on microtubules, it might have functions on the spindle and not just be a passive cargo.

There is much that we still do not understand. One question of particular interest is why the spindle induces Dlg and Pins crescents over one pole. How is the pole chosen? Are the spindle poles intrinsically different? A previous study showed that cortical enrichment of Dlg in *insc* mutants does sometimes occur over both spindle poles (Albertson and Doe, 2003), suggesting that the poles might not be different. Perhaps there is some underlying cortical polarity that influences which pole is active? Maybe contact with neighboring cells or cell shape play a role?

What is becoming clear from this study and many others is that there is extensive redundancy in generating cell polarity and linking it with downstream events: Par/Insc or Dlg/Khc-73/astral microtubules are sufficient for localizing Pins and they do so by different mechanisms. Similarly, there is functional redundancy between Par/Insc and Pins/Gα for generating spindle asymmetry (Cai et al. 2003). However, it is important to remember that these mechanisms are not completely redundant—there are both unique and overlapping roles. Redundancy can make it difficult to dissect the processes, but ensures that cellular events are reproducible. It is likely that we are only just beginning to uncover the checks and balances that exist to ensure that development is mistake free.

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