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REVIEW

Anterior-Posterior Polarity in *C. elegans* and *Drosophila*—PARallels and Differences

Jason Pellettieri and Geraldine Seydoux*

The eggs of *Caenorhabditis elegans* and *Drosophila* bear little similarity to each other, yet both depend on the *par* genes for control of anterior-posterior polarity. Here we explore possible common roles for the *par* genes (*pars*) in converting transient asymmetries into stably polarized axes. Although clear mechanistic parallels remain to be established, *par*-dependent regulation of microtubule dynamics and protein stability emerge as common themes.

The key to every biological problem must finally be sought in the cell, for every living organism is, or at some time has been, a cell.
E. B. Wilson, 1925 [(*1*), p. 1]

A major challenge in developmental biology is to understand how asymmetries are elaborated along the main body axes. How are heads made different from tails and everything in between? Remarkably, in many organisms these morphological differences can be traced back to the one-cell stage, where axis determinants localize to opposite ends of the egg. For many biologists, this realization has meant that to understand axis formation, one must first understand how asymmetries arise within a single cell: the egg.

Genetic screens in *Drosophila* and *C. elegans* have identified several regulators of egg polarity. These two models were long thought to bear little resemblance to one another. In *Drosophila*, polarization of the egg begins during oogenesis and requires microtubules. In contrast, in *C. elegans* polarization begins after fertilization and requires the actin cytoskeleton. The discovery of a group of genes essential for polarization of the *C. elegans* embryo ("*par*" genes) proved to be a turning point in the field (*2*). *par-3* and *par-6* encode two PDZ domain proteins, which together with the atypical protein kinase C PKC-3, form a complex in the anterior half of the *C. elegans* zygote (*3*–*5*). The serine threonine kinase PAR-1 and the ring finger protein PAR-2 occupy the posterior half (*6*, *7*). Two other genes, *par-4* and *par-5*, encode proteins that are uniformly distributed (*8*, *9*). Mutations in any one of these genes disrupt polarization of the zygote. Homologs of the *par* genes were soon discovered in mammals, where they regulate the polarization of epithelial cells, and in *Drosophila*, where they regulate epithelial and neuronal polarity [reviewed in (*10*, *11*)]. These observations prompted several groups to investigate whether the *par* genes might also regulate polarity in the *Drosophila* egg. Thus far, results indicate that this is the case. In fact, except for *par-2*, homologs of all the *par* genes have now been identified in *Drosophila* and are required for egg polarity (*12*–*20*). This remarkable conservation raises an apparent paradox: how can the same group of genes regulate polarity in such dissimilar cells (Fig. 1)? Here, we explore this issue by focusing on the role of the *par* genes in regulating anterior-posterior (A/P) polarity. We refer the reader to (*21*) and (*22*) for comprehensive reviews of axis formation in *Drosophila* and *C. elegans*.

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Establishment of A/P Polarity in *C. elegans*

In *C. elegans*, polarization of the egg begins after fertilization and is initiated by the sperm asters, which marks the future posterior end of the embryo (*23*–*26*). The first sign of polarity is seen when contractions of the egg cortex suddenly cease in a small area near the sperm pronucleus and internal cytoplasm begins to flow toward that area (*27*). The cue that initiates these rearrangements is not known but appears linked to the nucleation of microtubules by the sperm-derived centrosomes (*24*–*26*). The actin cytoskeleton is also involved: cytochalasin treatment and depletion of the nonmuscle myosin NMY-2 block polarization (*28*–*30*). Close contact between the sperm asters and the cortex has been correlated with the onset of polarity (*31*),

suggesting that intimate interactions between the sperm asters and the actin-rich cortex are what initiate the polarization process.

In response to the sperm aster signal, the PAR proteins, which start out uniformly distributed, rearrange dramatically. PAR-1 and PAR-2 localize to the posterior half of the cortex (*6*, *7*), whereas the PAR-3/PAR-6/PKC-3 complex localizes to a complementary region in the anterior (*3–6*). Localization of the PARs is interdependent: PAR-3/PAR-6/PKC-3 are required to localize PAR-2 to the posterior (*7*), and PAR-2 is required to localize PAR-3/PAR-6/PKC-3 to the anterior (*3–5*). All are required to localize PAR-1, but PAR-1 is not required for the initial localization of other PARs (*3*, *7*). Thus, antagonistic interactions between PAR-3/PAR-6/PKC-3 in the anterior and PAR-2 in the posterior create distinct cortical domains, which in turn regulate the placement of PAR-1. Time-lapse observations of green fluorescent protein (GFP)–tagged PAR-2 and PAR-6 (GFP:PAR-2 and GFP:PAR-6) were used recently to clarify the role of the sperm asters in this process (*30*). GFP:PAR-2 localizes to the posterior of wild-type embryos coincident with sperm aster formation, but fails to do so when expression of the anterior PARs is reduced by RNA-mediated interference (RNAi). In contrast, GFP:PAR-6 localizes to the anterior during sperm aster formation even in the absence of PAR-2. These observations indicate that the sperm aster signal functions primarily by excluding PAR-6 and the other anterior PARs from the posterior. Although GFP:PAR-6 initially localizes normally in the absence of PAR-2, it eventually reenters the posterior domain as the zygote prepares to divide. During that time, the microtubules form the mitotic spindle and are no longer restricted to the posterior. Thus, polarization of the cortex proceeds in two phases: an initial "establishment" phase regulated by the sperm asters, and a later "maintenance" phase regulated by PAR-2 (Fig. 2).

The PARs are required for all subsequent asymmetries, including the asymmetric segregation of P granules and the asymmetric placement of the first mitotic spindle. Thus, the establishment of distinct anterior and posterior PAR domains converts a transient cue (eccentrically placed sperm asters) into stable positional coordinates that are used to localize cytoplasmic factors. The mechanisms that restrict PAR proteins to specific cortical domains, however, remain unknown. Localization of PAR-2 and PAR-6 requires only coding sequences (*24*, *30*), suggesting that the mechanisms involved act on the proteins and not the RNAs. Ring finger domains such as the one in PAR-2 (*7*) have been found in E3 ubiquitin ligase subunits, raising the possibility that PAR-2 excludes anterior PARs by triggering their degradation in the posterior. Proteins involved in membrane trafficking also have been implicated in the establishment of PAR domains. POD-1 is a coronin-related protein required for secretion of the egg shell and for PAR asymmetry in the zygote (*32*). OOC-3 and the torsin-related protein OOC-5 are two endoplasmic reticulum (ER) proteins required for oocyte growth and PAR asymmetry in the two-cell stage (*33*, *34*). The PARs are not secreted proteins, and thus are unlikely direct targets for these factors. Targeted secretion of membrane proteins, however, could be used to localize anchorage sites for the PARs. An important task for the future will be to delineate domains in PAR proteins critical for localization and to identify factors that interact with these domains.

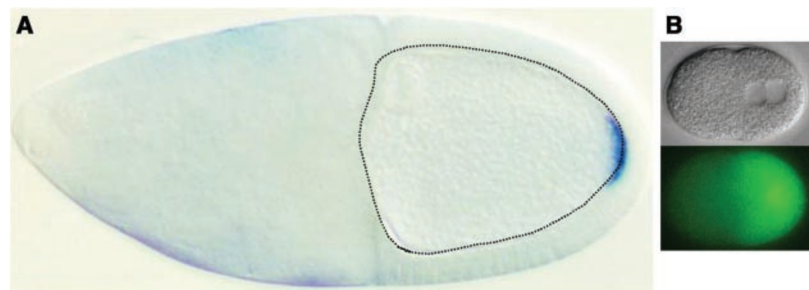

Fig. 1. Asymmetric localization of germline proteins in *Drosophila* and *C. elegans* eggs. (**A**) Stage 10 *Drosophila* egg chamber with *oskar* mRNA in blue and oocyte outlined. [Reprinted from (*14*), with permission from Elsevier Science] (**B**) Nomarski (top) and ultraviolet (UV) (bottom) exposures of a *C. elegans* zygote expressing PIE-1:GFP. Eggs are approximately to scale (*Drosophila* oocyte ≈ 100 μm; *C. elegans* embryo ≈ 50 μm), with anterior to the left and posterior to the right.

Establishment of A/P Polarity in *Drosophila*

In contrast to *C. elegans*, polarization of the *Drosophila* egg begins during oogenesis and is completed before fertilization. Asymmetries along the A/P axis are established during two distinct stages of oocyte development. The first polarization phase occurs early in oogenesis, during the process of oocyte specification. Later in oogenesis, the egg is repolarized, and it is during this second polarization that A/P determinants assume their final positions along the A/P axis [reviewed in (*21*)]. Here, we focus on the first polarization event and its dependence on the *par* genes.

Drosophila oogenesis begins with the division of a germline stem cell to form a 16-cell cyst, where sisters are interconnected by cytoplasmic bridges called ring canals. One of the 16 cells becomes the oocyte and the others become polyploid nurse cells. Which cell adopts the oocyte fate may depend on the asymmetric distribution of a membranous organelle called the fusome. The fusome is partitioned asymmetrically during cystoblast divisions and it has been hypothesized that the cell that inherits the most fusome material is the one that will become the oocyte (*35*, *36*). This hypothesis, however, has been difficult to test directly because the fusome disappears before the oocyte can be identified unambiguously. Differentiation of the oocyte is a gradual process that depends on the accumulation of several determinants, such as Orb (*37*), which travel to the oocyte on a polarized microtubule network that forms in a fusome-dependent manner throughout the cyst. The determinants initially accumulate at the anterior side of the oocyte and later shift to the posterior, coincident with relocalization of the oocyte microtubule organizing center (MTOC) to the posterior. This redistribution marks the first polarization phase of the oocyte and appears to be coupled to stable establishment of oocyte fate [reviewed in (*38*)].

Drosophila PAR-1 localizes to the fusome (*15*, *17*) and becomes restricted to the future oocyte in a microtubule dependent–fashion (*18*). In *par-1* null mutants, oocyte selection is delayed and transient (*15*) or does not occur at all (*17*), suggesting that PAR-1 may be one of the fusome-associated factors that participates in oocyte selection. PAR-1 is also required for polarization of the new oocyte: in the absence of PAR-1, the MTOC and Orb never relocalize to the posterior, Orb eventually disappears, and the oocyte reverts back to the nurse cell fate (*15*). Thus, as in *C. elegans*, PAR-1 appears to transform a transient asymmetry (fusome) into a stably polarized axis that is used to localize determinants, in this case required for oocyte fate.

Remarkably, the *Drosophila* homologs of *par-3* (*bazooka*, *baz*), *par-4* (*lkb1*), *par-5*, *par-6*, and *pkc-3* (*DaPKC*) are also required for anterior-to-posterior translocation of oocyte markers and for maintenance of oocyte fate (*13*, *16*, *19*, *20*). At first glance, the regulatory hierarchy that links these genes in *C. elegans* does not appear fully conserved in *Drosophila*. In *C. elegans*, localization of PAR-1 is dependent on all the other PARs. In contrast, in *Drosophila* localization of PAR-1 to the fusome is independent of Baz, PAR-6, and PAR-5 (*13*, *16*, *19*). In the oocyte, PAR-1 initially accumulates in the anterior and relocates to the posterior during polarization (*18*). In *baz* mutants, PAR-1 is present

early in the anterior but is lost after polarization and never appears in the posterior, suggesting that BAZ may regulate PAR-1 localization at this stage (*18*). The reverse, however, is also true: BAZ disappears from oocytes in *par-1* mutants (*18*). It is possible that these effects do not reflect true regulatory interactions. but rather are secondary to the failure to maintain an oocyte in these mutants. The inter-dependence of oocyte fate, oocyte polarization, and PAR asymmetry will certainly complicate the unraveling of any potential regulatory hierarchy. In established oocytes, BAZ and PAR-1 occupy complementary cortical domains (*18*) as they do in *C. elegans*, but the importance of this localization, which is observed after oocyte polarization, is not yet known. As in *C. elegans*, *par* genes in *Drosophila* function together to create a polarized axis, but their relative contributions remain to be determined.

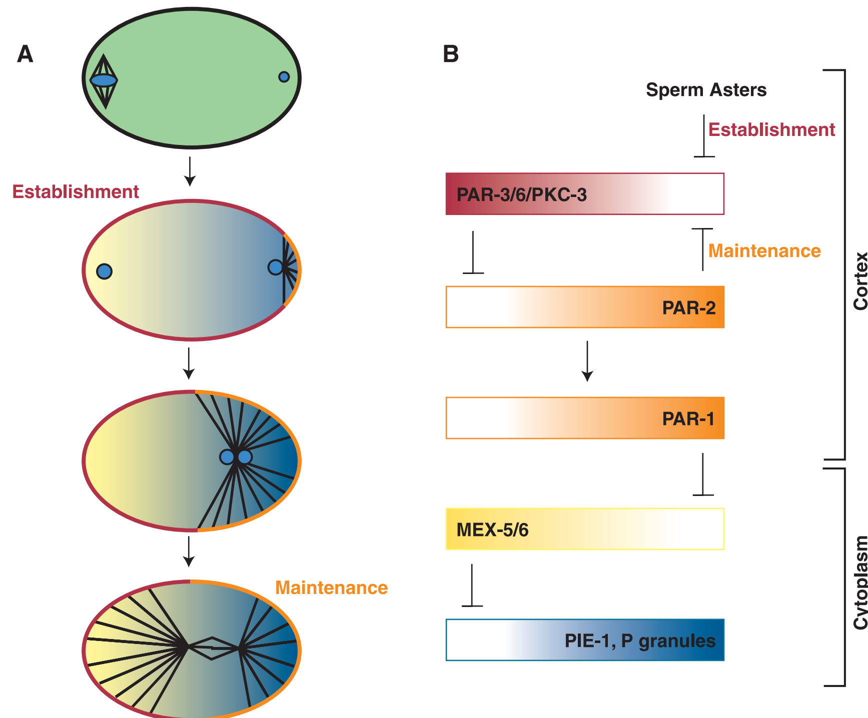

Fig. 2. The PAR hierarchy in *C. elegans*. (**A**) Anterior PARs, red; posterior PARs, orange. In the cytoplasm, PIE-1 and P granules, blue; MEX-5 and MEX-6, yellow. During meiosis (top), all are uniformly distributed throughout the zygote. Circles, pronuclei; black lines, microtubules. (**B**) Sequential repression model [modified from (*42*)]. Lines with bars, antagonistic interactions; lines with arrows, positive interactions.

Patterning of the A/P Axis in *C. elegans*

The next step after the creation of PAR domains on the cortex is to localize determinants in the cytoplasm. In *C. elegans*, the PAR most directly involved in this process appears to be PAR-1. PAR-1 is required for all cytoplasmic asymmetries but is not required for the initial localization of other PARs. Here we focus on PAR-1's role in localizing germ plasm components to the posterior end of the embryo. The germ plasm is a complex mixture of proteins (e.g., PIE-1) and RNA-rich organelles (P granules) essential for germline development. These factors start out uniformly distributed in oocytes and segregate to the posterior after fertilization coincident with the reorganization of the PAR proteins into two domains. In the absence of PAR-1, P granules and PIE-1 remain uniformly distributed and eventually disappear from all cells (*2*, *39*, *40*). PAR-1 does not act on P granules and PIE-1 directly, but instead functions through a pair of redundant intermediates. MEX-5 and MEX-6 are nearly identical cytoplasmic proteins, which, under the influence of PAR-1, localize to the anterior in a pattern opposite that of PIE-1 and P granules (*41*). In the absence of MEX-5 and MEX-6, P granules and PIE-1 remain uniformly distributed (*41*), suggesting a "sequential repression model" whereby PAR-1 excludes MEX-5 and MEX-6 from the posterior and MEX-5 and MEX-6, in turn, exclude P granules and PIE-1 from the anterior (*42*) (Fig. 2).

In principle, PAR-1 and MEX-5 and MEX-6 could affect protein localization by a number of mechanisms, including: (i) RNA localization, (ii) localized translation, (iii) protein transport, (iv) protein trapping, and (v) localized protein degradation. MEX-5 and MEX-6 belong to the CCCH finger family of RNA binding proteins, but their effect on PIE-1 localization is unlikely to be dependent on interactions with the *pie-1* RNA, because PIE-1 asymmetry is regulated at the protein level (*40*). PIE-1 localization involves two complementary mechanisms: a first mechanism that causes PIE-1 to become enriched in the posterior at the time of MEX-5 and MEX-6 localization in the anterior, and a second mechanism that degrades any PIE-1 left over in the anterior after division (*40*). P granule asymmetry also involves two mechanisms: movement toward the posterior and degradation in the anterior (*43*). In *par-1* mutants, P granules and PIE-1 are degraded in all cells (*2*, *39*, *40*), suggesting that PAR-1 creates an environment in the posterior that protects these factors from degradation. In *mex-5;mex-6* double mutants and *mex-5; mex-6;par-1* triple mutants, PIE-1 is maintained in all cells (*41*), consistent with the idea that MEX-5 and MEX-6 act downstream of PAR-1 to activate PIE-1 degradation in the anterior.

These data indicate that local inhibition of protein degradation is one of the PAR-1–dependent mechanisms used to maintain determinants in the germline. Whether protein transport and/or trapping also contribute to the initial segregation of PIE-1 and MEX-5 to opposite poles in the zygote remains an open question. One point, however, is clear. Microtubule-based transport is unlikely to be involved, because asymmetries arise before microtubules span the axis and because microtubule-depolymerizing drugs do not affect P granule or PIE-1 asymmetry (*28*, *40*).

Patterning of the A/P Axis in *Drosophila*

In contrast to *C. elegans*, segregation of determinants in the *Drosophila* oocyte depends on microtubules. Initial polarization of the oocyte during the oocyte-specification phase requires microtubules and correlates with the formation of a MTOC in the posterior of the cell. The posterior MTOC does not form in *par-1* mutants (*15*, *17*), but the role of PAR-1 in this process remains unclear. PAR-1 functions again later in oogenesis during repolarization of the oocyte (*12*, *14*). The asymmetry-generating mechanisms used in this second polarization are understood in more detail.

After oocyte specification, the 16-cell cyst becomes surrounded by somatic follicle cells. The oocyte is positioned at the posterior end of the cyst, in direct contact with follicle cells on all sides except at its anterior end, where it remains connected to the nurse cells via ring canals. This arrangement permits two essential processes.

First, messenger RNAs (mRNAs) encoding determinants important for embryonic development (e.g., *bicoid* and *oskar*) are transcribed in the nurse cells and transported into the oocyte via the ring canals. Second, the oocyte and the follicle cells exchange signals that define both the A/P and dorsal/ventral (D/V) axes. In particular, follicle cells at the posterior end of the cyst send a signal that repolarizes the oocyte: the posterior MTOC is lost and replaced by a new microtubule network that is essential to localize *bicoid* and *oskar* RNAs to opposite poles of the oocyte [reviewed in (*21*)].

Early studies led to a simple model for *bicoid* and *oskar* RNA localization. A β-galactosidase fusion of the plus-end–directed microtubule motor Kinesin localizes to the posterior pole of the oocyte, whereas a β-galactosidase fusion of the minus-end marker Nod localizes to the anterior pole of the oocyte (*44*, *45*). These localizations suggested that plus-end–directed motors might transport *oskar* mRNA to the posterior, whereas minus-end–directed motors might transport *bicoid* mRNA to the anterior. Consistent with this model, *oskar* RNA was reported to localize to the anterior in *kinesin I heavy chain* (*khc*) mutants (*46*).

A new study challenges this simple model. Reexamination of *oskar* localization in *khc* mutants showed that *oskar* mRNA is distributed over the entire cortex, not just the anterior (*47*). Depolymerization of microtubules leads to a similarly broad delocalization (*47*). Surprisingly, components of the γ-tubulin ring complex, which nucleates microtubules by associating with their minus ends, are uniformly distributed over the entire oocyte cortex; only the microtubule themselves are present at a lower density in the posterior (*47*). Together, these findings suggest an alternative explanation for why *oskar* becomes delocalized in the absence of Kinesin. According to the new model (*47*), Kinesin is not required to transport *oskar* to the posterior per se but rather to move it away from the microtubule-rich anterior and lateral cortices. The decreased density of microtubules at the posterior subsequently allows *oskar* access to the cortical actin network specifically in this region, thereby promoting its posterior localization.

These new findings imply that reduction of microtubule density at the posterior pole is a critical step in the localization of *oskar* to the posterior. How is this step regulated? Current evidence points to PAR-1. Partial loss-of-function mutants in *par-1* retain enough activity to polarize the oocyte early, but they fail to localize *oskar* during the late polarization phase (*12*, *14*). In these mutants, microtubule density remains uniform (*14*), and *oskar* mRNA accumulates in the center of the oocyte rather than the posterior (*12*, *14*), as predicted by the new model. These observations suggest that PAR-1 regulates *oskar* localization indirectly by destabilizing microtubules in the posterior (Fig. 3).

Downstream of PAR-1

Mammalian PAR-1 homologs destabilize microtubules by phosphorylating microtubule-associated proteins (*48*), suggesting that regulation of microtubule dynamics may be a conserved aspect of PAR-1 function. If so, is there any evidence that PAR-1 also regulates microtubule dynamics in *C. elegans*? PAR-1's effect on the localization of germline proteins is unlikely to be mediated by microtubules, but PAR-1 does play a role in the asymmetric positioning of the first spindle. During anaphase, the spindle becomes displaced toward the posterior as a result of asymmetric forces generated by interactions between the astral microtubules and the cortex (*49*). In *par-2* and *par-3* mutants, these forces are balanced and the spindle remains in the middle of the egg. In *par-1* mutants, spindle displacement is also compromised, although not as severely as in *par-2* and *par-3* mutants, suggesting that spindle asymmetry involves both *par-1*–dependent and *par-1*–independent processes. At first glance, these observations seem consistent with a possible role for PAR-1 in regulating microtubule dynamics. However, other observations suggest that PAR-1's effect on spindle placement may be secondary to its segregation functions. For example, *mex-5;mex-6* double mutants occasionally also misplace their spindle (*30*). Future studies will be required to distinguish whether PAR-1 influences microtubule dynamics directly or indirectly, by localizing other regulators.

Does this mean that *C. elegans* PAR-1 and *Drosophila* PAR-1 regulate egg polarity by completely different mechanisms? A new study reveals one possible area of overlap: control of protein stability (*50*). Oskar protein is a substrate for the PAR-1 kinase, and phosphorylation increases Oskar's stability in extracts (*50*). A moderate reduction in PAR-1 levels reduces Oskar protein levels in vivo, even when Oskar translation is uncoupled from localization. These observations suggest that, in addition to localizing *oskar* RNA, PAR-1 also protects Oskar protein from degradation. This function appears to be part of an amplifying feedback loop, because PAR-1 itself becomes restricted to the posterior in an Oskar-dependent manner (*14*). In *C. elegans*, PAR-1 stabilizes germ plasm proteins indirectly through regulation of other factors, whereas in *Drosophila*, PAR-1 stabilizes Oskar directly by phosphorylation. The two mechanisms, however, are not exclusive and could be used in concert for maximum efficiency.

The list of PAR-1 targets is unlikely to stop there. Rab11, a protein implicated in membrane trafficking, is required for efficient transport and anchoring of *oskar* mRNA to the posterior pole (*51*, *52*). PAR-1 homologs in yeast and mammalian cells have been implicated in the regulation of the exocytic machinery (*53*). Thus, polarized secretion may be yet another mechanism used by PAR-1 to localize determinants.

An important task for the future will be to identify all direct targets of PAR-1. Recently, PAR-1 was found to bind to 14-3-3 proteins (*19*), a conserved family of proteins that recognize phosphorylated pep-

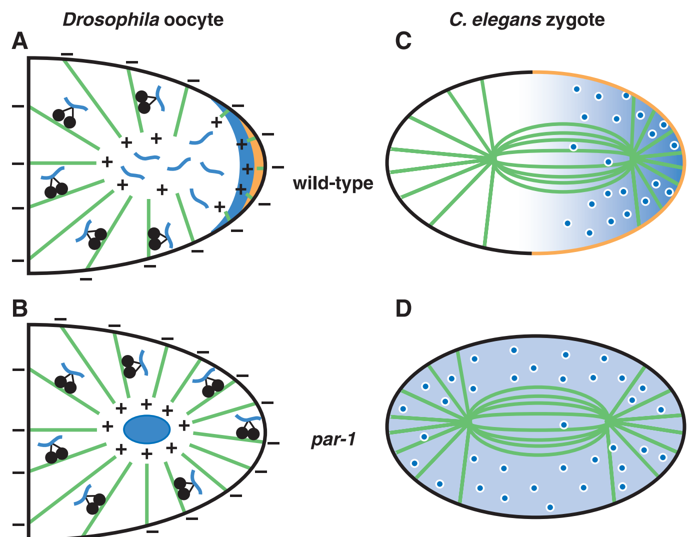

Fig. 3. Comparison of *par-1* phenotypes in *Drosophila* and *C. elegans* eggs. (**A**) Wild-type *Drosophila* oocytes: Microtubules (green) are nucleated around the entire cortex but at reduced density in the posterior due to PAR-1 (orange). *oskar* mRNA (blue) is transported away from the cortex by Kinesin (black circles), except in the posterior where the low density of microtubules allows access to the cortex. Oskar protein is stabilized in the posterior by PAR-1–dependent phosphorylation. (**B**) In the absence of *par-1*, microtubule density is uniform and *oskar* cannot access the cortex. (**C**) Wild-type *C. elegans* zygotes: PAR-1 (orange) in the posterior promotes enrichment of the germline determinants PIE-1 (blue) and P granules (blue dots) by a microtubule-independent mechanism. PAR-1 also contributes to the posterior displacement of the mitotic spindle (green) by an unknown mechanism. (**D**) In *par-1* mutant embryos, PIE-1 and P granules are uniformly distributed and spindle asymmetry is compromised.

tides (*54*). PAR-1 phosphorylation can create a binding site for 14-3-3, suggesting that PAR-1 substrates become bound to 14-3-3 after phosphorylation (*19*). *14-3-3* mutants in *Drosophila* have polarity defects identical to those seen in *par-1* mutants, consistent with the idea that 14-3-3 binding is essential for PAR-1 signal transduction (*19*). Remarkably, one of the *C. elegans 14-3-3* homologs is encoded by *par-5* (*9*), and PAR-5 protein binds to PAR-1 in a yeast two-hybrid assay (*19*). PAR-5, however, is unlikely to function only with PAR-1 in *C. elegans*, because it is required for the initial establishment of PAR domains, a process that is independent of PAR-1 (*9*, *30*). 14-3-3 proteins have been implicated in many cellular processes (*54*), including actin dynamics (*55*), and could potentially act multiple times in the PAR hierarchy. The identification of 14-3-3 proteins as potential mediators of PAR-1 function may facilitate the identification of PAR-1 substrates.

Conclusions

Three main themes emerge from a comparison of PAR functions in *Drosophila* and *C. elegans* eggs. First, PAR proteins act together to convert a transient polarity cue into a stably polarized axis. Second, of all the PARs, PAR-1 appears most directly involved in converting cortical polarity into cytoplasmic asymmetry. Last, PAR-1 orchestrates cytoplasmic asymmetries by impinging on diverse cellular functions, including microtubule dynamics, protein degradation, and, likely, many others. Thus, the secret to the *par* genes' remarkable adaptation to different cell types may lie in their ability to regulate a number of basic cellular processes. Although much has been learned, a complete picture awaits the identification of the essential cell machineries that interact with the PARs. As E. B. Wilson predicted, the key to this problem also lies in the cell biology of the egg.

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REVIEW

Shaping the Vertebrate Body Plan by Polarized Embryonic Cell Movements

Ray Keller

Polarized cell movements shape the major features of the vertebrate body plan during development. The head-to-tail body axis of vertebrates is elongated in embryonic stages by "convergent extension" tissue movements. During these movements cells intercalate between one another transverse to the elongating body axis to form a narrower, longer array. Recent discoveries show that these polarized cell movements are controlled by homologs of genes that control the polarity of epithelial cells in the developing wing and eye of the fruit fly, *Drosophila*.

How the body plan is shaped from a cohesive aggregate of individual cells during embryogenesis is an enduring mystery. A major breakthrough is the recent discovery that homologs of genes controlling the polarity of hairs on the epidermal cells of *Drosophila* (fruit fly) wings also control the polarized cell motility underlying the morphogenic movements that shape the vertebrate body plan. These movements, known as "convergence and extension" or "convergent extension," narrow (converge) the mediolateral aspect and elongate (extend) the anterior-posterior aspect of the vertebrate embryo and thereby establish its morphological and func-

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