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PARsing Embryonic Polarity

Minireview

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Current understanding of the way in which embryonic polarity is established relies heavily on studies of maternal effect lethal mutants in *D. melanogaster* and *C. elegans* (St. Johnston and Nüsslein-Volhard, 1992; Rose and Kemphues, 1998). Although the analysis in worms began in earnest about a decade after the explosion of information from flies, we now know enough about both systems to make comparison meaningful (Bowerman, 1995, 1998), and to ask whether there are conserved mechanisms used for establishing embryonic polarity. Thus far, the single common feature is translational repression, which has been shown to localize important fate regulators in both systems (see Evans et al., 1994). Now, however, in this issue of *Cell*, Shulman and colleagues (2000) report an analysis in *D. melanogaster* of the first molecule to play an important and perhaps conserved role in both animals, PAR-1.

Establishing Embryonic Polarity in C. elegans

Embryogenesis in *C. elegans* begins with a series of five asymmetric cleavages that create six founder blastomeres, each of which produces a clone of cells with a distinct behavior and set of cell fates (Figure 1A). Although cell–cell signaling plays a role in specifying some founder fates, most of the differences are due to the cell-intrinsic action of molecules that are distributed asymmetrically along the anterior/posterior (A/P) axis (Figure 1B). Three classes of polarized "fate-determining" proteins can be distinguished: an anterior group, enriched in AB and its descendents (MEX-3, GLP-1), a posterior group, enriched in P_1 and its descendents (SKN-1, PAL-1), and a germline group, stably expressed only in blastomeres in the lineage leading to germline formation (PIE-1, MEX-1, POS-1, P granule components). With the exception of GLP-1, which mediates cell–cell interactions, these proteins appear to act cell-intrinsically and combinatorially to specify the fates of founder cells (Bowerman, 1998; Rose and Kemphues, 1998). Correct placement of the fate-determining molecules depends upon an earlier establishment of A/P polarity. A/P polarity in worms arises in the one-cell embryo. The initial cue is provided by the sperm, whose position sets the posterior pole. The nature of this polarity cue is not known, but the favored hypothesis is that the sperm centrosomes signal to the cell cortex promoting actomyosin-based cytoskeletal changes that drive polarity.

Genetic analyses have identified many maternally acting genes with essential roles in early embryogenesis. The genes fall into two major classes: the cell-fate-determining genes like *skn-1* mentioned above, and polarity genes, which are required to establish or maintain embryonic polarity. The polarity genes with the strongest effects are the *par* genes (*par*titioning defective). Mutations in these genes lead to similar but somewhat gene-specific defects in early embryonic asymmetries (Bowerman, 1998; Rose and Kemphues, 1998). Par mutant embryos have cleavage pattern defects and alterations in the fates of the founder cells that can be attributed to the mislocalization of some or all of the fate-determining proteins. Molecular analyses of the *par* genes have revealed that they encode proteins with functional domains that could act in intracellular signaling. These include two proteins with serine/threonine kinase domains (PAR-1 and PAR-4), two proteins with PDZ domains (PAR-3 and PAR-6), and a protein with a "ring finger" zinc binding domain (PAR-2). Four of the PAR proteins are asymmetrically distributed in asymmetrically dividing cells of the early germline lineage (P_0, P_1, P_2, P_3). PAR-1 and PAR-2 become enriched at the posterior periphery of the zygote and PAR-3 and PAR-6 become enriched at the anterior periphery. Another protein, PKC-3, an atypical protein kinase C, colocalizes with PAR-3 and PAR-6 and has a similar loss-of-function phenotype. The current model is that PAR proteins act in the one-cell embryo to interpret the polarity cue provided by the sperm, mediating the local changes in cytoplasm that establish the A/P axis.

Much remains to be understood about how the PAR proteins function. Perhaps the most significant gap in

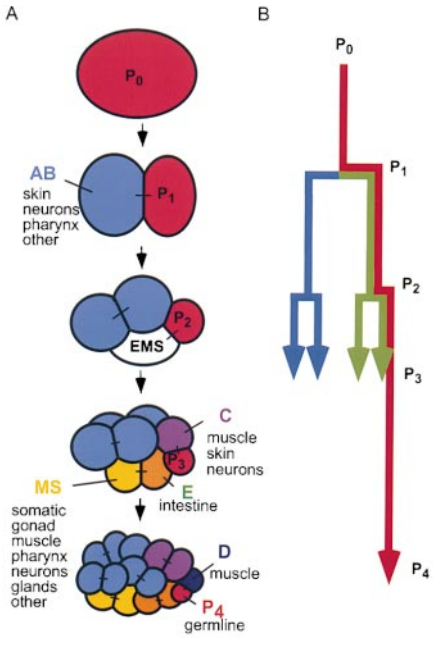

Figure 1. *C. elegans* Early Embryogenesis

(A) Founder cells and their fates.

(B) Distribution of cell fate regulators. Blue: Anterior group (GLP-1, MEX-3); green: posterior group (SKN-1, PAL-1); red: germline group (POS-1, MEX-1, PIE-1). Panel (B) is adapted from Schubert et al. (2000).

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our understanding has been information linking the PAR proteins to downstream localization events. That is, how do the PAR proteins mediate asymmetric distribution of other molecules? Because the maternally supplied mRNAs for most of the localized proteins are distributed uniformly throughout the embryo, polarized distributions must arise by movement of proteins or by asymmetric translation or protein turnover. Studies of GLP-1, a protein whose restriction to the AB lineage in early embryos is dependent upon the PAR proteins (Crittenden et al., 1997) have indicated that one output of PAR protein action is differential translational regulation. GLP-1 mRNA is present throughout the early embryo, but translation is repressed in the P_1 lineage. This translational repression cannot be a general mechanism of action of the PAR proteins, however, because the proteins mediate differential accumulation of SKN-1, PIE-1, and P granule components in P_1.

Recently, Schubert and colleagues (2000) filled a gap downstream of the PAR proteins through their identification and analysis of a partially redundant pair of proteins, MEX-5 and MEX-6. Homozygous *mex-5* mutant mothers produce embryos that arrest without undergoing morphogenesis and with an *ex*cess of *m*uscle cells, most of which arise ectopically from cells in the anterior, a phenotype that is shared by mutations in *mex-1* and the *par*s. However, *mex-5* embryos do not share the cleavage defects characteristic of mutations in previously identified polarity genes.

The *mex-5* gene encodes a novel protein with two copies of a CCCH "finger motif" that is found in several proteins thought to interact with RNA. Database searches revealed another nearly identical gene in the *C. elegans* genome, *mex-6*, that Schubert and colleagues showed functioned redundantly with *mex-5*. MEX-5 protein distribution is asymmetric and dynamic, consistent with its proposed role in establishing polarity (Figure 2). The distribution is complex, but is essentially the reciprocal of the distribution of the germline proteins.

Schubert and colleagues were able to place MEX-5/6 function downstream of PAR activity and upstream of localization of the determinants using a series of protein distribution analyses. In embryos from *mex-5;mex-6* mothers, PAR proteins localize normally in P_0, but in *par* mutants MEX-5 is no longer asymmetric. Thus, at least in the one-cell embryo, MEX-5 acts downstream of the PAR proteins. Embryos from *mex-5;mex-6* mothers exhibit uniform distributions of posteriorly localized proteins SKN-1 and PAL-1 and the germline-localized proteins PIE-1, MEX-1, and POS-1. Anteriorly localized proteins are also abnormal in *mex-5;mex-6* mutants; these embryos lack GLP-1 and have mislocalized and reduced levels of MEX-3. Thus, MEX-5/6 appears to act upstream of the localization of the fate-determining proteins.

Ectopic expression experiments showed that MEX-5 restricts the distribution of the germline proteins by repressing their expression. These results lead to a model (Figure 2) in which the PAR-1 and PAR-3 proteins act to restrict MEX-5/6 proteins to the anterior of the one-cell embryo and MEX-5/6 restrict germline proteins (PIE-1, MEX-1, POS-1, and other unknown proteins) to the posterior. The relationship of MEX-5 to SKN-1, PAL-1, and GLP-1 distributions is less clear. Although

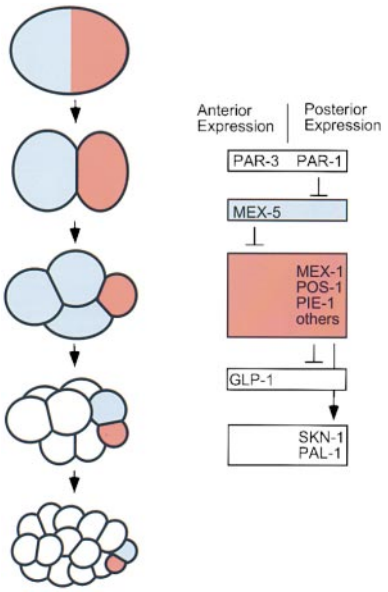

Figure 2. Distribution of MEX-5 and Sequential Repression Model for Establishing Polarity in *C. elegans*

Simplified distribution of MEX-5 (green) and germline localized proteins (pink) is shown on the left. In the model on the right, distributions of proteins along the A/P axis are indicated by their position in the boxes and repression of protein expression is indicated by the perpendicular bars. The "other" germline-localized molecules are hypothetical and would include proteins that function to repress GLP-1 translation and proteins that restrict the distribution of the posterior group genes.

other interpretations are possible, in the model proposed here MEX-5/6 affects SKN-1, PAL-1, and GLP-1 distributions indirectly (see Schubert et al., 2000 for a discussion).

Of course, plugging MEX-5/6 into the gap between the PARs and the localization of cell fate determinants creates two new gaps that need to be filled: how the PAR proteins restrict MEX-5/6 to the anterior, and how MEX-5/6 repress the expression of the germline proteins. The presence of CCCH finger motifs in MEX-5/6 and other proteins with likely interactions with RNA raises the possibility that MEX-5/6 might bind to and prevent translation of the messages for the germline proteins. Alternatively, MEX-5 may promote their degradation. Identifying the biochemical function and binding partners of MEX-5/6 should help to distinguish these possibilities. Hopefully, in the next few years, all the gaps will be filled and it will be possible to describe the complete series of events that polarizes the embryo.

PAR-1 and Polarity in the Drosophila *Embryo*

The initial events in polarizing the fly embryo have been well-studied and are quite different from those in worms (Figure 3; van Eeden and St Johnston, 1999). A/P polarity in flies is established during oogenesis based on a cue from the asymmetrically placed egg nucleus. This cue is in the form of an extracellular signal (Gurken) which is positioned by the oocyte nucleus that specifies the fate of posterior follicle cells. The posterior follicle cells then signal back to the egg via an unknown mechanism to trigger a reorganization of the microtubule cytoskeleton. In the reorganization, a posterior microtubule-

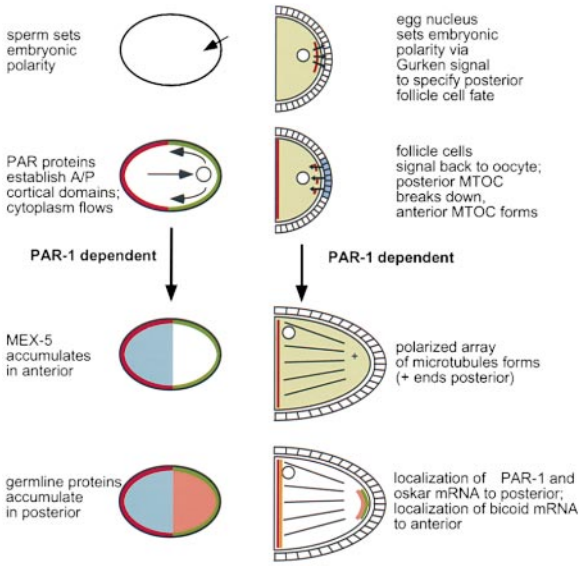

Figure 3. Comparison of Polarity Establishment in *C. elegans* (left) and *D. melanogaster* (right)

Green, PAR-1 and *Drosophila* PAR-1; red, anterior PAR proteins; light blue, MEX-5; pink, germline proteins MEX-1, POS-1, PIE-1 (left), germplasm components *oskar* mRNA, Stau (right); dark blue, posterior follicle cells; brown, microtubule-organizing centers (MTOC), orange, *bicoid* mRNA.

organizing center (MTOC) is replaced by an anterior MTOC that nucleates a polarized array of microtubules with plus ends at the posterior. As a consequence of the polarized microtubule array, mRNA for the morphogen Bicoid accumulates in the anterior, and pole plasm, carrying mRNA for the morphogen Nanos and determinants of germline fate, accumulates in the posterior. After fertilization, the polarized anterior and posterior morphogens interact with other maternal factors to establish gradients that regulate differential transcription of the embryonic genome along the A/P axis. The differences between flies and worms are striking. Worms polarize after fertilization, flies before; the polarity cue in worms comes from the sperm and acts cell-intrinsically, in flies from the oocyte nucleus and acts through the follicle cells; polarity in worms appears microfilament based, polarity in flies is microtubule based. Furthermore, in spite of extensive genetic analysis in both systems no common molecule has been identified.

Thus, it is with some surprise that we learn from Shulman and colleagues in this issue of *Cell* that A/P polarity in flies depends upon a homolog of *C. elegans* PAR-1 that, like PAR-1, becomes localized asymmetrically at the posterior cell periphery during the time that polarity is established. Both molecules are members of the PAR-1/MARK/KIN1 family of serine/threonine kinases (Drewes et al., 1997). This family of kinases has a high degree of similarity both within the kinase domain and within an extreme carboxy-terminal domain. PAR-1 and KIN1 have roles in polarity in *C. elegans* and *S. pombe*, respectively, and rat MARK1 and MARK2 were identified on the basis of their ability to phosphorylate microtubule binding proteins and were shown to disrupt the microtubule cytoskeleton when overexpressed (references can be found in Shulman et al., 2000).

The *D. melanogaster par-1* gene is a complex transcription unit with at least five identifiable protein isoforms in ovaries. Antibodies recognizing a region of the protein common to all isoforms revealed that *Drosophila* PAR-1 is expressed early in both somatic follicle cells and germline cells in the ovary. In early oocytes (stages 1–8) *Drosophila* PAR-1 is not visibly asymmetric. It is transiently enriched at the anterior cortex in early stage 9 oocytes but then accumulates at the cortex at the posterior pole during stages 9 and 10. This distribution is identical to the pole plasm components *oskar* mRNA and Staufen protein as verified by colocalization of *Drosophila* PAR-1 with GFP:Stau fusion protein. However, unlike these two pole plasm components, *Drosophila* PAR-1 is no longer detectable in early embryos.

Deletion of *Drosophila par-1* appears to be zygotic lethal and germline clones result in arrest of oocytes prior to axis formation. However, two P element insertions result in partially penetrant maternal effect lethality in which embryos are produced with abdominal pattern defects and loss of pole cells. The basis of these defects is a disruption of the localization of pole plasm components. Pole plasm forms during oogenesis through the step-wise accumulation of a number of components (Rongo and Lehmann, 1996). The first step is the localization of *oskar* mRNA and Staufen protein to the posterior. In *Drosophila* PAR-1 mutants, *osk* mRNA accumulates normally in the early oocyte, but at stage 9, instead of localizing to the posterior pole, along with Staufen protein it accumulates centrally in a tight sphere or remains delocalized. Interestingly, *Drosophila* PAR-1 fails to accumulate at the posterior in *oskar* protein null mutants, indicating a codependence.

Chasing these defects back to a more proximal cause, Shulman and colleagues (2000) examined the organization of the oocyte microtubule cytoskeleton in stage 9 and 10 egg chambers and found that microtubule organization was disrupted. Instead of the wild type arrangement with an anterior MTOC and an A/P gradient of microtubules with plus ends toward the posterior, microtubules are distributed uniformly around the oocyte periphery and arranged with plus ends oriented toward the center of the cell. This arrangement easily explains the central accumulation of *oskar* mRNA and Staufen protein.

The basis for this new organization appears not to involve the failure to eliminate the posterior MTOC, as is the case with other mutants that mislocalize *oskar* mRNA to the center of the oocyte. Instead, *Drosophila par-1* embryos no longer have a focus of microtubules in the posterior. Neither does the new organization arise because the anterior MTOC fails to form. *bcd* mRNA, a marker for the anterior MTOC, is localized normally in the *Drosophila par-1* mutants. Therefore, the *Drosophila par-1* mutant phenotype reveals that switching the location of the MTOC is not sufficient to repolarize the oocyte. A second process, dependent on *Drosophila* PAR-1, is required to focus the plus ends of microtubules to the posterior. This could be mediated by a direct effect of *Drosophila* PAR-1 on microtubule dynamics as suggested by the ability of mammalian family members MARK1 and MARK2 to phosphorylate microtubule-associated proteins containing Tau repeats.

Although the enrichment of *Drosophila* PAR-1 at the

posterior pole might lead to the inference that its localization to the posterior was a key component of its function, in fact, at the time of reorganization in stage 8 oocytes, *Drosophila* PAR-1 is not detectably asymmetric. Thus, posterior accumulation of active kinase does not seem to be its mode of action. An alternative hypothesis proposed by Shulman and colleagues is that the PAR-1 function is localized through the action of its kinase. MARK kinase activity depends upon phosphorylation of regulatory sites within the kinase domain that are conserved in *Drosophila* PAR-1.

Conservation of PAR Function in Polarity

Thus, in spite of widely divergent developmental mechanisms, worms and flies both use a member of the PAR-1/MARK/KIN1 family during an early step in polarity establishment. What does this mean? One possibility is that this is a case of a conserved function being independently co-opted by evolution for use in polarity. That is, conserved features of this family of kinases (e.g., a regulatable kinase activity and a C-terminal domain with a hypothetical role in localization) have been selected independently for a role in polarity during evolution of the two animals. In this case, the method of activation, the substrates, and the output would be expected to be different. The more interesting possibility, however, is that this family of kinases is part of a biochemical pathway that has been conserved for its role in setting polarity in a variety of organisms and cell types. If so, then we clearly have much to learn about this new pathway in both systems.

Several observations are consistent with co-option. First is the failure to identify other conserved components after extensive genetic analysis in each system. Second is the difference in the apparent output of kinase activity. Although *Drosophila* PAR-1 seems to most directly affect microtubule organization, there is no evidence that *C. elegans* PAR-1 affects microtubule organization. In fact, most aspects of *C. elegans* polarity are resistant to microtubule-inhibiting drugs and sensitive to microfilament-inhibiting drugs. Third, although PAR-1 localization depends upon PAR-3, the PAR-3 homolog, Bazooka (see below), does not have a role in localizing *Drosophila* PAR-1. Similarly, although *Drosophila* PAR-1 localization depends upon *oskar*, there is no recognizable *oskar* homolog in worms.

On the other hand, none of these observations rule out conservation of a core cassette of interacting proteins with a fundamental role in polarity. It is possible that the direct activators and targets of PAR-1/MARK/KIN1 family members are conserved but have fundamental roles throughout development and therefore, like *Drosophila* PAR-1, will only be discovered by reverse genetic methods. In addition, the output of PAR-1 in the two systems may not be as different as it appears; the role of microtubules in *C. elegans* polarity is still an open question. Other observations more positively support a conserved cassette. Conservation of PAR-1 fits well with the apparent conservation of function of germline granules in flies and worms. Perhaps most compelling is the consistent coincidence of PAR-1/MARK/KIN1 family kinases with polarity systems in other organisms and cell types. KIN1 has a clear role in polarity in yeast and *Drosophila* PAR-1, in addition to its polar distribution in oocytes, is restricted basolaterally in follicle cells, a distribution that has also been seen for mammalian PAR-1 in polarized epithelial cells (see Shulman et al., 2000).

Possible conservation of PAR function in polarity is not limited to PAR-1. Homologs of PAR-3 have been identified and are associated with polarity in flies and in mammals. A PAR-3 homolog in *Drosophila*, Bazooka, has recently been found to be localized apically in embryonic epithelial cells, is required for maintenance of the epithelium, and plays a key role in cell polarity during the asymmetric divisions of neuroblasts (See Jan and Jan, 2000). Although the function of mammalian PAR-3 is not clearly known, it is also localized apically in epithelial cells and like PAR-3, biochemically interacts with an atypical protein kinase C (Izumi et al., 1998).

Although the extent to which PAR function is conserved between animals remains to be seen, it seems pretty clear that PARs are polar and that understanding the function of this interesting class of proteins promises to yield insights into establishment of polarity in a wide range of systems.

Selected Reading

Bowerman, B. (1995). Bioessays *17*, 405–414.

Bowerman, B. (1998). Curr. Top. Dev. Biol. *39*, 73–117.

Crittenden, S.L., Rudel, D., Binder, J., Evans, T.C., and Kimble, J. (1997). Dev. Biol. *181*, 36–46.

Drewes, G., Ebneth, A., Preuss, U., Mandelkow, E.M., and Mandelkow, E. (1997). Cell *89*, 297–308.

Evans, T.C., Crittenden, S.L., Kodoyianni, V., and Kimble, J. (1994). Cell *77*, 183–194.

Izumi, Y., Hirose, T., Tamai, Y., Hirai, S., Nagashima, Y., Fujimoto, T., Tabuse, Y., Kemphues, K., and Ohno, S. (1998). J. Cell Biol. *143*, 95–106.

Jan, Y.-N., and Jan, L.Y. (2000). Cell *100*, 599–602.

Rongo, C., and Lehmann, R. (1996). Trends Genet. *12*, 102–109.

Rose, L.S., and Kemphues, K.J. (1998). Annu. Rev. Genet. *32*, 521–545.

Schubert, C.M., Lin, R., de Vries, C.J., Plasterk, R.H.A., and Priess, J.R. (2000). Mol. Cell *5*, 671–682.

Shulman, J.M., Benton, R., and St Johnston, D. (2000). Cell *101*, this issue, 377–388.

St Johnston, D., and Nüsslein-Volhard, C. (1992). Cell *68*, 201–219.

van Eeden, F., and St Johnston, D. (1999). Curr. Opin. Genet. Dev. *9*, 396–404.