

# Embryonic Development: A New SPN Dispatch on Cell Fate Specification

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The recent identification and characterization of the *Caenorhabditis elegans* gene *spn-4* has shed new light on the mechanisms that link embryonic polarity to the specification of cell fates.

One of the keys to understanding how cell diversity is generated in early development is to determine how embryonic polarity, once established, leads to the specification of diverse cell fates. The nematode *Caenorhabditis elegans* is an extraordinarily useful model for studying early development, but the mechanisms that link embryonic polarity to the specification of cell fates are still poorly understood (reviewed in [1]). The polarization of the antero-posterior axis in *C. elegans* embryos depends on the activity of the *par* genes, named for their roles in cytoplasmic partitioning [2]. The *par* genes encode cortical cytoplasmic proteins, several of which are localized along the antero-posterior axis of the embryo [1]. By largely unknown mechanisms, the PAR proteins affect the localization of proteins such as GLP-1, SKN-1, PAL-1 and PIE-1, which are enriched in certain cells and specify cell fates in the early embryo (Figure 1) [1,3].

The molecular identities of the PAR proteins have not yet given clear answers as to how they function, although the presence of kinase and PDZ domains has suggested that some PAR proteins have roles in intracellular signaling. Interest in this issue has grown as the functions of many of the PAR proteins in cell polarity have been found to be conserved in other organisms — fly, frog, mouse and humans (reviewed in [4]). Recently, three groups of *C. elegans* researchers ([5,6] and K. Ogura, personal communication), using three different approaches, have converged on a key piece of this puzzle — a novel gene called *spn-4*.

A handful of gene products were previously known to have roles in transducing polarity cues, including the RNA-binding protein MEX-3, and the CCCH-type zinc-finger proteins POS-1, MEX-1 and the partially redundant MEX-5/6 [7–12]. These gene products are localized at either the anterior (MEX-3, MEX-5) or the posterior (POS-1, MEX-1) end of the embryo and, at least in the case of MEX-3 and MEX-5, this localization depends on *par* gene activity. Consistent with roles for their products in transducing polarity, loss of function mutations in these genes can cause the mislocalization of several cell-fate specification proteins, resulting in embryonic cell-fate transformations. These transformations do not, however, fully recapitulate those caused by mutations in the *par* genes, suggesting that

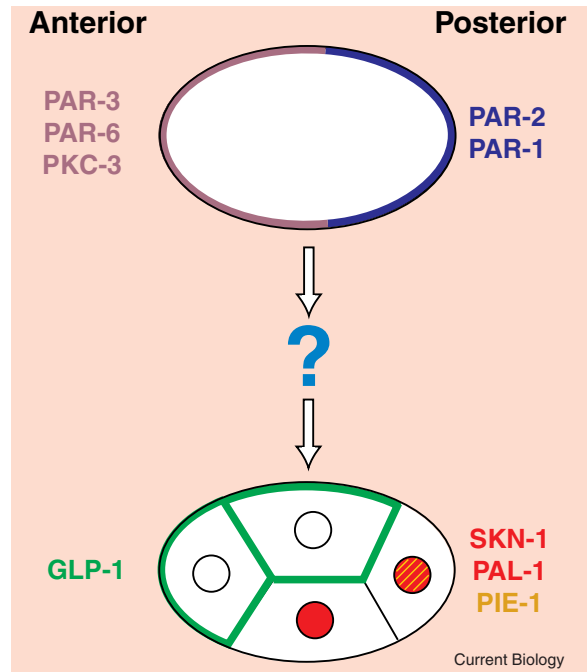


Figure 1. Polarization and cell-fate specification in the early *C. elegans* embryo.

The polarity of the early *C. elegans* embryo is established by the PAR proteins, which are localized along the antero-posterior axis of the cortical cytoplasm. The PAR proteins affect the localization of proteins involved in cell fate specification, such as GLP-1, PAL-1, SKN-1 and PIE-1. Although genes such as *pos-1*, *mex-3*, *mex-5/6* and *mex-1* are known to transduce this polarity signal, the precise mechanism through which this is accomplished remains elusive.

additional genes are likely to be involved in transducing polarity cues to the cell-fate specification genes. The *spn-4* gene product turns out to be one of the key molecules in this process.

Gomes and colleagues [5] identified *spn-4* in a screen for mutants defective in mitotic spindle orientation in the posterior cell of the two-cell stage embryo. The *spn-4* mutants have a dizzying array of defects besides their spindle orientation defect, including a partial lack of intestine, a lack of pharyngeal muscle cells, an excess of body wall muscle cells, and an excess of germline cells. Despite this, the careful analysis reported by Gomes *et al.* [5] indicates that most of the cell-fate changes are caused by the mislocalization of the cell-fate specifying proteins SKN-1, PAL-1 and PIE-1. These three proteins have been shown to function in the specification of the two posterior blastomeres of the four-cell stage embryo (Figure 1) [8,13–15]. SPN-4 affects the activity of these proteins in both anterior and posterior cell lineages. Gomes *et al.* [5] demonstrated that *spn-4* and *skn-1* partially complement each other for the specification of mesoderm and endoderm, and that the excess body wall

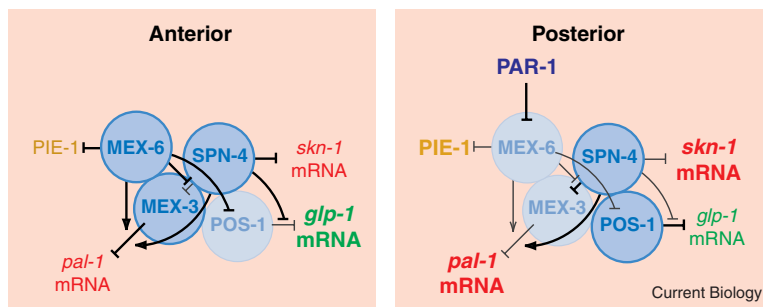


Figure 2. Genetic interactions and physical interactions in the anterior and posterior sides of the early embryo.

In this model (based on data in [5,6] and K.Ogura and Y. Kohara, personal communication), lines represent genetic interactions, as active (thick lines) or inactive (thin lines). Members of putative polarity-transducing complexes are represented as circles, with circles touching each other where direct interactions have been found. Circles are colored according to whether they are enriched (dark blue) or at low levels/absent (light blue) in the

anterior and posterior cell of the two-cell stage embryo. Cell fate specification mRNAs and proteins are represented in bold in the place where each is expressed. This conceptual model is not meant to imply that these proteins are known to be linked simultaneously in these putative complexes. Relevant results not represented here include that *par-4* modulates some of the activities shown [6], and that *spn-4* and *skn-1* cooperate in the specification of endoderm [5].

muscle in *spn-4* mutants is the result of mislocalization of *pal-1* activity. They also showed that *spn-4* affects the levels of PIE-1 protein, possibly the cause of the inappropriate germline blastomere divisions observed in *spn-4* mutants.

Huang and colleagues [6] identified SPN-4 while looking for proteins that can interact with MEX-3 in a yeast two-hybrid screen. Their screen identified a total of 14 genes that encode MEX-3-interacting proteins. Huang *et al.* [6] cleverly used RNA interference [16] of each of these genes to narrow the long list down to those that also have roles, as MEX-3 itself does, in localizing PAL-1 protein to the posterior blastomeres. The final list included SPN-4 and some familiar proteins — POS-1, MEX-6 and MEX-3 itself. Huang *et al.* [6] demonstrated that SPN-4 and MEX-6 act downstream of the PAR proteins to regulate the localization and levels of MEX-3 and PAL-1. At this point, the precise mechanism by which SPN-4 regulates these activities is not known, although it is likely to involve interactions with both protein and mRNA.

Ogura and colleagues (K. Ogura and Y. Kohara, personal communication) have identified SPN-4 in yet a third way, while looking for proteins that could interact with POS-1. They found that SPN-4 and POS-1 work together to regulate *glp-1* mRNA translation. They also found that *spn-4* mRNA and SPN-4 protein are present in all blastomeres of the early embryo, and later become localized to posterior lineages, at the end of the four-cell stage. This suggests that the activity of SPN-4 is not regulated by its localization early in embryonic development.

SPN-4 thus participates in diverse ways in patterning the early *C. elegans* embryo: regulating the localization, levels and/or activity of MEX-3, PAL-1, GLP-1, SKN-1 and PIE-1; orienting a mitotic spindle; and cooperating with SKN-1 in the specification of mesoderm (Figure 2). How can a single protein do so much? One answer to this question might lie in the molecular nature of SPN-4, an RNA-binding protein with broad specificity predicted to interact with several classes of RNAs, including mRNAs (reviewed in [17]). As pointed out by all three groups ([5,6] and K. Ogura, personal communication), this might allow SPN-4 to bind and regulate the translation of several maternal mRNAs that are present in the early embryo.

Such a mechanism is consistent with unpublished data of N. Kishimoto and Y. Kohara (Y. Kohara, personal communication) indicating that SPN-4 can directly interact not only with the 3' UTR of *glp-1* mRNA, but also with the 3' UTRs of *skn-1* and *pal-1* mRNA.

Finding direct interactions between some of the key proteins that transduce polarity has begun to link some of the pieces of this puzzle. It is likely that the diversity of SPN-4 functions might result not only from its predicted ability to bind diverse RNAs, but also from its involvement as part of various protein complexes. For instance, members of these putative protein complexes might interact with each other in a way that could modulate their individual activities, or the activity of the complex as a whole. It will be of interest to learn about additional members of these putative complexes, in order to further understand how they transduce polarity. It will also be of interest to determine whether similar proteins play roles in transducing polarity in other organisms.

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