Cell, Vol. 95, 225–235, October 16, 1998, Copyright ©1998 by Cell Press

Partner of Numb Colocalizes with Numb during Mitosis and Directs Numb Asymmetric Localization in *Drosophila* Neural and Muscle Progenitors

Bingwei Lu, Michael Rothenberg, Lily Y. Jan, and Yuh Nung Jan*
Howard Hughes Medical Institute
Departments of Physiology and Biochemistry
University of California, San Francisco
San Francisco, California 94143–0725

Summary

During mitosis of multiple types of precursor cells in *Drosophila*, Numb is asymmetrically distributed between the two daughter cells and confers distinct daughter cell fates. Here we report the identification of a novel gene product, Partner of Numb (PON), based on its physical interaction with Numb. PON is asymmetrically localized during mitosis and colocalizes with Numb. Loss of *pon* function disrupts Numb localization in muscle progenitors and delays Numb crescent formation in neural precursors. Moreover, ectopically expressed PON responds to the apical-basal polarity of epithelial cells and is sufficient to localize Numb basally. We propose that PON is one component of a multimolecular machinery that localizes Numb by responding to polarity cues conserved in neural precursors and epithelial cells.

Introduction

How the diverse cell types of a multicellular organism are specified is a fundamental question in developmental biology. One important means by which cellular diversity is achieved is through asymmetric cell divisions (reviewed in Horvitz and Herskowitz, 1992; Chang and Drubin, 1996; Guo and Kemphues, 1996a; Shapiro and Losick, 1997; Jan and Jan, 1998). Some asymmetric cell divisions involve intrinsic determinants, where the mother cell is polarized and the cell fate determinants are differentially distributed between the two daughter cells. The Numb protein is one such determinant and plays crucial roles in the asymmetric cell divisions during *Drosophila* nervous system and muscle development (Uemura et al., 1989; Rhyu et al., 1994; Spana et al., 1995; Gomez and Bate, 1997; Carmena et al., 1998).

Numb is a cell membrane–associated protein that contains a phosphotyrosine-binding (PTB) domain. During mitosis of multiple types of progenitor cells, Numb becomes localized asymmetrically as a crescent on one side of the cortex and segregates preferentially into one of the two daughter cells (Rhyu et al., 1994; Knoblich et al., 1995; Spana et al., 1995; Kraut et al., 1996; Gomez and Bate, 1997; Carmena et al., 1998). Asymmetrically distributed Numb confers distinct daughter cell fates. In the absence of *numb* function, the daughter cell that normally inherits Numb is transformed into its sister cell fate, whereas overexpression of *numb* causes the opposite cell fate transformation (Uemura et al., 1989; Rhyu et al., 1994; Spana et al., 1995; Gomez and Bate, 1997; Carmena et al., 1998). Recent studies indicate that intrinsically inherited Numb interacts with the cell surface receptor Notch and a serine-threonine kinase NAK (Guo et al., 1996; Chien et al., 1998) and that Numb functions at least in part by antagonizing Notch activity (Frise et al., 1996; Guo et al., 1996; Spana and Doe, 1996; Gomez and Bate, 1997).

Another cell fate determinant that is asymmetrically distributed during neural precursor division in *Drosophila* is Prospero, a homeodomain-containing transcription factor (Hirata et al., 1995; Knoblich et al., 1995; Spana and Doe, 1995). Prospero is required for ganglion mother cell (GMC) fate specification during neuroblast division and for neuronal differentiation in the central nervous system (CNS) and the peripheral nervous system (PNS) (Doe et al., 1991; Vaessin et al., 1991; Matsuzaki et al., 1992). Like Prospero protein, *prospero* RNA is also asymmetrically localized during neuroblast mitosis (Li et al., 1997; Broadus et al., 1998).

The Numb and Prospero crescents always colocalize and are tightly coordinated with the position of the centrosomes and orientation of the mitotic spindle (Knoblich et al., 1995; Spana and Doe, 1995). Inscuteable (Insc), a novel protein that is localized to the apical side of the neuroblast membrane during mitosis, is required for such coordination (Kraut and Campos-Ortega, 1996; Kraut et al., 1996). In the absence of *insc* function, the mitotic spindle fails to align along the apical-basal axis in dividing neuroblasts and cells in the procephalic neurogenic region (PNR), and both Numb and Prospero are mislocalized (Kraut et al., 1996). Furthermore, the asymmetric localization of *prospero* RNA is also defective in *insc* mutants (Li et al., 1997). In addition to its role in the CNS, Insc also functions during *Drosophila* myogenesis, where it orients asymmetric muscle progenitor cell divisions and is required for the asymmetric localization of Numb in these divisions (Burchard et al., 1995; Gomez and Bate, 1997; Knirr et al., 1997; Carmena et al., 1998).

The role of Insc in Numb and Prospero localization is to provide positional information but not to localize them directly. Misexpression of *insc* in epithelial cells is not sufficient to asymmetrically localize Numb, although it is sufficient to reorient the mitotic spindles along the apical-basal axis (Kraut et al., 1996). Moreover, the *inscuteable* mutant phenotype differs in the PNR, the CNS, and the PNS. In cells of the PNR and the CNS, Numb/Prospero localization is affected; however, no defects in Numb/Prospero localization are readily detectable in SOP cells of the PNS (Kraut et al., 1996). These results suggest that other organizing activities besides Insc are required in the PNS to orient the localization of Numb/Prospero and that, in the CNS and PNR, Insc controls Numb/Prospero localization indirectly through other adaptor-like molecules. Miranda is one such adaptor protein that directly localizes Prospero. Miranda was

*To whom correspondence should be addressed (e-mail: ynjan@itsa.ucsf.edu).

Figure 1. Primary Sequence of PON

The predicted amino acid sequence of PON protein is shown. Numbers indicate amino acid positions. In vitro translation of this sequence gives rise to a protein identical in size to the PON protein detected in *Drosophila* embryos. The NPXX sequences, putative PTB binding motifs, in the N terminus of the protein are underlined.

identified by virtue of its direct interaction with the localization domain of Prospero and encodes a novel coiled-coil domain containing protein that is asymmetrically localized. Loss of *miranda* function affects Prospero crescent formation and results in the uniform distribution of Prospero during neuroblast divisions (Ikeshima-Kataoka et al., 1997; Shen et al., 1997). Although Miranda also interacts with Numb in vitro, the asymmetric localization of Numb is not affected in *miranda* zygotic mutant embryos (Shen et al., 1997).

To identify cellular components that are required to localize Numb directly, we have searched for proteins that interact with Numb in a yeast two-hybrid screen. Previous structure-function analysis indicated that the N terminus of the Numb protein, containing a membrane-targeting domain and the PTB domain, is sufficient to direct the asymmetric localization of a heterologous protein (Knoblich et al., 1997). Here we report the identification of a novel gene, named *partner of numb* (*pon*), whose product interacts with the PTB domain of Numb. The expression and asymmetric localization of PON coincides with asymmetric cell division and Numb localization. Our analyses of Numb localization under *pon* loss-of-function and ectopic expression situations indicate that PON is an important component of the cellular machinery that localizes Numb by responding to polarity cues conserved in neuroblasts and epithelial cells.

Results

Identification of *pon*

We used the yeast two-hybrid system (Bartel et al., 1993) to isolate proteins that interact with the Numb PTB domain. From approximately ten million cDNAs screened, positive clones representing five different genes that show specific interaction with the Numb PTB domain were isolated. One gene, *pon*, was chosen for further analysis based on its RNA expression pattern.

The full-length cDNA encodes a protein of 672 amino acids, with a predicted molecular weight of 76,122 daltons (Figure 1). Database searches with *pon* sequences did not reveal any gene that shows significant homology. Structure prediction indicates that the central part of PON may adopt a coiled-coil structure. Since PON interacts with the PTB domain of Numb, we searched for the known PTB domain–binding motif ϕXNPXY (where ϕ stands for a hydrophobic amino acid, X for any amino acid, and N, P, and Y stand for Asn, Pro, and Tyr, respectively) in PON sequence and did not find such a motif. Instead, we found nine NPXX motifs in the N-terminal portion of the protein, four of which fit the ϕXNPXX consensus. Since certain PTB domain-containing proteins can bind to the NPXY motif in the absence of tyrosine phosphorylation or when the Y residue is mutated (Borg et al., 1996), the NPXX motifs in PON protein could be binding sites for the Numb PTB domain.

Characterization of the Numb/PON Interaction

To confirm that PON and Numb physically interact with each other, we tested whether these two proteins can form a complex in solution. In vitro translation products of Numb and PON were mixed and subjected to immunoprecipitation with anti-Numb antibody. As shown in Figure 2A, the anti-Numb antibody can precipitate both Numb and PON, demonstrating that Numb and PON bind to each other in solution.

We used the yeast two-hybrid assay to characterize further the interaction between Numb and PON (Table 1). First, deletion variants of PON were tested for their interactions with the Numb PTB domain to map the region in PON responsible for interaction with Numb. The PON-A construct (amino acids 1–222), which includes all the NPXX motifs, retains the ability to interact with the PTB domain of Numb. This suggests that the N terminus of PON contains a domain that binds Numb. However, we cannot exclude the possibility that a second domain in PON can also interact with Numb. Such a domain may have been broken by our deletions. We next addressed the specificity of Numb-PON interaction by testing mutant forms of Numb for their ability to interact with PON. Based on studies of the interaction between mammalian Shc PTB domain and its binding motif ϕXNPXY (Zhou et al., 1995; Borg et al., 1996), we introduced point mutations in conserved residues of Numb PTB domain and tested the abilities of these mutant forms of Numb to interact with PON. Mutations in Ser-148 and Arg-165 of Numb PTB domain (corresponding to residues Ser-151 and Arg-169 in Shc, critical for binding to the phosphotyrosine residue) did not affect its interaction with PON. However, mutation of Phe-195 in the PTB domain of Numb (corresponding to Phe-198 in Shc that contacts the ϕ and N residues) dramatically reduced its interaction with PON. This suggests that the interaction between Numb and PON probably involves specific contacts between Phe-195 in the Numb PTB domain and possibly the ϕ and N residues in the NPXX motifs of PON. This analysis demonstrates the specificity of the Numb-PON interaction.

Numb and PON Form a Complex In Vivo

To study the physiological relevance of the Numb-PON interaction detected in vitro, we tested whether the two proteins interact in *Drosophila* embryos. A C-terminal

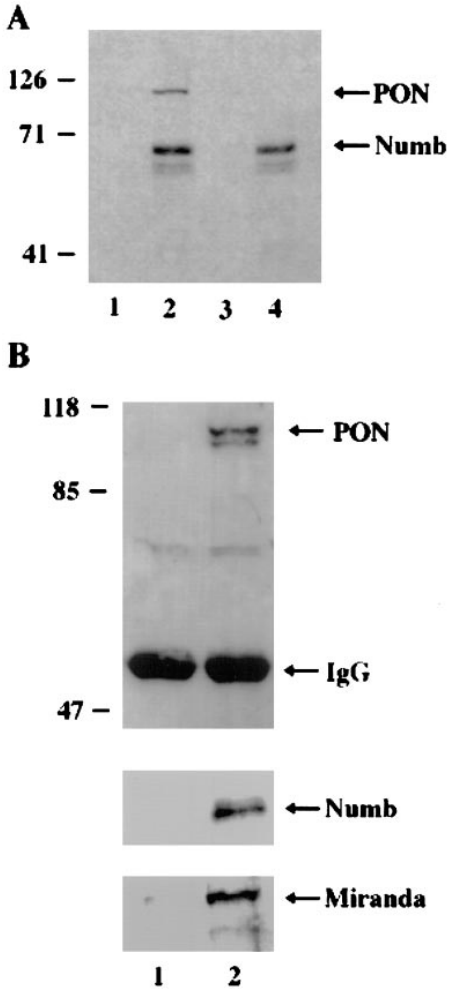

Figure 2. Characterization of the Numb and PON Interaction

(A) PON and Numb interact in vitro. In vitro translated, ^{35}S-labeled PON and Numb proteins were mixed and then subjected to immunoprecipitation with preimmune serum (lane 1) or anti-Numb antibody (lane 2). In vitro translated PON alone (lane 3) or Numb alone (lane 4) were also immunoprecipitated with anti-Numb antibody.

(B) PON interacts with Numb and Miranda in vivo. Myc-tagged PON expressed from a transgene was immunoprecipitated from embryonic extracts with monoclonal anti-Myc antibody 9E10 (lane 2). Equal amounts of protein extracts made from wild-type fly embryos were similarly immunoprecipitated with anti-Myc antibody as a control (lane 1). Immunoprecipitated proteins were separated by SDS-PAGE, blotted, and probed with anti-PON, anti-Numb, or anti-Miranda antibodies. Sizes of molecular weight markers are shown on the left.

Myc-tagged version of PON was expressed in *Drosophila* embryos using the *UAS-GAL4* system (Brand and Perrimon, 1993). Protein extracts made from transgenic fly embryos were immunoprecipitated with anti-Myc antibody. As shown in Figure 2B, PON protein is present in the immunoprecipitate, as expected. Probing the immunoblot with Numb antibody detected Numb in the immunocomplex. Thus, the interaction between Numb and PON detected in vitro also occurs in vivo.

Probing of the immunocomplex with antibodies against other proteins known to be involved in protein localization showed that Miranda is also present in the complex (Figure 2B). This coimmunoprecipitation of PON and Miranda may result, at least partly, from their direct binding, as protein–protein interaction between the two has been detected in the yeast two-hybrid assay (data not shown). Since Numb and Miranda also interact in vitro (Shen et al., 1997), our result suggests that in vivo, Numb, PON, and Miranda may associate with one another in a complex.

Table 1. Interaction of Numb PTB Domain and PON in the Yeast Two-Hybrid Assay

LexA DNA–Binding Domain Fusion	GAL4 Activation Domain Fusion	Beta-Galactosidase Activity
1. PON FL	Numb PTB	++
2. PON A (1–222)	Numb PTB	++
3. PON B (222–500)	Numb PTB	−−
4. PON C (500–672)	Numb PTB	−−
5. WT PTB	PON	++
6. PTBm1 (S148A)	PON	++
7. PTBm2 (R165Q)	PON	++
8. PTBm3 (F195V)	PON	−−

Full-length and deletion variants of PON (PON FL, PON A, PON B, and PON C) or wild-type and mutant forms of Numb PTB domain (WT PTB, PTBm1, PTBm2, and PTBm3) cloned in pBHA vector were cotransformed with pGAD10-NumbPTB or pGAD10-PON, respectively, into yeast L40 strain. Transformants were tested for LacZ activity using the filter lift assay. ++ indicates positive interaction, −− indicates negative interaction.

Colocalization of PON and Numb during Mitosis of Neural and Muscle Precursor Cells

Our RNA in situ hybridization analysis indicates that *pon* is expressed in neuroblasts and sensory organ precursor (SOP) cells in the nervous system and muscle progenitor cells in the mesoderm (data not shown). To determine the subcellular localization of PON, we generated anti-PON antibodies for immunofluorescence studies. Two antibodies raised against different regions of the protein give similar staining patterns, and the staining is absent in deficiency embryos missing the *pon* gene. In neuroblasts PON is first detected in prophase, where it is localized as a crescent on the basal side of the cell cortex (Figure 3A). The PON crescent persists through metaphase and anaphase and becomes more concentrated at the basal cortex. At the end of telophase, PON is segregated predominantly into the GMC daughter cell, where it is uniformly distributed on the cortex (Figures 3B-3D). In the SOP cells PON is also asymmetrically localized in a fashion similar to that in neuroblasts. Double labeling of Numb and PON shows that the two proteins are colocalized during mitosis of SOP cells (Figures 3E-3H). We also examined PON localization in imaginal disc tissues. In imaginal discs at puparium formation, PON is expressed in SOP cells for the adult sensory organs and is asymmetrically distributed and colocalized with Numb during mitosis (data not shown).

We next examined the localization of PON in muscle progenitors. We used the even-skipped (Eve) antibody to identify a subset of the muscle progenitor cells (P2

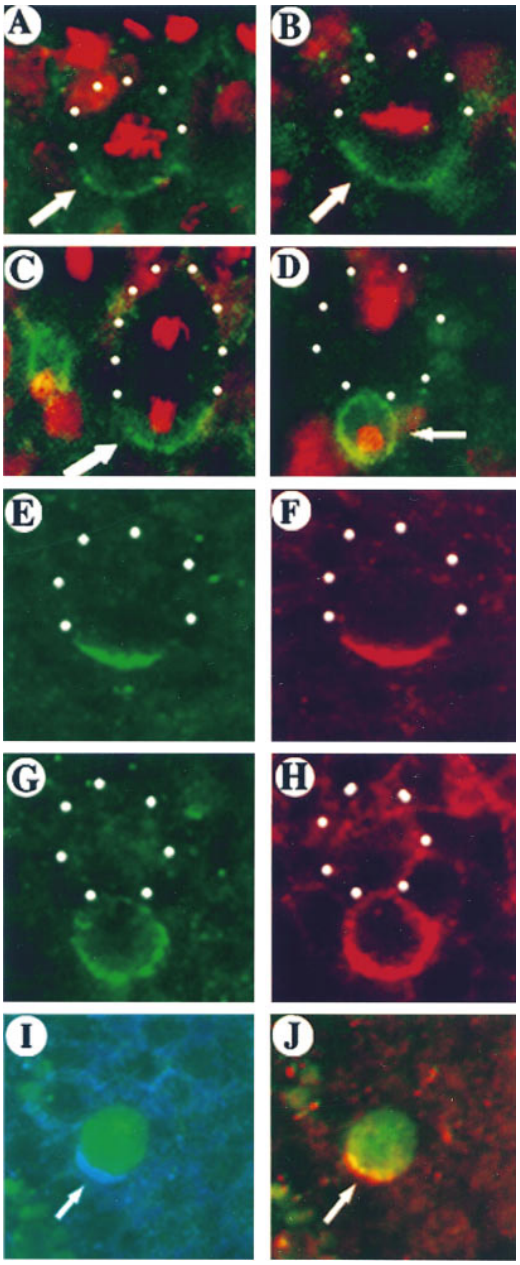

Figure 3. Colocalization of PON and Numb in Mitotic Neural and Muscle Precursor Cells

(A-D) Asymmetric localization of PON in neuroblasts during mitosis. Wild-type embryos were stained with anti-PON antibody (green) and propidium iodide (red, for DNA). Four neuroblasts at different stages of the cell cycle are shown: (A) late prophase, (B) metaphase, (C) anaphase, and (D) telophase. The basal sides of the neuroblasts are facing down in this and all subsequent figures. PON starts to form a crescent at prophase, and after cell division it is segregated into the basal GMC cell. PON crescents are marked by arrows, and the dots outline cell boundaries.

(E-H) Numb and PON colocalize in embryonic SOP cells during mitosis. Stage 13–14 wild-type embryos were stained with rabbit anti-PON antibody (E and G) and guinea pig anti-Numb antibody (F and H). (E and F) colocalization of Numb and PON in a metaphase SOP cell. (G and H) after cell division, Numb and PON are segregated to one of the two daughter cells.

(I and J) Numb and PON colocalize in muscle progenitor cells. Stage 10–11 wild-type embryos were stained with anti-Eve (green) antibody to identify the muscle progenitor cells (P2 and P15). The cell shown in (I) and (J) is a P2 cell, which divides first. The cytoplasmic staining of nuclear protein Eve indicates that this cell is undergoing mitosis. In this mitotic P2 cell, both Numb (blue) and PON (red) form a crescent (marked with arrows) and the two crescents colocalize.

and P15) at stage 10 embryos. When these cells are undergoing mitosis, as indicated by the cytoplasmic distribution of the nuclear protein Eve and DNA staining, PON forms a crescent starting at late prophase and is segregated predominantly to one of the two daughter cells after cell division. Double labeling of Numb and PON shows that they colocalize in these mitotic muscle progenitor cells (Figures 3I and 3J). We conclude that in dividing neuroblasts, SOP cells, and muscle progenitor cells, PON and Numb are colocalized during their asymmetric localization.

Differential Influence of Insc on PON Localization in Different Cell Types

To understand the relationship between *pon* and other genes known to be involved in asymmetric cell division, we examined the localization of PON in *numb*, *prospero*, *miranda*, and *insc* mutants. In embryos homozygous for null alleles of *numb* (Figure 4B), *prospero*, or *miranda* (data not shown), the localization of PON in dividing neuroblasts is similar to that in wild-type embryos. PON localization in SOP cells of these mutant embryos also appears normal (Figure 4E; data not shown). Thus, zygotic *numb*, *prospero*, and *miranda* are not required for the asymmetric localization of PON.

In embryos homozygous for a null allele of *insc*, we observed different effects on the localization of PON in different cell types. In dividing neuroblasts of *insc* mutant embryos, PON either forms crescents that are randomly positioned and not aligned with the mitotic spindle or are present all around the membrane (Figure 4C). However, in dividing SOP cells of *insc* mutants, PON crescent formation and the alignment of PON crescent with the mitotic spindle appear normal, with the crescent always positioned over one of the spindle poles (Figure 4F). Similarly, the localization of Numb is also not affected in SOP cells of *insc* mutants (data not shown). Therefore, Insc is required for the localization of PON in neuroblasts of the CNS but is not necessary for PON localization in SOP cells of the PNS.

Altered Numb Localization Caused by *pon* Loss of Function

Given that Numb is not required for the localization of PON, we tested whether PON is required for the localization of Numb. We used a transposon mutagenesis approach to generate two mutant alleles of *pon*, pon^{P65} and pon^{P26}, both of which appear to be null or strong hypomorphic mutants (see Experimental Procedures). We also obtained the deficiency strain *Df(1)bi-D3*, which contains a small chromosomal deficiency from 4C5–6 to 4C7–8 that uncovers the *pon* gene. Numb localization was examined in both *pon* mutants and deficiency embryos, and similar results were obtained.

In muscle precursor cells of *pon* mutant or deficiency

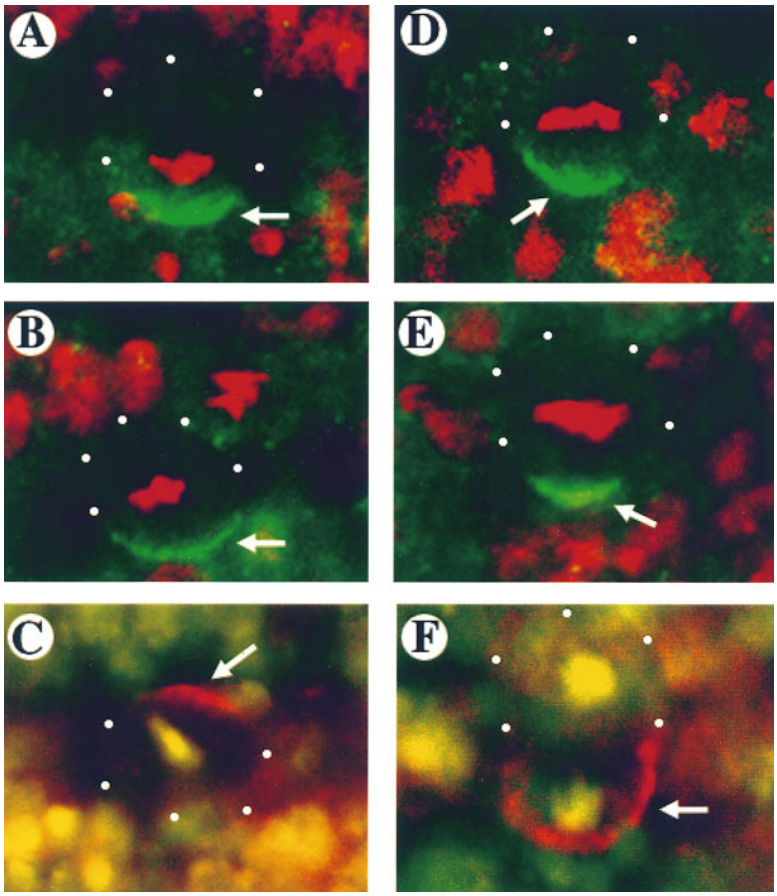

Figure 4. PON Localization in *numb* and *insc* Mutant Embryos

(A-C) PON localization in neuroblasts is normal in a *numb* mutant background but is affected in *insc* mutant. Metaphase neuroblasts from wild-type (A), $numb^{1}$ (B), and $insc^{P72}$ (C) embryos were stained with anti-PON antibody (green in A and B, red in C) and propidium iodide (red in A and B) or Sytoxgreen (C). Note that in *insc* mutant, the PON crescent is mislocalized apically and the mitotic spindle (as deduced from the chromosome positioning) is not aligned apical-basally.

(D-F) PON localization in SOP cells is normal in *numb* and *insc* mutants. Metaphase SOP cells from wild-type (D), $numb^{1}$ (E), and a telophase SOP cell from $insc^{P72}$ (F) embryos were stained with anti-PON antibody (green in D and E, red in F) and propidium iodide (red in D and E) or Sytoxgreen (F). PON localization in *numb* and *insc* mutants is similar to that in wild type, with the crescent always positioned over one of the spindle poles. PON crescents are marked by arrows, and the dots outline cell boundaries.

embryos, we observed that the asymmetric localization of Numb is affected. In mitotic muscle precursor cells of stage 10–11 wild-type embryos, both PON and Numb are localized as a crescent at late prophase, the crescent persists through metaphase and anaphase and is segregated predominantly to one of the daughter cells after cell division (Figures 5A and 5B). However, in mitotic muscle precursors of *pon* mutant embryos, Numb is uniformly distributed at prophase and metaphase in all the embryos examined (Figure 5C). After division, Numb is equally distributed to both daughter cells in approximately 50% of the cells examined (Figure 5D). In the rest of the cells, we observed either predominant segregation of Numb to one of the daughter cell, as observed in wild-type cells, or moderately polarized distribution of Numb, with one daughter cell receiving noticeably higher amounts of Numb than the other cell.

In neuroblasts and SOP cells of *pon* mutants, we observed that there is a delay in Numb crescent formation. At late prophase, Numb is present all around the cell cortex (Figure 6A), whereas in wild-type embryos Numb has already formed a crescent at this time (Figure 6E). At metaphase, most of the neuroblasts still show uniform distribution of Numb (Figure 6B), while others (approximately 30%) show polarized distribution of Numb, where Numb is distributed along the basal and lateral sides of the membrane. At anaphase and telophase, however, the majority of Numb protein is concentrated at the basal side of the membrane to form a crescent. After cell division, Numb protein is eventually segregated predominantly to one of the two daughter cells (Figures 6C and 6D). This delay of crescent formation is specific to Numb, as the localization of Prospero is normal (Figure 6J). The localization of Miranda and Insc are also not affected in *pon* mutants (data not shown).

To confirm that loss of *pon* function is responsible for the observed defects in Numb localization, we tested whether a *pon* transgene can rescue the mutant phenotypes. The *UAS-GAL4* system was used to express a *UAS-pon* transgene in a tissue-specific manner in *pon* mutant embryos. To rescue the defects in muscle precursors, we used the *twist-GAL4* line to drive *pon* expression in mesodermal cells. In *pon* mutant embryos that have acquired the transgene-provided PON protein, Numb is localized normally in all mitotic muscle precursor cells examined, where Numb starts to form a crescent at late prophase and is distributed predominantly to one of the daughter cells after division (Figures 5E and 5F). Similarly, we used the *scabrous-GAL4* line, which drives gene expression in neuroblasts and SOP cells, to rescue the delay of Numb crescent formation in neural precursor cells. In mutant embryos that have acquired the transgene-provided PON protein, the delay of Numb localization is corrected and Numb is localized in a fashion indistinguishable from that in wild-type embryos (Figures 6G and 6H). This demonstrates that the observed Numb localization defects in *pon* mutant embryos are specific to the loss of *pon* function.

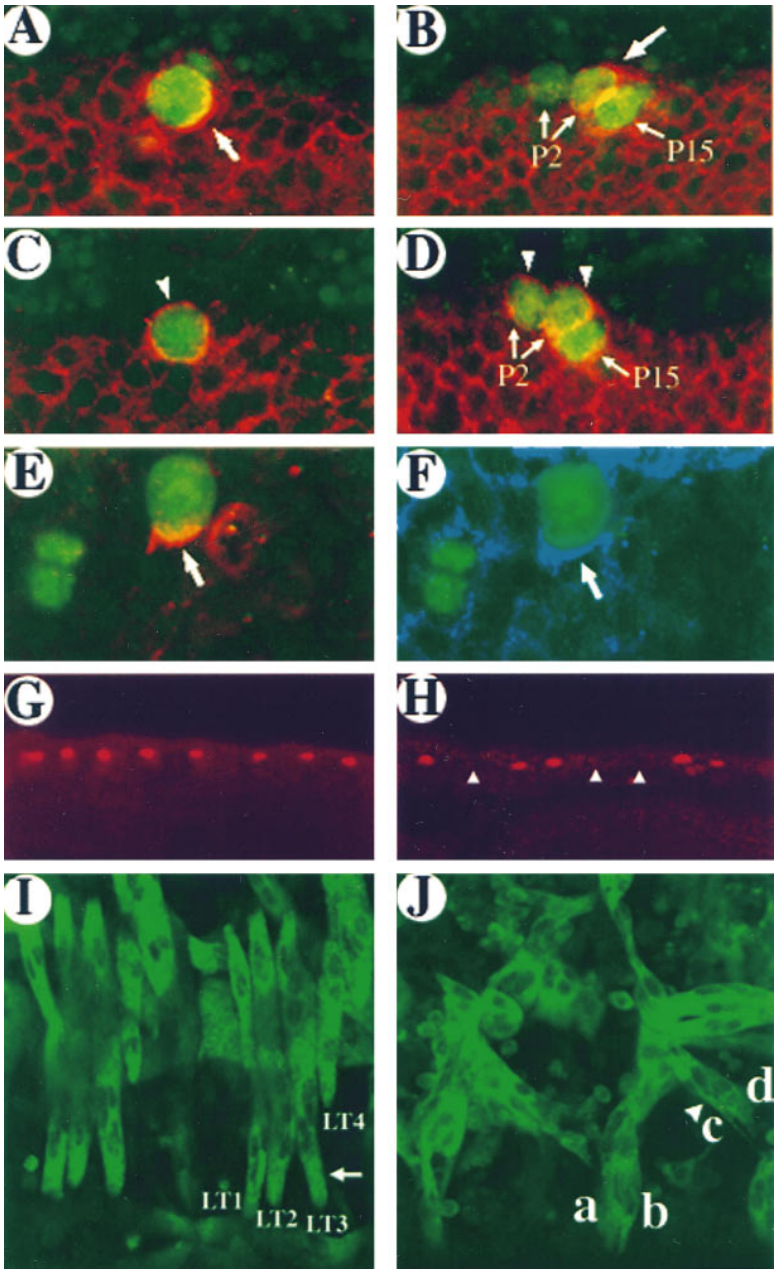

Figure 5. Numb Localization Is Altered in Muscle Precursors of *pon* Loss-of-Function Mutants

(A and B) Asymmetric localization of Numb in dividing muscle progenitor cells of wild-type embryos. Stage 10–11 wild-type embryos were stained with anti-Eve (green) and anti-Numb (red). In a metaphase P2 cell (A), Numb is localized as a crescent. After division (B), Numb is distributed predominantly to one of the daughter cells (marked with an arrow). Note that P15 appears after P2 has divided (in both B and D). In similarly staged pon^{P65} mutant embryos, Numb is uniformly distributed during mitosis (C), and after division (D), Numb is distributed to both daughter cells (marked by arrowheads). A mitotic muscle precursor cell of a *pon* mutant embryo that obtains PON from a transgene (shown in red, E) is able to asymmetrically localize Numb (shown in blue, F). (G and H) Loss of *pon* function causes a reduction of EPCs. Stage 16 wild-type (G) and pon^{P65} mutant (H) embryos were stained with anti-Eve antibody to identify EPCs. In a wild-type embryo, there are generally two EPC cells per hemisegment. In *pon* mutant embryos, a loss of EPC is frequently observed (indicated with arrowheads) in some segments. (I and J) Lateral muscles in wild-type and *pon* mutants. Stage 16 wild-type (I) and pon^{P65} mutant (J) embryos were stained with MAb6D5. Lateral muscles LT1-LT4 of a wild-type embryo are indicated (I). Note that the LT3 muscle (indicated with an arrow in I) is normally ventrally attached, whereas the LT4 muscle is more dorsally attached. The lateral muscles of a similarly stage pon^{P65} mutant embryo (J) are labeled a-d. Based on the morphology of these muscle fibers, a and b are designated as LT1 and LT2, and both c and d are designated as LT4. The arrowhead indicates a transformation of LT3 into LT4.

Mesodermal Phenotypes Associated with *pon* Loss of Function

The role of Numb in specifying certain cell fates in the myogenic lineage has been defined previously. To test whether the mislocalization of Numb caused by loss of *pon* function affects muscle development, we analyzed *pon* mutants with molecular markers such as anti-Eve, which stains EPC (Eve^+ pericardial cells), the descendents of the P2 progenitor, and MAb6D5, which stains somatic muscles. In *pon* mutants, there is a reduction in the number of EPCs (Figure 5H), a phenotype observed when Numb is mislocalized or ectopically expressed in the progenitors of these cells (Gomez and Bate, 1997; Carmena et al., 1998). Staining with MAb6D5 showed that in *pon* mutants there is a severe reduction and disorganization of somatic muscles. We focused our analysis on the lateral set of muscles (LT3 and LT4), as these muscles show clear transformations under *numb* loss-of-function and ectopic expression conditions. In a *numb* mutant, LT4 is transformed into LT3 and ectopic expression of *numb* transforms LT3 into LT4 (Gomez and Bate, 1997). In *pon* mutants embryos, we frequently observe lateral muscle phenotypes suggesting LT3 being transformed into LT4 (Figure 5J), as judged by the morphology of these muscle fibers. Further analysis with appropriate molecular markers specific to LT3 or LT4 may confirm this transformation.

Ectopic Expression of PON Confers Asymmetric Numb Localization in Epithelial Cells

The observed defects of Numb localization under *pon* loss-of-function conditions indicate that PON is necessary for the correct localization of Numb. We then tested whether misexpression of PON is sufficient to direct

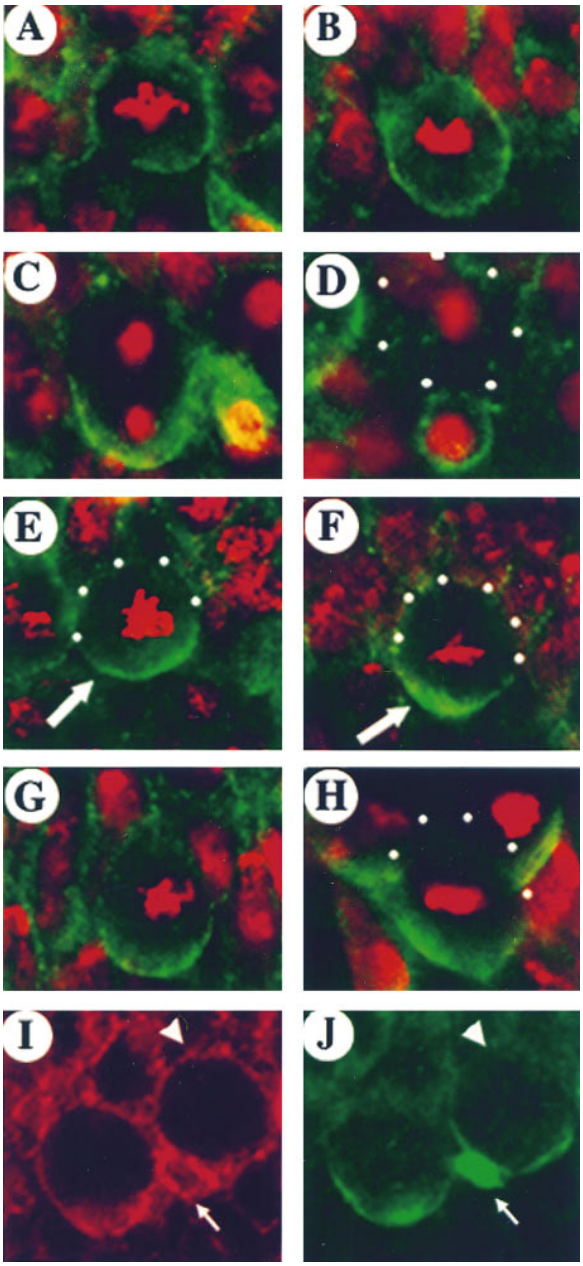

Figure 6. Numb Localization Is Delayed in Neural Precursors of *pon* Mutants

(A–D) Numb localization in *Df(1)bi-D3* neuroblasts during mitosis. Four neuroblasts at different stages of the cell cycle are shown: (A) late prophase, (B) metaphase, (C) anaphase, and (D) telophase. Numb is shown in green and DNA in red. Note that in prophase and metaphase neuroblasts, Numb crescents do not form. At anaphase, Numb crescents start to form, and at the end of cell division, Numb is distributed predominantly to the GMC cell.

(E and F) Numb localization in wild-type neuroblasts. A late prophase (E) and a metaphase (F) neuroblast are stained with Numb and propidium iodide. Note that Numb forms a crescent at both stages.

(G and H) The delayed Numb crescent formation phenotype in neuroblasts can be rescued by introducing a *pon* transgene. Two neuroblasts from a rescued embryo, one at prophase (G) and another at metaphase (H), are stained with anti-Numb antibody (green) and propidium iodide (red).

(I and J) The crescent formation of Prospero is normal in *pon* mutant embryos. Two metaphase neuroblasts are shown. In both neuroblasts, Prospero (J) forms basal crescents while Numb (I) is uniformly distributed. Arrowheads point to the apical domain of one neuroblast where Numb but not Prospero staining is present. Arrows mark a GMC cell from the previous division.

Numb localization in cell types in which Numb is normally uniformly distributed. The expression pattern of Numb in embryos is broader than that of PON, and in cells types where Numb but not PON is expressed, such as epithelial cells, Numb is uniformly distributed during mitosis. Therefore, we used the *hairy-GAL4* enhancer trap line to drive *pon* expression in a pair-rule pattern in epithelial cells of the embryonic epidermis. In mitotic epithelial cells of a stage 12 embryo that ectopically express PON, we observed that ectopic PON provided by the transgene is localized as basal crescents (Figure 7A). In the mitotic epithelial cells that show basal localization of PON, Numb also forms a crescent and is colocalized with PON (Figures 7B and 7C). This result shows that PON can respond to epithelial polarity and is sufficient to drive the asymmetric localization of Numb in epithelial cells. Since Insc is not expressed in epithelial cells (Kraut and Campos-Ortega, 1996), this result demonstrates that PON is sufficient to asymmetrically localize Numb independent of Insc.

Discussion

We have identified a novel gene product, PON, which physically interacts with Numb both in vitro and in vivo. Our analyses of Numb localization under *pon* loss-of-function and ectopic expression conditions indicate that PON is an important component of the cellular machinery that localizes Numb. Moreover, our findings have several implications on asymmetric cell divisions. First, previous studies showed that Numb appears to have a fairly universal function as an intrinsic factor to make two daughter cells different. It functions in the fly PNS, CNS, and muscle in much the same way (Uemura et al., 1989; Rhyu et al., 1994; Spana et al., 1995; Guo et al., 1996; Gomez and Bate, 1997; Carmena et al., 1998; Dye et al., 1998; Skeath and Doe, 1998). The present study shows that the mechanisms for asymmetrically localizing Numb may also be shared between the nervous system and muscle, with PON as a common component. Second, previous work showed that Miranda functions as an adaptor to localize Prospero and *prospero* mRNA in mitotic neuroblasts (Ikeshima-Kataoka et al., 1997; Shen et al., 1997; Schuldt et al., 1998; Shen et al., 1998). This work shows that PON serves an analogous role for localizing Numb. Third, previous work revealed that Insc is essential in some cell types (e.g., neuroblasts and muscle progenitor cells) but not in others (e.g., SOP cells) for controlling Numb localization (Kraut et al., 1996; Carmena et al., 1998). There are, therefore, Insc-dependent and Insc-independent mechanisms for localizing Numb. This study suggests that both Insc-dependent and -independent mechanisms act through PON. Thus, PON is a converging point in the Numb localization pathway.

PON Functions in the Localization of Numb in the Nervous System and the Muscle Lineage

Our analyses of *pon* gene expression and protein localization suggest that it is expressed in the expected developmental stages and subcellular locations in order for it to have a role in the asymmetric localization of

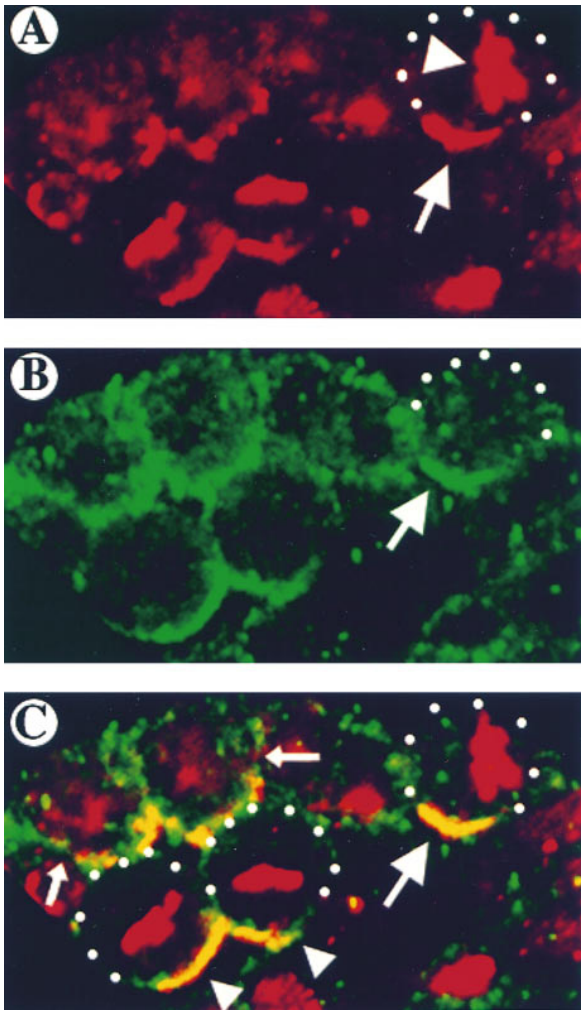

Figure 7. Ectopic Expression of *pon* in the Epidermis Can Direct Numb Crescent Formation

UAS-PONmyc transgenic flies were crossed to *hairy-GAL4* flies, and embryos were stained with anti-Myc (red), anti-Numb (green), and propidium iodide (DNA, red).

(A) In dividing epithelial cells of a stage 12 embryo, ectopic PON provided by the transgene is localized to the basal cortex. The arrow indicates the PON crescent, and the arrowhead marks the chromosomes of a mitotic epithelial cell. The cell boundary is outlined with dots.

(B) In the same mitotic epithelial cell in which PON forms a crescent, Numb also forms a crescent as indicated by the arrow.

(C) A merged image of (A) and (B). The arrow indicates that Numb and PON crescents colocalize. In neighboring epithelial cells (small arrows) where PON crescent is not obvious, Numb is not asymmetrically localized. Two dividing neuroblasts (arrowheads) that also express the *pon* transgene are present below the epithelial cells (Note that the division plane of the dividing epithelial cell is perpendicular to those of the neuroblasts.)

Numb. Evidence for the function of PON in Numb localization comes from analyses of Numb localization under *pon* ectopic expression and loss-of-function conditions. When *pon* is misexpressed in epithelial cells of the ectoderm, it is asymmetrically localized to the basal side of the membrane during mitosis and is sufficient to direct the basal Numb crescent formation in these cells. In contrast, misexpression of Insc in epithelial cells fails to localize Numb asymmetrically, even though these cells are capable of localizing misexpressed Insc apically (Kraut et al., 1996). These results are consistent with the notion that Insc controls Numb localization indirectly via intermediate molecules (or adaptors) such as PON. That PON functions in localizing Numb is further supported by analysis of Numb localization when *pon* function is missing. In dividing muscle precursor cells of stage 10–11 *pon* mutant embryos, the asymmetric localization of Numb is disrupted in approximately 50% of the cells examined and Numb is distributed to both daughter cells. The effect of loss of *pon* function on Numb localization in neuroblasts and SOP cells is less severe. In these cells a delay of Numb crescent formation is observed. Nevertheless, the mutant phenotypes suggest that PON is necessary for the correct localization of Numb in both muscle and neural progenitor cells.

There are several possible explanations for the partial penetrance of Numb localization defects in *pon* mutants. First, maternal contribution of *pon* RNA or protein may be the source of activity that localizes Numb in *pon* mutant embryos. Our RNA in situ hybridization analysis suggests that there is maternal contribution of *pon* RNA that may persist during early stages of embryonic myogenesis and neurogenesis (data not shown). Although we have not detected maternally contributed PON protein, this could be due to a low abundance of the protein or a low sensitivity of our antibody. Removal of maternal contribution by making germ-line mitotic clones will test this possibility. Second, the asymmetric localization of Numb may involve a combination of PON anchoring and other mechanisms, such as aggregation of Numb by a capping mechanism or selective degradation of Numb in one domain of the membrane. Third, there may be functional redundancy in the Numb localization process, and other protein(s) may be involved. Previous structure-function analysis of Numb localization supports this scenario. Although the Numb N-terminal sequence containing a membrane targeting signal and the PTB domain is sufficient to direct the asymmetric localization of a heterologous protein (Knoblich et al., 1997), deletion of the PTB domain in the context of the full-length Numb protein does not eliminate the asymmetric localization of Numb in neuroblasts (Frise et al., 1996). This suggests that a separate domain can also direct the asymmetric localization of Numb in neuroblasts. Further mapping of the alternative localization domain in Numb and identifying proteins that associate with it will shed more light on the complex process of Numb localization.

The differential effects of loss of *pon* function on Numb localization in muscle progenitors and neural precursors suggest that either the redundant function is not expressed in muscle progenitors or that it plays a less important role there. We have tested whether *miranda* and *pon* play redundant functions in Numb localization by constructing double mutants. It appears that Numb localization in early stage double mutant embryos is similar to that in *pon* single mutant (B. Lu, unpublished data). This suggests that other yet-to-be-identified factors may be involved in Numb localization. However, we can not exclude the possibility that *miranda* and *pon* play redundant functions in Numb localization but the maternal contributions of both genes mask their loss-of-function phenotypes.

Multimolecular Complexes in Mediating Asymmetric Protein Localization

From the analyses of molecules that are localized during asymmetric divisions in *Drosophila*, three distinct localization patterns in the neuroblast have emerged. These are the basal localization of Numb and PON, the apical localization of Insc, and the initial apical then basal localization of Prospero, Miranda, Staufen, and *prospero* RNA. There is evidence suggesting that Insc may nucleate an apical multicomponent complex that mediates at least three functions: asymmetric RNA localization, spindle orientation, and asymmetric protein localization. One component of the complex is Staufen, which physically interacts with Insc and together with Insc is required for the asymmetric localization of *prospero* RNA (Li et al., 1997).

The colocalization of Numb, PON, Prospero, and Miranda crescents to the basal cortex of neuroblasts in wild-type embryos or at random positions in *insc* mutant points to the existence of a basal complex. Data from this and previous studies have demonstrated protein–protein interactions between Numb-PON, Numb-Miranda, PON-Miranda, Miranda-Staufen, and Prospero-Miranda (Ikeshima-Kataoka et al., 1997; Shen et al., 1997, 1998; Schuldt et al., 1998). The in vivo coimmunoprecipitation of Numb and Miranda with PON supports the existence of a basal complex. Although we have not detected Prospero in the complex, this could be due to a lack of direct interaction between PON and Prospero or the transient nature of Prospero-Miranda interaction. It will be interesting to find out whether the basal complex contains additional components such as cytoskeletal elements or motor proteins. Further characterization of this basal complex should provide insights into the mechanisms of asymmetric cell division.

Polarity Cues that Direct the Localization of PON in Different Cell Types

Our observation that misexpressed PON in epithelial cells is asymmetrically localized to the basal cortex suggests that PON can respond to the apical-basal polarity cues of epithelial cells. This process is independent of Insc, since Insc is not expressed in these cells (Kraut and Campos-Ortega, 1996). Furthermore, when Insc is ectopically expressed in epidermal cells, it is localized to the apical cortex (Kraut et al., 1996), independently of PON. We postulate that the same apical-basal polarity cue is present in all ectodermal cells and is inherited by neuroblasts, which are derived from neuroepithelial cells of the proneural cluster. It is possible that the apical localization of Insc also responds to this initial apical-basal polarity cue. Mislocalization of PON in neuroblasts of *insc* mutant suggests that the initial apical-basal polarity cue is further modified or reinforced by Insc in neuroblasts. We hypothesize that after neuroblasts delaminate from the neuroepithelial layer, they lose some of the standard epithelial polarity information and that Insc is required to preserve the remaining apical-basal polarity cues to set up neuroblast-specific cellular polarity.

The normal localization of PON in SOP cells of *insc* mutant implies that, unlike neuroblasts, SOP cells use polarity information specified by factors other than Insc to localize PON. SOP cells do differ from neuroblasts in certain aspects of their cellular polarity; for example, SOP cells generally divide with their spindles oriented parallel to the surface (Bodmer et al., 1989). It is possible that Frizzled signaling, which has been shown to control planar polarity in *Drosophila* and *C. elegans* (Adler, 1992; Sawa et al., 1996; Rocheleau et al., 1997; Thorpe et al., 1997), is involved in regulating the activity or localization of an unknown polarity organizing factor in SOP cells, and PON is responding to this factor. Consistent with this notion, Gho and Schweisguth recently showed that the orientation of Numb crescents in SOP cells requires *frizzled* and the Wnt signaling pathway component *dsh* (Gho and Schweisguth, 1998). An important implication of our results is that Numb localization depends on either Insc-mediated apical-basal polarity (as in the case of neuroblasts) or Insc-independent planar polarity (as in the case of SOP cells) and that PON is a common component in both processes. It will be very interesting to find out how Insc and the Frizzled-Wnt signaling pathway impinge on PON to control Numb localization.

Experimental Procedures

Yeast Two-Hybrid Assay

A *Drosophila* embryonic cDNA library, constructed by cloning 3–12 hr embryonic cDNAs into the GAL4 activation domain vector pGAD10, was screened according to the method of Bartel et al. (1993) using a LexA DNA–binding domain-Numb PTB domain fusion (pBHA-PTB) as the bait. Approximately ten million colonies were selected for *His*$^+$ and β-gal activity and 34 positive clones were recovered, representing five different genes upon hybridization and sequencing analysis. One clone is derived from *pon*.

To map the domain in PON that interacts with Numb, deletion constructs *pon*A, *pon*B, *pon*C, and full-length *pon* were cloned into the pBHA vector and cotransformed with pGAD10-NumbPTB plasmid into yeast strain L40. Transformants were tested for His and β-gal activities. To test the interaction between mutant forms of Numb PTB domain and PON, PTBm1, PTBm2, PTBm3, and wild-type PTB cloned in the pBHA vector were cotransformed with pGAD10-PON into yeast strain L40, and transformants were tested for β-gal activity.

Molecular Cloning

Full-length *pon* cDNAs were isolated by screening a *Drosophila* embryonic cDNA library. Mutant forms of the Numb PTB domain were generated by a PCR-based site-directed mutagenesis protocol (ExSite site-directed mutagenesis kit, Stratagene) using pBHA-PTB as the template. The point mutations introduced into the Numb PTB domain are PTBm1 (S148 to A), PTBm2 (R165 to Q), and PTBm3 (F195 to V).

The pUAS-PONmyc plasmid was constructed by PCR amplification of the entire coding region of *pon* using a primer pair that results in a PCR product containing the full-length *pon* cDNA plus a 3′ Myc tag coding sequence. Myc-tagged full-length *pon* cDNA was then subcloned into the pUAST vector to generate pUAS-PONmyc.

Immunoprecipitation Assays

For the in vitro coimmunoprecipitation assay, full-length Numb and PON proteins were produced by the coupled in vitro transcription/translation system (Promega) in the presence of ^{35}S-methionine and subject to immunoprecipitation as described (Guo and Kemphues, 1996b). For in vivo coimmunoprecipitation, *UAS-PONmyc* transgenic flies were crossed to *scabrous-GAL4* enhancer trap flies to produce embryos expressing Myc-tagged PON proteins. The embryos were collected and aged for 5–6 hr, dechorionated, and processed for immunoprecipitation. Extracts prepared from wild-type embryos were processed similarly as a control.

Fly Stocks and Genetics

To generate *pon* mutants by transposon mutagenesis, we adopted the mutant screen scheme of Kaiser and Goodwin (1990) using the EP55 line (Rørth, 1996) as the starting point. From approximately 3000 flies we obtained one line that contains a new P insertion approximately 700 bases upstream of the AUG start codon. Since this new insertion does not seem to disrupt *pon* function, we then remobilized this P element and generated two *pon* mutant alleles by imprecise excision. Both of these alleles, pon^{P65} and pon^{P26}, are protein-null based on immunostaining. Further analysis by Southern hybridization and single embryo PCR with nested primer pairs showed that both alleles contain deletions that remove the translation initiation codon of the *pon* coding region.

UAS-PONmyc transgenic flies were obtained by injecting pUAS-PONmyc DNA into *w* embryos. For the misexpression experiment, *UAS-PONmyc* flies were crossed to *hairy-Gal4* enhancer trap line and embryos were collected in a 2 hr interval at 25°C and aged at 30°C for 4–5 hr before antibody staining. For the muscle precursor cell rescue experiment, females of the genotype pon^{P65}/+; *twi-GAL4*/+ were crossed to homozygous *UAS-PONmyc* male flies, and embryos from this cross were triple labeled with anti-PON, anti-Numb, and anti-Eve antibodies. Embryos of the genotype *twi-GAL4/UAS-PONmyc;* pon^{P65}/Y were identified based on the presence of PON staining in the mesoderm and its absence in the CNS and analyzed for rescue of Numb localization. To rescue the Numb localization delay phenotype in neural precursors, *Df(1)bi-D3*/+; *scabrous-Gal4*/+ female flies were crossed to *FM7c, ftz-LacZ/Y* male flies to generate *Df(1)bi-D3/FM7c, ftz-LacZ; scabrous-Gal4*/+ females, which were then crossed to *FM7c, ftz-LacZ/Y; UAS-PONmyc*/+ male flies. *Df(1)bi-D3/Y; UAS-PONmyc/scabrous-GAL4* embryos from the above cross were analyzed for the rescue.

Antibody Production and Staining

Polyclonal antibodies were raised in rabbits against a peptide (amino acids 13–43 of PON) or against a glutathione-S-transferase (GST) fusion protein (amino acids 6–378 of PON). A guinea pig anti-Numb antibody was obtained by injecting guinea pig with the same peptide used to immunize rabbits by Rhyu et al. (1994). Immunostaining of embryos was performed as described (Rhyu et al., 1994). The rabbit antibody against PON was preabsorbed against wild-type embryos and used at a dilution of 1:1000. Other antibodies used in this study were MAb6D5 (1:5), guinea pig anti-Numb (1:1000), rabbit anti-Numb (1:1000), guinea pig anti-Asense (1:2000) (Rhyu et al., 1994), mouse anti-Prospero (1:5) (Spana and Doe, 1995), rabbit anti-Eve (1:2000), mouse monoclonal antibody (9E10) against c-Myc (1:300) (Santa Cruz Biotechnology, Inc.), and mouse anti-β-galactosidase (1:200) (Promega). Propidium iodide or Sytoxgreen (Molecular Probes) were used to counterstain DNA.

Acknowledgments

We are grateful to Yee-Ming Chan, Marcus Schonemann, Juergen Knoblich, and Su Guo for comments on the manuscript and members of the Jan lab for discussions. We thank Larry Ackerman, Cheng-ting Chien, Emily Coven, Susan Younger-Shepherd, and Sandy Barbel for advice and technical supports. We are grateful to Chris Doe and Manfred Frasch for providing antibodies and to Pernille Rørth and the Bloomington stock center for fly strains. B. Lu wishes to thank Su Guo for advice and encouragement. B. Lu is a postdoctoral fellow, and L. Y. Jan and Y. N. Jan are investigators of the Howard Hughes Medical Institute.

Received June 26, 1998; revised August 25, 1998.

References

Adler, P.N. (1992). The genetic control of tissue polarity in *Drosophila*. Bioessays *14*, 735–741.

Bartel, P.L., Chien, C.-T., Sternglanz, R., and Fields, S. (1993). Elimination of false positives that arise in using the two-hybrid system. Biotechniques *14*, 920–924.

Bodmer, R., Carretto, R., and Jan, Y.N. (1989). Neurogenesis of the peripheral nervous system in *Drosophila melangaster* embryos: DNA replication patterns and cell lineages. Neuron *3*, 21–32.

Borg, J.P., Ooi, J., Levy, E., and Margolis, B. (1996). The phosphotyrosine interaction domain of X11 and FE65 bind to distinct sites on the YENPTY motif of amyloid precursor protein. Mol. Cell. Biol. *16*, 6229–6241.

Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development *118*, 401–415.

Broadus, J., Fuerstenberg, S., and Doe, C.Q. (1998). Staufen-dependent localization of *prospero* mRNA contributes to neuroblast daughter-cell fate. Nature *319*, 792–795.

Burchard, S., Paululat, A., Hinz, U., and Renkawitz-Pohl, R. (1995). The mutant *not enough muscle (nem*) reveals reduction of the *Drosophila* embryonic muscle pattern. J. Cell Sci. *108*, 1443–1454.

Carmena, A., Murugasu-Oei, B., Menon, D., Jimenez, F., and Chia, W. (1998). *insc* and *numb* mediate asymmetric muscle progenitor cell divisions during *Drosophila* myogenesis. Genes Dev. *12*, 304–315.

Chang, F., and Drubin, D.G. (1996). Cell division: why daughters cannot be like their mothers. Curr. Biol. *6*, 651–654.

Chien, C.-T., Wang, S., Rothenberg, M., Jan, L.Y., and Jan, Y.N. (1998). Numb-associated kinase interacts with the phosphotyrosine binding domain of Numb and antagonizes the function of Numb in vivo. Mol. Cell. Biol. *18*, 598–607.

Doe, C.Q., Chu-LaGraff, Q., Wright, D.M., and Scott, M.P. (1991). The *prospero* gene specifies cell fates in the *Drosophila* central nervous system. Cell *65*, 451–465.

Dye, C.A., Lee, J-K., Atkinson, R.C., Brewster, R., Han, P-L., and Bellen, H.J. (1998). The *Drosophila sanpodo* gene controls sibling cell fate and encodes a tropomodulin homolog, an actin/tropomyosin-associated protein. Development *125*, 1845–1856.

Frise, E., Knoblich, J.A., Younger-Shepherd, S., Jan, L.Y., and Jan, Y.N. (1996). The *Drosophila* Numb protein inhibits signaling of the Notch receptor during cell-cell interaction in sensory organ lineage. Proc. Natl. Acad. Sci. USA *93*, 11925–11932.

Gho, M., and Schweisguth, F. (1998). Frizzled signaling controls orientation of asymmetric sense organ precursor cell divisions in *Drosophila*. Nature *393*, 178–181.

Gomez, M.R., and Bate, M. (1997). Segregation of myogenic lineages in *Drosophila* requires Numb. Development *124*, 4857–4866.

Guo, M., Jan, L.Y., and Jan, Y.N. (1996). Control of daughter cell fates during asymmetric division: interaction of Numb and Notch. Neuron *17*, 27–41.

Guo, S., and Kemphues, K.J. (1996a). Molecular genetics of asymmetric cleavage in the early Caenorhabditis elegans embryo. Curr. Opin. Genet. Dev. *6*, 408–415.

Guo, S., and Kemphues, K.J. (1996b). A non-muscle myosin required for embryonic polarity in Caenorhabditis elegans. Nature, *382*, 455–458.

Hirata, J., Nakagoshi, H., Nabeshima, Y., and Matsuzaki, F. (1995). Asymmetric segregation of a hemeoprotein, Prospero, during cell divisions in neural and endodermal development. Nature *377*, 627–630.

Horvitz, H.R., and Herskowitz, I. (1992). Mechanisms of asymmetric cell division: two Bs or not two Bs, that is the question. Cell *68*, 237–255.

Ikeshima-Kataoka, H., Skeath, J.B., Nabeshima, Y., Doe, C.Q., and Matsuzaki, F. (1997). Miranda directs Prospero to a daughter cell during *Drosophila* asymmetric divisions. Nature *390*, 625–629.

Jan, Y.N., and Jan, L.Y. (1998). Asymmetric cell division. Nature *392*, 775–778.

Kaiser, K., and Goodwin, S.F. (1990). "Site-selected" transposon mutagenesis of *Drosophila*. Proc. Natl. Acad. Sci. USA *87*, 1686–1690.

Knirr, S., Breuer, S., Paululat, A., and Renkawitz-Pohl, R. (1997). Somatic mesoderm differentiation and the development of a subset of pericardial cells depends on the *not enough muscle (nem)* locus, which contains the *insc* gene and the intron located gene, *skittles*. Mech. Dev. *67*, 69–81.

Knoblich, J.A., Jan, L.Y., and Jan, Y.N. (1995). Asymmetric segregation of Numb and Prospero during cell division. Nature *377*, 624–627.

Knoblich, J.A., Jan, L.Y., and Jan, Y.N. (1997). The N terminus of *Drosophila* Numb protein directs membrane association and actin-dependent asymmetric localization. Proc. Natl. Acad. Sci. USA *94*, 13005–13010.

Kraut, R., and Campos-Ortega, J.A. (1996). *insc*, a neural precursor gene of *Drosophila*, encodes a candidate for a cytoskeleton adaptor protein. Dev. Biol. *174*, 65–81.

Kraut, R., Chia, W., Jan, L.Y., Jan, Y.N., and Knoblich, J.A. (1996). Role of *insc* in orienting asymmetric cell division in *Drosophila*. Nature *383*, 50–55.

Li, P., Yang, X., Wasser, M., Cai, Y., and Chia, W. (1997). Insc and Staufen mediate asymmetric localization and segregation of *prospero* RNA during *Drosophila* neuroblast cell divisions. Cell *90*, 437–447.

Matsuzaki, F., Koizumi, K., Hama, C., Yoshioka, T., and Nabeshima, Y. (1992). Cloning of the *Drosophila prospero* gene and its expression in ganglion mother cells. Biochem. Biophys. Res. Comm. *182*, 1326–1332.

Rhyu, M.S., Jan, L.Y., and Jan, Y.N. (1994). Asymmetric distribution of Numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. Cell *76*, 477–491.

Rocheleau, C.E., Downs, W.D., Lin, R., Wittmann, C., Bei, Y., Cha, Y.-H., Ali, M., Priess, J.R., and Mello, C.C. (1997). Wnt signaling and an APC-related gene specify endoderm in early C. elegans embryos. Cell *90*, 707–716.

Rørth, P. (1996). A modular misexpression screen in *Drosophila* detecting tissue-specific phenotypes. Proc. Natl. Acad. Sci. USA *93*, 12418–12422.

Sawa, H., Lobel, L., and Horvitz, H.R. (1996). The *C. elegans* gene *lin-17*, which is required for certain asymmetric cell divisions, encodes a putative seven-transmembrane protein similar to the *Drosophila* Frizzled protein. Genes Dev. *10*, 2189–2197.

Schuldt, A.J., Adams, J.H.J., Davidson, C.M., Micklem, D.R., Haseloff, J., St. Johnston, D., and Brand, A.H. (1998). Miranda mediates asymmetric protein and RNA localization in the developing nervous system. Genes Dev. *12*, 1847–1857.

Shapiro, L., and Losick, R. (1997). Protein localization and cell fates in bacteria. Science *276*, 712–718.

Shen, C-P., Jan, L.Y., and Jan, Y.N. (1997). Miranda is required for the asymmetric localization of Prospero during mitosis in *Drosophila*. Cell *90*, 449–458.

Shen, C-P., Knoblich, J.A., Chan Y-M., Jiang, M-M., Jan, L.Y., and Jan, Y.N. (1998). Miranda as a multidomain adaptor linking apically localized Insc and basally localized Staufen and Prospero during asymmetric cell division in *Drosophila*. Genes Dev. *12*, 1837–1846.

Skeath, J.B., and Doe, C.Q. (1998). Sanpodo and Notch act in opposition to Numb to distinguish sibling neuron fates in the *Drosophila* CNS. Development *125*, 1857–1865.

Spana, E.P., and Doe, C.Q. (1995). The Prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in *Drosophila*. Development *121*, 3187–3195.

Spana, E.P., Kopczynski, C., Goodman, C.S., and Doe, C.Q. (1995). Asymmetric localization of Numb autonomously determines sibling neuron identity in *Drosophila* CNS. Development *121*, 3489–3494.

Spana, E.P., and Doe, C.Q. (1996). Numb antagonizes Notch signaling to specify sibling neuron cell fates. Neuron *17*, 21–26.

Thorpe, C.J., Schlesinger, A., Carter, J.C., and Bowerman, B. (1997). Wnt signaling polarizes an early C. elegans blastomere to distinguish endoderm from mesoderm. Cell *90*, 695–705.

Uemura, T., Shepherd, S., Ackerman, L., Jan, L.Y., and Jan, Y.N. (1989). *numb*, a gene required in determination of cell fate during sensory organ formation in Drosophila embryos. Cell *58*, 349–360.

Vaessin, H., Grell, E., Wolff, E., Bier, E., Jan, L.Y., and Jan, Y.N. (1991). *prospero* is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in Drosophila. Cell *67*, 942–953.

Zhou, M.M., Ravichandran, K.S., Olejniczak, E.T., Petros, A.M., Meadows, R.P., Sattler, M., Harlan, J.E., Wade, W.S., Burakoff, S.J., and Fesik, S.W. (1995). Structure and ligand recognition of the phosphotyrosine binding domain of Shc. Nature *378*, 584–592.

GenBank Accession Number

The GenBank accession number for the *pon* gene reported in this text is AF091050.