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The *Drosophila sanpodo* gene controls sibling cell fate and encodes a tropomodulin homolog, an actin/tropomyosin-associated protein

Catherine A. Dye[1,*], Ja-Kyeong Lee[1,*,†], Richard C. Atkinson[1], Rachel Brewster[2], Pyung-Lim Han[1,‡] and Hugo J. Bellen[1,§]

[1]Department of Cell Biology, Howard Hughes Medical Institute, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA
2Department of Biology, University of Michigan, Ann Arbor, MI 48109, USA
*These authors contributed equally to the work
†Present address: Aju University, Medical School, Suwon, South Korea
‡Present address: Hanhyo Institute of Technology, Taejon, South Korea
§Author for correspondence (e-mail: hbellen@bcm.tmc.edu)

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SUMMARY

Notch signaling is required in many invertebrate and vertebrate cells to promote proper cell fate determination. Mutations in *sanpodo* cause many different neuronal peripheral nervous system precursor cells to generate two identical daughter neurons, instead of a neuron and sibling cell. This phenotype is similar to that observed when Notch function is lost late in embryonic development and opposite to the *numb* loss-of-function phenotype. Genetic interaction studies show that *sanpodo* is epistatic to *numb*. *sanpodo* encodes a homolog of tropomodulin, an actin/tropomyosin-associated protein. Loss of *sanpodo* leads to an aberrant F-actin distribution and causes differentiation defects of actin-containing sensory structures. Our data suggest that an actin-based process is involved in Notch signaling.

Key words: Tropomodulin, Actin, Neurogenesis, *numb*, Notch, Glia, *sanpodo*, *Drosophila*

INTRODUCTION

How cell fate specification occurs is a fundamental question in developmental biology. This event is mediated in part through asymmetric cell divisions, the process by which one cell divides to give rise to two daughter cells with distinctly different fates (for review see Horvitz and Herskowitz, 1992). A genetic dissection of the development of the sensory structures of the *Drosophila* peripheral nervous system (PNS) has provided important insights into the molecular mechanisms governing asymmetric divisions (Jan and Jan, 1995; Posakony, 1994). In the external sensory (es) organ lineage (Fig. 1A,B), the primary sensory organ precursor (SOPI) divides asymmetrically to produce two daughter cells, SOPIIa and SOPIIb. These divide to give rise to a hair and socket cell, and to a neuron and glial cell, respectively (Bodmer et al., 1989; Brewster and Bodmer, 1995). Many genes that play a role in specifying the fate of PNS cells and neurons have been placed in the Notch signaling pathway: *Notch, Delta, Suppressor of Hairless*, *Enhancer of split, mastermind*, *kuzbanian* and *numb* (for review see Artavanis-Tsakonas et al., 1995; Campos-Ortega, 1995; Jan and Jan, 1995; Posakony, 1994; Rooke et al., 1996; Schweisguth et al., 1996; Pan and Rubin, 1997). Notch is a transmembrane receptor for the ligand Delta (Fehon et al., 1990; Heitzler and Simpson, 1991; Kidd et al., 1986; Wharton et al., 1985) and contains an intracellular domain which binds several proteins, including Suppressor of Hairless, a transcription factor (Lecourtois and Schweisguth, 1995; Schweisguth and Posakony, 1992). The functions of these proteins are conserved in many species (Chitnis et al., 1995; Fortini and Artavanis-Tsakonas, 1994; Jarriault et al., 1995; Lu and Lux, 1996; Roehl et al., 1996; Schweisguth and Posakony, 1992; Wettstein et al., 1997). Loss of *Delta*, *Notch*, *Supressor of Hairless* or *Kuzbanian* lead to an increase in the number of neurons, the neurogenic phenotype (Lehmann et al., 1981; Rooke et al., 1996; Schweisguth and Posakony, 1992; Vaessin et al., 1987; Pan and Rubin, 1997), whereas overexpression of the cytoplasmic portion of Notch results in a loss of neurons (Fortini et al., 1993; Lieber et al., 1993; Struhl et al., 1993). Recently, another component, Numb, has been integrated into the Notch signaling pathway. Numb is a cytoplasmic protein that has been shown to bind to the intracellular domain of Notch and to repress Notch function (Guo et al., 1996; Spana and Doe, 1996). Interestingly, in the SOPI of es organs the Numb protein is localized to a crescent at the cell surface (Rhyu et al., 1994). Upon SOPI division, the Numb protein segregates into the SOPIIb daughter cell. The resulting daughter-cell-specific repression of Notch signaling is necessary for the SOPIIa and b cells to adopt different fates.

Numb is then again segregated preferentially into the neuron, to repress Notch signaling in this cell. Cells that lack Numb, e.g. SOPIIa and glia, express Notch responsive genes, like *tramtrack*, allowing non-neuronal differentiation to proceed (Frise et al., 1996; Guo et al., 1996; Rhyu et al., 1994; Salzberg et al., 1994). Hence, extrinsic cues like Delta-Notch signaling, and intrinsic cues like Numb, cross-talk to specify cell fate.

We have carried out extensive screens to isolate mutations in genes that affect the developmental pattern of the embryonic PNS (Salzberg et al., 1994, 1997; Kania et al., 1995). Here, we report that mutations in *sanpodo* (*spdo*) affect the asymmetric cell division of the SOPIIb. Instead of generating a neuron and a glial cell, the SOPIIb produces two identical neurons. In addition, we show that *spdo* is required in several other PNS lineages where its absence equalizes the fate of sibling cells. We present evidence that *spdo* is epistatic to *numb* and encodes a homolog of tropomodulin, a vertebrate actin/tropomyosin-associated protein (Fowler, 1987). Tropomodulin caps the pointed end of actin filaments and may regulate the length of actin polymers (Gregorio et al., 1995). Our data suggest that an actin-based process is involved in cell fate determination. In addition, SPDO is also required for proper morphology and F-actin distribution in PNS external sensory structures, indicating a role for SPDO in F-actin biology.

MATERIALS AND METHODS

Isolation and mapping of *sanpodo* mutations

The characterization of the spdo phenotype and subsequent cloning of the gene relied on several alleles generated in four separate screens. The first two alleles identified, $spdo^{C55}$ and $spdo^{K433}$, are ethyl methane sulfonate (EMS)-induced mutations isolated in a screen to identify genes required for peripheral nervous system (PNS) development (Salzberg et al., 1994). The $spdo^{1309/10}$ allele was identified in a similar screen using P elements as mutagens instead of EMS (Salzberg et al., 1997). However, molecular characterization revealed that the molecular defect in $spdo^{1309/10}$ was not caused by a P-element insertion, but rather by a deletion of a portion of the *spdo* gene, as previously observed in P-element screens (Kania et al., 1995; Salzberg et al., 1997). The *P{lacZ,* w^+*}*$spdo^{H7}$ insertion was generated in order to isolate and clone the gene by mobilization of a viable insertion mapping at 99D-F: *P{lacZ, w+}1447* (FlyBase, 1994, see below). This allele, $spdo^{H7}$, was mapped to 99F6-9, in agreement with the meiotic mapping reported by Salzberg et al. (1994). *P{lacZ, w+}*$spdo^{H7}$ failed to complement all other alleles described here and exhibits a severe *spdo* phenotype. Lastly, two EMS alleles, $spdo^{ZZ27}$ and $spdo^{G104}$, were isolated in a screen to identify genes affecting sibling cell fate in the CNS (Skeath and Doe, 1997). All *spdo* mutations fail to complement each other and *Df(3R)tll*^{G} *ca* [99E; 100B3-4], a deficiency that uncovers *spdo*. All alleles, in *trans* to the deficiency, show similar phenotypes and are therefore severe loss-of-function mutations.

Cloning of *spdo*

Initially, an 11 kb *Hin*dIII genomic fragment spanning either side of the *H7* insertion was used as a probe to isolate cDNAs from a 9- to 12-hour-old embryonic cDNA library (Zinn et al., 1988). This permitted isolation of two cDNAs: *K7* and *K8* (Fig. 4A). Both cDNAs as well as the 11 kb *Hin*dIII fragment were sequenced. Embryonic in situ hybridization experiments with sense and antisense probes showed that neither cDNA is expressed in the tissues/cells of the PNS that display a *sanpodo* phenotype (data not shown). In addition, neither *K7* nor *K8* is expressed in a pattern resembling the β-galactosidase expression pattern observed in *P{lacZ,* w^+*}*$spdo^{H7}$ /+ embryos, suggesting that they do not correspond to the *spdo* gene. In addition to the *K7* and *K8* cDNAs isolated from a 9- to 12-hour-old embryonic library (Zinn et al., 1988), we isolated nine other cDNAs (which correspond to *spdo*) from a 3- to 12-hour-old embryonic library (*E* and *A* clones) (a gift from Larry Kauvar and Tom Kornberg). Two cDNAs were sequenced: *E42* and *A14*. To determine the structure of *spdo*, the genomic sequences to which *spdo* hybridized were subcloned and sequenced, except for the 3′UTR region. Northern analysis was conducted as described in Sambrook et al. (1989). The sequence of *spdo* has been deposited in Genbank under accession number U92490.

Overexpression of *spdo* and fly strains

To overexpress SPDO, the *E42* cDNA was subcloned into the p-Casper-hs vector (Pirrotta, 1988) and five transgenic lines were obtained. Two of the five P elements were crossed into the *P{lacZ,* w^+*}*$spdo^{H7}$ line to test for rescue of the spdo phenotype. Embryos were collected on grape juice agar plates for 2 hours, aged for 5 hours and transferred to a prewarmed juice plate kept at 37°C. The plate was then floated on the surface of a 37°C water bath, covered by the other half of the Petri plate. Embryos were heat shocked for 30 minutes and aged for 10 hours in a moist chamber, fixed and stained with anti-β-galactosidase and mAb 22C10 or anti-β-galactosidase, anti-Prospero and mAb BP102. *P{lacZ,* w^+*}*$spdo^{H7}$ /*P{lacZ,* w^+*}*$spdo^{H7}$ embryos were identified by the CNS phenotype as revealed by BP102 staining.

Two independent numb;spdo stocks were utilized and similar results were obtained: $numb^1$*, Bc / CyO, P{wg-LacZ};* $spdo^{G104}$*/TM3, P{ftz-lacZ}* and $numb^1$ *Bc/ CyO, P{wg-lacZ}; P{LacZ,* w^+*}*$spdo^{H7}$ */TM6, P{Ubx-lacZ}.*

Immunohistochemical staining of embryos and in situ hybridizations

Immunohistochemical staining was carried out as described by Salzberg et al. (1994). The following concentrations were used for the primary antibodies: mAb 22C10, 1:50; mAb anti-ELAV, 1:50; mAb anti-Pros (MR1A), 1:4; rabbit polyclonal anti-Bar (S12), 1:1,000; rat anti-RK2/REPO (RK2-5′c), 1:1000; mAb BP102, 1:50; mAb anti-β-galactosidase (Promega), 1:5000; mAb 1D4, 1:50. Mutant embryos were identified by lack of β-galactosidase staining from the balancer chromosome. In situ hybridizations were carried out as described by Ingham et al. (1991). To assay β-galactosidase activity from the *gcm* enhancer trap, the protocol of Klambt et al. (1991) was followed.

Actin staining

Embryonic F-actin was stained using 2.5 μg/ml TRITC-conjugated phalloidin (Sigma) in PBS, pH 7.2. Optimal staining of the late embryonic PNS and musculature was obtained by manually dechorionating embryos, puncturing them and manually removing the vitelline membrane in freshly prepared 8% formaldehyde in PBS pH 7.6. The embryos were left in the fixative for 15 minutes. After washing in PBS containing 0.3% Triton X-100 for 1 hour, the embryos were stained with phalloidin. This protocol reveals reduced levels of F-actin staining in the PNS and altered F-actin staining in muscles of mutant embryos. For staining of the CNS, we dechorionated the embryos with 50% bleach. After washing and neutralization, the embryos were fixed in 8% formaldehyde and heptane for 15 minutes, devitellinized by vigorously shaking for 2 minutes after replacing the aqueous phase with equal volumes of 95% ethanol, followed by staining as above. This protocol showed similar levels of F-actin staining in mutant and wild-type CNS.

RESULTS

sanpodo equalizes the fate of sibling cells

The *spdo* gene was identified by Salzberg et al. (1994), and in subsequent screens [(Salzberg et al., 1997); Material and Methods] many additional alleles were isolated, which were used in this study. All *spdo* alleles exhibit similar phenotypes and are either strong hypomorphic or null alleles. All mutants are embryonic lethal. Staining with mAb 22C10 and anti-ELAV (Fig. 1C,D), neuronal markers, demonstrate that *spdo* embryos display an approximate doubling of the number of neurons in the PNS when compared to wild-type embryos (Salzberg et al., 1994). In spite of this defect, the neurons are distributed among the four typical clusters within each segment and, unlike other neurogenic mutants (e.g. *Notch, Delta*), the overall morphology of *spdo* embryos is not affected. In addition, no defects in axon pathfinding were observed in the PNS.

An increased number of md or es neurons, as observed in *spdo* mutants, can be obtained through several mechanisms: an increased recruitment of SOPs, extra divisions of the SOPI, SOPII or neuron, or transformation of the es hair, socket or glial cells into neurons. Previously, we have shown that there are no extra SOPs recruited, and that the total number of cells within the dorsal and lateral clusters of the PNS is not increased significantly. We therefore concluded that the supernumerary neurons must result from a fate change of cells in the same lineage and proposed that the two sibling cells of the SOPIIb (neuron and glia) took the same fate, i.e. neurons (Salzberg et al., 1994).

To establish if glial cells are present in *spdo* mutants, we immunocytochemically stained embryos with antisera that label the glial support cells of PNS organs in stage 16: anti-Prospero antiserum (Doe et al., 1991; Vaessin et al., 1991), which labels 21 glial support cells per hemisegment (Fig. 1 E,F), and anti-Bar antiserum (Higashijima et al., 1992), which labels the es glial support cells, a total of 14 cells per hemisegment (Fig. 1G,H). As shown in Fig. 1F,H, *spdo* mutant embryos lack virtually all Prospero and Bar-positive glial support cells in the PNS. We conclude that the two sibling cells derived from the es SOPIIb take the same neuronal fate in *spdo* mutants.

To determine if other cell lineages are affected, we examined the fates of two classes of multiple dendritic (md) neurons: those that are known to have sibling cells, the solo mds, e.g. the ventral multiple dendritic neurons (vmd5) and dorsal bipolar dendritic (dbd) neurons, and an md neuron that is not known to have a sibling cell, the vpda (Brewster and Bodmer, 1995). Mutant embryos containing the E7-2-36 P element, which specifically expresses *lacZ* in md neurons (Bier et al., 1989), were stained with X-gal. As shown in Fig. 2A, this analysis revealed the presence of supernumerary neurons in all clusters. For example, the vmd5 are typically doubled in number when stained with neuronal markers (see Figs 1C,D, 2I,J; Discussion). The vmd5 solo-md neurons have no associated glial support cells and have previously been proposed to have a sibling ectodermal cell (Bodmer et al., 1989). It is therefore likely that the extra neurons associated with the vmd5 are sibling ectodermal cells that are transformed into md neurons. Similar conclusions were reached after analysis of the dbd lineage in wild-type and *spdo* mutant embryos**.** The dbd lineage is the most simple and well-characterized asymmetric cell division in the PNS (Fig. 2 K): the precursor cell divides asymmetrically to give rise to an md neuron and a glial cell (Brewster and Bodmer, 1995). As shown in Fig. 2L, the dbd neuron is often duplicated in *spdo* embryos (E7-2-36 staining shows that more than 80% of the dorsal clusters have duplicated dbd neurons). Staining with neuronal markers (mAb 22C10, anti-ELAV, E7-2-36, anti-CPO) show two dbd neurons in *spdo* mutants, whereas glial markers (anti-RK2/REPO, anti-Prospero) show no glia associated with the duplicated dbd neurons (see Fig. 2M). Hence, as in the vmd lineage, the sibling cell is transformed into an md neuron. This transformation is opposite to the numb phenotype, which consist of two glial cells instead of two dbd neurons (Fig. 2M; Brewster and Bodmer, 1995). This suggests that for those md neurons that have a lineage-related sibling, the non-neuronal cells as well as the neuronal cell adopt the neural fate in *spdo* mutants. Further support for this hypothesis is provided by the observation that the vpda neuron, an md neuron with no sibling (Brewster and Bodmer, 1995), is not duplicated in *spdo* mutants (Fig. 2I,J).

Staining with the E7-2-36 md-specific marker revealed another feature of *spdo* mutants. As shown in Fig. 2H, the v′es2 and its associated glial cell ectopically express the E7-2-36 md marker in mutant embryos. This suggests that es neurons are programmed to become md neurons in *spdo* mutants. Although it is difficult to establish if the same change in cell type occurs in all es lineages, the number of E7-2-36-positive cells in each cluster supports the idea that many if not all es neurons have become md neurons. This observation is particularly interesting because Vervoort et al. (1997) have recently documented the same phenotype in *Notch* mutants, suggesting that Notch signaling is impaired in *spdo* mutants.

Lack of *spdo* results in glial loss in the CNS

Although most glial CNS cell lineages are not characterized, we wished to establish if CNS glia are also affected by mutations in *sanpodo*. As shown in Fig. 3B, staining with anti-RK2/REPO, a glial marker (Campbell et al., 1994; Xiong et al., 1991), revealed a severe loss of glial cells in the CNS. Analysis of the expression of another glial marker, *glial cells missing* (*gcm*) (Hosoya et al., 1995; Jones et al., 1995; Kania et al., 1995; Vincent et al., 1996), showed that lack of *spdo* causes a severe decrease in the number of *gcm*-positive cells in stage 15 embryos (Fig. 3C,D). Because *gcm* loss-of-function mutations transform glial cells into neurons and overexpression of *gcm* transforms neurons into glia, *gcm* is considered to be a glial identity gene (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996). Thus, the absence of *gcm* expression in *spdo* mutants suggests that a subset of CNS glia have not adopted a glial fate. The lineages of most CNS glia are not well defined and it is not known if these cells have siblings. As a result, we can not determine if the absence of *gcm* expression in *spdo* mutants results from a cell fate switch, as is occurring in the PNS, or from a failure to maintain *gcm* expression.

As seen in Fig. 3C-F, staining of axons in the CNS with mAb BP102 (Klambt et al., 1991) and mAb 1D4 (Van Vactor et al., 1993) shows that mutations in *spdo* cause very noticeable disruptions of the longitudinal tracts. The anterior and posterior commissures form and separate properly, but they appear thicker and less condensed than normal. The phenotype in the

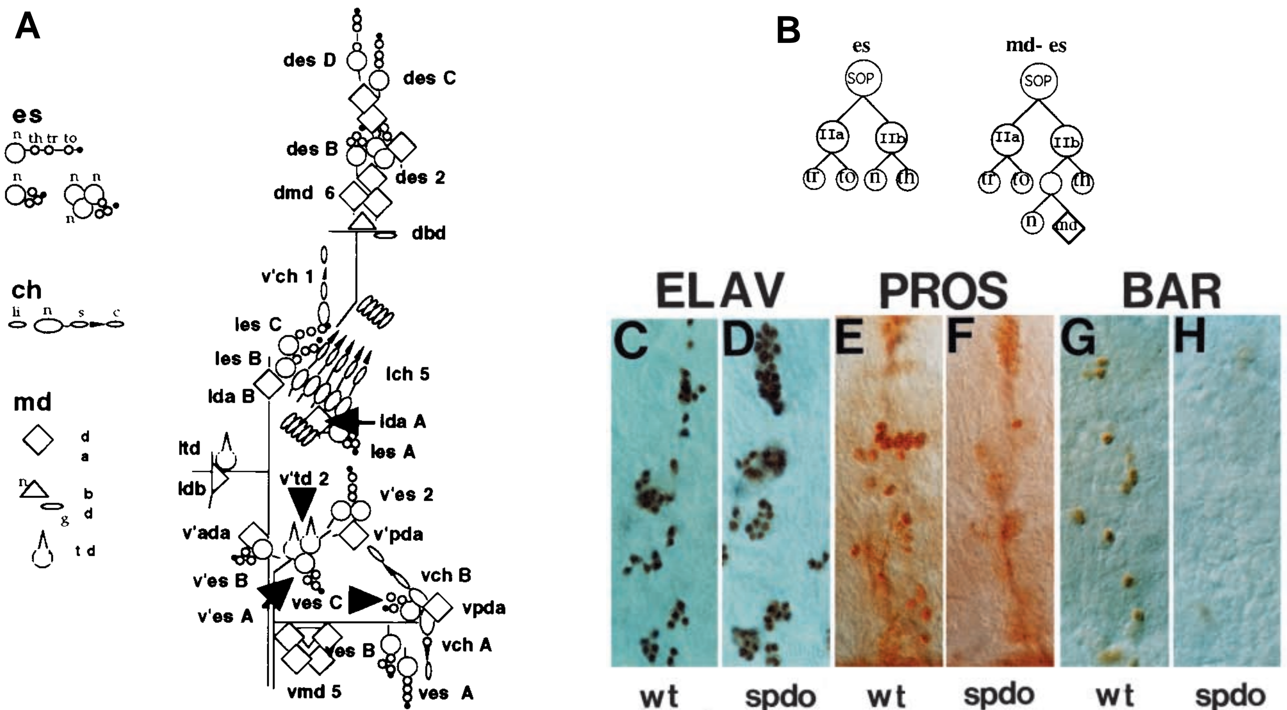


Fig. 1. *spdo* mutations cause a transformation of sibling cells into neurons. Anterior is left, dorsal is up. (A) Diagrammatic representation of the PNS cells of one abdominal hemisegment. es, external sensory organ; n, neuron; th, thecogen or glial cell; tr, tricogen or hair cell; to, tormogen or socket cell; ch, chordotonal organ; l, ligament cell; s, sheath cell; c, cap cell; n, neuron; md, multiple dendritic neuron; da, neuron with large dendritic arbors (diamond shape); bd, bipolar dendritic neuron (triangle shape); n, neuron; g, glial cell; td, tracheal innervating neuron (drop shape); d, dorsal; l, lateral; v, ventral; v′, ventral prime; a, anterior; p, posterior. (B) Diagrammatic representation of wild-type solo es and md-es lineages. (C,D) Lateral views of one abdominal hemisegment of wild-type (C) or *P{lacZ, w⁺}spdo*^{H7} mutant embryos (D) immunohistochemically stained with anti-ELAV antibody. ELAV is a nuclear neuronal-specific antigen. Note the doubling of the number of neurons in most clusters. (E,F) Anti-Prospero staining. Prospero expression is detected in the nuclei of 21 glial cells per hemisegment in stage 16 wild-type embryos (E), whereas in *P{lacZ, w⁺}spdo*^{H7} embryos (F) only one to four faintly stained nuclei per abdominal hemisegment are seen. (G,H) Anti-Bar staining. Bar is normally present in the nuclei of fourteen es-associated glia (Fig. 1G). *spdo*^{C55} embryos lack all but one es glial cell, which stains very faintly (H). In C-H, a portion or all of the dorsal cluster of cells is not shown.

ventral nerve cord in *spdo* mutants is strikingly similar to the phenotype reported for *gcm* loss-of-function mutations (Hosoya et al., 1995; Jones et al., 1995; Vincent et. al., 1996). These observations suggest that loss of *spdo* alters cellular identity in the CNS as well.

spdo encodes a *Drosophila* homolog of tropomodulin, a microfilament-associated protein

Since the original *spdo* mutations were induced with chemical mutagens, we used a local hopping strategy (Zhang and Spradling, 1993) with a homozygous viable P-element enhancer detector, which maps at 99F1-2, *P{lacZ, w⁺}1447* (Bloomington Stock Center and S. Wasserman, unpublished data), to isolate a P element that failed to complement the *spdo*^{C55} mutation. This new *spdo* allele, *P{lacZ, w⁺}spdo*^{H7}, causes embryonic defects that are very similar to those caused by other *spdo* alleles. β-galactosidase expression in *P{lacZ, w⁺}spdo*^{H7} /+ embryos is detected in all cells, but is conspicuously present in the support cells of the es organs of the PNS in stage 13-16 embryos, as well as in cells of the CNS, the visceral mesoderm and somatic musculature (data not shown). This P-element insertion is responsible for the *spdo* phenotype, as precise or near precise excisions revert both the lethality and the phenotypes associated with the insertion.

To clone *spdo*, a genomic DNA fragment flanking the *spdo*^{H7} P element was isolated, and used as a probe to initiate a genomic walk shown in Fig. 4A. Screening of two different embryonic libraries with a 2 kb fragment spanning either side of the P-element insertion permitted isolation of nine putative *spdo* cDNAs, two of which, *E42* and *A14,* were sequenced. Both cDNAs are virtually identical and differ only in the length of their terminal 5′ UTR and 3′ UTR sequences. Sequence analysis of the cDNAs and genomic DNA revealed the structure of the gene shown in Fig. 4A. These cDNAs map entirely within the second intron of another unrelated gene (see Experimental Procedures). The *spdo*^{H7} P element is inserted in the first intron of *spdo*, in the vicinity of the first exon (Fig. 4A,B).

Sequencing of cDNAs identified a single ORF, encoding a protein of 367 amino acids (Fig. 4C). Database searches (BLAST) yielded highly significant probability scores to human, chicken, mouse and rat E-tropomodulin (E-tmod) (Babcock and Fowler, 1994; Ito et al., 1995; Sung et al., 1992) and a neural-specific N-tropomodulin (N-tmod) (Watakabe et al., 1996). The protein encoded by the ORF shows 36% identity (I) and approximately 70% similarity (S) to the two vertebrate tropomodulins over the entire length of the protein and significant but lower levels of homology to a *C. elegans* ORF (Fig. 4C and D).

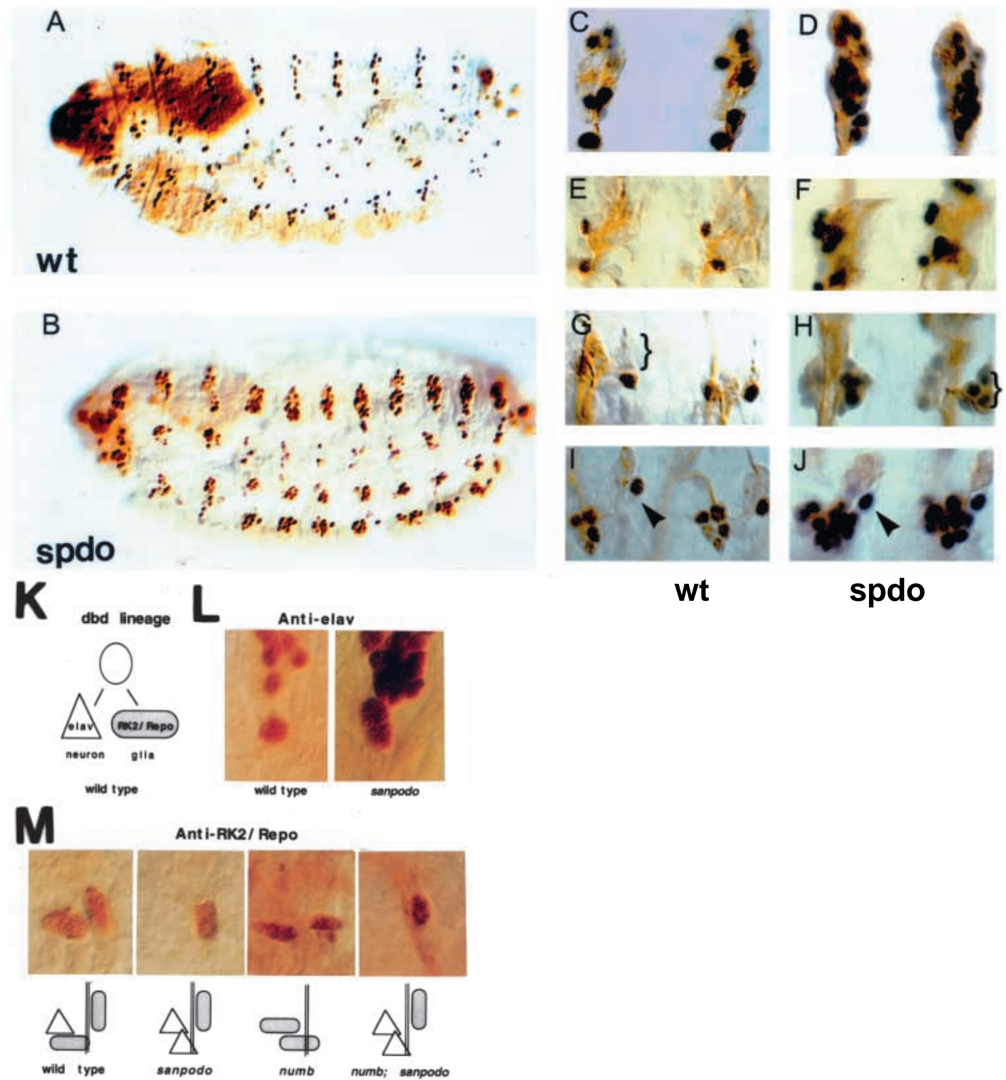


Fig. 2. The number of md neurons is increased in *spdo* mutants. (A) Wild-type stage 16 embryo containing the md-specific E7-2-36 enhancer detector stained with anti-β-galactosidase antibody. (B) Similarly stained E7-2-26; *spdo* mutant embryo. The number of md neurons is increased in all PNS clusters of *spdo* mutants when compared to wild type. (C-J) These panels are stained with anti-β-galactosidase antibody (black) and mAb 22C10 (brown). Panels on the left are wild type, those on the right are mutant. (C,D) Dorsal clusters. (E,F) Lateral clusters. (G,H) Ventral prime (v′) cluster. (I,J) Ventral cluster. The brackets in G and H indicate the position of neurons and glial cell of a polyinnervated es organ (v′es2) that is closely associated with a β-galactosidase-positive md neuron (v′pda). (H) The number of stained cells is increased in *spdo* mutants, partly because the neurons and the presumptive glial cell of v′es2 now express β-galactosidase. (I,J) Ventral (v) clusters. Arrowheads point to the vpda md neuron. (J) While the number of md neurons is increased in the vmd5 cluster (average is about 10), there is only a single vpda neuron in *spdo* mutants. (K) The dbd lineage is simple: a precursor cell divides to give rise to the dbd neuron and its associated glial cell. (L) Anti-ELAV (and other neural markers like mAb 22C10, E7-2-36, not shown) staining shows two dbd neurons in *spdo* mutants at the ventral area of the dorsal cluster (see Fig. 1A for position of dbd neuron and glial cell). (M) Anti-RK2/REPO labels glial cells and the sibling cell of the dbd neuron. In wild-type embryos, there is one horizontal glial cell, which is associated with the dbd neuron, and one vertical peripheral glial cell, which is associated with the axons of the neurons of the dorsal cluster. This peripheral glial cell is not related to the dbd lineage, but is a convenient marker. In *spdo* mutants, the dbd-associated glial cell is absent. In *numb*^{1} mutants, we observe the opposite phenotype: there are two horizontal glial cells, no neurons (not shown) and the peripheral glial cell associated with the axon bundles is also missing (see also Brewster and Bodmer, 1995). In double mutant embryos *numb*^{1}*; P{lacZ, w*$^+$*}spdo*^{H7}, we observe the *spdo* phenotype: a single peripheral glial cell associated with a thick bundle of axons (here faintly labeled with mAb 22C10) and two neurons (as indicated by ELAV staining). Thus *spdo* fully suppresses the *numb* phenotype in the dbd lineage.

As shown in Fig. 5, the RNA detected with the *E42* cDNA is expressed in embryos at all developmental stages. At cellularization, we detect transcript in most or all cells, and this ubiquitous/basal expression remains throughout embryogenesis, concomitant with several bursts of transcription in discrete cells or tissues. During germ-band extension, expression is initiated in the CNS precursors and a set of uncharacterized dorsally located cells (Fig. 5A). Enhanced staining in the PNS is first detected at stage 12.3 (Fig. 5D). Initially, only four to five cells per segment can be distinguished, then, at completion of germ-band retraction (stage 13; 9 hours. AEL), there is a sudden marked increase in both levels of expression and the number of cells transcribing message, and expression in PNS cells becomes obvious (Fig. 5B,C). Many cells of the lateral cluster initially express slightly higher levels of *E42* than the surrounding cells, but elevated levels of expression are maintained only in the es support cells in stage 14-16 embryos (Fig. 5C,H). The highest concentration of mRNA is typically found in the areas where the two support cells are in contact (Fig. 5H). As shown in the developmental northern blot in Fig. 5I, this burst of expression corresponds with the appearance of a second transcript of 3.6 kb.

The following observations demonstrate that the *E42* cDNA corresponds to the *spdo* gene. First, the expression pattern of *P{lacZ, w*$^+$*}spdo*^{H7} /+ is quite similar to the *E42* cDNA expression pattern (data not shown) and the P element is inserted in the proximity of the first coding exon (Fig.

4A,B). Second, the *spdo* phenotype associated with *P{lacZ, w⁺}spdo*^{H7} is revertible upon precise or near precise excision of the P element. Third, imprecise excisions of *P{lacZ, w⁺}spdo*^{H7} that are associated with small deletions affecting only the first exon of *E42* are severe *spdo* alleles (data not shown). These mutant embryos, as well as embryos that are homozygous for *P{lacZ, w⁺}spdo*^{H7}, lack only the basal, ubiquitous expression when probed with the *E42* cDNA (Fig. 5E), yet exhibit a severe *sanpodo* phenotype. This suggests that the expression of *E42* in ectodermal cells is functionally relevant for specification of cell fate in the PNS (see discussion). Fourth, mutant *spdo*^{ZZ27} and *spdo*$^{1309/10}$ embryos lack any *E42* hybridization signals (Fig. 5F), including the support-cell-specific expression, and likely are null alleles. Fifth, in situ hybridization with the *K7* transcript shows no changes in the expression of this transcript in mutant embryos when compared to wild type (data not shown). Lastly, as shown in Fig. 5K,L, ubiquitous expression of the *E42* cDNA induced by heat shock in 5- to 7-hour-old embryos permits a highly consistent but partial rescue of many Prospero-positive cells in *spdo* mutant embryos. Combined, these observations demonstrate that the *E42* cDNA corresponds to a transcript of the *spdo* gene and that ubiquitous expression of SPDO is sufficient to partially rescue the phenotype.

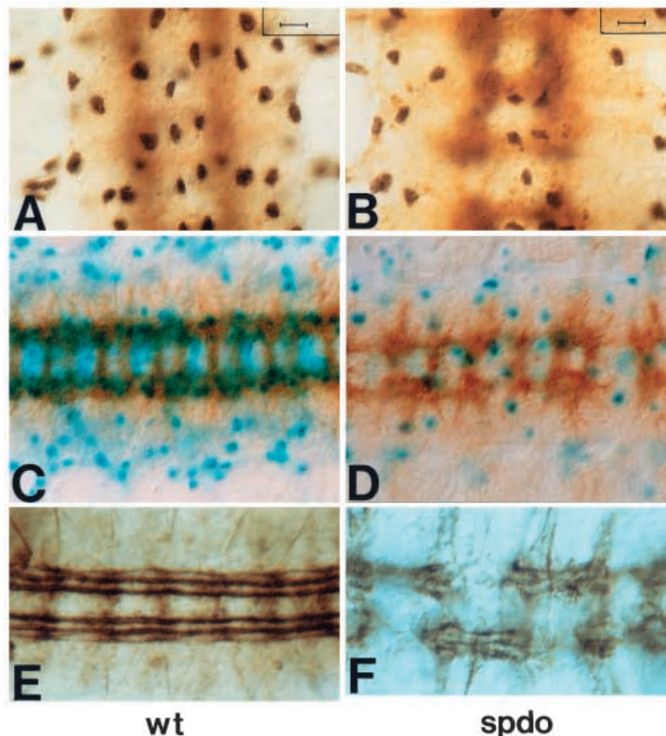


Fig. 3. CNS glia are missing in *spdo* embryos. Dorsal view of stage 15/16 wild-type (A,C,E) or *spdo* mutant (B,D,F) ventral nerve cords (VNC), anterior is top in A and B. Scale bar represents approximately 5 µm. (A,B) anti-REPO/RK2 and mAb BP102 staining. The REPO/RK2 protein is expressed in the nuclei of virtually all CNS glia, with the exception of the midline glia. mAb BP102 recognizes most axons of the VNC. (A) Wild-type embryos have approximately 25-30 RK2/REPO-positive glial cells per hemisegment, only one third of which are visible in this focal plane. This number is reduced approximately three-fold in *spdo*^{C55} embryos (B). (C,D) β-galactosidase and BP102 staining. (C) An enhancer detector insertion in the *gcm* gene (rA87), (Klambt et al., 1991) drives β-galactosidase expression in all glial cells expressing *gcm* transcripts (Jones et al., 1995). (D) *spdo*^{ZZ27} mutants exhibit approximately 30-40% of the number of β-galactosidase-positive cells observed in wild-type embryos. mAb BP102 was used to highlight the longitudinal tracts and the commissures. (E,F) Staining with mAb 1D4 recognizes a subset of axons within the longitudinal tracts (Van Vactor et al., 1993). Many sections of the longitudinal tracts are absent in the VNC of *spdo*^{C55} mutant embryos (F, see also B and D) and the commissural axon bundles appear somewhat thicker than in wild-type embryos (E).

sanpodo disrupts external sensory organ hair differentiation

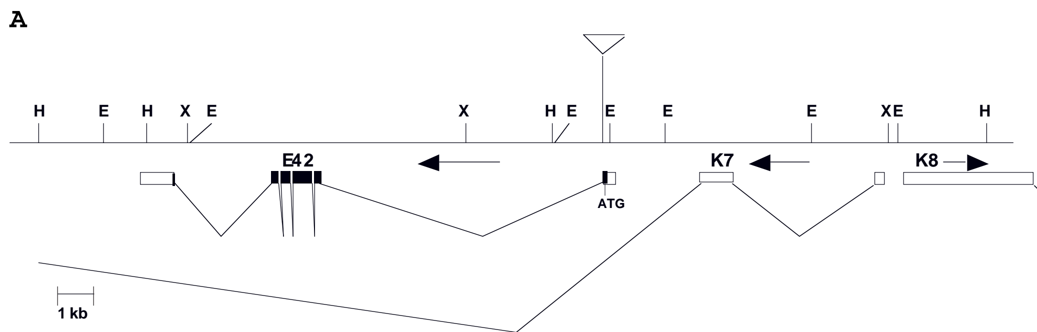
The hairs of external sensory organs have been shown to be reduced in size and altered in morphology in *singed* and *chickadee* mutants. These genes encode, respectively, an actin-bundling protein, Fascin, and G-actin binding protein, Profilin (Cant et al., 1994; Verheyen and Cooley, 1994). Because *spdo* is a putative cytoskeletal protein, we wished to determine if lack of *spdo* also causes any defects in the morphology of the es hairs of first instar larvae. Examination of cuticle preparations of pharate first instar larvae revealed that the three hairs of Keilin organs, a cluster of external sensory organs in the thoracic segments, are almost always affected in *spdo* mutants (Fig. 6A-D). When compared to wild type (Fig. 6A), the hairs are most often reduced in size or absent (Fig. 6B). In addition, the morphology of the socket cells is often abnormal (Fig. 6D). In approximately 10% of the mutant embryos, we observe extra hair cells and a corresponding lack of socket cells, suggesting that the socket cells are transformed into hair cells. We also fail to observe the typical structures corresponding to the ventral Kolbchen, a refractive club-like sensory structure, in the thoracic segments that appear as round structures in the top of wild-type embryos in Fig. 6A and C. Hairs in the abdominal segments are often present, albeit reduced in size (data not shown). We therefore conclude that the differentiation of the external structures of many PNS organs are affected in *spdo* mutants and that these defects resemble those associated with mutations in genes encoding actin binding proteins. As shown in Fig. 6E-H, staining with phalloidin reveals a severe decrease in F-actin staining in Keilin organs and Kolbchen organs in *spdo* mutants (Fig. 6G,H) when compared to wild type (Fig. 6E,F). This decrease in F-actin staining is observed for the external structures of the entire PNS in mutants (data not shown).

sanpodo is epistatic to *numb*

It is thought that in *numb* mutant embryos there are increased levels of Notch signaling (Guo et al., 1996; Spana and Doe, 1996), resulting in a transformation of SOPIIb cells into SOPIIa cells. Consequently, loss of *numb* reduces the number of PNS neurons (Uemura et al., 1989). Because this phenotype is opposite to that of *spdo* mutants, we were interested in determining the epistatic relationship between *numb* and *spdo*. We analyzed *numb;spdo* double homozygous embryos with both neuronal and glial markers to determine if the phenotype caused by loss of *numb* required the presence of SPDO or vice versa. When compared to wild-type (Fig.

7A), *spdo* mutants exhibit roughly twice the normal amount of neurons (Fig. 7B, see also Fig. 1D); embryos that lack only *numb* function (Fig. 7C) have 3-8 neurons per hemisegment (compared to approximately 35 in wild-type). Double mutants (Fig. 7D) never exhibit the *numb* phenotype, rather, the number of neurons is more similar to that seen in *spdo* embryos, showing that loss of *spdo* partially suppresses the *numb* phenotype.

Analysis of the epistatic interactions between *numb* and *spdo* within a single PNS lineage also demonstrates that *spdo* is epistatic to *numb*. The dbd lineage of the PNS consists of one neuron and one glial cell derived from a single precursor cell (Fig. 2K). In the absence of *numb*, two dbd glial cells are seen; in *spdo* mutants, the dbd glial cell is absent. In double mutants, the dbd glial cell is missing, as is seen in *spdo* embryos (Fig. 2M). These observations suggest that *spdo* functions downstream of, or antagonistically to, *numb* in this cell lineage to specify cell fate.



B

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CATGGAG  gt  tagtagtgttgcaattgttgctgccgctcaagtgtctaaattgtttttcattgatgtttttttttttgtgggctgtt ..(P-ELEMENT
INSERTION) tgccttgaatgatttcatttcattaatttagtttttgtttgcctc  7 kb intron  ..tttaagtatatattttataagataatgtaatcct
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ttttc ag ACTTCTGCCACCACAAAGAC
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C

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RAT-E                              MSYRRELEKYRDLDEDEILGALTEEELRTLENELDELDPDNALLPAGLRQKDQTTKAPTGPFKREELLDHLEKQAKEFKD
DROSOPHILA  METSATTKTTTLTTPAKLYGKDLSEYDDVDVESLLAQLSPEEITIL---AKEVDPDDNFLPPDQRNSYECTKEATGPLNRKQLIEHINKQAIETPD
RAT-N                            MALPFQKGLEKYKNIDEDELLGKLSEEELKQLENVLDDLDPESATLPAGFRQKDQTQKAATGPFDREHLLMYLEKEALEQKD

RAT-E       REDLVPY-TGEKRGKIWVP---KQKPMDP-VLESVTLE--PELEEALANASDAELCDIAAILGMHTLMSNQQYYQALGSSSIVNKEGLNSVIKPTQ
DROSOPHILA  QPEFEPFVQGKVRGKKWVPPPRDARDIEA--EEQIAIDMGEEYEHALNDATQEEIIDLAAILGFHSMMNQDQYHASLLNKGQPVGLGWDGITKSTQ
RAT-N       REDFVPF-TGEKKGRVFIP---KEKPVETRKEEKVTLD--PELEEALASASDTELYDLAAVLGVHNLLNNPKFDE-ETTNGQGRKGPVRNVVKGEK

RAT-E       YKPVPDEEPNPTDVEETLERIKNNDPELEEVNLNNIRNIPIPTLKAYAESLKENSYVKKFSIVGTRSNDPVAFALAEMLKVNKVLKTLNVESNFIS
DROSOPHILA  QKLFPMDPPNNTDVEESIKRVKDDDSKLIDLNLNNIKNISDEKLEQLFAALPQNEHLEVLSLTNVGLTDKTALLLAAAIEKSKTLRVLNVETNFIS
RAT-N       AKPVFEEPPNPTNVEASLQQMKANDPSLQEVNLNNIKNIPIPTLKEFAKALETNTHVRKFSLAATRSNDPVALAFAEMLKVNKTLKSLNVESNFIT

RAT-E       GAGILCLVEALPHNTSLVELKIDNQSQP-LGNKVEMEIVNMLEKNTTLLKFGYHFTQQGPRLRASNAMMNNNDLVRKRRLADLTGPIIPKCRSGV
DROSOPHILA  PPVIVKLVQALLKCHTIEEFRASNQRSAVLGNKIEMEITDLVEKNSSLLRLGLHLEFNDARHRVAAHLQRNIDRIRVKRLNQRK
RAT-N       GAGILALVEALRENDTLTEIKIDNQRQ-QLGTAVEMEIAQMLEENSRILKFGYQFTKQGPRTRVAAAITKNNDLVRKKRVEGDRR
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D.

% IDENTICAL (SIMILAR)

		# Amino Acids	N	E	D	C1	C2
N	n-tropomodulin(rat)	351		60(92)	34(80)	16(71)	29(76)
E.	e-tropomodulin(rat)	359	[345]		35(71)	13(77)	27(78)
D.	sanpodo (Drosophila)	363	[355]	[350]		15(72)	28(49)
C1	C. elegans	319	[252]	[318]	[215]		20(80)
C2	C. elegans	687	[206]	[226]	[332]	[209]	

[#AMINO ACIDS IN ALIGNED REGION]

Fig. 4. *spdo* encodes a homolog of tropomodulin. (A) A 26 kb genomic walk (restriction enzymes are E, *Eco*RI, H, *Hin*dIII, and X, *Xba*I) and approximate mapping position of three cDNAs, *K8*, *K7* and *E42*, which map in the vicinity of the *P{lac-Z, w+}spdo*^{H7} (stalked triangle). The precise mapping position of the P element is shown in B. *E42* corresponds to *spdo*, spans 12.4 kb and is localized in the second intron of the *K7* gene. The *E42* cDNA has at least six exons, five of which are coding. ORFs are shown with closed boxes except for *K7*. The introns are 7 kb, 62 bp, 61 bp, 72 bp and 2.5 kb. (B) Genomic sequence surrounding the site of the P-element insertion. The P element is inserted in the first intron, in proximity of the first exon of the cDNA. Lower case, genomic sequence; upper case, sequence of cDNAs; bold, ORF. (C) Alignment of SPDO with rat N (neural) and E (erythrocyte) tropomodulin. The predicted SPDO protein is estimated to be 42 kDa. SPDO shows similar homology (35-6% I; 69-75% S) to two known vertebrate Tropomodulins. (D) Alignments of sequences using the PIMA program (Human Genome Center, Baylor College of Medicine) indicate that proteins with regions of very high similarity to SPDO are found in organisms ranging from *Caenorhabditis elegans* to higher vertebrates. The sequence of *spdo* has the GenBank accession number U92490.

DISCUSSION

Sanpodo is required for asymmetric cell fate specification

Here, we describe the characterization of *spdo*, a gene required for identity of sibling cells. In the absence of SPDO, sibling cells of the PNS take the same fate, i.e. instead of producing two different daughter cells, a precursor cell gives birth to two identical neurons. The following evidence shows that the PNS glial cells in the es lineages are transformed into neurons: first, the number of SOPI and II is not altered in *spdo* mutants (Salzberg et al., 1994); second, with the exception of some cells associated with solo-md neurons (Brewster and Bodmer, 1995), the total number of PNS cells is not increased, indicating that no extra divisions occur in *spdo* mutants; third, molecular markers that identify glial cells reveal that they are absent in the PNS. A similar transformation of glia into neurons occurs in an md cell lineage as the sibling glial cell associated with the dbd neuron also takes a neuronal fate. The above data clearly show that Sanpodo plays a key role in cell fate determination of glial cells that are siblings of neuronal cells. However, Sanpodo is not only required in glial cells as we observe a doubling of some solo md neurons. The solo md neurons are derived from a precursor cell that gives rise to an md neuron and an ectodermal cell (Bodmer et al., 1989). As we observe no extra recruitment of PNS precursors (Salzberg

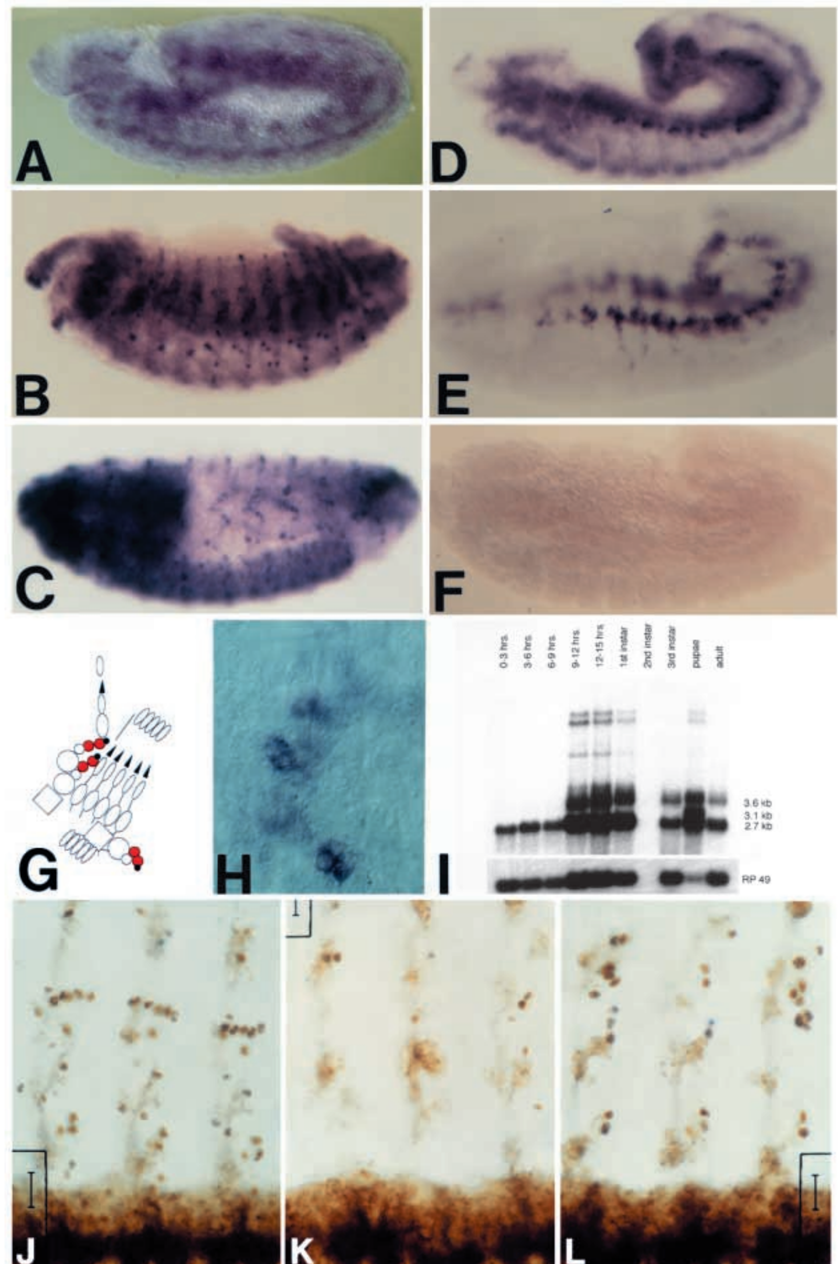

Fig. 5. *spdo* is expressed in most embryonic cells throughout development and the spdo phenotype can be rescued by overexpression of SPDO protein. (A-F,H) RNA in situ hybridization of the *E42* cDNA to wild-type and mutant embryos. All embryos are shown in a lateral view, with anterior to the left, and dorsal up. (A) Early stage 12. Expression can be seen throughout the embryo. (B) Stage 13. The ubiquitous ectodermal staining and enhanced expression in specific PNS cells, most noticeably the es-associated cells, can now be seen. (C) Stage 16 embryo. Expression is more abundant in the CNS and some PNS cells. (D) Stage 12-13 of a wild-type embryo. Note the higher levels of expression in the CNS, the visceral mesoderm and clusters of unknown cells beneath the dorsal ridge of the ectoderm. (E) Stage 12-13 *P{lacZ, w⁺}spdo* $^{\Delta H7}$ (allele #1035). Expression of *spdo* is limited to those cells that express higher levels of transcripts initiated during late stage 12, early stage 13 in wild-type embryos. (F) Stage 12-13 $spdo^{ZZ27}$ / $spdo^{ZZ27}$ embryo. No zygotic *spdo* expression is detected in these embryos. (G) Diagrammatic representation of *SPDO* expression (indicated in red) in the es support cells (hair and socket cell) of the lateral cluster of one abdominal hemisegment of a mature wild-type embryo. (H) Lateral, high magnification view of the lateral cluster of one abdominal hemisegment of a stage 16 wild type embryo. (I) Developmental northern analysis using *E42* as a probe. Three abundant transcripts can be seen; one constitutive transcript of 2.7 kb, one induced at 9-12 hours AEL of approximately 3.6 kb, and one pupal-specific transcript of 3.1 kb. Three high molecular weight transcripts of 5.8, 7.3 and 7.9 kb are also observed beginning at 9-12 hours and continuing through the pupal stage. The RP49 probe was used as a loading control. Transcript lengths were determined with RNA standards. (J-K) Lateral view of three abdominal hemisegments of dissected stage 15 embryos (anterior is left, dorsal on top), stained with anti-Prospero and mAb BP102 antisera. Scale bar represents approximately 12.5 µm. A heat-shocked wild-type embryo (J) has 21 Prospero-positive cells per hemisegment (not all cells are in the same focal plane and only 16-17 can be seen here). In *P{lacZ, w⁺}*$spdo^{H7}$ mutant embryos that received heat shock (K), there are 1-4 cells per hemisegment. In hs-E42; *P{lacZ, w⁺}*$spdo^{H7}$ embryos, heat shocked at 5-7 hours AEL and aged for 10 hours (L), there are 6-10 cells per hemisegment, showing that the *E42* cDNA can partially rescue the number of Prospero-positive cells and thus corresponds to the *spdo* gene.

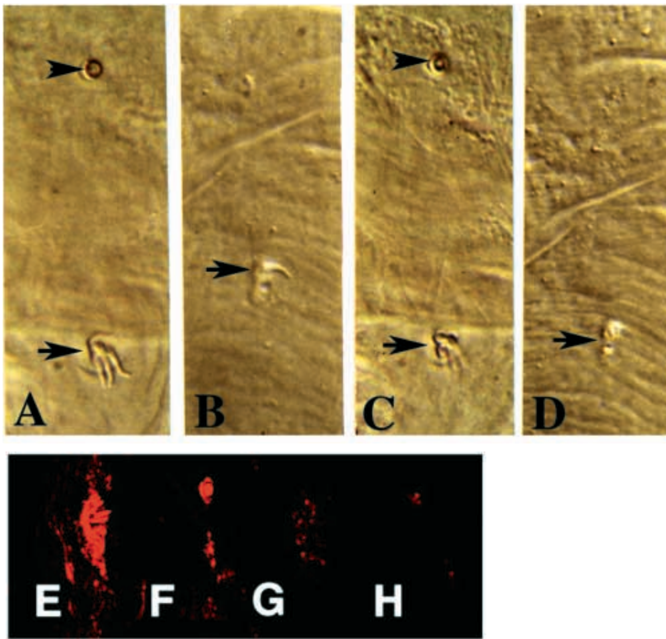

Fig. 6. Loss of SPDO disrupts ES support cell differentiation and actin organization. (A-H) Ventrolateral views of the third thoracic segment of first instar larvae with focus on the Keilin organs and ventral Kolbchen (Dambly-Chaudiere and Ghysen, 1986) in wild-type (A,C,E,F) and *spdo*^{ZZ27} mutants (B,D,G,H). (A,D) Wild-type Keilin organs are composed of three hairs emanating from sockets (arrow). Note the single short hair (B) and the absence of a socket cell in the mutant Keilin organ (arrow in D). *spdo* mutants are also missing the ventral Kolbchen (the refractive round structures indicated by arrowheads in A and C). (E-H) Phalloidin staining of F-actin in embryos. (E) Wild-type Keilin organ and (F) Kolbchen. (G) *spdo* Keilin organ and (H) Kolbchen. Note the reduced F-actin staining in both hair and other cells of the Keilin organ and cells of the Kolbchen when compared to wild-type staining.

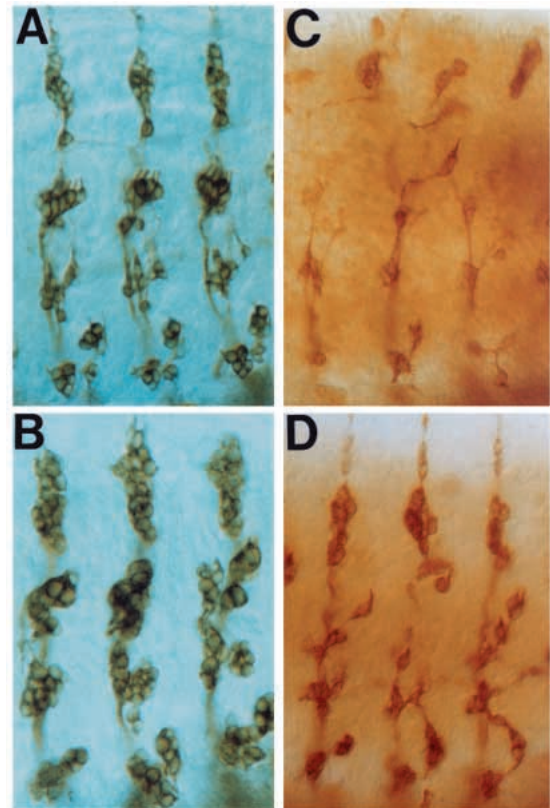

Fig. 7. *spdo* is Epistatic to *numb*. (A-D) Lateral view of three abdominal hemisegments of stage 15-16 embryos oriented with anterior left, dorsal up, after immunohistochemical staining with mAb 22C10. (A) Wild-type embryo. (B) *P{lacZ, w*$^+$*}spdo*^{H7} mutant embryo. (C) *numb*^{1} embryo. (D) *numb*^{1}*; P{lacZ, w*$^+$*}spdo*^{H7} embryos exhibit a significant increase in the number of neurons when compared to those that only lack *numb*.

et al., 1994), and as we and others (Skeath and Doe, 1997) observe highly specific cell fate changes in many siblings of other cell lineages of the CNS of *spdo* mutants, we propose that a lineage related cell of solo-md neurons is transformed into a neuron. Finally, an md neuron which has no known sibling, the vpda, is not duplicated in *spdo* mutants. We therefore conclude that SPDO is required to specify the fate of sibling cells in PNS neuronal cell lineages, and in *spdo*'s absence the siblings of neurons follow the default or neuronal fate.

Changes in cell fate may also underlie the severe disruption of the longitudinal tracts in the CNS, since many RK2/REPO-positive glial cells along the longitudinal tracts are lacking. Disruptions in the longitudinal tracts are also observed in *gcm* mutants, a gene that controls RK2/REPO expression (Akiyama et al., 1996) and that has been proposed to act as a binary switch of neuronal versus glial cell fate (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996). Interestingly, specific cells that normally express *gcm* in the CNS fail to do so late in embryonic development in *spdo* mutants. However, we cannot determine if the lack of *spdo* results in a cell fate switch in the CNS, as is occurring in the PNS, or that failure to maintain *gcm* expression causes a cell fate switch.

spdo encodes a tropomodulin homolog

Cloning and sequencing has revealed that *spdo* encodes a tropomodulin (tmod) homolog. Tmod was first characterized in human erythrocytes as a monomeric 43 kDa tropomyosin binding protein (Fowler, 1987). Subsequent analysis revealed that tmod was the first identified capping protein of the slow growing (pointed) end of actin filaments (Weber et al., 1994). This capping function is necessary to maintain filament length in cardiac myocytes and presumably in other tissues in which it is expressed (Gregorio et al., 1995). Tmod is widely expressed in mouse embryos in neuronal and non-neuronal tissues, including the cells that give rise to the PNS (Ito et al., 1995). Recently, a neural-specific tmod gene (N-tmod) was isolated by virtue of its ability to bind a neural-specific tropomyosin isoform (Watakabe et al., 1996). Vertebrate N-tmod and E-tmod are quite divergent for cytoskeletal proteins since they exhibit only 60% identity (87% similarity). Interestingly, SPDO shares similar homology to both proteins (Fig. 4C and D), suggesting that N-tmod and E-tmod have evolved from the same ancestral protein, a SPDO-like protein. Given the relatively low homology between the vertebrate E- and N-tropomodulins, the 35% identity/ 70% similarity of SPDO with these proteins must be regarded as highly significant. We found no SPDO homolog in yeast, suggesting

that tropomodulins originated in higher eukaryotes, possibly because of the need for a more complex control of the cytoskeletal network.

Is SPDO required to maintain actin filament length in *Drosophila* as proposed in vertebrates (Gregorio et al., 1995)? Although we do not know the precise answer, the fact that *spdo* embryos display defects in the es hair cells, including severely decreased levels of F-actin, suggests that SPDO and the actin cytoskeleton have an intimate relationship. Mutations in other actin binding proteins such as Profilin (Verheyen and Cooley, 1994), Fascin (Cant et al., 1994) and Actin Capping Protein (Hopmann et al., 1996) have also been found to affect bristle length and morphology. Our data indicate a role for *spdo* in the control of actin polymerization and organization, but this role needs to be explored in more detail.

Ubiquitous expression of SPDO is required for proper cell fate specification

When, and in which cells, is *spdo* required during development? The ubiquitous and continuous expression of SPDO does not permit us to assign a temporal or spatial restriction to its requirement. Although an enhanced cell-specific expression in the PNS (and some other tissues or cells) is observed during late stage 12, several observations suggest that this expression is not relevant to the cell lineage defects. First, the P-element insertion mutant, *P{lacZ, w+}spdo*^{H7}, causes a complete absence of the ubiquitous expression of *spdo*, but expression in support cells is not affected (Fig. 5E). Yet, homozygous mutant *P{lacZ, w+}spdo*^{H7} embryos lack glial cells. Second, the elevated levels of *spdo* in the PNS occur too late in development to play a role in cell fate determination. The earliest time point that we observe higher levels of *spdo* expression in the PNS is during late stage 12, when all divisions giving rise to the PNS organs have already occurred (Bodmer et al., 1989). Third, the message is enriched only in the hair and socket of the es organs, but not in the glial cells. We therefore conclude that the ubiquitous expression prior to stage 12.3 is required for proper lineage specification, and that the support cell-specific expression indicates a temporally distinct function of SPDO. Hence, Sanpodo expression resembles that of most neurogenic genes, e.g. *Notch*, *Delta* and *Su(H)*, during neurogenesis.

SPDO functions downstream of Numb and is a putative component of the Notch signaling pathway

Within the es lineage, *numb* function is required in both the SOPIIb and its daughter cells to correctly specify cell fate. *spdo* function is also required within the es lineage, but in a manner opposite to that of *numb*. To place *spdo* in the Numb/Notch pathway, we undertook an analysis of *numb*;*spdo* double mutants. Our data show that *spdo* function is likely downstream of *numb*, because *numb*;*spdo* embryos have many more neurons than those that only lack *numb*. Alternatively, SPDO functions as an antagonist of Numb. Based on the epistatic interaction studies, we propose that the transformation of SOPIIb into SOPIIa brought about by loss of *numb* is often blocked in the absence of *spdo*. Therefore, SPDO must function in the SOPIIb in addition to its function in the es glia and neuron. Because loss of Numb in the SOPIIb leads to increased Notch signaling, Notch activation and/or signaling may require SPDO function. A role for SPDO in Notch signaling is supported by the finding that reducing Notch protein function at the time of SOPIIa division decreases the number of es glial cells and increases the number of es neurons (Guo et al., 1996). In addition, the es neurons express md-specific markers in *spdo* mutants, as observed in *Notch* mutants (Vervoort et al., 1997). It is therefore unlikely that SPDO functions in an independent pathway, although at the present time this possibility can not be formally excluded.

Is SPDO required in the SOPIIa cell? A requirement for SPDO in this cell and its progeny is less obvious. Morphological analysis of mutant pharate first instar larvae (see Fig. 7) and immunohistochemical staining with anti-CPO (Bellen et al., 1992) and anti-Cut (Blochlinger et al., 1990) clearly show that support cells are present in *spdo* mutants, albeit morphologically impaired. In addition, we observe occasional socket-to-hair transformations, suggesting that SPDO plays a role in these lineages as well. The phenotypic consequences of *spdo* loss in the SOPIIa cell may affect these cells less due to different requirements for SPDO protein or to cell-specific differences in the perdurance of maternally provided protein. To clarify this issue, the consequences of removal of maternally provided SPDO and removal of SPDO in adult tissues will have to be established.

SPDO plays two roles in development

In conclusion, we propose that SPDO plays two different roles in development. One function is indicated by the findings that *spdo* affects bristle and papilla morphogenesis, that it causes a reduction of F-actin in bristle and papilla, and that it encodes a tropomodulin homolog. This function is associated with late differentiation of the PNS structures, possibly because of lack of maintenance of F-actin filaments or their subsequent assembly into larger structures. At present, we can not rule out the possibility that the morphological defects that we observe in support cells are due to improper cell fate determination. However, we believe this is not the case because many hairs are present, indicating that, in some cases, cell fate has been correctly specified. Secondly, these hairs have been shown to have disruptions in polymerized actin, a phenotype often associated with mutations in actin binding proteins.

The second function of SPDO is to mediate cell signaling to specify sibling cell identity in asymmetric lineages. The current model of es determination is that upon ligand binding to the Notch heterodimer, the Suppressor of Hairless (Su(H)) protein becomes activated by forming a complex with the Notch ankyrin repeats (Blaumueller et al., 1997; Wettstein et al., 1997). In cells that inherit Numb, there is decreased activation of Notch. The Notch cytoplasmic domain, after being cleaved off, is thought to be translocated to the nucleus together with Su(H) where it activates target genes (Kopan et al., 1996; Kopan and Turner, 1996). However, it should be noted that processed forms of Notch have not yet been detected in the nucleus upon ligand binding. Based on both the phenotype and the predicted homology to an actin capping protein, one plausible function of the *spdo* product is to serve as a regulatory constituent within a multiprotein complex. This cytoskeletal-based clustering may be required for the proper spatial and temporal control of the activation of the Su(H)-Notch complex at the cell cortex. In vitro studies with cultured CNS neuroblasts clearly indicate that the cortical actin network is required for asymmetric protein localization (Broadus and

Doe, 1997). It is possible that similar mechanisms are utilized in the PNS to regulate Notch signaling activity to control cell fate.

Alternatively, a specific array of microfilaments and microfilament-associated proteins might be used to initiate transport or propel the Su(H)-Notch complex towards the nucleus. In this scenario, SPDO could function to stabilize such a cytoskeletal array or perhaps regulate the association of transport proteins with the actin filament in response to extracellular signals. The identification of proteins that interact with SPDO will serve as a useful entry point to begin to address these issues.

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