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Prospero distinguishes sibling cell fate without asymmetric localization in the *Drosophila* adult external sense organ lineage

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SUMMARY

The adult external sense organ precursor (SOP) lineage is a model system for studying asymmetric cell division. Adult SOPs divide asymmetrically to produce IIa and IIb daughter cells; IIa generates the external socket (tormogen) and hair (trichogen) cells, while IIb generates the internal neuron and sheath (thecogen) cells. Here we investigate the expression and function of *prospero* in the adult SOP lineage. Although Prospero is asymmetrically localized in embryonic SOP lineage, this is not observed in the adult SOP lineage: Prospero is first detected in the IIb nucleus and, during IIb division, it is cytoplasmic and inherited by both neuron and sheath cells. Subsequently, Prospero is downregulated in the neuron but maintained in the sheath cell. Loss of *prospero* function leads to 'double bristle' sense organs (reflecting a IIb-to-IIa transformation) or 'single bristle' sense organs with abnormal neuronal differentiation (reflecting defective IIb development). Conversely, ectopic *prospero* expression results in duplicate neurons and sheath cells and a complete absence of hair/socket cells (reflecting a IIa-to-IIb transformation). We conclude that (1) despite the absence of asymmetric protein localization, *prospero* expression is restricted to the IIb cell but not its IIa sibling, (2) *prospero* promotes IIb cell fate and inhibits IIa cell fate, and (3) *prospero* is required for proper axon and dendrite morphology of the neuron derived from the IIb cell. Thus, *prospero* plays a fundamental role in establishing binary IIa/IIb sibling cell fates without being asymmetrically localized during SOP division. Finally, in contrast to previous studies, we find that the IIb cell divides prior to the IIa cell in the SOP lineage.

Key words: SOP, Microchaete, Macrochaete, Axon outgrowth, *Drosophila*, Prospero, Sense organ, Cell lineage

INTRODUCTION

The development of the external sensory organs in the adult *Drosophila* notum begins with the formation of sensory organ precursors (SOP) in the wing disc epithelium. The SOP divides along the anteroposterior (AP) axis to produce the IIa and IIb secondary precursors; the posterior IIa cell generates the external socket (tormogen) and hair (trichogen) cells, while the anterior IIb cell gives rise to the internal neuron and sheath (thecogen) cells (Gho et al., 1996; Gho and Schweisguth, 1998; Hartenstein and Posakony, 1989; Wang et al., 1997). Macrochaete SOPs begin dividing around 0-1 hours after pupal formation (APF) and the microchaete SOPs divide at 14-16 hours APF (Hartenstein and Posakony, 1989). A careful orchestration of intrinsic and extrinsic cues is required for the specification of cell fate in the SOP lineage (Campos-Ortega, 1996; Jan and Jan, 1995; Posakony, 1994). Extrinsic signaling is mediated by the Notch pathway, in which Delta or Serrate ligand activates the transmembrane Notch receptor, resulting in nuclear translocation of an intracellular domain of Notch together with the Suppressor of Hairless (SuH) transcription factor (reviewed in Bray, 1998). Loss or reduction of Notch signaling can transform IIa into IIb, socket into hair or sheath cell into neuron; an activated Notch receptor can produce the opposite cell fate transformations for each of these cell types (reviewed in Campos-Ortega, 1996). Thus, regulating Notch activity is essential for establishing distinct sibling cell fates at each division in the SOP lineage.

Asymmetric localization of the membrane-associated Numb protein is a primary mechanism for restricting Notch signaling activity to just one of two sibling cells. Numb is segregated into the IIb cell during mitosis (Rhyu et al., 1994) and *numb* mutants have a transformation of IIb into IIa cell fate that is dependent on Notch function (Guo et al., 1995; Uemura et al., 1989). These results suggest that Numb antagonizes Notch signaling to confer IIb cell fate. A similar relationship of Numb inhibition of Notch signaling also controls hair/socket and neuron/sheath sibling fates at the next step in the SOP lineage (Jan and Jan, 1995; Posakony, 1994) and many or all sibling neuron fates in the CNS (Skeath and Doe, 1998; Spana and Doe, 1996; Spana et al., 1995).

The *prospero* gene encodes a divergent homeodomain transcription factor that is asymmetrically localized in CNS, PNS and non-neural lineages in the embryo (Hirata et al., 1995; Knoblich et al., 1995; Spana and Doe, 1995). In the embryonic CNS, neuroblasts divide asymmetrically to produce smaller ganglion mother cells (GMCs), with each

GMC generating a pair of neurons and/or glia (Goodman and Doe, 1993). Prospero protein is asymmetrically localized into the daughter GMC (Hirata et al., 1995; Knoblich et al., 1995; Spana and Doe, 1995), and *prospero* mutants fail to establish GMC-specific gene expression and show defects in neuron axon outgrowth (Doe et al., 1991; Vaessin et al., 1991). In the embryonic SOP lineage, Prospero protein is asymmetrically localized in the dividing SOP and partitioned into the IIb cell. Following IIb division, Prospero is transiently detected in the neuron and persists in the sheath cell throughout embryogenesis (Knoblich et al., 1995; Spana and Doe, 1995). *prospero* mutants show no cell fate defects in the embryonic SOP lineage, but there are clear defects in axon outgrowth (Doe et al., 1991; Vaessin et al., 1991). *prospero* is also expressed in the R7 photoreceptor neuron, where it is required for proper axon connectivity in the medulla (Kauffmann et al., 1996).

The *Drosophila* adult external SOP lineage is a model system for the study of asymmetric cell division (Campos-Ortega, 1996; Jan and Jan, 1995; Posakony, 1994), yet the expression and function of *prospero* in this lineage has not been determined. Here we investigate the role of *prospero* in the adult external sense organ lineage.

MATERIALS AND METHODS

Drosophila strains

All *Drosophila* strains were raised on standard cornmeal, yeast and agar food. For identifying cells of the SOP lineage, we used A101 (an enhancer trap insert into the *neuralized* gene that expresses *lacZ* in all cells of the SOP lineage) and A1-2nd-29 (an enhancer trap line expressing *lacZ* in the hair and socket cell; Hartenstein and Jan, 1992). For generating *prospero* loss-of-function clones, we crossed *hs-FLP*/+; *FRT82B pros*^{17}/+ males (kindly provided by V. Reddy and V. Rodrigues) to *y w, P[ry+ hs-neo FRT] 82B, P[w+ hs-πM] 87E, Sb, P[ry+ y+] 96E* / TM6B, *Hu, Tb* females and the progeny were heat shocked at 39°C for 60 minutes at second or third larval instar to induce FLP-mediated recombination. For generating misexpression of *prospero* in all cells of the SOP lineage, we used two methods: (1) we crossed *y w*; 109-68GAL4 / CyO (an enhancer trap insertion in the *scabrous* gene that expresses GAL4 in all cells of the SOP lineage; kindly provided by Y. N. Jan) to *w*; *w+ UAS-prospero* 17K-2 (kindly provided by Fumio Matsuzaki); when grown at 30°C the progeny from this cross show strong *prospero* expression in the SOP lineage at all stages assayed. (2) Larvae from the *w*; *w+, UAS-hsp70-prospero* 24H2 line (also kindly provided by Fumio Matsuzaki) were heat shocked at 39°C for 60 minutes at 14 hours APF to transiently induce *prospero* expression in all cells.

Colcemid treatment and immunohistochemistry

Dissections of 14-18 hours APF pupae were done in PBT (1× PBS, 1% BSA, 0.1% Triton), and the wing discs were either fixed for 40 minutes in fresh 4% formaldehyde diluted in PEM (100 mM Pipes, 2 mM EGTA, 1 mM $MgSO_4$) or treated for 2 hours in 5 mg/ml Colcemid (Sigma) in Schneider's medium and then fixed as described above. Primary antibodies were applied for 2 hours at room temperature and the tissue was rinsed with PBT. Primary antibodies used were: fluorescein-conjugated anti-HRP (1:200; Jackson ImmunoRes.), rat anti-Elav 7E8A10 (1:10; Developmental Studies Hybridoma Bank at the University of Iowa), rabbit anti-β-galactosidase (1:3000; Cappel), rabbit anti-phosphohistone H3 (1:500; Upstate Biotechnology), mouse anti-Prospero MR2A (1:4; Spana and Doe, 1995) and rat anti-Supressor of Hairless (1:1000; kindly provided by F. Schweisguth). DTAF-, LRSC- or Cy5-conjugated secondary antibodies (1:300; Jackson ImmunoRes.) were applied for 2 hours at room temperature; the tissue was rinsed in PBT, mounted in 95% glycerol with 1% n-propylgallate (Sigma) and viewed on a BioRad MRC-1024 confocal microscope.

Cuticle analysis

Adults were mounted in Hoyers medium and baked in a 55°C oven overnight and photographed on a Zeiss Axioplan. Scanning electron micrographs were taken of frozen specimens on a Hitachi microscope (kindly acquired by Dr John Bozzola at the Southern Illinois University electron microscope facility).

RESULTS

Expression of Prospero in the adult external SOP lineage

We used the A101 enhancer trap line to label all cells of the SOP lineage; A101 encodes a nuclear β-galactosidase (β-gal) protein (Huang et al., 1991; Usui and Kimura, 1993). To identify specific cells within the SOP lineage, we used the A1-2nd-29 enhancer trap line to mark the hair and socket cells (Hartenstein and Jan, 1992), anti-Elav to mark the pre-divisional IIb cell and the neuron (Bier et al., 1988), anti-BarH1 to mark the sheath cell (Higashijima et al., 1992; Leviten and Posakony, 1996), anti-phosphohistone H3 or condensed DNA to mark cells in mitosis (Ajiro et al., 1996; Broadus and Doe, 1997; Hendzel et al., 1997; Mahadevan et al., 1991), and external morphology to identify hair and socket cells; in addition, we use the dA10 enhancer trap line to mark hair and socket cells of the SOP lineage at the 4-cell stage (Gho and Schweisguth, 1998), and we use the stereotyped position of cells along the anteroposterior axis to confirm cell identity assignments (Gho et al., 1996; Gho and Schweisguth, 1998; Wang et al., 1997).

Using these cell-type-specific markers, we first detect Prospero in the nucleus of the IIb cell; it is cytoplasmic during IIb mitosis and then distributed to both neuron and sheath cell progeny. It is maintained at high level in the sheath cell, but only transiently detected in the neuron. Prospero is never detected in the SOP, IIa, socket and hair cells (Figs 1, 7A). In addition, we detect the IIb cell dividing before the IIa cell (Figs 1, 2). These results are surprising for two reasons. Previous reports claim that IIa divides before IIb (Gho et al., 1996; Gho and Schweisguth, 1998; Hartenstein and Posakony, 1989; Wang et al., 1997), but our genotypes show IIb dividing ahead of IIa (see Discussion). Also, the embryonic SOP localizes Prospero into the IIb cell (Knoblich et al., 1995; Spana and Doe, 1995), but the adult SOP does not localize Prospero protein, although Prospero is ultimately detected in IIb but not IIa.

To confirm the lack of Prospero asymmetric localization in the adult SOP lineage, we treated A101 imaginal discs with Colcemid to arrest SOP or IIb cells in mitosis. Colcemid treatment in embryos or in vitro primary embryonic cell cultures results in the accumulation of Prospero protein in asymmetric cortical crescents in mitotically arrested neuroblasts or SOPs (Broadus and Doe, 1997; Knoblich et al., 1995; Spana and Doe, 1995). We found that mitotically arrested SOPs did not have detectable Prospero protein (Fig. 2A). We observed mitotically arrested IIb cells in which the more posterior IIa cell was not yet in mitosis (Fig. 2B), and mitotically arrested IIb and IIa cells (data not shown); in both

cases, the IIb cell showed cytoplasmic Prospero localization, consistent with our observations in the wild-type SOP lineage. These results strongly support our conclusion that the IIb cell divides before the IIa cell, and also confirm our finding that Prospero protein is not asymmetrically localized in the mitotic SOP or IIb cells.

Loss of *prospero* gives a partial IIb-to-IIa cell fate transformation and failure of neuronal differentiation

To remove *prospero* function in the adult SOP lineage, we used the FLP/FRT system (Golic and Lindquist, 1989) to create *prospero* mutant clones in the wing and eye imaginal discs (see Materials and Methods); sense organs developing in the center of these mutant clones will be termed '*prospero*$^-$ sense organs'. We used morphological criteria to score the fate of the external bristle and socket cell types in *prospero*$^-$ sense organs. In females, *prospero*$^-$ clones in the notum contain 86% single bristles and 14% double bristles (two bristle shafts emerging from a single or fused double socket) (Table 1; Fig. 3); similar phenotypes are seen in males but with lower frequency (Table 1). A more penetrant phenotype is seen in eye clones, where there are 54% double bristles and 46% single bristles. Double bristle sense organs in notum and eye are usually composed of one morphologically normal bristle and one stunted bristle (Table 1; Fig. 3). We were unable to accurately quantitate whether there are one or two socket cells in the *prospero*$^-$ sense organs because two socket cells can fuse to form a single socket (Shiomi et al., 1994); however, double sockets are occasionally observed in *prospero*$^-$ sense organs (Reddy and Rodrigues, 1999). Our results are consistent with the normal differentiation of the IIa cell (into bristle and socket) and a partial transformation of the IIb cell into a IIa cell (in the sense organs with double bristles).

To score the fate of the IIb cell in adult *prospero*$^-$ sense organs, we assayed neuronal identity. Wild-type sense organs have a single associated neuron that has an axonal process projecting into the CNS and a dendrite that attaches to the base of the bristle/socket structure (Fig. 4A,B). In *prospero*$^-$ single bristle sense organs, a neuron is detected just internal to the bristle/socket cells, but the axon is stunted and often has a circular trajectory, whereas the dendrite is extremely stunted and usually fails to connect with the base of the bristle/socket cells (Fig. 4C,D). Our results suggest that loss of *prospero* results in abnormal neuronal differentiation in *prospero*$^-$ single bristle sense organs. In *prospero*$^-$ double bristle sense organs, there is a complete loss of neuronal staining (Reddy and Rodrigues, 1999). These results are consistent with a transformation of IIb towards a IIa cell fate in the double bristle *prospero*$^-$ sense organs.

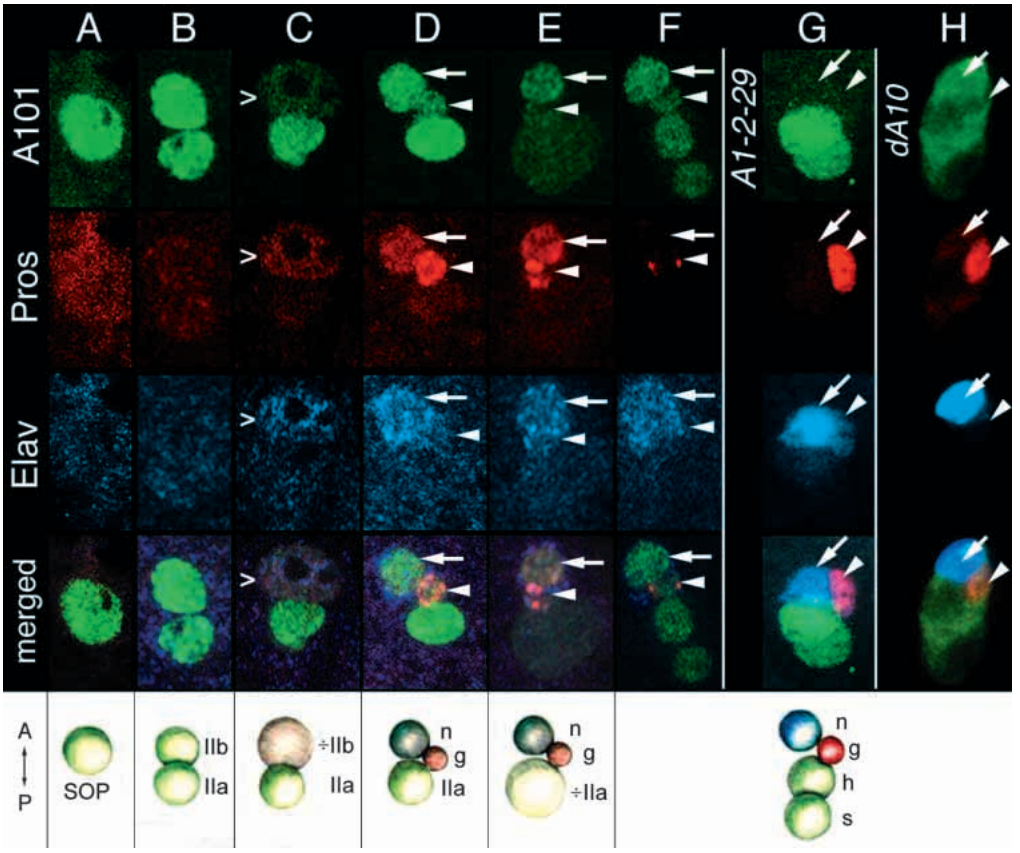

Fig. 1. Distribution of Prospero protein during the adult external SOP lineage. A101 labels all the entire SOP lineage with nuclear β-gal (green; A-F); 1-2-29 labels the socket and hair cells with nuclear β-gal (green; G); dA10 labels the neuron, sheath, socket and hair cells with cytoplasmic β-gal (green, H); Prospero (red; A-H); Elav (blue; A-H). Anterior is up in all panels. Images are from the dorsocentral region of the notum between 16 and 24 hours APF. (A) 1-cell stage. The SOP is Prospero$^-$ and Elav$^-$. (B) 2-cell stage: IIa, IIb. Both cells are Prospero$^-$ and Elav$^-$. (C) Late 2-cell stage: IIa, mitotic IIb. The anteriorly located IIb cell (>) is in mitosis as judged by condensed DNA (dark spots in cytoplasm; Broadus and Doe, 1997), large size, and cytoplasmic β-gal and Prospero proteins. The IIb cell is weakly Prospero$^+$ and Elav$^+$. (D) 3-cell stage: IIa, neuron (arrow, n), sheath cell (arrowhead, g). The posteriorly located IIa cell is Prospero$^-$ and Elav$^-$, while the neuron and sheath cell are Prospero$^+$ and weakly Elav$^+$. (E) Late 3-cell stage: mitotic IIa, neuron (arrow, n), sheath cell (arrowhead, g). All cells express A101; the neuron and sheath cell are Prospero$^+$ and weakly Elav$^+$. The posteriorly located IIa cell (unlabeled) is in mitosis as judged by condensed DNA (dark spots in cytoplasm; Broadus and Doe, 1997), large size, and cytoplasmic β-gal protein. (F) 4-cell stage: socket (s), hair (h), neuron (arrow, n) and sheath cell (arrowhead, g). All cells express A101; the neuron is weakly Elav$^+$ and the sheath cell is Prospero$^+$. (G) 4-cell stage: socket (s), hair (h), neuron (arrow, n) and sheath cell (arrowhead, g). The socket and hair cells express A1-2nd-29 (green), the neuron is Elav$^+$ and the sheath cell is Prospero$^+$. (H) 4-cell stage: socket (s), hair (h), neuron (arrow, n) and sheath cell (arrowhead, g). All cells express dA10; the neuron is Elav$^+$ and the sheath cell is Prospero$^+$.

Table 1. Loss or misexpression of *prospero* affects adult SOP development

	0 bristle 0 socket *n* (%)	0 bristle 1 socket *n* (%)	1 bristle 1 socket *n* (%)	2 bristles + socket* *n* (%)	3 bristles 1 socket *n* (%)
wild type					
microchaetes	0 (0)	0 (0)	1160 (100)	0 (0)	0 (0)
macrochaetes	0 (0)	0 (0)	110 (100)	0 (0)	0 (0)
eye bristles	0 (0)	0 (0)	40 (100)	0 (0)	0 (0)
prospero^{-} / *prospero*$^{-}$‡					
notum (female)	0 (0)	0 (0)	21 (86)	3 (14)	0 (0)
notum (male)	0 (0)	0 (0)	89 (97)	2 (3)	0 (0)
eye	0 (0)	0 (0)	18 (44)	22 (56)	0 (0)
hs-*prospero*§					
microchaetes	474 (68)	19 (3)	198 (28)	7 (1)	3 (0)
GAL4/*UAS-prospero*¶					
microchaetes	200 (100)	0 (0)	0 (0)	0 (0)	0 (0)
macrochaetes	50 (100)	0 (0)	0 (0)	0 (0)	0 (0)
eye bristles	889 (99)	0 (0)	11 (1)	0 (0)	0 (0)

*One socket or fused double socket are not distinguished.
‡Both microchaetes and macrochaetes scored.
§Heat shock done at 14 hours APF and thus macrochaete are not significantly affected.
¶109-68GAL4 × *UAS-prospero* gives continuous *prospero* expression in the SOP lineage.

prospero misexpression gives a IIa-to-IIb cell fate transformation

To determine whether misexpression of *prospero* in the IIa cell, or its hair and socket daughter cells, is sufficient to alter their cell fate or differentiation, we selectively misexpressed *prospero* throughout the adult SOP lineage. We crossed 109-68GAL4 flies (which express GAL4 in all cells of the adult SOP lineage) with flies containing a *UAS-prospero* transgene (which allows GAL4-inducible expression of the full length Prospero protein; see Materials and Methods). The 109-68GAL4/*UAS-prospero* flies are lethal as pharate adults, and are virtually bald, showing less than 1% of the normal external sense organ bristles in the notum and eye (Fig. 5A-D; Table 1). These results show that *prospero* misexpression within the SOP lineage is incompatible with the normal development of the IIa cell and/or its bristle and socket progeny.

To determine whether the IIa cell is transformed into a IIb cell following *prospero* misexpression, we stained wild-type and 109-68GAL4/*UAS-prospero* pupal nota for cell-type-specific markers: neuron, Elav; sheath cell, BarH1; socket cell, SuH; there is no marker for the hair cell (Fig. 5E-H). In wild-type nota, each SOP lineage contains one Elav^{+} neuron, one BarH1^{+} sheath cell and one SuH^{+} socket cell (Fig. 5E,G). In 109-68GAL4/*UAS-prospero* nota, we observe clusters expressing BarH1 (sheath marker) and/or Elav (neuron marker) but never SuH (socket marker). They were predominantly either 4-cell clusters (29/54) or 2-cell clusters (21/54); a small fraction were 3- or 5-cell clusters (2 of each). Among the 4-cell clusters, the most common phenotype is one cell strongly BarH1^{+}, one cell strongly Elav^{+} and two cells weakly expressing both BarH1 and Elav (15/29; Fig. 5F, arrowhead); other phenotypes are four cells weakly BarH1^{+} and one cell weakly Elav^{+} (5/29; Fig. 5F, arrow), and four cells weakly BarH1^{+} and two cells weakly Elav^{+} (4/29; data not shown). Among 2-cell clusters, the most common phenotype is that both cells have strong Elav and weak or no BarH1 (20/21; Fig. 5F, inset); the remaining cluster shows one Elav^{+} cell and one BarH1^{+} cell (data not shown). Within each cell, we observe either inverse levels of BarH1 and Elav, or mutually poor levels, suggesting that BarH1 and Elav negatively regulate each other. Our results show that misexpression of *prospero* blocks IIa cell differentiation, and can lead to duplications of IIb cell progeny. Taken together with the external hair/socket phenotype, we conclude that misexpression of *prospero* in the SOP lineage can produce a IIa-to-IIb cell fate transformation, resulting in a loss of IIa progeny (bristle/socket) and a duplication of IIb progeny (neuron/sheath cell). In addition, clusters with three or four BarH1^{+} sheath cells and only one Elav+ neuron indicate that

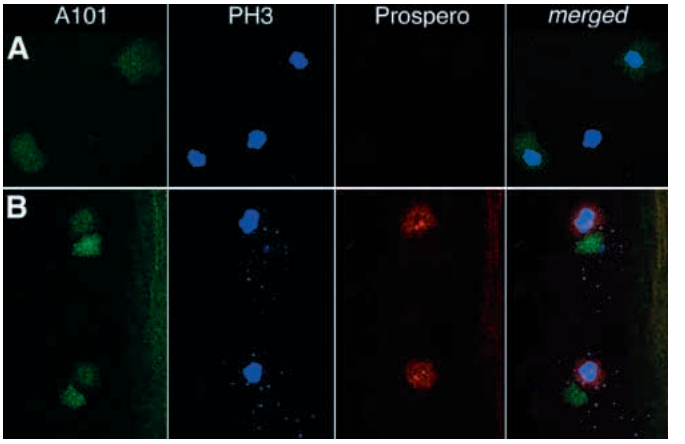

Fig. 2. Prospero is not asymmetrically localized in the adult external SOP lineage. Imaginal discs were treated with Colcemid to arrest cells in mitosis (see Materials and Methods) and stained for Prospero (red); A101 nuclear β-gal (green); and phosphohistone H3 (blue; a marker for mitotic cells). Anterior is up; images are from the dorsocentral notum. (A) Two mitotic SOPs are shown, both are Prospero^{-}. (B) A pair of IIA/IIB cell clusters are shown; in each the anterior IIB cell is mitotic and has cytoplasmic Prospero protein.

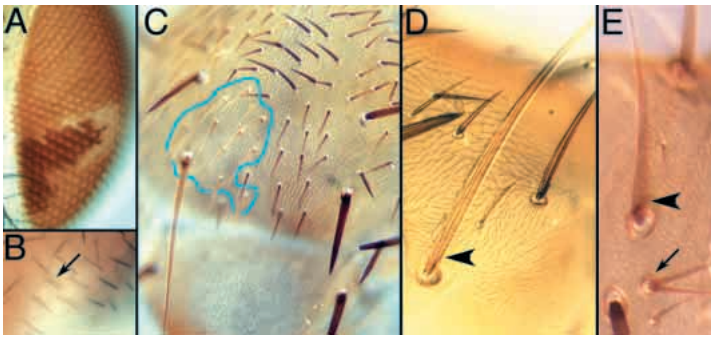

Fig. 3. Loss of *prospero* in the adult external SOP lineage results in single or twin bristles. Homozygous $pros^{17}/pros^{17}$ null clones in the notum and eye; $pros^{17}/pros^{17}$ clones are y^{-} Sb^{+} in the notum and w^{-} in the eye (see methods). (A,B) Eye clones show more sense organs with double bristles (56%; arrow) than with single bristles (44%). (C-E) Notum clones (blue outline in C) show predominantly single bristle sense organs (arrowheads); double bristles (shown here on the head by an arrow) occur in only 5% of the sense organs.

misexpression of *prospero* may induce a low frequency neuron to sheath cell transformation.

In addition to the 109-68GAL4/*UAS-prospero* misexpression studies, we used a *hsp70-prospero* transgene to misexpress *prospero* at specific times during the SOP lineage as well as in all surrounding cells (see Materials and Methods). There is fairly severe balding in the notum, with 90% of the heat-shocked flies missing ~20% of the microchaete (Fig. 6; Table 1). We also noticed an 'edge effect' along the perimeter of the bald areas in which double bristles are observed and a very low frequency of triple bristles (Fig. 6E). Internal cell fates under the bald patches of notum include a duplication of $Elav^{+}$ neurons within each SOP lineage (Fig. 6F), consistent with a IIa-to-IIb cell fate transformation of SOP lineages in the bald regions of the notum.

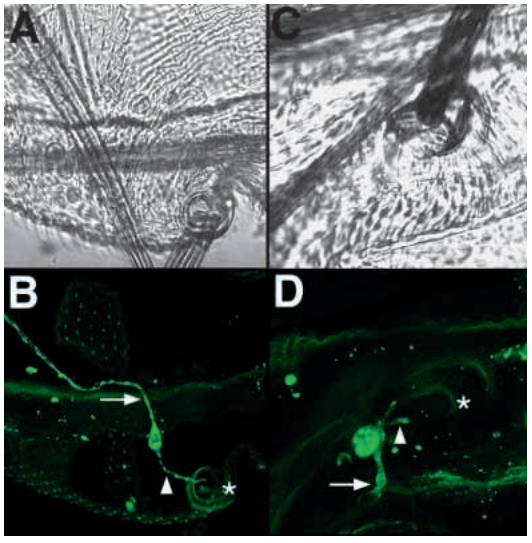

Fig. 4. Loss of *prospero* in the adult external SOP lineage results in defects in neuronal differentiation. (A,B) Wild-type adult external sense organ. (A) External bristle and socket. (B) The neuron associated with this exact bristle is detected by anti-HRP staining (green); the axon (arrow) extends straight into the CNS, and the dendrite (arrowhead) connects with the bristle/socket structure (asterisk). (C,D) $pros^{17}/pros^{17}$ adult external sense organ. (C) External bristle and socket. (D) The neuron associated with this exact bristle is detected by anti-HRP staining (green); the axon (arrow) is stunted and twisted, and the dendrite (arrowhead) fails to connect with the bristle/socket structure (asterisk).

DISCUSSION

The adult *Drosophila* external SOP lineage is a model system for the study of asymmetric cell division, in which both intrinsic and extrinsic mechanisms interact to control binary cell fate (Campos-Ortega, 1996; Jan and Jan, 1995; Posakony, 1994). The Prospero homeodomain protein is one of the first identified and best understood intrinsic determinants, yet until now, the expression and function of *prospero* in the adult external sense organ lineage has not been determined. We show that Prospero is first detected in the IIb nucleus, and during IIb mitosis, it is distributed to both neuron and sheath daughter cells. Loss of *prospero* results in either a IIb-to-IIa transformation or the defective development of the IIb neuronal progeny (Fig. 7B); ectopic *prospero* produces IIa-to-IIb cell fate transformations (Fig. 7C). Our observations suggest that Prospero plays an important role in specifying sibling cell differences, even if it is not asymmetrically localized. These findings have implications for the function of *prospero* homologues in other organisms, where the Prospero 'asymmetric localization domain' (Hirata et al., 1995) does not appear to be conserved.

The adult SOP lineage: IIb divides before IIa

The adult external SOP produces the IIa and IIb cells; IIa generates the external socket and hair cells, while IIb generates the internal neuron and sheath cells. Previous reports claim that IIa divides ahead of IIb (Gho et al., 1996; Gho and Schweisguth, 1998; Hartenstein and Posakony, 1989; Wang et al., 1997). However, in this and the accompanying paper (Reddy and Rodrigues, 1999), we find that the IIb cell divides before the IIa cell (Fig. 1). Although this has never been observed previously, we feel that our lineage is accurate for the following reasons. (1) At the 2-cell stage, we are sure the anterior cell is the IIb cell. Previous studies conclude that the anterior cell is the IIb cell (Gho et al., 1996; Gho and Schweisguth, 1998; Wang et al., 1997), and we show that anterior cell expresses Elav and Prospero, well-characterized markers for the neuron and sheath cells, which are progeny of the IIb cell (Bier et al., 1988; Bodmer et al., 1987; Gho et al., 1996; Gho and Schweisguth, 1998; Higashijima et al., 1992; Nakamura et al., 1994; Wang et al., 1997). (2) At the 2-cell stage, we are sure the anterior cell divides first. Mitotic cells are unambiguously identified using established markers: anti-phosphohistone (Ajiro et al., 1996; Hendzel et al., 1997; Mahadevan et al., 1991), condensed DNA (Broadus and Doe, 1997), or anti-tubulin in the accompanying paper (Reddy and

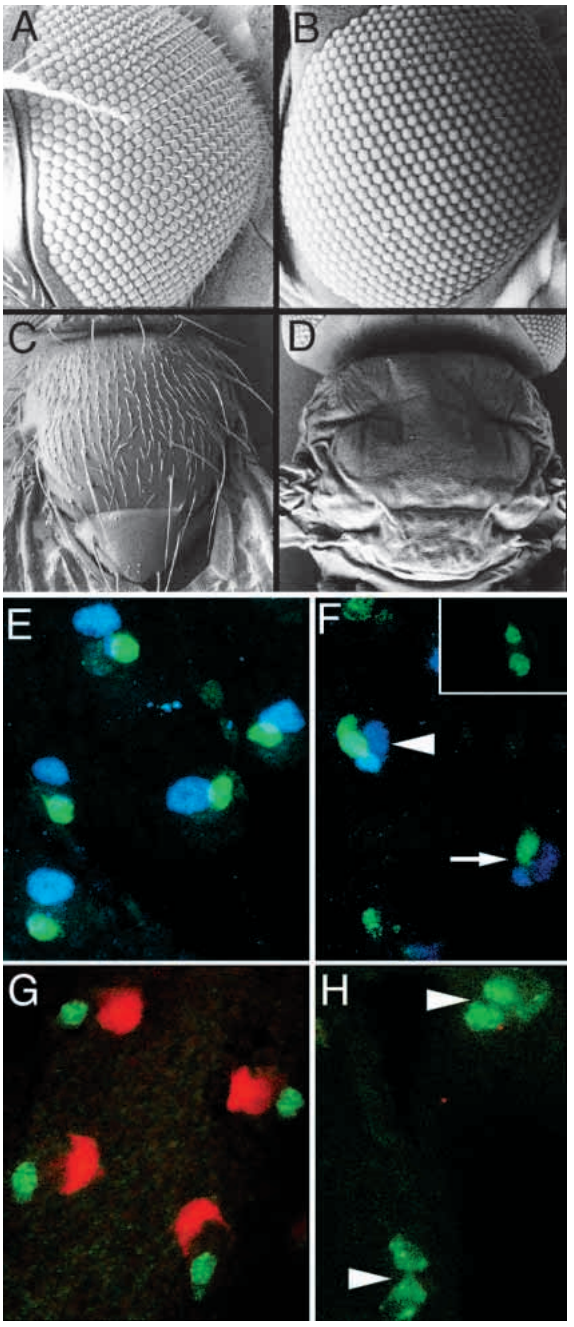

Fig. 5. Continuous ectopic *prospero* in the adult external SOP lineage results in duplication of IIb progeny (neuron/sheath cell) at the expense of IIa progeny (hair/socket cells). (A,C,E,G) Wild-type flies; (B,D,F,H) 109-68GAL4;*UAS-prospero* flies in which *prospero* is misexpressed specifically in the adult external SOP lineage. Anterior is at right (A,B) or at top (C-H). (A) Wild-type eyes show a single interommatidial sense organ bristle; (B) 109-68GAL4;*UAS-prospero* flies have very few interommatidial sense organ bristles. (C) Wild-type notum with normal single bristle sense organs; (D) 109-68GAL4;*UAS-prospero* notum lacks nearly all sense organ bristles. (E-H) Pupal nota at 14-18 h APF. (E,F) Staining for the sheath cells (BarH1; blue) and neurons (Elav; green). (E) In wild-type nota, there is one BarH1$^+$ sheath cell and one Elav$^+$ neuron; (F) in 109-68GAL4;*UAS-prospero* nota, we can detect three BarH1$^+$ sheath cells and one Elav$^+$ neuron (arrow); or two BarH1$^+$ sheath cells and two Elav$^+$ neurons (arrowhead); or just a pair of cells that are both strongly Elav$^+$ and weakly BarH1$^+$ (inset). (G, H) Staining for neurons (Elav; green) and socket cells (SuH; red). (G) In wild-type nota, there is one Elav$^+$ neuron and one SuH$^+$ socket cell; (H) in 109-68GAL4;*UAS-prospero* nota, there are often two Elav$^+$ neurons (arrowhead) and never any SuH$^+$ socket cells.

Rodrigues, 1999). (3) We detect the neuron and sheath cells (IIb progeny) prior to IIa mitosis. At the 3-cell stage, we observe Elav and Prospero in the two anteriormost cells, which are thus the neuron and glia, derived from the IIb cell. The posterior third cell is the undivided IIa cell. (4) Finally, Colcemid-treated discs show A101$^+$ 2-cell stages in which only the anterior cell is in mitosis; this clearly shows that the anterior cell, IIb, enters mitosis before the posterior cell, IIa.

How can we explain the previous findings showing that IIa divides before IIb? The initial description of IIa/IIb division timing was done by BrdU labeling (Hartenstein and Posakony, 1989); BrdU labeling detects the time of DNA replication (S phase) but not the time of mitosis, and it is possible that IIa goes through S phase before IIb, even though IIb enters mitosis before IIa. A more recent analysis was performed using tubulin staining to mark mitotic cells; this study appears to show the posterior IIa cell dividing prior to the anterior IIb cell (Gho and Schweisguth, 1998). We can imagine three possible explanations to resolve the difference between our results and that of Gho and Schweisguth (1998). (1) There may be genetic background differences between the A101 lines used in each study, with IIa dividing first in their genotype and IIb dividing first in our genotype. However, we detect IIb dividing first in several genotypes (data not shown; Rodrigues and Reddy, 1999). (2) It may be that the IIb cell divides first to produce two previously unrecognized daughters: a 'IIIb' tertiary precursor cell, that will later generate the neuron and sheath cells, and the previously identified 'fifth cell', the origin of which is unknown (Hartenstein and Posakony, 1989; Usui and Kimura, 1993). This hypothesis requires that the 'fifth cell' is Prospero$^+$ and rapidly migrates away. If so, the remaining IIa/IIIb might be mistaken for a IIa/IIb pair, with IIa dividing first to produce the socket/hair cells and the IIIb dividing later to generate the neuron/sheath cells. (3) The '2-cell stage' with the anterior cell dividing first (observed by Gho and Schweisguth, 1998) may actually be a 3-cell stage in which the sheath cell was not identified. Although we think this is unlikely, the sheath cell does have a low level of A101 β-gal and is often positioned internal to the neuron and IIa cell; without a sheath cell marker this cell might be missed.

Prospero is not asymmetrically localized, yet it is detected in IIb but not IIa

In the embryonic SOP lineage, Prospero is asymmetrically partitioned into the IIb cell during the SOP division (Hirata et al., 1995; Knoblich et al., 1995; Spana and Doe, 1995), but we show that asymmetric localization of Prospero does not occur in the adult SOP lineage. Prospero is not detected in the adult SOP; it is first seen in the IIb cell but not in the IIa cell. It is unlikely that there is low level of Prospero protein present in the SOP and asymmetrically partitioned to the IIb cell, because prolonged mitotic arrest of the SOP fails to reveal an accumulation of cortical asymmetric Prospero protein (similar treatment leads to a robust cortical crescent of Prospero in

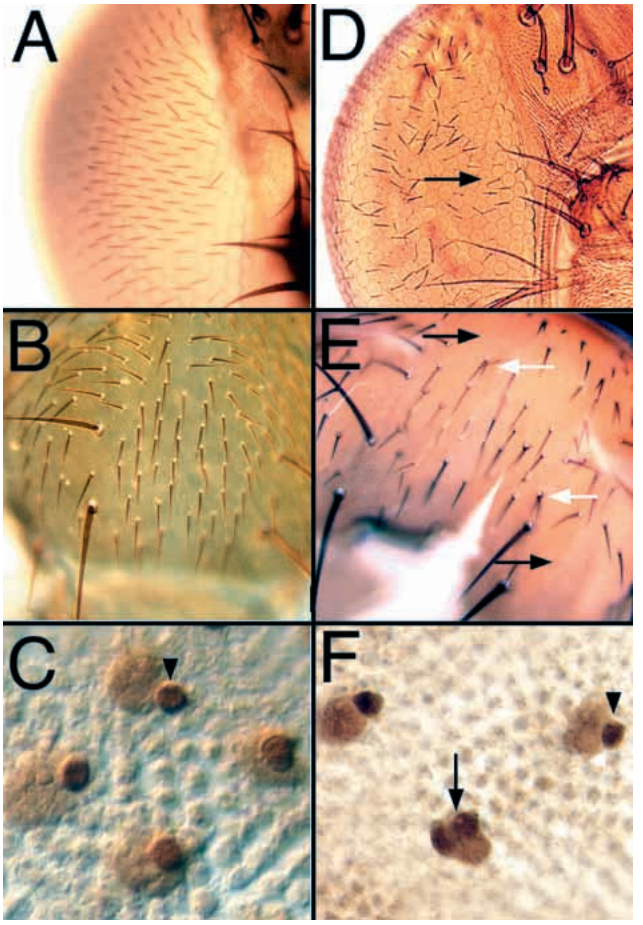

Fig. 6. Transient ectopic *prospero* expression results in partial duplication of IIb progeny (neuron) at the expense of IIa progeny (hair/socket cells). (A-C) Wild-type adults have (A) a single interommatidial bristle, (B) a single bristle in each sense organ on the notum and (C) a single neuron expressing high levels of Elav underlying each bristle (arrowhead). (D-F) *hsp70-prospero* flies heat shocked for 1 hour at 14 hours APF and allowed to develop into adults show (D) 'bald' patches in the eye lacking interommatidial bristles and (E) 'bald' patches in the notum lacking bristles; in both tissues, there are occasionally double bristles at the edges of the bald areas (white arrows). (F) There are frequent duplication of $Elav^{+}$ neurons under the 'bald' cuticle of the notum (arrow).

embryonic SOPs and neuroblasts; Broadus and Doe, 1997). In addition, Prospero protein is present in the cytoplasm of the mitotic IIb cell, and is distributed to both the neuron and sheath daughter cells; it subsequently becomes nuclear in the sheath cell and disappears from the neuron. Finally, Miranda protein is required to anchor Prospero protein at the cortex of mitotic neuroblasts and SOPs in the embryo (Ikeshima-Kataoka et al., 1997; Shen et al., 1997), but there is no detectable Miranda protein in the SOP lineage (Fuerstenberg et al., 1998). Taken together, these observations show that accumulation of Prospero in the IIb cell is not the initial cause of IIb cell fate, but rather a secondary step in the pathway of IIb cell specification. Similarly, elimination of Prospero from the neuron is an intermediate step in neuronal differentiation, rather than a consequence of partitioning Prospero into the sheath cell and out of the neuron.

What mechanisms lead to *prospero* expression in the IIb cell but not in the IIa cell? Specification of IIa/IIb cell fates is determined by the relative activity of Notch signaling. Productive Notch signaling results in IIa cell fate; asymmetric localization of Numb protein into the IIb cell blocks Notch signaling and results in the IIb cell fate (reviewed in Campos-Ortega, 1996). We propose that productive Notch signaling prevents *prospero* expression in the IIa cell, whereas lack of Notch signaling is allows *prospero* expression in the IIb cell. Consistent with this model, SOP lineages with unregulated Notch signaling produce a pair of IIa cells that both fail to express *prospero*, while SOP lineages lacking Notch function produce two IIb cells that both express *prospero* (Reddy and Rodrigues, 1999). One effector of Notch signaling in the IIa cell is the zinc-finger transcriptional repressor Tramtrack, which may directly or indirectly repress *prospero* expression (Guo et al., 1995). Interestingly, *prospero* is expressed in the R7 neuron during eye development (Kauffmann et al., 1996; Spana and Doe, 1995) and *tramtrack* mutants have supernumerary R7 neurons while *tramtrack* misexpression reduces R7 differentiation (Li et al., 1997; Tang et al., 1997). Thus, a similar *Notch*-, *tramtrack*-dependent pathway may repress *prospero* expression in both the R7 photoreceptor neuron and the IIa cell. It should be noted that a somewhat different mechanism must be involved in repressing *prospero* in the neuron but not sheath cell; in this case, Notch signaling is required for sheath cell fate (Guo et al., 1996; Hartenstein and Posakony, 1990), the cell that maintains *prospero* expression. The lack of Notch-mediated repression of *prospero* expression in the sheath cell may reflect the fact that Notch signaling is SuH-dependent in the IIa cell, but SuH-independent in the sheath cell (Wang et al., 1997).

prospero is essential for distinguishing IIa and IIb cell fates

We demonstrate a role for *prospero* in establishing different IIa/IIb cell fates based on both loss-of-function and misexpression experiments. A significant fraction of the SOP lineages lacking *prospero* function show a duplication of the external bristle (a progeny of the IIa cell; Fig. 3, Table 1) and a loss of the neuron (a progeny of the IIb cell) (Reddy and Rodrigues, 1999). We were unable to accurately score socket cell fate, because multiple socket cells can generate a single, fused socket structure. The simplest interpretation of the double bristle $prospero^{-}$ sense organs is that the IIb cell has become partially or fully transformed into a IIa cell, resulting in duplicate hair/sockets and loss of neuron/sheath cell. We think it is unlikely, but cannot rule out, the possibility that the neuron is transformed into a duplicate hair cell and the sheath cell is unaffected. In both notum and eye, however, there are still many 'single bristle' sense organs that have an associated neuron and, in these sense organs, the IIb cell must have been specified relatively normally. Thus *prospero* is not strictly necessary for IIb cell specification, but its function is important for the high-fidelity specification of IIb cell fate.

While the presence of *prospero* in the IIb cell is important for reliable IIb cell specification, the absence of *prospero* from the IIa cell is absolutely essential for IIa cell specification. Misexpression of *prospero* in the IIa cell and its progeny results in a fully penetrant loss of a socket cell

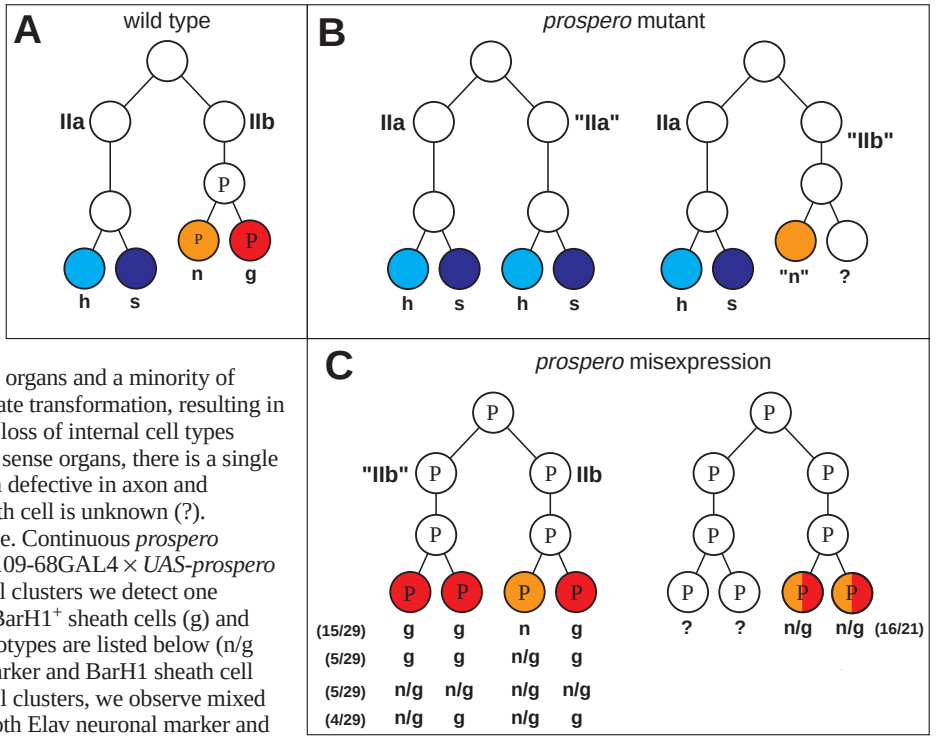

Fig. 7. Summary of *prospero* expression and function in the adult external SOP lineage. (A) Wild-type SOP lineage: the SOP produces the IIa and IIb precursors; IIb divides first to generate a neuron (orange, n) and a sheath cell (red, g); IIa divides soon after to generate a hair cell (turquoise, h) and socket cell (blue, s). Prospero (P) is first detected at low levels in the IIb cell just prior to its division and it is inherited by both neuron and sheath cell; the sheath cell maintains a high level of Prospero (P), while the neuron has transient Prospero (small P). (B) *prospero* mutant SOP lineage. (Left) In a majority of eye sense organs and a minority of notum sense organs, there is a IIb-to-IIa cell fate transformation, resulting in duplicate external cell types (hair/socket) and loss of internal cell types (neuron/sheath cell). (Right) In the remaining sense organs, there is a single external hair/socket with an associated neuron defective in axon and dendrite morphology (n). The fate of the sheath cell is unknown (?). (C) *prospero* misexpression in the SOP lineage. Continuous *prospero* expression (P) within the SOP lineage in the 109-68GAL4 × *UAS-prospero* genotype. (Left) 4 cell clusters. In 15/29 4-cell clusters we detect one strongly BarH1^{+} sheath cell (g), two weakly BarH1^{+} sheath cells (g) and one strongly Elav^{+} neuron (n). Minority phenotypes are listed below (n/g indicates expression of both Elav neuronal marker and BarH1 sheath cell marker). (Right) 2 cell clusters. In 16/21 2-cell clusters, we observe mixed fate 'sheath/neuron' cells (n/g) that express both Elav neuronal marker and BarH1 sheath cell marker.

marker (SuH) as well as the morphological external socket and hair structures; there is a corresponding increase in the internal Elav^{+} neurons and BarH1^{+} sheath cells. Our misexpression experiments show that absence of Prospero in the IIa cell is required for normal IIa development, and that presence of Prospero in the IIa transforms it partially or fully to the IIb cell fate. Thus, differential expression of *prospero* between IIa and IIb siblings is essential for normal SOP development. Similar results were observed using transient heat-shock-induced misexpression of *prospero* although, in these experiments, we observed a very low frequency of double and triple bristle sense organs at the borders of the bald areas. The cell lineage of these rare sense organs is unknown.

Asymmetry in sibling cells: *prospero* uses different mechanisms in neuroblast and SOP lineages

It is interesting to consider the different mechanisms by which *prospero* acts to distinguish sibling cell fate. During embryonic neuroblast cell division, localization of Prospero into the daughter GMC is necessary for GMC development, but exclusion of Prospero from the neuroblast is relatively unimportant for neuroblast development (since neuroblast development is fairly normal in *miranda* mutants where Prospero remains in the neuroblast; C. Q. D., unpublished results). In contrast, during the adult SOP lineage, it appears equally important to remove Prospero from the IIa cell as well as provide it to the IIb cell. Another key difference between the adult SOP lineage and the embryonic SOP and neuroblast lineages is the timing of cell divisions. There are several hours between each cell division in the adult SOP lineage, considerably longer than the 40-60 minutes cell cycle of embryonic neuroblasts and SOPs (Bodmer et al., 1989; Hartenstein et al., 1987). The shorter cell cycles of the embryonic lineages may require asymmetric localization of Prospero for efficient specification of sibling cell fate, whereas the longer adult SOP cell cycles may provide time for the action of other regulatory mechanisms (e.g. Notch-mediated repression of *prospero* expression).

prospero is essential for proper neurite outgrowth

In single bristle *prospero*$^{-}$ sense organs, we observe a single neuron with profound defects in neurite outgrowth. The defects in axon and dendrite outgrowth and connectivity could be due to lack of *prospero* function in the IIb cell, a non-autonomous effect due to lack of *prospero* function in the sheath cell, or the absence of *prospero* function in the neuron itself. We think the first possibility is unlikely because axon outgrowth defects can be observed in R7 neurons, which do not arise from a Prospero^{+} precursor cell. We think the second possibility is unlikely because lack of sheath cells (in *glial cells missing* embryos) does not generate similar axon outgrowth defects (Jones et al., 1995). We favor the third model, in which *prospero* has a direct function in the neuron, because many neurons with different origins (CNS, PNS, eye) transiently express *prospero* and all show a similar *prospero* mutant phenotype: stunted and misrouted axons (Doe et al., 1991; Vaessin et al., 1991; Kauffmann et al., 1996).

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