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Sibling cell fate in the *Drosophila* adult external sense organ lineage is specified by Prospero function, which is regulated by Numb and Notch

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SUMMARY

Specification of cell fate in the adult sensory organs is known to be dependent on intrinsic and extrinsic signals. We show that the homeodomain transcription factor Prospero (Pros) acts as an intrinsic signal for the specification of cell fates within the mechanosensory lineage. The sensory organ precursors divide to give rise to two secondary progenitors – PIIa and PIIb. Pros is expressed in PIIb, which gives rise to the neuron and thecogen cells. Loss of Pros function affects the identity of PIIb and neurons fail to differentiate. Pros misexpression is sufficient for the transformation of PIIa to PIIb fate. The expression of Pros in the normal PIIb cell appears to be regulated by Notch signaling.

Key words: Sensory organ, Cell lineage, Machrochaetae, Microchaetae, Prospero, Notch, Numb, *Drosophila*

INTRODUCTION

The mechanosensory bristles on the notum of the adult fly have been utilized extensively to study both pattern formation and cell fate specification (Jan and Jan, 1993). The microchaetae are arranged in uniformly spaced longitudinal rows while the macrochaetae are located at invariant positions on the epidermal surface. Each sensory structure is composed of four cells with distinct fates – two outer support cells, which form the trichogen and tormogen, and two subepidermal cells, the neuron and thecogen (Fig. 1). These cells arise from a single sensory organ progenitor (the SOP), which divides to give rise to two secondary progenitors, PIIa and PIIb; PIIa gives rise to the trichogen and tormogen, and PIIb gives rise to the neuron and thecogen (Hartenstein and Posakony, 1989). The fates of different cells within the lineage are decided by a combination of extrinsic and intrinsic cues (reviewed in Horowitz and Herskowitz, 1992; Jan and Jan, 1995).

The most well-studied extrinsic cues are generated by the transmembrane receptor Notch, which together with the ligands Delta and Serrate mediate a binary choice between two cell fates (Simpson, 1990; Hartenstein and Posakony, 1990; Zeng et al., 1998). After division of the SOP, Notch signaling influences the choice of the PIIa cell. Loss of *Notch* function results in a conversion of PIIa to PIIb, resulting in the formation of two thecogen cells and two neurons (Artavanis-Tsakonas et al., 1995; Guo et al., 1996; Wang et al., 1997). Since Notch is ubiquitously expressed, a bias must exist immediately after division of the SOP to allow signaling in PIIa and not PIIb. Such a role is fulfilled by Numb, a membrane-associated protein, which is asymmetrically localized in the SOP itself and is segregated to one of the cells following division (Rhyu et al., 1994). Numb negatively regulates Notch by direct protein-protein interaction involving the phosphotyrosine-binding domain (PTB) of Numb (Guo et al., 1996; Frise et al., 1996). The cell that inherits Numb therefore has lowered Notch signaling and becomes PIIb; Numb loss of function results in a PIIb-to-PIIa conversion.

Binary cell fate decisions mediated by Notch and Numb occur again at the next level in the choice between thecogen or neuron and tormogen or trichogen. Hence loss of Notch function throughout the lineage results in the conversion of all cells of the sensory organ to neurons (Hartenstein and Posakony, 1990). Gain of Notch function induced by expressing $Notch^{intra}$ results in the conversion of all four cells of the lineage to sockets (Guo et al., 1996). Loss of Numb function in the hypomorphic allele nb^{SW} resulted in duplication of sockets and thecogen demonstrating its role in both PIIa and PIIb lineages (Wang et al., 1997).

Activation of Notch results in release of Suppressor of Hairless [Su(H)] from its binding site on the cytoplasmic domain of Notch and its translocation into the nucleus (Fortini and Artavanis-Tsakonas, 1994). Su(H) protein accumulates in the nucleus of the tormogen cell shortly after division of PIIa (Gho et al., 1996; Guo et al., 1996). Thus it is likely that Su(H) is involved in the differentiation of the tormogen cell. Numb, which is presumably synthesized de novo in PIIa, is asymmetrically segregated to the trichogen cell where it serves to downregulate Notch signaling. Wang et al. (1997) have provided convincing evidence that the choice between neuron and thecogen cell occurs by a Su(H)-independent mechanism. Recent data shows that the intracellular domain of Notch itself enters the nucleus in the response to ligand and may be directly involved in the activation of target genes (Struhl and Adachi, 1998).

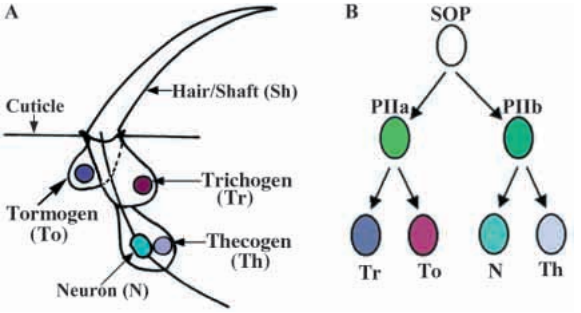

Fig. 1. Schematic diagram showing the cells of the mechanosensory organ (A) and their lineage (B). Each sense organ develops from a sensory organ precursor (SOP) following two rounds of division. The first division gives rise to the secondary progenitors – PIIa and PIIb. PIIa divides to give rise to Trichogen (Tr) and Tormogen (To) and PIIb divides to form the neuron (N) and thecogen (Th).

One of the downstream targets of Notch signaling in the peripheral nervous system (PNS) is the zinc finger protein Tramtrack (Ttk) (Guo et al., 1995). Loss of *ttk* function in the embryonic PNS affects the identity of PIIa. Factors that affect the differentiation of PIIb are less well characterized. The homeodomain transcription factor Prospero (Pros) has been shown to be asymmetrically located in the SOPs of the embryonic PNS and is subsequently detected in the nucleus of PIIb. Pros expression is seen within the thecogen cell of the sensory lineage (Vaessin et al., 1991; Doe et al., 1991). The function of this molecule in PNS development is still unclear. In the central nervous system (CNS), Pros has been shown to distribute asymmetrically, with Numb, from the neuroblast into the ganglion mother cell (GMC) (Spana and Doe, 1995; Hirata et al., 1995; Knoblich et al., 1995). Pros function is believed to be necessary for the determination of GMC identity and, in mutant animals, the axonal outgrowth and pathfinding of several central neurons is affected (Doe et al., 1991; Vaessin et al., 1991).

In this paper, we investigate the role of Pros during the development of the adult external sensory organs. We show that Pros is first detected within the nucleus of the PIIb and is symmetrically distributed to both its progeny following cell division. Expression is very transient in the neuron but is maintained in the thecogen cell. Loss-of-function as well as gain-of-function studies of Pros activity suggest a role in the determination of the fate of PIIb. The expression in PIIb appears to be dependent on Numb and Notch function.

MATERIALS AND METHODS

Fly strains

The null allele of *pros* (*pros*^{17}) and the UAS-numb strains were obtained from Chris Doe, University of Illinois. UAS-pros and hs-pros strains were generated by F. Matsukazi, National Institute of Neuroscience, Tokyo. The UAS-Notch^{intra} strain was kindly provided by Marc Haenlin, IGBMC, Strasbourg. Sensory cells were visualized during development using the enhancer trap line A101; the reporter enzyme β-galactosidase is expressed in progenitor cells of sense organs as well as in all their progeny (Huang et al., 1991). The GAL4 enhancer trap line P309 bears an insertion in *scabrous* (*sca*) and was used as the driver for all the UAS constructs. The strain was provided by Scott Selleck, Univ of Arizona. The second chromosome hs-FLP (*pr pwn* hs-FLP^{38}; *Ki kar*^{2} *ry*^{506}) and the third chromosome FRT (*pr pwn*; FRT 82B *kar*^{2} *ry*^{506} *bx*^{34e} Dp(2;3)P^{32} /FRT82B *kar*^{2} *ry*^{506}) strains were obtained from Pat Simpson and Pascal Heitzler (IGBMC, Strasbourg). Details of markers are listed in Lindsley and Zimm (1992). For staging, white pupae (0 hours after puparium formation; APF) were collected and allowed to develop further on a moist filter paper at 25°C. This stage lasts for 1 hour, hence the error in staging is ±30 minutes.

Mosaic analysis

pros mutant patches were generated by site-specific recombination at FRT sites induced by hs-flipase (Golic and Lindquist, 1989). Flies of genotype *pr pwn* P[ry^{+}, hs-FLP]^{38}/*CyO*; *Ki kar*^{2} *ry*^{506}/*Ki kar*^{2} *ry*^{506} males were crossed to *pros*^{17} P[ry^{+}, hs-neo, FRT]82B /TM3 *Sb ry*^{RK} females. The non-*Cy*, non-*Sb*, *Ki* flies obtained in the progeny were mated to *pr pwn/pr pwn*; P[ry^{+}, hs-neo, FRT]82B Dp(2;3)P^{32}/ P[ry^{+}, hs-neo, FRT]82B Dp(2;3)P^{32} virgin females. Progeny were collected 24-72 hours after egg laying (AEL) and pulsed at 38°C for 1 hour to induce mitotic recombination. Adults were allowed to emerge and flies of phenotype *pr Ki* $^{+}$*pros*^{17} were screened for the presence of clones, which will be indicated by the *pwn* marker.

Misexpression analysis

Misexpression of *pros*, *Numb* and *Notch*^{intra} was carried out using either heat-shock transgenes or the GAL4/UAS system (Brand and Perrimon, 1993). GAL4^{P309} was used to drive UAS-Pros, UAS-Numb and UAS-Notch^{intra} in the sensory organ lineage. In most of the experiments, animals were reared at 18°C until white prepupal stage and subsequently transferred to 25°C till pharate adult stage. In order to visualize the cells within the sensory lineage, a chromosome bearing the A101 enhancer-trap insertion was introduced into the appropriate UAS strain. UAS-Pros (or UAS-Numb, UAS-Notch^{intra}) were crossed to *Bc/Bc*; *Kg*^{v} A101/TM6c *ry* and the Black cell (*Bc*) and tubby (Kg^{v} A101) phenotypes obtained from this cross were mated to GAL4^{P309}/GAL4^{P309} animals. Non-Bc, but Tubby pupae carry the A101 marker, UAS-Pros and P(Gal4).

Scanning electron microscopy

The adult flies were dehydrated in a graded series of acetone and incubated in absolute acetone at 60°C for 1 hour for fast evaporation. Dried specimen were mounted on metal grids and sputter coated with gold. They were observed under JEOL scanning electron microscope and photographed on Kodak 125ASA black and white film.

Immunohistochemistry

Samples were dissected, fixed as described previously and incubated in mAb22C10 diluted 1:100 in phosphate-buffered saline (PBS) containing 0.5% Triton X-100 (0.5% PTX) and 10% normal goat serum (NGS). The primary antibodies used were: mouse-anti-Pros (1:4; a kind gift from Chris Doe), rat-anti-Elav 7E8A10 (1:2000; Developmental Studies Hybridoma Bank at University of Iowa), anti-β-tubulin (1:4) and rabbit-anti-β-galactosidase (1:4000, Cappel). In all cases, reaction was carried out at 4°C overnight. Samples were washed in 0.1% PTX, treated with suitable secondary antibodies (1:200) either coupled to fluorophors (DATF; LRSC, Cy3; Jackson ImmunoRes; or FITC, Molecular Probes) or biotinylated (Vector labs, UK). The fluorescently labeled preparations were mounted in glycerol and viewed on a BioRad MRC1024 confocal microscope. Where biotinylated secondary antibodies were used, samples were incubated in streptavidin/biotin-horseradish peroxidase (HRP) and cells were visualized by performing DAB reaction.

Colcemid treatment

Dissected pupal tissues were incubated in 5 μg/ml colcemid in Schneider's insect medium for 60-90 minutes. Tissues were then fixed and processed for immunohistochemisty as described above.

RESULTS

Pros is expressed during development of the external sense organs on the adult notum

The development of a single sense organ can be followed using the enhancer-trap marker A101, which labels the SOP and its progeny (Huang et al., 1991). Sensory cells were visualised by staining with antibodies to β-galactosidase (red) and mitotic spindles with an antibody against β-tubulin (green) The division of the SOP occurs in an anteroposterior orientation (Fig. 2A-C; Gho and Schweisguth, 1998) to give rise to two secondary precursors, PIIa and PIIb; PIIa is located more posteriorly and divides to form the trichogen and tormogen cell, while the more anteriorly located PIIb gives rise to the neuron and thecogen cell. Since the secondary progenitors do not divide synchronously, clusters composed of two cells, three cells and four cells can be visualized between 17 and 19 hours APF during development of the notum (Fig. 2). In all 2-cell clusters examined (*n*=25), the more anteriorly located cell (PIIb) was observed to be in mitosis before the posterior cell (PIIa) (Fig. 2D-I). The division was concluded to occur in an apical-basal plane since two spindle poles cannot be observed in the same planes of focus (compare Fig. 2E and H). and one of the progeny cells occupies a more basal position compared to its sibling (arrow in Fig. 2J,L). Mitotic figures can be discerned in the posteriorly located cell (the PIIa) when the cluster is already composed of three cells (Fig. 3, arrowheads in Fig. 2J-L). These data suggest that during development of the sense organ, the secondary progenitor PIIb divides before PIIa. This completely unexpected result is difficult to reconcile in the light of existing data and needs to be investigated in more detail. However, the cell division order between PIIa and PIIb is not germane to the issues being discussed in this paper.

We examined the expression of Pros (green in Fig. 3) in the cells (red in Fig. 3) of the developing thoracic microchaetae between 17 and 19 hours APF. Cell division was monitored by staining with the DNA dyes DAPI (data not shown) and with propidium iodide (blue in Fig. 3). The SOPs did not express Pros at any stage of the cell cycle (data not shown). Pros expression was first detected in the nucleus of the more anteriorly located cell (PIIb) among the secondary progenitors (Fig. 3A-C) (Gho and Schweisguth, 1998). Interestingly, this was the first cell to divide in all cases examined (*n*=60) (Fig. 3D-G, arrowheads). Prior to cell division, Pros becomes uniformly distributed on the cortical membrane and throughout the cytosol (Fig. 3E,G). Unlike the findings in the central nervous system (Spana and Doe, 1995; Knoblich et al., 1995), Pros was not asymmetrically localised in cells at any stage of the lineage. We failed to observe asymmetric crescents of Pros immunoreactivity even after blocking mitosis with colcemid. These data, interpreted in the light of results from Gho et al. (1996), suggest that Pros is expressed first in the nucleus and then generally in the cytosol of PIIb.

In clusters composed of three cells, we observed Pros immunoreactivity in two cells (*n*=63), both of which are located anteriorly, although one is located below the plane of the other (arrow in Fig. 3H,I,K). We believe that these cells are the progeny of PIIb, which in our experiments divides before the PIIa (Fig. 2). Immunoreactivity in one of the cells appears to decay fairly rapidly (Fig. 3K). While it is formally possible

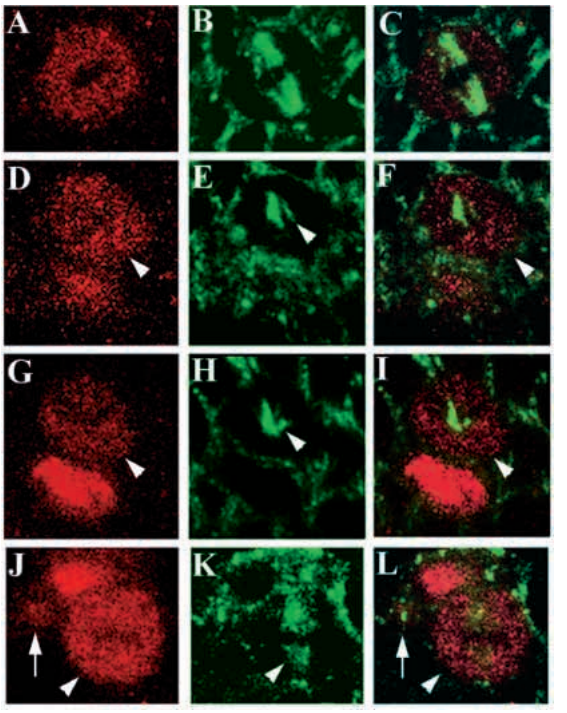

Fig. 2. Mitotic figures in the cells of the sensory lineage at 17-19 hours APF. Sensory cells were visualized by monitoring β-galactosidase expression (red) in the A101 strain (first row); and mitotic figures with an antibody against β-tubulin (green) (second row). Last row shows merged pictures; anterior is to top. (A-C) SOPs; (D-I) 2-cell clusters showing mitotic spindles in the anterior cell. The spindle can be visualised in two planes of focus (D-F) and (G-I) suggesting an apical-basal division. (J-L) In 3-cell cluster, one of the PIIb progeny occupies a subepidermal position compared to its sibling (arrow). The posterior cell is seen in mitosis (arrowhead).

that one of the two $Pros^+$ cells is not part of the sensory lineage but lies adjacent to these cells, this seems unlikely since both Pros-positive cells are also labelled by the reporter of the A101 line (Fig. 3H,K). The posterior cell never expresses Pros and was observed to be dividing in the 3-cell cluster (Fig. 3J,K arrowheads).

In all the 4-cell clusters examined at 17-19 hours APF (*n*=85), we observed Pros staining in two cells (Fig. 3L-N). Expression in one of the cells is very transient and at 30 hours APF only one of the four cells expresses Pros (Fig. 3W). Double labeling with anti-Elav antibody, which recognizes neuronal cells, allows us to conclude that expression is excluded from the neuron at 30 hours APF (Fig. 3V-X). In some clusters, the cell that expressed lower levels of Pros (arrowhead in Fig. 3O) undergoes one more round of mitosis (Fig. 3Q,R). This ‘extra’ division could account for the small number of clusters composed of five cells (Fig. 3O,S-U); Pros immunoreactivity could be observed in three cells at 17-19 hours APF (Fig. 3T,U). The nature of this rare fifth cell associated with the mechanosensory organ is not clear, although presence of such clusters was previously observed by Hartenstein and Posakony (1989). This ‘extra’ cell division merits closer attention.

In summary, we have detected Pros immunoreactivity in the nucleus of PIIb just prior to division, which is segregated

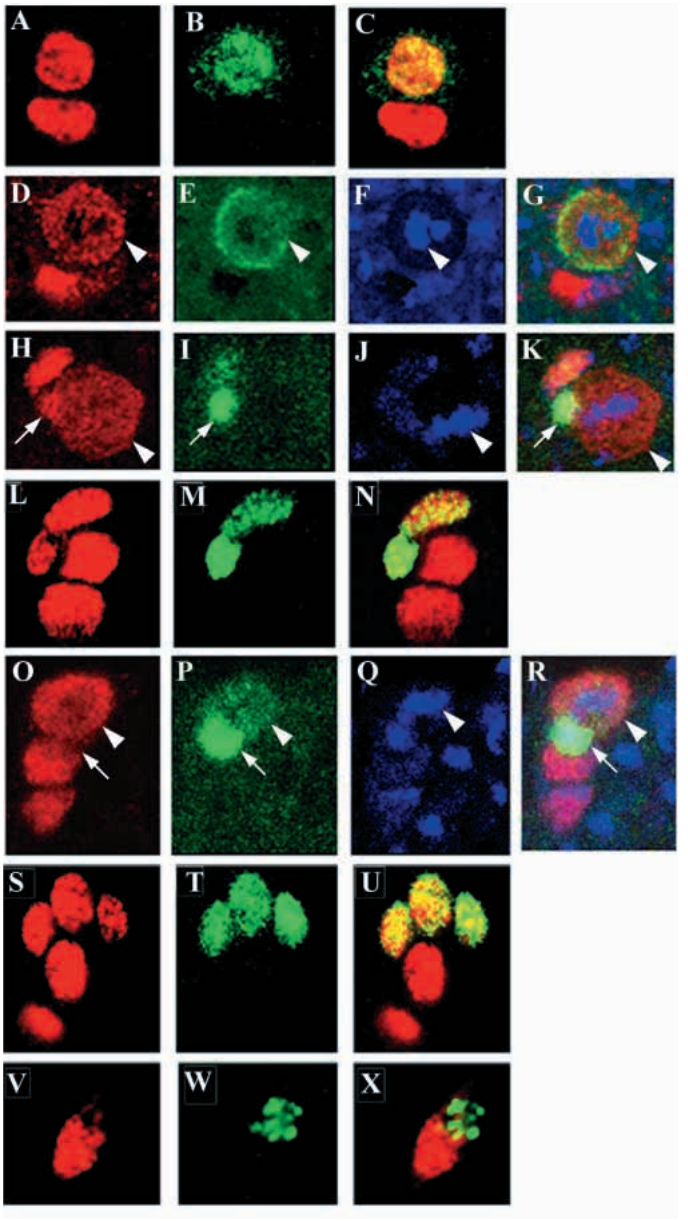

Fig. 3. Expression pattern of Pros during development of the sensory organ. Pros immunoreactivity (green) was visualized in A101 where sensory cells were labeled by anti β-galactosidase (red) and DNA by propidium iodide (blue) (F,J,Q). The rightmost panel shows the merged images. Anterior is top and arrowheads indicate dividing cell. (A-C) 2-cell clusters showing complete overlap between nuclear localized β-galactosidase and Pros in the anterior cell (C). (D-G) The anterior cell (arrowhead) is seen undergoing division and Pros is located uniformly in the cytosol. (H-K) 3-cell cluster composed of progeny of PIIb and PIIa (arrowheads in H,J,K). PIIa is undergoing division at this stage (arrowheads in H,J,K) but does not express Pros. Pros expression is significantly stronger in one of the PIIb progeny (arrow in H,I,K). (L-N) PIIa and PIIb have completed division to form a 4-cell cluster. Both the anterior cells express Pros although expression is stronger in one of the two cells. (O-R) In some 4-cell clusters, one of the progeny of PIIb divides; this is usually the cell in which the Pros expression had decayed (arrowhead). (S-U) A 5-cell cluster where three cells express Pros. (V-X) The 30 hour APF notum stained using antibodies against Pros (green) and Elav (red). Pros expression is seen in a single cell which is closely associated with the cell expressing the neuronal marker Elav.

equally to its daughters, the neuron and thecogen cell. The expression is transient in the neuron but is maintained in the thecogen cell until late in development, suggesting that there is de novo expression in this cell.

Loss of Pros function alters PIIb development

In order to test the function of Pros in the PIIb lineage, we generated *pros*$^{-}$ clones during sensory development. Mitotic clones of the null allele *pros*^{17} were generated by temperature pulses to a hs-flp/FRT strain at a time well before the appearance of SOPs; clones were visualized using the bristle marker – *pawn* (*pwn*) (Lindsley and Zimm, 1992). The *pwn* mutation leads to a truncated shaft, but does not affect the differentiation of the tormogen or neuronal cell (data not shown). Since all the cells of a sensory organ are related by lineage, when mitotic recombination is induced prior to SOP formation, appearance of the *pwn* marker indicates that all the sensory cells are *pros*$^{-}$.

We analyzed mutant clones covering the notum, head and compound eye. External cells were monitored by visual inspection of the socket and shaft of each sensory structure and the underlying neurons were examined by staining with mAb22C10. With the exception of a few sensory organs, most *pros*$^{-}$ sensilla on the notum and the head showed a normal complement of external cells – the tormogen and trichogen (Table 1). We stained such clones with mAb22C10 thus visualizing the neuronal cells lying beneath the epidermis (arrowheads in Fig. 4A,B). No mAb22C10-stained cells were observed within the *pros*$^{-}$ sensory structures which are marked by the *pwn* phenotype (arrows in Fig. 4A,B). In a small number of cases (Table 1), the cell body was present but it lacked an apical dendrite (arrow in Fig. 4C,D).

These defects could mean that *pros* function is required either for the differentiation of the neuronal cell itself, or of its progenitor – the PIIb. Several clones, notably those covering the mechanosensory structures on the compound eye and the head, also showed defects in external cells – the trichogen and the tormogen. Of the 152 sensory organs examined on the notum and head, one had twinned shafts and two sockets (Fig. 4E), one had two sockets with a single shaft (Fig. 4F) and four had two shafts arising from a single socket (Fig. 4G). The predominant phenotype of clones covering the mechanosensory organs on the compound eye (36 out of 61 cases) was the twinning of shafts (Fig. 4H). Several sensory organs with twinned shafts revealed the absence of an underlying neuron as assessed by staining with mAb22C10 (Table 1).

The most highly penetrant phenotype of a complete loss of *pros* function was an absence of the neuron or a lack of axonal outgrowth as visualized by staining with mAb22C10. These phenotypes suggest that *pros* function is essential for the differentiation of the PIIb cell, hence affecting its daughter – the neuron. The duplication of external cells can be explained by a partial conversion of the PIIb to PIIa suggesting that PIIa determination does not result as a simple consequence of *pros* absence.

Table 1. Analysis of the clones of the null allele of *pros* (*pros*^{17}) on the notum and head

	Microchaetae	Macrochaetae	Head bristles
Total number of *pros* mutant hairs analyzed	98	29	25
Mutant sensory organs devoid of mAb22C10 staining	92	18	17
Mutant sensory organs with mAb22C10 staining but no apical dendrites	3	3	2
Mutant sensory organs with twinned shafts and a single socket and devoid of mAb22C10 staining	1	0	3
Mutant sensory organs with twinned shafts and sockets and devoid of mAb22C10 staining	0	0	1
Mutant sensory organs with a single shaft and two sockets and with mAb22C10 staining	0	0	1
Mutant sensory organs with normal external structure and mAb22C10 staining	2	8	1

Clones were stained with the monoclonal antibody 22C10 to visualize neurons. External phenotypes were observed by examining the socket and shaft cells.

Gain-of-function experiments suggest that Pros function is sufficient following SOP division for the formation of PIIb

In order to test whether pros function was sufficient to switch the PIIa to a PIIb fate, we misexpressed Pros in the cells of the sensory cluster using the *hs-pros* transgene as well as the GAL4/UAS system (Brand and Perrimon, 1993). GAL4^{P309} is an insertion in *sca*, which initially expresses reporter activity in proneural domains and continues to express uniformly in all four cells of the lineage (Sudipto Roy, personal communication). Misexpression of Pros using GAL4^{309} resulted in a bald notum where both the external structures, the shaft and the socket were absent (Fig. 5B). Partial balding of the notum was discerned when Pros was misexpressed between 15 and 20 hours APF using a *hs-pros* transgene (data not shown). The misexpression experiments were carried out in the background of an A101 strain hence allowing the visualization of the cells in the lineage by β-galactosidase expression (Table 2).

We examined sensory clusters at 30 hour APF by staining A101 notum with antibodies against β-galactosidase (red) and Elav (green) (Fig. 5). Elav is a well-known marker for post-mitotic neurons; however, more recently Manning and Doe (1999) have shown that it is also expressed in the secondary progenitor cell PIIb and its progeny, the neuron and thecogen. Expression decays rapidly from the thecogen cell and is enhanced in the neuron. At 30 hours APF, most of the clusters in the wild-type notum are composed of four cells and only of one of these cells expresses Elav. Analysis of the 4-cell clusters in GAL4^{P309}/UAS-Pros; *Kg*^{v} A101/+ preparations revealed several (14 out of 34; Table 2) apparently 'normal' clusters where only one cell expressed Elav (Fig. 5C-E). In 12 clusters, (Table 2) two out of four cells expressed Elav suggesting a conversion of an additional cell to the neuronal fate (Fig. 5I-K). Staining with mAb22C10 demonstrated that these additional neurons did send out projections comparable to the normal neurons (see inset in Fig. 5B). The cells that did not express Elav in these clusters were recognizable as thecogen

Table 2. Pros misexpression phenotypes in differentiated sensory clusters of mechanosensory bristles on the notum

Number of Elav expressing cells per cluster	Number of A101-positive cells/sensory cluster			
	1	2	3	4
1	26	4	2	14
2	–	31	0	12
3	–	–	0	2
4	–	–	–	5
0	4	3	–	1

Misexpression was induced by rearing Gal-4^{P309}/UAS-Pros/*Kg*^{v} A101 animals at 25°C. Pupal notum from 30 hour APF animals were dissected and stained with antibodies against β-galactosidase and Elav. A total of 104 clusters were analyzed.

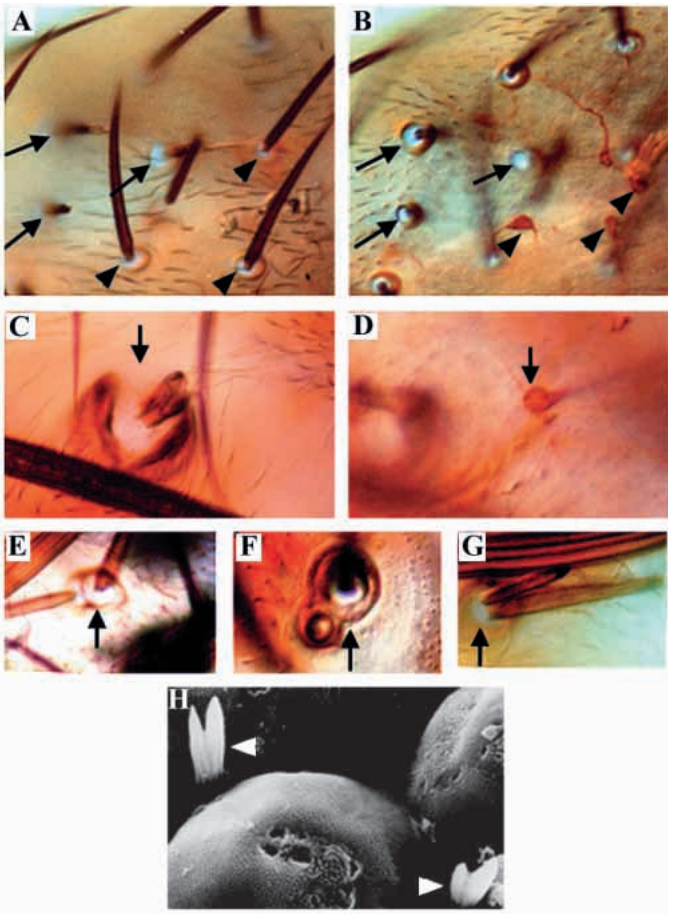

Fig. 4. Phenotypes of clones of the null allele of *pros*, *pros*^{17}. (A,B) The same thorax at two focal planes. (A) *pros*$^{-}$ clones marked by *pwn* show stunted bristles (arrows) compared to the wild type (arrowheads). (B) Clones stained with mAb22C10, which stains neurons (arrowheads). Staining is absent below the pros^{-} bristles marked by *pwn* phenotype (arrows). (C,D). In rare cases, a neuron was detected below a *pwn* bristle but did not exhibit normal axonal projections (arrow). (E-H). External phenotypes observed in *pros*$^{-}$ clones on the head. (E) Twinned shaft; (F) duplication of sockets with a single shaft; (G) twinned shafts with a single socket. (H) Clones covering the compound eye revealed a large proportion of twinned shafts (arrowheads).

cells based on their smaller size and close apposition to the neurons (Fig. 5K). Hence these phenotypes are consistent with a duplication of the PIIb cell resulting in two thecogen and two neuronal cells. A small proportion of clusters (see Table 2) showed Elav immunoreactivity in all the cells (Fig. 5F-H) or in three of the four cells (not shown). These rare types could represent newly divided secondary progenitors before the expression of Elav in the thecogen has decayed.

Somewhat surprisingly, we observed single cells (26 out of 106) and 2-cell clusters (38 out of 106; Table 2) at a time when most clusters are expected to be composed of four cells. The observation of such clusters could imply that overexpression of Pros acts to arrest development in some cases; it is of course also possible that some of the sensory cells in these clusters have been eliminated by cell death. Interestingly the 2-cell clusters (Fig. 5L-N) as well as the single cells (Fig. 5O-Q) expressed Elav; PIIb expresses Elav and it is tempting to speculate that Pros misexpression in these cells converts them to a PIIb fate.

All the phenotypes induced by Pros overexpression are consistent with a conversion of PIIa to PIIb. Hence Pros function is sufficient for causing a PIIa-to-PIIb switch in cell fate.

Loss of function of Notch or Gain of function of Numb results in ectopic expression of Pros in the sensory lineage

Notch function has been shown to be required at several stages in the development of the sensory organ. Apart from its role in lateral inhibition Notch also acts in the determination of fate among the sibling cells of the mechanosensory lineage. Loss of *Notch* function after SOP selection results in conversion of all cells to neurons indicating a conversion of PIIa to PIIb and a subsequent conversion of thecogen cells to neurons (Hartenstein and Posakony, 1990). Similar phenotypes were generated by gain of function of *numb* (Rhyu et al., 1994). Since *pros* function is required for PIIb identity, we examined whether the reduction in Notch signaling would influence Pros expression in both the secondary progenitors.

White pupae from the *Notch$^{ts\text{-}1}$* strain were collected and reared at 22°C for 18.5 hours (equivalent to 13 hours at 25°C) and then pulsed at 30°C for 6 hours. When pupae treated in this manner were allowed to grow to adults at 22°C they showed absence of external structures of several notal microchaetae as described previously by Hartenstein and Posakony (1990) (Fig. 6B,A). Since the macrochaetae develop earlier, they are unaffected by *Notch* loss of function at this stage. In a parallel series of experiments, *Notch$^{ts\text{-}1}$* females were mated to *Kg^{v}* A101/TM3-Sb males; progeny were sexed as larvae and pulsed as described above. Pupae were dissected an hour after the pulse and examined for Pros immunoreactivity (green) using A101 reporter activity (red) as a marker for the cells of the lineage (Fig. 6). The females (*Notch$^{ts\text{-}1}$*/+; *Kg^{v}* A101/+) served as internal controls and were comparable to wild type (Fig. 6D-F). It must be noted that *Notch^{+}* function is also required for lateral inhibition and its removal results in the selection of ectopic SOPs. Since development of the sensory organs across the notum is staggered in time, it is possible to obtain single SOPs that have apparently 'escaped' the effect of *Notch* loss of function on lateral inhibition and these were selected for lineage analysis as described below.

In control pupae, only the anteriorly located of the secondary progenitors expresses Pros (arrows in Fig. 6D-F). In heat-pulsed *Notch$^{ts\text{-}1}$* pupae, we detected Pros expression in both cells of the cluster (arrows in Fig. 6G-I). We also observed several 4-cell clusters (n=12) where Pros expression was apparent in all the cells (arrowheads in Fig. 6G-I). These results are consistent with a conversion of PIIa to PIIb in *Notch^{ts}* animals resulting in an absence of the external cells of the lineage as described previously by Hartenstein and Posakony (1990).

The membrane-associated molecule Numb has been shown to be expressed in the SOP and is segregated asymmetrically

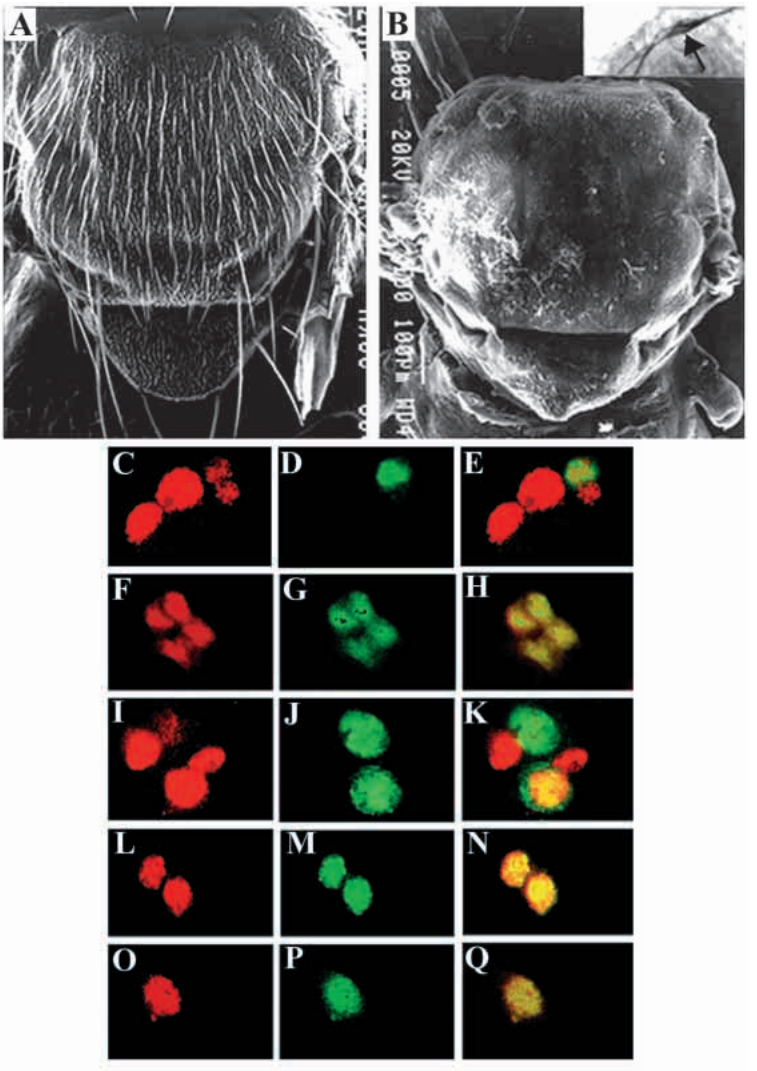

Fig. 5. Overexpression of Pros in the notum leads to loss of external structures. (A) Wild-type notum; (B) notum from GAL4^{309}/UAS-Pros. strain reared at 25°C; (C-Q) 30 hours APF GAL4^{P309}/UAS-Pros; *Kg^{v}* A101/+ notum stained with anti-β-galactosidase (red) and anti-Elav (green). (C-E) Several 4-cell clusters were 'normal' showing only 1 Elav-positive cell (C-E). (F-H). 4-cell cluster where all the A101 cells also expressed Elav. (I-K) 4-cell cluster where two cells expressed Elav. Several 2-cell clusters (L-N) and single cells (O-Q) were observed in the notum of GAL4^{P309}/UAS-Pros; *Kg^{v}* A101/+ pupae. These cells were all positive for Elav.

to PIIb where it serves to downregulate Notch signaling in this cell (Guo et al., 1996). We misexpressed Numb in all the cells of the lineage using GAL-4^{P309} and UAS-Numb. Notum of such flies were almost totally devoid of external sensory structures (Fig. 6C). We stained sensory clusters of GAL-4^{P309}/+;UAS-Numb/*Kg*^{v} A101 animals to examine if Numb gain of function affected the expression of Pros. Internal controls (Bc/+; UAS-Numb/*Kg*^{v} A101) were indistinguishable from the wild type (Fig. 6D-F). In the case of 2-cell clusters, we chose only those where one of the cells was undergoing division as evidenced by staining with DAPI (not shown). As described above, in our experiments, the more anteriorly located cell (PIIb) was always the first to divide. In GAL4^{P309}/+; UAS-Numb/*Kg*^{v} A101, however, we found that this was not always the case. Of the 35 clusters examined, we observed only 13 where the anterior cell divided first, 21 where the posterior cell divided first and 1 case where both cells underwent division together. This suggests that the differential division times of the two secondary progenitors reflects their different fates and Numb misexpression makes both cells equivalent. This would more or less randomize the division times of the two cells. In all cases both cells in the 2-cell cluster expressed Pros, consistent with them being similar to PIIb in this respect (Fig. 6J-L). Similarly all cells in the 3-cell clusters (21/23) and 4-cell clusters (29/30) expressed Pros.

Notch loss of function and *numb* gain of function results in ectopic expression of Pros in PIIa. This suggests a negative regulation of Pros by Notch signaling in PIIa. While it is possible that Numb could independently act to influence Pros expression, the simplest explanation is that the effect of Numb overexpression is a consequence of its effect on Notch signaling.

Notch gain of function downregulates Pros expression from the sensory lineage

Results summarized above suggest that inhibition of Notch signaling results in expression of Pros in PIIa. In this case gain of function of Notch would be expected to decrease Pros levels in PIIb. We expressed Notch^{intra} in the cells of the sensory lineage using GAL4^{P309}/UAS-Notch^{intra}. Flies were reared at 18°C throughout development and raised to 25°C at the beginning of pupation. The most highly penetrant phenotype in the adult was the duplication of tormogen leading to double sockets, at the expense of trichogen cells (Fig. 7A). We observed three-socket (Fig. 7B) and four-socket (Fig. 7C) phenotypes although at lesser frequencies.

In order to study the effect of Notch gain of function on Pros expression, we stained GAL4^{P309}/+; UAS-Notch^{intra}/*Kg*^{v} A101 notum with anti-Pros (green) and selected 2-, 3- and 4-cell clusters for analysis. Only those 2-cell clusters in which one cell was undergoing division were selected. Siblings from this cross (Bc/+; UAS-Notch^{intra}/*Kg*^{v} A101) served as internal controls and developed normally (Fig. 7D-F). Misexpression of Notch^{intra} resulted in several cases where Pros expression failed to occur in 2-cell (arrowheads in Fig. 7G-I) 3-cell (arrows in Fig. 7G-I) and 4-cell clusters (arrows in Fig. 7J-L). Of the 40 4-cell clusters analyzed, we observed a lack of Pros expression in 22 cases.

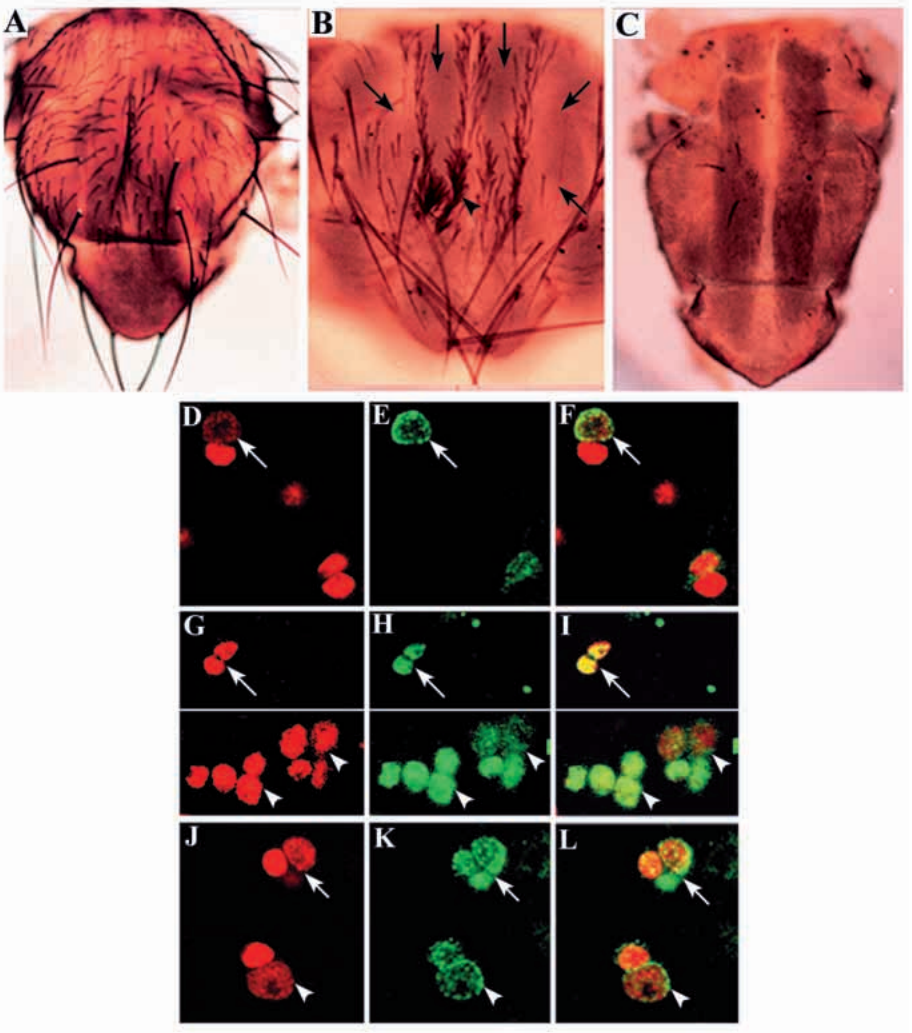

Fig. 6. Effect of loss of *Notch* function and gain of *Nb* function on Pros expression in sensory cells. (A) Wild-type notum; (B) notum from *N*$^{ts-1}$ pulsed at 30°C for 6 hours beginning at 13 hours APF. External sensory structures are missing (arrows) and there are cases of ectopic bristles (arrowheads). (C) Misexpression by GAL4^{P309}; UAS-Nb resulted in a lack of most external structures. (D-L) 17-19 hours APF notum stained with anti-Pros (green) and anti-β-galactosidase (red). (D-F) 2-cell cluster from *Bc*/+;UAS-Nb/*Kg*^{v} A101 controls; similar patterns were observed in Notch^{ts-1}/+; *Kg*^{v} A101/+ notum. The anteriorly located cell undergoes division and expresses cytosolic Pros. (G-I) Notch^{ts-1}; *Kg*^{v} A101/+. 2-cell clusters (arrows) and 4-cell clusters (arrowheads) expressing Pros (arrows); (J-L) GAL4^{P309}/+; UAS-Nb/*Kg*^{v} A101 3-cell cluster with Pros expression in all cells (arrows). Note that in the 2-cell cluster (arrowhead), the posteriorly located cell is undergoing division. The split panels in G-I indicate cell clusters obtained from different preparations.

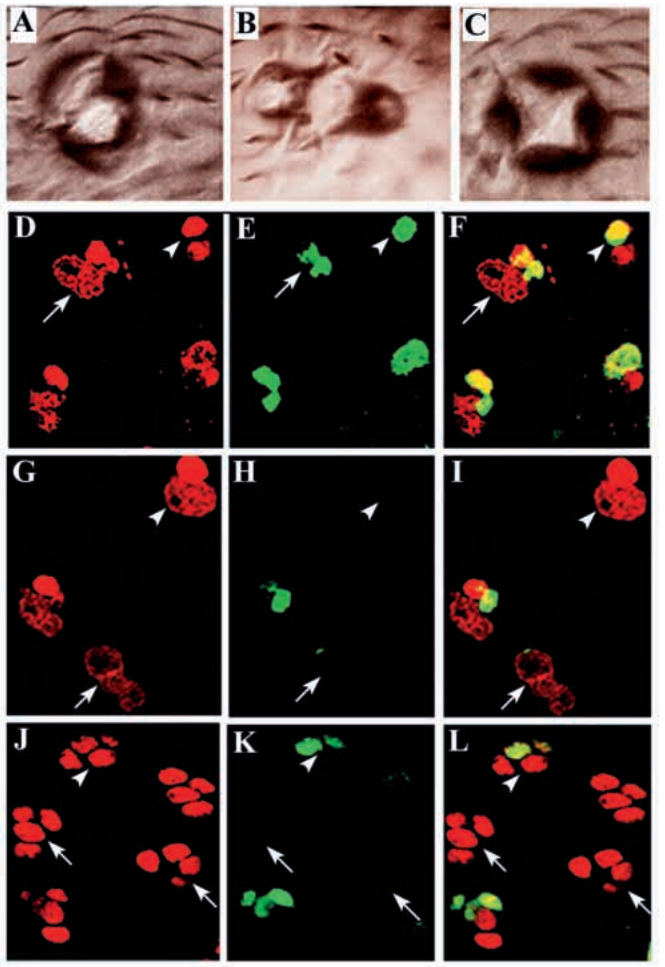

Fig. 7. Misexpression of activated *Notch* in the sensory lineage using GAL4^{P309} and UAS-Notch^{intra}. (A) Duplication of sockets; (B) three sockets; (C) four sockets. (D-L) 17-19 hour APF notum stained with anti-β-galactosidase (red) and with anti-Pros (green). (D-F) *Bc*/+; UAS-Notch^{intra}/*Kg^{v}* A101 controls show normal Pros immunoreactivity; anterior cell in 2-cell clusters express Pros (arrowheads) while both siblings express Pros in 3-cell clusters (arrows). (G-L) Notch^{intra} misexpression resulted in downregulation of Pros expression in 2-cell clusters (arrowheads), 3-cell clusters (arrows), as well as 4-cell clusters (arrows in J-L). Some 4-cell clusters expressed Pros in two cells despite Notch^{intra} expression (arrowheads in J-L).

Some of the remaining cases revealed strong expression in two cells of the cluster. This can be explained by Notch^{intra} expression in the progeny of the PIIb resulting in a neuron to thecogen cell conversion as previously reported by Guo et al. (1996).

In summary, expression of an activated Notch protein leads to a conversion of PIIb to PIIa. We also observed downregulation of Pros in the secondary progenitors suggesting that expression is influenced directly or indirectly by Notch signaling.

DISCUSSION

Pros is expressed in PIIb and is required for the determination of its identity and differentiation

Pros is expressed in the nucleus of the secondary progenitor PIIb and is subsequently distributed to both its daughters – the

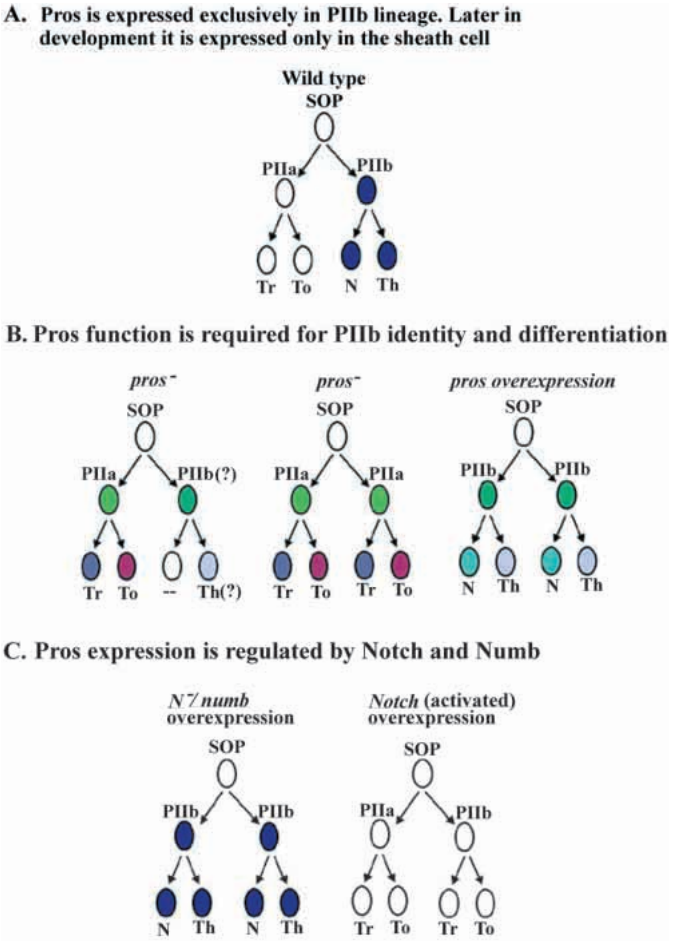

Fig. 8. Schematic diagram summarizing the role of Pros in the sensory lineage. (A) Pros expresses in PIIb and its progeny. (B) Loss of Pros function causes failure of neuronal differentiation and rarer duplications of external cells. Pros misexpression leads to duplication of internal cells and absence of external cells. (C) Loss of *N* function or gain of *Nb* function causes ectopic Pros expression; while dominantly active Notch represses Pros expression. To, tormogen; Tr, trichogen; Th, thecogen; N, neuron.

neuron and the thecogen. The neuron expresses Pros only transiently but expression in the nucleus of the thecogen cell is sustained late in development perhaps until the cells of the sensory organ are differentiated (schematized in Fig. 8A). Loss of *pros* function results in defects either in the formation of the neuron or its axonal outgrowth. Since Pros is only transiently expressed in neurons, we suggest that these phenotypes are due to differentiation defects in the progenitor cell – PIIb (see model in Fig. 8B). This is reminiscent of the finding in the CNS where Pros is required for GMC identity and affects proper differentiation of the sibling neurons (Doe et al., 1991; Vaessin et al., 1991; Spana and Doe, 1995). In a small number of *pros* null clones, we detected cases of twinned shafts arising from a single socket or double sockets with a single shaft. Such sense organs lacked the underlying neuron suggesting that these phenotypes are due to conversion of PIIb to PIIa. The low penetrance of these defects demonstrates that Pros does not act alone in a binary choice between PIIb and PIIa but additional factors are required for PIIa differentiation. These observations together with gain-of-function experiments, which result in a

conversion of external cells to internal cells, suggest that Pros is essential and sufficient for PIIb identity and differentiation.

Notch and Numb appear to regulate the expression of Pros in PIIb

Previous studies in the embryonic and adult PNS have shown that Numb plays a key role in generating asymmetry in the SOP lineage (Uemura et al., 1989; Rhyu et al., 1994). Numb has been shown to be inherited by PIIb where it reduces Notch signaling therefore creating a bias between the secondary progenitors. Hence PIIa identity is a result of Notch signaling and results from the role of its effector targets. PIIb, on the contrary, is selected as a result of the lack *Notch* function in this cell. We have identified Pros as one of the factors that controls PIIb identity and differentiation and have shown that its expression occurs in the absence of signaling by Notch. Loss of Notch function generated using either a conditional mutant allele or by misexpressing Numb protein results in the ectopic expression of Pros in PIIa (Fig. 8C). This observation is consistent with a PIIa-to-PIIb conversion by *Notch* loss of function or *nb* gain of function. It is not clear whether the apparent negative regulation of Pros by Notch is a direct effect or merely reflects the altered fate of the cells that is caused by other molecular factors.

In the developing compound eye of *Drosophila*, Pros is first expressed in cells competent to become R7, the R7 equivalence group, and subsequently in R7. Pros function is required for proper development of the R7 photoreceptor (Kaufmann et al., 1996). The low level activation of Pros in the R7 equivalence group requires activities of Ras1 as well as MAP-kinase-responsive ETS transcription factors Pointed and Yan. Subsequent upregulation of Pros in R7 occurs through the interaction of Ras1 with Phyllopod (Phyl) and Sina. The regulation is brought about by a complex of Phyl and Sina proteins (Dickinson et al., 1995; Kaufman et al., 1996). The Phyl/Sina complex, in addition, binds to Ttk to bring about its proteolysis (Tang et al., 1997).

In the compound eye, *ttk* suppresses neuronal identity while, in the mechanosensory lineage, it is required for the activity of *Notch* in PIIa (Guo et al., 1995). We propose that *ttk* could function as a negative regulator of *pros* in PIIa. In this scenario, the downregulation of *ttk* in PIIb could be brought about by *phyl*/*sina* activity. Analysis of *phyl*$^{-}$ clones covering the notum and the eye showed twining of the macrochaete and microchaete shafts with loss of underlying neurons (Chang et al., 1995). Similarly the loss-of-function allele of *sina* shows several instances of two shafts emerging from a single socket (Carthew and Rubin, 1990). This is consistent with a role for *phyl*/*sina* in PIIb where loss of function results in its conversion to PIIa.

Pros is not necessary for thecogen cell identity

Pros is not asymmetrically localized in PIIb; following division of this cell, the protein is detected in both the progeny. This is strikingly different from findings in the CNS, where Pros together with Numb and Miranda are localized asymmetrically in the neuroblasts and inherited by the GMC (Broadus and Doe, 1997; Jan and Jan, 1998). The asymmetric partitioning is controlled by Inscuteable (Insc), which appears to co-ordinate protein localization and spindle orientation (Kraut et al., 1996). Interestingly, neither loss of function, nor gain of function of *insc* affects the development of the adult mechanosensory organs (X. Yang and W. Chia, personal communication). Following division of PIIb, Pros is inherited by nuclei of both progeny. Expression in one of the siblings decays rapidly and this cell differentiates as the neuron. Pros immunoreactivity is sustained in the thecogen cell possibly due to de novo synthesis. The requirement for *pros* function in PIIb precludes analysis of its later role after division.

In experiments where Pros was misexpressed in all four cells of the sensory organ, we observed a conversion of external to internal cells, consistent with a PIIa-to-PIIb transformation. Neuronal cells formed normally despite the fact that they expressed the thecogen cell marker. Similarly, Notch loss of function and Numb gain of function results in a conversion of all four cells of the lineage to neurons. We found Pros expression in all these cells under these conditions. These observations together demonstrate that *pros* activity is not sufficient for identity of the thecogen cell and that neuronal cell differentiation can occur normally inspite of Pros expression. The elucidation of *pros* function in the thecogen cell awaits the availability of a hypomorphic mutant allele that could allow loss of function in the thecogen cell without affecting the secondary progenitors. Pros has been shown to be expressed in several CNS- as well as PNS-associated glial cells and it is possible that it plays a role in the later differentiation and /or function of these cells.

In the normal mechanosensory lineage, Notch is involved in the binary choice between thecogen and neuron (Guo et al., 1996). Here Notch signaling is experienced by the cell that ultimately becomes the thecogen cell. This is distinct from the scenario at the secondary progenitor stage where the cell that experiences Notch signaling does not express Pros. This means that, unlike in PIIb, Notch signaling does not downregulate Pros in the thecogen cell. There are several possible explanations for this finding. One possibility is that Pros protein is merely partitioned to the daughters of the PIIb after division. It therefore serves no role in the binary choice of thecogen versus neuron but is synthesized de novo after these cells have acquired their identity. At this later time point, the thecogen cell is no longer experiencing the Notch signal.

Another possibility lies in the different effector mechanisms utilized for Notch activity. During lateral signaling, PIIa-PIIb choice, as well as in the PIIa lineage, Notch activation results in release of Su(H) from its binding site on the cytoplasmic domain of Notch and its translocation to the nucleus. Su(H) protein can be detected in the nucleus of the socket cell; however, the role of *Su(H)* cannot be seen in the PIIb lineage. Extra copies of Su(H) did not produce thecogen-to-neuron transformations suggesting that Notch signaling in the thecogen/neuron choice occurs by a Su(H)-independent mechanism (Schweisguth and Posakony, 1994; Wang et al., 1997). Thus the regulation of Pros expression could be mediated by Notch through a Su(H)-independent event.

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