Revisiting the *Drosophila* microchaete lineage: a novel intrinsically asymmetric cell division generates a glial cell

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SUMMARY

The bristle mechanosensory organs of the adult fly are composed of four different cells that originate from a single precursor cell, pI, via two rounds of asymmetric cell division. Here, we have examined the pattern of cell divisions in this lineage by time-lapse confocal microscopy using GFP imaging and by immunostaining analysis. pI divided within the plane of the epithelium and along the anteroposterior axis to give rise to an anterior cell, pIIb, and a posterior cell, pIIa. pIIb divided prior to pIIa to generate a small subepithelial cell and a larger daughter cell, named pIIIb. This unequal division, oriented perpendicularly to the epithelium plane, has not been described previously. pIIa divided after pIIb, within the plane of the epithelium and along the AP axis, to produce a posterior socket cell and an anterior shaft cell. Then pIIIb divided perpendicularly to the epithelium plane to generate a basal neurone and an apical sheath cell. The small subepithelial pIIb daughter cell was identified as a sense organ glial cell: it expressed *glial cell missing*, a selector gene for the glial fate and migrated away from the sensory cluster along extending axons. We propose that mechanosensory organ glial cells, the origin of which was until now unknown, are generated by the asymmetric division of pIIb cells. Both Numb and Prospero segregated specifically into the basal glial and neuronal cells during the pIIb and pIIIb divisions, respectively. This revised description of the sense organ lineage provides the basis for future studies on how polarity and fate are regulated in asymmetrically dividing cells.

Key words: Polarity, Cell fate, Sensory organ, Numb, Prospero, Glial cell missing, *Drosophila*

INTRODUCTION

During animal development, cell fate diversity is generated in part by intrinsically asymmetric cell division, in which a mother cell divides to generate daughters of different developmental potential (Horvitz and Herskowitz, 1992). This process requires the coordination of two events: the mother cell must have an uneven distribution of cell-fate determinants and the mitotic spindle must be oriented so that, upon division, daughter cells receive different amounts of these determinants (Rhyu and Knoblich, 1995). In many cases, the orientation of asymmetric cell divisions is also determined relative to the body and/or tissue axes.

The cell lineage generating the mechanosensory bristle of the adult fly provides a genetic model system to study the process of oriented asymmetric cell division at the molecular level (Posakony, 1994). In the notum, each bristle mechanosensory organ is composed of four different cells that originate from a single precursor cell (pI) after two rounds of asymmetric divisions (Hartenstein and Posakony, 1989; Fig. 1). Microchaete pI cells are specified in a regular pattern of rows in the dorsocentral region of the notum, around 6-9 hours after puparium formation (APF) (Usui and Kimura, 1993) and

divide asymmetrically around 16 hours APF to generate two secondary precursors, pIIa and pIIb. The pIIa/pIIb fate decision is regulated by the differential activation of the Notch signaling pathway, which is activated in pIIa and inhibited in pIIb (Hartenstein and Posakony, 1990; Posakony, 1994). A negative regulator of Notch signalling, the membrane-associated Numb protein, is localized in a cortical crescent in mitotic pI and asymmetrically segregates into one of its daughter cells (Rhyu et al., 1994; Guo et al., 1996). Notch signalling is specifically inhibited in the cell that inherits Numb. This cell therefore adopts a pIIb fate.

According to the lineage proposed by Hartenstein and Posakony (1989), pIIa divides first to generate the socket and shaft cells, while pIIb divides later to produce the sheath cell and neurone (Fig. 1). The identities of the pIIa and pIIb daughter cells are again regulated by the unequal segregation of Numb at mitosis and the inhibition of Notch signalling in the cells that inherit Numb. Notch signalling is activated in the socket and sheath cells, and inhibited in the shaft cell and neurone, which specifically inherit Numb (Zeng et al., 1998; Wang et al., 1997; Posakony, 1994; Gho and Schweisguth, 1998). Recently, Prospero, a divergent homeodomain transcription factor expressed in pIIb, but not in pIIa, has also
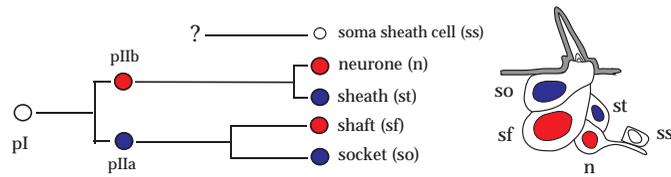
Fig. 1. A proposed model for the bristle sense organ lineage. Schematic representation of cell divisions in the adult mechanosensory organ lineage, as first proposed by Hartenstein and Posakony (1989). The daughter cells that specifically inherit Numb at mitosis and in which Notch signalling is inhibited are in red. See Introduction for a detailed description.

been shown to participate in the pIIa/pIIb fate decision, possibly downstream of Notch signalling (Manning and Doe, 1999; Reddy and Rodrigues, 1999).

The sense organ lineage is thought to produce four different cells that together form a mechanosensory organ. In addition to these four cells, a fifth cell is associated with the sense organ. This cell has been described as a small subepithelial cell associated with the neurone and has been named the soma-sheath cell (Hartenstein and Posakony, 1989). The origin of this cell is unknown.

Each of these asymmetric divisions is spatially oriented relative to the fly body axis (Gho and Schweisguth, 1998). pI divides within the plane of the epithelium, along the anteroposterior (AP) axis, with Numb localized to the anterior pole of pI, to produce pIIa posteriorly and pIIb anteriorly. The orientation of this division is regulated by the activity of the Frizzled (Fz) receptor. The division of pIIa is again oriented parallel to the AP axis, with Numb localized to the anterior pole of pIIa, to generate the socket cell posteriorly and the shaft cell anteriorly. Lastly, pIIb divides perpendicularly to the socket-shaft axis, with Numb segregating to the lateralmost cell, which will become a neurone. The mechanisms regulating the orientation of pIIa and pIIb do not involve Fz signalling (Gho and Schweisguth, 1998) and remain to be studied.

In this study, we have examined the pattern of oriented cell divisions in the microchaete lineage by time-lapse confocal microscopy in living pupae and immunodetection on dissected nota. This analysis has revealed the existence of a previously undescribed division in this lineage. Thus, five cells, and not four as previously thought, are produced by each pI cell. This novel unequal division generates a glial cell. This study has also revealed that two divisions are polarized along the apicobasal axis. Together, these observations have led us to propose a novel model for the microchaete lineage.

MATERIALS AND METHODS

Drosophila stocks

The A101 line carries a P[*lacZ*, *ry*⁺] enhancer-trap allele of *neuralized* that specifically expresses nuclear β-galactosidase in pI and its progeny cells (Usui and Kimura, 1993). The dA-10 transformant line carries a *deadpan-lacZ* construct expressing cytoplasmic β-galactosidase (Emery and Bier, 1995; a gift from E. Bier). The rA87 line carries a P[*lacZ*, *ry*⁺] enhancer-trap allele of *glial cell missing* (Jones et al., 1995; gift of C. Klaembt). A chromosome carrying a Pgal4 inserted at the *scabrous* locus (gift from D. Busson) and a UAS-nls-GFP transgene (Shiga et al., 1996; gift from N. Perrimon) was

obtained by recombination. Flies homozygous for this second chromosome were phenotypically wild type.

Time-lapse confocal microscopy

Pupae were stuck on double-sided tape at 15 hours APF, with the notum facing up. The pupal case was gently removed over the head and the notum. A coverslip supported on one side by a capillary was placed onto the pupa. This coverslip was coated with Voltalef 10S oil on its bottom side to increase optical resolution. Presumptive pI cells were identified based on their high level of nlsGFP accumulation, their regular pattern of distribution in rows and on the large size of their nucleus compared to neighbouring epidermal cells. Confocal images were acquired every 4 minutes on a Leica TCS 4D confocal microscope using a oil-immersion 63× objective. Laser exposure had no detectable effect on microchaete formation. Time-lapse movies were assembled using NIH image and Avid Videoshop softwares.

Immunohistology

Dissected nota from pupae at 15-24 hours APF were processed as described in Gho et al. (1996). The analysis of the sense organ lineage was restricted to the dorsocentral region of the pupal notum (microchaete rows 1 to 3). The following primary antibodies were used: rat anti-α-tubulin (Serotec, 1:500); rabbit anti-γ-tubulin (gift from C. Gonzalez; 1:500); mouse anti-Prospero (MR1A, gift from C. Doe 1:5); rabbit anti-Numb (gift of Y.-N. Jan 1:500); rabbit anti-β-galactosidase (Cappel, 1:1000); mouse anti-β-galactosidase (Promega, 1:2000); rat anti-β-galactosidase (gift of C. Doe, 1:5); rabbit anti-HRP (gift from J.-R. Martin 1:1000); mouse anti-Cut (DSHB, 1:500). Secondary FITC-, LRSC- or Cy5-conjugated antibodies anti-mouse, rat or rabbit were purchased from Jackson and used at 1:500 for the FITC- and LRSC-conjugated antibodies or 1:2000 for the Cy5 antibodies. Images were obtained on a Leica TCS 4D confocal microscope and were processed with NIH Image and Photoshop software.

Measuring mitotic spindle orientation

Staged A101 pupae were stained for β-galactosidase, α-tubulin and γ-tubulin and analyzed by confocal microscopy. Cell division was only analyzed in regions mounted flat, as seen by apical α-tubulin immunostaining in epithelial cells. Mitotic sense organ cells, located between rows 1 and 3 and above the anterior dorsocentral macrochaetes, were identified by the presence of mitotic spindles. The identity of the dividing cell was determined by its position and the number of β-galactosidase-positive cells in the cluster (pI, 1-cell stage; pIIb, anterior cell at the 2-cell stage; pIIa, posterior cell at the 3-cell stage; pIIIb, anterior cell at the 4-cell stage). A z-series between the upper and lower centrosomes was acquired and used to determine the position of the two centrosomes in the following coordinate system. The origin of the coordinate system was fixed at the centrosome that was not associated with the Numb crescent. It corresponds to the posterior centrosome in pI and pIIa (Gho and Schweisguth, 1998), and to the upper centrosome in pIIb and pIIIb (this study). The x- and y-axis correspond to the mediolateral and AP axes, respectively, and the z-axis corresponds to the apicobasal axis of the epithelium. The plane of the epithelium corresponds to the xy plane. Based on the coordinates of the two centrosomes (0,0,0 and x_c,y_c,z_c), the orientation of the mitotic spindle was fully characterized by two angles, α_{plan} and α_{AP} (Fig. 2). α_{plan} is the angle between the mitotic spindle and the plane of the epithelium. The value of α_{plan} is determined using tangent $(\alpha_{plan})=z_c/\sqrt{(x_c^2+y_c^2)}$. This angle was measured in the trigonometric orientation looking toward the midline. When the cells divide perpendicularly to the AP axis, α_{plan} was measured in the trigonometric orientation looking toward the head. α_{AP} is the angle between the mitotic spindle projected in the xy plane and the AP axis of the notum. The value of α_{AP} is determined using tangent $(\alpha_{AP})=x_c/y_c$. This angle is the one measured by Gho and Schweisguth (1998) for the pI and pIIa divisions. α_{AP} can only be defined when α_{plan} is not 90°.
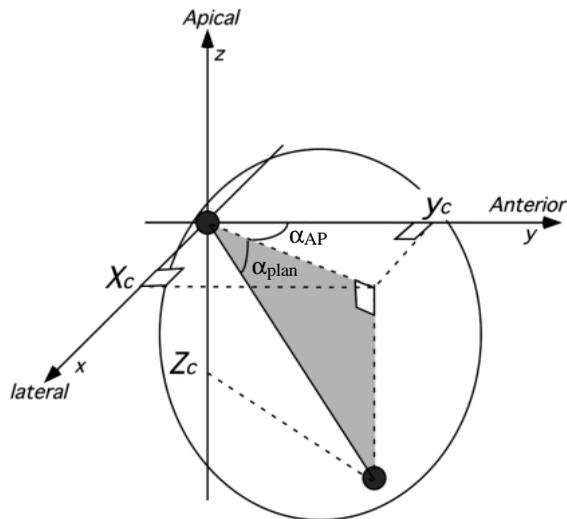
Fig. 2. Definition of the α_{plan} and α_{AP} angles. Diagrammatic representation of the angles α_{plan} and α_{AP} as measured in a mitotic cell, represented here as a sphere. The centrosomes are shown by small dots. See Materials and Methods for a detailed description.

RESULTS

Identification of a novel cell division by time-lapse microscopy in living pupae

The pattern of cell division in the sense organ lineage was analyzed in living pupae using GFP imaging. A nuclear GFP fusion protein (nlsGFP) was used to follow the relative position of the sense organ cells in the pupal notum. Both entry into and exit from mitosis could easily be detected via the changes in nlsGFP distribution associated with nuclear breakdown and nuclear membrane reformation. High levels of nlsGFP protein accumulation in sense organ cells were obtained using the Gal4-UAS expression system (Brand and Perrimon, 1993). A Pgal4 insertion line, sca-gal4, was chosen because it led to detectable levels of nlsGFP accumulation in pI prior to its division. In the dorsocentral region of the notum, sca-nlsGFP was also expressed in many epidermal cells around microchaete rows 1 and 5. However, between row 2 and 4, sca-nlsGFP expression was mostly restricted to pI cells.

Time-lapse confocal microscopy was used to study the pattern of cell divisions in the notum of sca-nlsGFP pupae. The complete lineage of a microchaete sense organ is shown in Fig. 3. The nuclear breakdown of pI was used as the reference time point. The pI cell divided to generate an anterior pIIb cell and a posterior pIIa cell [Fig. 3A at 0 and 1:08 after pI division (APD)]. These two cells appeared to remain within the plane of the epithelium (Fig. 3B at 1:08 APD; from hereafter, the size and position of nlsGFP-positive cells have been estimated from the size and position of their nucleus).

The anterior pIIb daughter divided next (Fig. 3A at 2:44 APD). This division generated a larger cell that appeared to remain within the epithelium and a smaller subepithelial cell (Fig. 3B at 3:28 APD). This small subepithelial cell was identified as a glial cell (see below) and was named sense organ glial cell (g). We have called its sister cell the tertiary b precursor cell (pIIIb) because it underwent one additional round of cell division (see below).

The posterior pIIa cell then divided, at 3:40 APD, to generate an anterior and a posterior cells (Fig. 3A at 3:40 and 4:24 APD). While these two pIIa daughter cells were found within the same plane as pIIIb, the small pIIb daughter cell maintained its subepithelial position (Fig. 3B at 4:24 APD). The origin and relative position of these two daughters along the AP axis indicated that these were the shaft (sf) and socket (so) cells (Gho and Schweisguth, 1998; Gho et al., 1996). Finally, at 5 hours APD, pIIIb divided to generate two cells of identical size (Fig. 3A at 5:00 and 5:32 APD). Upon differentiation, these two cells delaminated under the socket and shaft cells (Fig. 2B at 5:32), suggesting that these were the neurone (n) and sheath cell (st).

A similar pattern of cell division was observed in 8 out of 8 lineages followed by time-lapse confocal microscopy. We conclude that a previously undescribed cell division occurs in the microchaete sense organ lineage: pIIb divides prior to pIIa to generate a tertiary precursor cell, pIIIb, and a smaller subepithelial cell. A total of five sense organ cells, and not four as previously thought, are generated from a single pI precursor cell.

Analysis of four asymmetric and spatially oriented cell divisions in the sense organ lineage

Because the lineage inferred from time-lapse GFP imaging contradicted the current model (Fig. 1), it was important to define the orientation and polarity of all cell divisions in this lineage. Orientation of cell divisions was analyzed in dissected A101 pupae immunostained with α- and γ-tubulin antibodies to reveal mitotic spindles and centrosomes, respectively (Fig. 4). The polarity of dividing cells was studied using anti-Numb and anti-Prospero antibodies (Fig. 5). We also followed the pIIa and pIIb sublineages using Prospero and dA-10 as distinct sublineage markers (Fig. 6).

#### pI divides along the AP axis and within the plane of the epithelium

pI was found to divide within the plane of the epithelium and parallel to the AP axis [$\alpha_{plan}=-1°\pm13$ and $\alpha_{AP}=1°\pm29$; Fig. 4A-A‴; α_{plan} is the angle between the mitotic spindle and the plane of the epithelium; α_{AP} represents the angle between the mitotic spindle and the AP axis of the notum (see Fig. 2)]. At mitosis, Numb localized to the anterior pole of pI. No accumulation of Prospero was detected at this stage (Fig. 5A,A′).

pIIb divides prior to pIIa and perpendicularly to the plane of the epithelium to generate a small subepithelial Prospero-positive cell

At the 2-cell stage, pIIa and pIIb were identified using dA-10 and Prospero as markers. In dA-10 pupae, cytoplasmic β-galactosidase accumulated at a low level in pIIb and at a high level in pIIa. The nuclear transcription factor Prospero was specifically expressed in pIIb, and was not detectable in pIIa (Fig. 6A,A′; see also Manning and Doe, 1999 and Reddy and Rodrigues, 1999). At the 2-cell stage, we observed that the anterior, Prospero-positive and dA-10-negative pIIb cell always divided prior to the posterior, Prospero-negative and dA-10-positive pIIa cell (Fig. 6A-B′).

The pIIb cell divided perpendicularly to the plane of the

Fig. 3. Time-lapse analysis of the microchaete lineage in living
pupae. A live pupa expressing nlsGFP under the control of sca-
gal4 was observed by time-lapse confocal microscopy. The
divisions of a row 3 microchaete pI cell were followed. Images
are presented at the times indicated after pI division (APD).
(A) Pattern of cell divisions in surface views. Four divisions
were observed over a total recording time of 11:20 hours (from
t=−2:20 to +9:00; the breakdown of the nuclear envelope of the
pI cell was chosen as the 0 reference time point). The pI cell
divided at 0:00 to generate two cells aligned along the AP axis;
pIIa and pIIb were identified as the posterior and anterior
daughters, respectively. The pIIb cell divided next at 2:44 to
generate two cells of unequal size: the large cell was named
pIIIb and the small cell g. This latter cell produced a weaker
immunofluorescence signal, probably because of its smaller
size. The posterior pIIa cell divided after pIIb, at 3:40, and
anterior pIIIb at 5:00. pIIa generates the socket (so) and shaft
(sf) cells, and pIIIb produces the neurone (n) and sheath (st)
cells. The socket cell was identified as the posteriormost cell.
Three criteria were used to tentatively identify the neurone and
the sheath cells in this recording: following pIIIb division, the
neurone was the most basal (not shown) and the most lateral
pIIIb daughter, and was found close to the small pIIb daughter
cell (g). No additional division was observed until the end of
recording (i.e. 3.5 hours after pIIIb division). It took about 25
minutes from nuclear envelope breakdown in the mother cell to
nuclear import of nlsGFP into newly reformed nuclei in the
daughter cells. Since recording was done at 23°C, development
was slightly slower than is usually observed under normal
culture conditions at 25°C. Each image corresponds to the
merge of 6-8 horizontal confocal optical sections. As a
consequence, the relative position of each nucleus relative to the
apicobasal axis is lost. Asterisks indicate non-lineage related
cells, which probably correspond to epidermal cells. Bar, 5 μm.
Anterior (A) is up and lateral (L) is to the left. A quick-time movie
is available at http://www.biologie.ens.fr/fr/droso/droso.html.
(B) Relative positions of the nuclei in lateral views. Each view
corresponds to a lateral projection from eleven horizontal optical
sections. The nucleus of the small pIIb daughter cell occupies a
basal position. Following pIIIb division, the sheath nucleus moved
underneath the shaft nucleus. Anterior (A) is to the left, apical
(Ap) is up.
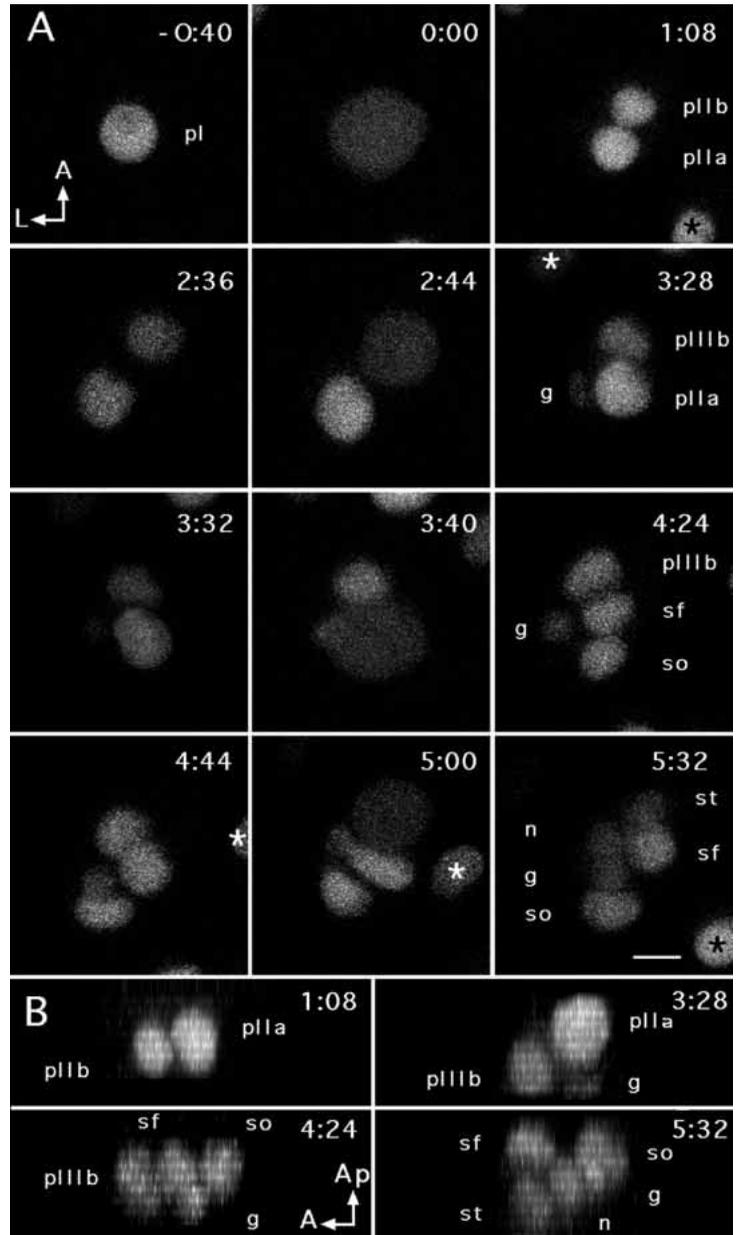
epithelium (α_{plan}=−94°±22 in Fig. 4B-B‴; see also Fig.
6A,B′). This division generated a small subepithelial cell and
a large cell that remained in the plane of the epithelium. Based
on their size and position, these two cells corresponded to the
glial (g) and pIIIb cells identified by time-lapse microscopy
(Fig. 3).

At pIIb mitosis, Prospero localized at the cell cortex and
preferentially accumulated at the basal pole at telophase (Figs
5B,B′, 6A′). Following pIIb division, but prior to the
translocation of Prospero into reforming nuclei, a higher level
of Prospero accumulation was detected in the small
subepithelial cell relative to its larger sister cell (Fig. 6B′). In
dividing pIIb, Numb accumulated at the basal cortex and
along the region of cell contact between pIIb and pIIa (Fig.
5B,B′). Numb was later found to unequally segregate into the
small subepithelial daughter. Therefore, although both Numb
and Prospero co-segregated into the small subepithelial

daughter, they did not exactly co-localize in dividing pIIb
(Fig. 5B,B′).

pIIa divides after pIIb, within the plane of the epithelium and along the AP axis

At the 3-cell stage, pIIa was identified as a Prospero-negative
and strongly dA-10-positive cell; cytoplasmic β-galactosidase
accumulated at a lower level in the weakly Prospero-positive
pIIIb and was not detectable in the strongly Prospero-positive
small subepithelial pIIb daughter cell (Fig. 6C-D′). Because the
two pIIb daughter cells were always detected when pIIa
divided (see the pIIIb and g cells in Fig. 6C-D′), we conclude
that the posterior, dA-10-positive and Prospero-negative pIIa
cell divides after pIIb. Previous studies have shown that the
anterior pIIa daughter cell adopts a shaft fate, while its
posterior daughter adopts a socket fate (Gho and Schweisguth,
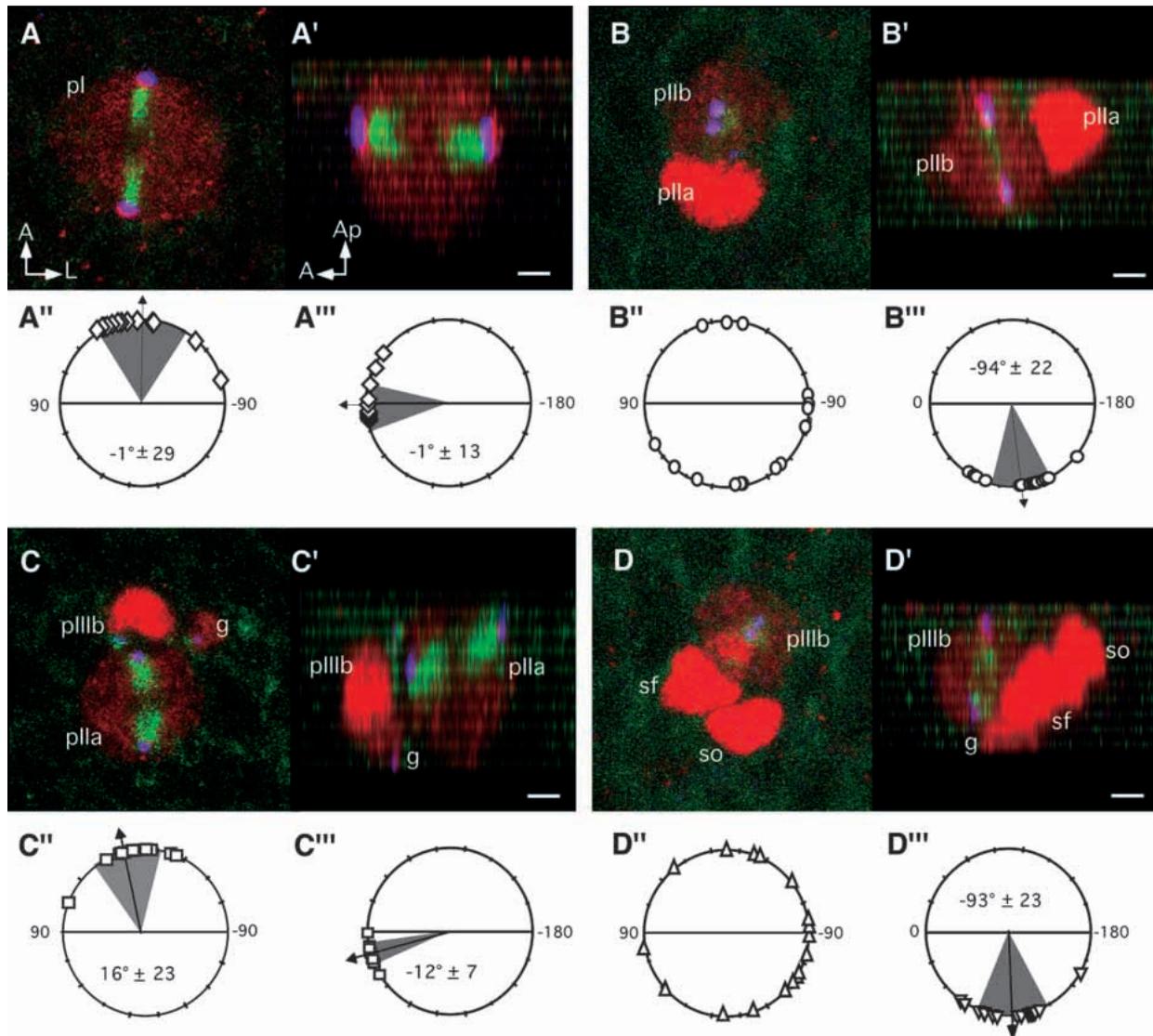1998; Gho et al., 1996).

Fig. 4. Orientation of cell division in the microchaete lineage. The orientation of cell divisions was analyzed in confocal images of nota dissected from staged A101 pupae immunostained with α- and γ-tubulin to reveal mitotic spindles and centrosomes, respectively. The nuclear β-galactosidase A101 marker was used to identify sense organ cells. Nuclear β-galactosidase is in red, α-tubulin in green and γ-tubulin in blue (centrosomes appeared pink-colored because, in this experiment, Cy5 fluorescence was also detected in the red channel). Dividing cells (pI: A-A‴, *n*=14; pIIb: B-B‴, *n*=17; pIIa: C-C‴, *n*=16; pIIIb: D-D‴, *n*=18) were identified by the presence of a mitotic spindle. In A-D, horizontal confocal sections are shown. In A′-D′, vertical optical reconstructions show the plane containing both centrosomes. The values of the angle between the projected horizontal view of the mitotic spindle and the AP axis (α_{AP}) are plotted in A″-D″ (0° corresponds to a division parallel to the AP axis). The values of the angle between the mitotic spindle and the plane of the epithelium (α_{plan}) are plotted in A‴-D‴ (−90° corresponds to a division perpendicular to the plane of the epithelium). pI and pIIa divide along the AP axis (A″,C″), within the plane of the epithelium (A‴,C‴), while pIIb and pIIIb divide perpendicularly to the plane of the epithelium (B‴,C‴). In each plot, the arrow indicates the mean value and the shaded area shows the standard deviation. 20° intervals are shown on each circular plot. The angle values of α_{plan} and α_{AP} were determined as described in Materials and Methods. Bar, 2 μm.

In contrast with pIIb, pIIa divided parallel to the AP axis and within the plane of the epithelium (α_{AP}=16°±23 and α_{plan}=−12°±7 in Fig. 4C-C″; see also the position of the mitotic spindle in Fig. 6C-D′). We also noted that the anterior centrosome often adopted a basal position (*n*=15 out of 16; Fig. 4C′-C‴).

A crescent of Numb formed at the anterior cortex of dividing pIIa (Fig. 5C,C′), resulting in its segregation into the anterior shaft cell. By contrast to Numb, Prospero was not detectable in dividing pIIa (Figs 5C,C′, 6C-D′).

pIIIb divides last, perpendicularly to the plane of the epithelium

At the 4-cell stage, the anterior and weakly Prospero-positive pIIIb cell divided to generate two cells of equal size (Fig. 6E-G). Based on their size and position, these cells correspond to the neurone and sheath cell. They can be identified at 23 hours APF as the HRP- and Elav-positive neurone and Prospero-positive sheath cell (see below). Like pIIb, pIIIb divided perpendicularly to the plane of the epithelium (α_{plan}=−93°±23 in Fig. 4D-D‴; see also Fig. 6E′). In most cases (*n*=13 out of
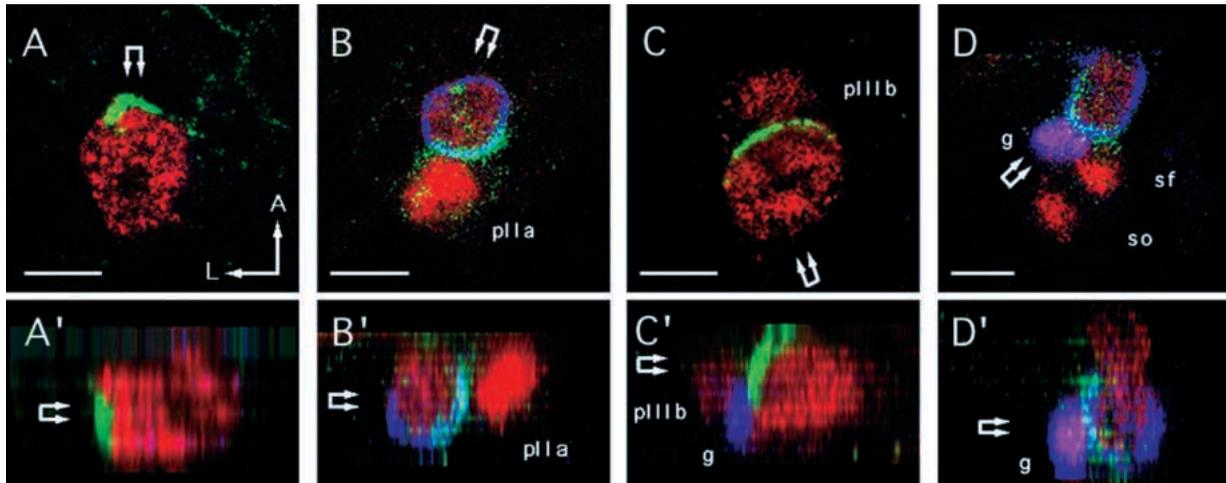
Fig. 5. Unequal segregation of Prospero and Numb. Confocal images of nota dissected from staged A101 pupae showing pI (A,A′), pIIb (B,B′), pIIa (C,C′) and pIIIb (D,D′) divisions. Nuclear β-galactosidase is in red, Numb in green and Prospero in blue. Dividing cells were identified via the detection of cytoplasmic β-galactosidase in the entire cell volume as the nuclear envelope broke down. In each pair, the top panel shows a horizontal confocal section, and the bottom panels a vertical optical reconstruction. The position of the slice used for vertical reconstruction and of the horizontal section is shown by a double arrow in A-D and A′-D′, respectively. In dividing pI (A,A′) and pIIa cells (C,C′), Numb was localized in an anterior crescent and Prospero was not detectable. In dividing pIIb (B,B′) and pIIIb cells (D,D′), Prospero protein localized at the basal pole, while Numb accumulated both basally and at the site of contact with pIIa and glial cells, respectively. In A-D, anterior is up; in A′-D′, apical is up. Bar, 5 μm.

18), the division of pIIIb was slightly tilted along the mediolateral axis, with the basal centrosome adopting a lateral position (Fig. 4D″).

At mitosis, Prospero was seen to accumulate at the basal cortex of pIIIb, while Numb localized at the basal cortex and along the region of cell contact between pIIIb and the small pIIb daughter cell (Fig. 5D,D′). Both fate determinants were found to segregate into the basal daughter cell (Fig. 5D′).

It is generally thought that Numb acts cell autonomously during asymmetric cell divisions by inhibiting Notch signalling in the cell that specifically inherits Numb (Guo et al., 1996; Wang et al., 1997; Spana et al., 1995; Rhyu et al., 1994). Thus, since Numb was found to segregate into the basal pIIIb daughter (Fig. 5D,D′), we infer that Notch signalling is inhibited in this cell. Because *Notch* activity is required to specify the sheath fate (Hartenstein and Posakony, 1990), we propose that the basal pIIIb daughter, which inherits Numb, will become a neurone. This implies that Prospero is also unequally inherited by the neurone. However, at 23 hours APF, Prospero was mostly detected in the sheath cell (Fig. 6G). Thus, our hypothesis that the basal cell will become a neurone implies that the accumulation of Prospero in this cell should only be transient. Therefore, we would expect to detect 5-cell clusters in which three cells accumulated Prospero: the small dA-10-negative pIIb daughter cell should be strongly Prospero-positive, and the two dA-10-positive neurone and sheath cells should accumulate a low amount of Prospero: this was indeed observed (Fig. 6F).

At the 5-cell stage, the nucleus of the shaft cell appeared to move underneath the socket cell nucleus and the small pIIb daughter cell associated with the neurone and its growing axon (Fig. 6G).

These results are entirely consistent with the pattern of cell divisions observed in vivo by time-lapse microscopy. Both sets of results show that sensory clusters are composed of five cells: a small subepithelial cell generated by the unequal division of pIIb, the socket and shaft cells produced by the asymmetric division of pIIa, and the sheath cell and neurone generated by the asymmetric division of pIIIb.

The small subepithelial pIIb daughter cell is a glial cell

To determine the fate of the small subepithelial pIIb daughter cell, we followed the position of this cell relative to the four other sense organ cells by time-lapse confocal microscopy in sca-nlsGFP pupae. Following the pIIIb division, the socket, shaft, sheath and neurone cells, identified here based on their recorded lineage origin, remain clustered over a period of 200 minutes. By contrast, the small pIIb daughter cell moved in a posterior direction over a distance of 40-60 μm (Fig. 7). This provides direct evidence that this cell migrates away from the sensory cluster. This migratory behaviour explains why differentiated microchaete sense organs only comprise four cells and lack the small subepithelial pIIb daughter cells.

The position of the small pIIb daughter cell relative to sense organ cells was also determined in fixed nota. Small subepithelial pIIb daughter cells, identified as dA-10-negative and Prospero-positive cells in 23-24 hours APF dA-10 pupae, were found associated with axons growing from sense organs composed of only four dA-10-positive cells (Figs 8A, 6G). Similarly, small pIIb daughter cells, identified as subepithelial, A101- and Prospero-positive cells, were found along axonal tracts, some distance away from sense organs comprised of only four cells (arrow in Fig. 8B). We conclude that the small subepithelial pIIb daughter cell migrates along the extending axon around 23-24 hours APF. This suggests that this cell has acquired a glial fate (Giangrande, 1994).
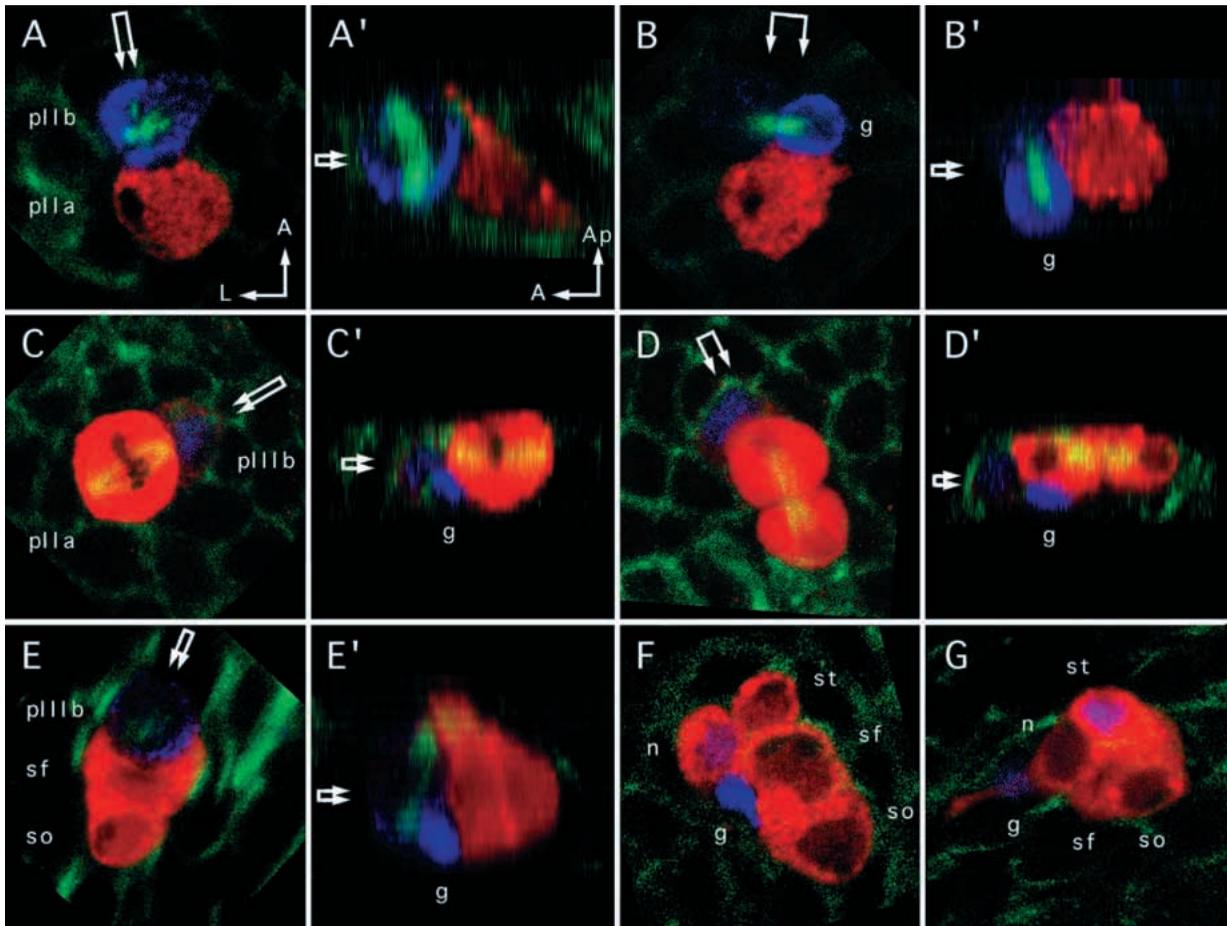
Fig. 6. Pattern of microchaete cell divisions in the notum. Confocal images of nota dissected from staged dA-10 pupae showing pIIb (A-B′), pIIa (C-D′) and pIIIb (E,E′) divisions, and a sensory cluster at 23 hours APF (F,G). Cytoplasmic β-galactosidase is in red, α-tubulin in green and Prospero in blue. Dividing cells were identified by the presence of either a mitotic spindle at metaphase/anaphase (A,A′,C,C′,E,E′) or a midbody at telophase (B,B′,D,D′). Each pair from A-E′ shows a horizontal confocal section and a vertical optical reconstruction from eleven horizontal sections. A double arrow shows the position of the slice used for vertical reconstruction (A-E) and of the horizontal section (A′-E′). The level of expression of the dA-10 β-galactosidase marker in pI was too low to allow the identification of dividing pI cells in this experiment. pIIb was identified at the 2-cell stage as the anterior, Prospero-positive and dA-10-negative cell. pIIb divided perpendicularly to the epithelium plane. Prospero accumulated predominantly in the basal daughter cell (A-B′). pIIa, identified as the posterior, Prospero-negative and dA-10-positive cell, divided next within the plane of the epithelium (C-D′). At the time pIIa divided, two Prospero-positive cells, corresponding to the two pIIb daughter cells, were always detected. The socket (so) and shaft (sf) cells were identified as the posterior and anterior pIIa daughter cells, respectively. These two cells were usually found to remain within the epithelium until the division of pIIIb. pIIIb divided last, perpendicularly to the plane of the epithelium (E,E′). Prospero localized predominantly in the basal daughter cell (E′). At 23 hours APF, following pIIIb divisions, five cells were identified (F,G). The small pIIb daughter cell (g) was subepithelial, dA-10-negative, strongly Prospero-positive, and closely associated with the extending axon (G). At the 5-cell stage, two types of sensory clusters were observed: in most cases, one of the two pIIIb daughter cells accumulated a high level of Prospero accumulation (G). This pIIIb daughter, which was always located opposite to the small pIIb daughter cell, was the sheath (st) cell. Occasionally, the two pIIIb daughter cells were weakly Prospero-positive (F). We suggest that this may represent an earlier situation, in which the neurone (n) close to the small pIIb daughter cell still contains detectable levels of Prospero inherited from its pIIIb mother, and the sheath cell starts to accumulate Prospero.

In *Drosophila*, glial cells are specified by the expression of a key selector gene, *glial cell missing* (*gcm*) (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996). Glial cells associated with sensory axons have previously been identified in pupae based on the expression of the rA87 *lacZ* enhancer-trap inserted at the *gcm* locus (Giangrande et al., 1993). We have used this *gcm*-enhancer-trap line to identify glial cells in the pupal notum. No rA87 expression was detectable prior to pIIIb division (not shown). At 23 hours APF, expression of rA87 was first detected in one of the five

sensory organ cells. The size and the position of this cell indicate that it corresponds to the small subepithelial pIIb daughter cell (Fig. 8C). Furthermore, this rA87-positive cell was associated with axons (data not shown), and accumulated Prospero (Fig. 8D). Interestingly, small A101-positive but Prospero-negative cells were also seen associated with axonal tracts (arrowhead in Fig. 8B); likewise, small rA87-positive but Prospero-negative cells were also detected, but only at some distance from sensory organs (Fig. 8D). We propose that these rA87- or A101-

Fig. 7. Migration of the small pIIb daughter cell. Microchaete sense organ cells were observed by time-lapse confocal microscopy. Recording was initiated at t=−250 minutes, just after pI division. The image shown in A (t=0) was taken 40 minutes after the division of pIIIb in the anterior cluster. Two microchaete sense organs from the dorsocentral row 3 were analysed. The identity of each sense organ cell was determined based on their lineage origin. The migration of two small pIIb daughter cells was analyzed over a period of 180 minutes, during which they moved a net linear distance of 40 and 60 μm. (A) A schematic diagram recapitulating the migration of these two pIIb daughter cells. During the period of observation, the 20 minute intervals are color-coded as indicated on the time-scale bar shown at the bottom. Two sensory clusters from row 2 can also be observed (on the left). Asterisks indicate non-lineage related cells, that probably correspond to epidermal cells expressing nlsGFP. (B) Selected images are presented at the indicated time points. Anterior (A) is at the top, lateral (L) is on the left. Bar, 5 μm. A quick-time movie is available at http://www.biologie.ens.fr/fr/droso/droso.html.
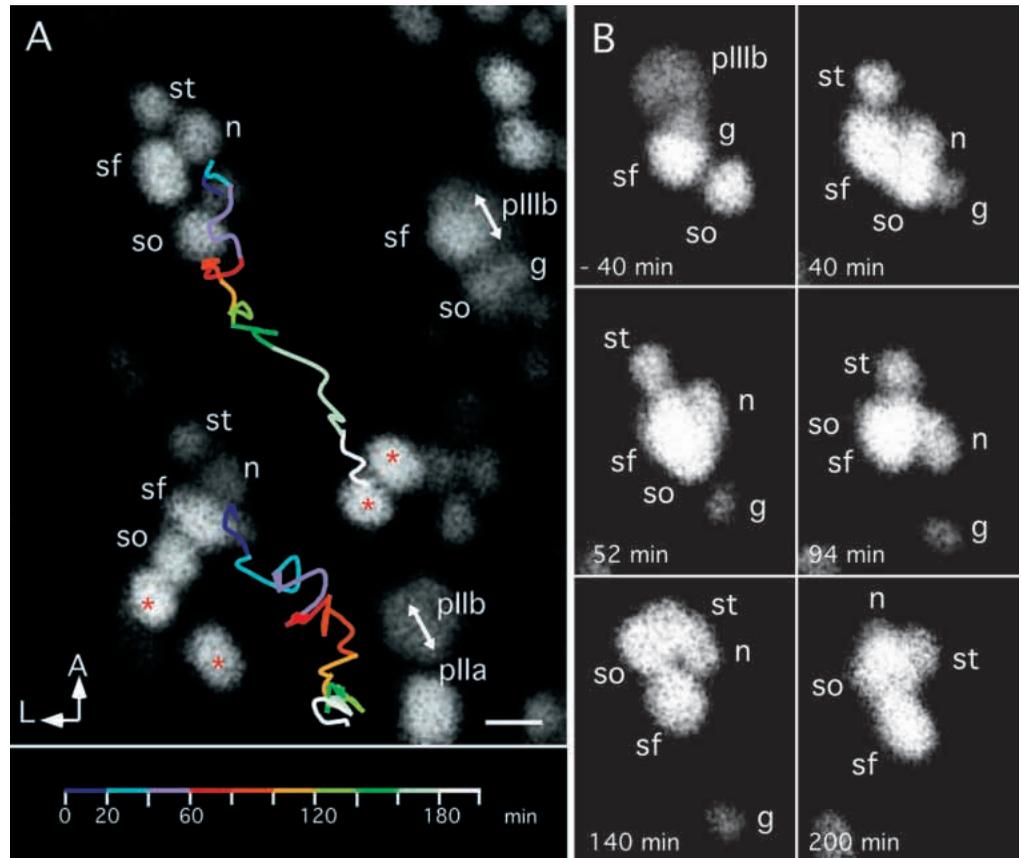
positive cells are small subepithelial pIIb daughter cells that lost Prospero immunoreactivity as they migrated away from the sensory clusters.

We conclude that the small pIIb daughter cell acquires a glial fate, and that, in the notum, sense organ glial cells are generated by the unequal division of pIIb.

DISCUSSION

In this study, we have analyzed the pattern of oriented cell divisions in the microchaete sense organ lineage by time-lapse confocal microscopy on living pupae and by immunostaining analysis of dissected and fixed nota. Our results are summarized in Fig. 9: pI divides asymmetrically, within the plane of the epithelium and along the AP axis, to give rise to two secondary precursor cells, pIIa and pIIb. Then, prior to the division of pIIa, pIIb divides unequally and perpendicularly to the plane of the epithelium to generate a large tertiary precursor cell, pIIIb, and a small glial cell. Following the pIIb division, pIIa divides along the AP axis, within the plane of the epithelium, to produce the socket and shaft cells. Finally, pIIIb divides perpendicularly to the plane of the epithelium to generate the sheath cell and the neurone. Therefore, the microchaete lineage generates five cells. Finally, we showed that Numb is asymmetrically localized in mitotic pI, pIIa, pIIb and pIIIb, and that Prospero is asymmetrically distributed in mitotic pIIb and pIIIb, and unequally inherited by the glial and the basal pIIIb daughter.

A novel division in the microchaete lineage of *Drosophila*

The first detailed description of the sense organ lineage in the pupal notum of *D. melanogaster* proposed that four cells are generated from a single pI cell via two rounds of asymmetric divisions (Hartenstein and Posakony, 1989; see Fig. 1). This description relied on BrdU pulse-labelling experiments which showed the following. (1) The sheath and neurone cells were sisters, born from a common precursor cell, called pIIb (note that pIIb in Hartenstein and Posakony, 1989 corresponds to the pIIIb described in this study). This pIIb cell was the last one to replicate its DNA. (2) The socket and shaft cells were also sister cells, and were generated by the division of a common precursor cell, called pIIa. This pIIa cell incorporated BrdU just prior to pIIb (defined here as the precursor of the neurone and sheath cell). (3) All four cells were derived from a unique primary precursor cell, pI. This study also indicated the presence of a small BrdU-positive soma-sheath cell associated with the four BrdU-labelled sense-organ cells. Because this cell was often seen at some distance from the sensory cluster, it was inferred that it derived from an unknown precursor, which also carried out its terminal DNA replication at approximately 16 hours APF. Soma sheath cells have previously been described in adult external sense organs as small, subepithelial, A101-positive cells found associated with the neurone and/or its axon (Hartenstein and Posakony, 1989; Usui and Kimura, 1993; Huang et al., 1991). Our data indicate that this soma-sheath cell most likely corresponds to the small
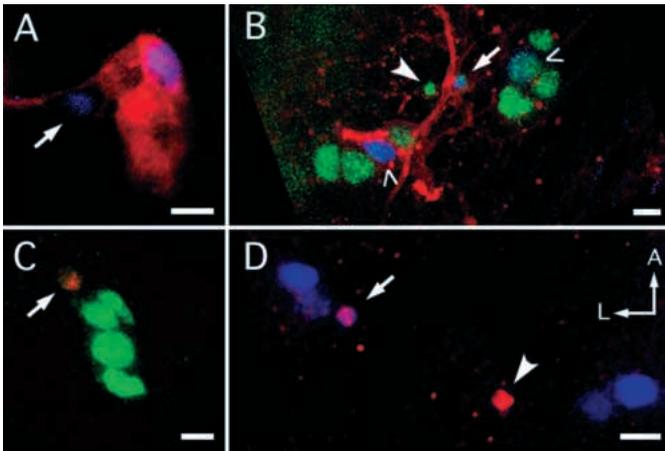
Fig. 8. The small pIIb daughter cell is a glial cell. Confocal images of nota dissected from staged dA-10 (A), A101 (B) and rA87 (C,D) 23 hours APF pupae. (A) The small pIIb daughter cell, identified as a small Prospero-positive, dA-10-negative cell, is indicated by an arrow. It was found associated with the outgrowing axon. The dA-10-positive, Prospero-positive cell is the sheath cell. Cytoplasmic β-galactosidase in dA-10 is shown in red, Prospero in blue. (B) Two small A101-positive cells, one Prospero-positive (arrow), the other Prospero-negative (arrowhead), were found associated with a large axonal tract, close to two sense organs composed of four cells, each including a Prospero-positive cell identified as the sheath cell based on its size and position (open arrowheads). Nuclear β-galactosidase is shown in green, membrane-associated HRP in red, Prospero in blue. (C) A small Cut-positive cell, identified as the small pIIb daughter cell based on its size and position, expressed nuclear β-galactosidase in a rA87 pupa (arrow). Nuclear Cut is shown in green, nuclear β-galactosidase in rA87 in red. (D) Two small β-galactosidase-positive cells were detected close to two developing microchaetes in a rA87 pupa. The one on the left (arrow) was still located in close proximity to a neurone and a sheath cells, detected here by their low- and high-level of Prospero accumulation, respectively. The second, on the right (arrowhead), has migrated some distance away, and does not express Prospero any more. Nuclear β-galactosidase in rA87 is shown in red, Prospero in blue. Anterior is at the top. Bar, 5 μm.
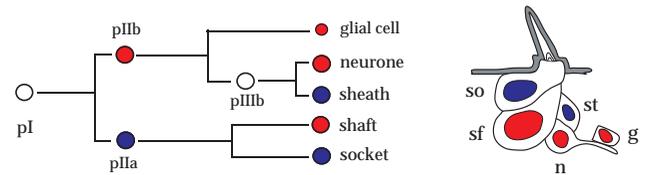

Fig. 9. A proposed model for the microchaete bristle sense organ lineage. Schematic representation of cell divisions in the lineage generating the mechanosensory microchaetes of the notum in *D. melanogaster*. The daughter cells that specifically inherit Numb at mitosis are in red. The cells in which Notch signalling is activated are in blue. Whether Notch is activated in pIIIb is unknown. See text for a detailed description.

pIIb daughter cell that differentiates as a glial cell. Our revised description of the lineage is therefore entirely consistent with the results from these BrdU pulse-labelling experiments. However, we now suggest that, because sense organ and sense organ glial (soma-sheath) cells originate from a common precursor, they all incorporated BrdU at the same time, around 16 hours APF.

These BrdU pulse-labelling experiments indicated that the precursor of the shaft and socket cells, pIIa, replicated its DNA before the precursor of the neurone and sheath cells, named pIIb in this study (Hartenstein and Posakony, 1989). However, more recent studies indicated that the anterior Prospero-positive daughter cell, pIIb, enters mitosis prior to pIIa (Manning and Doe, 1999; Reddy and Rodrigues, 1999). The model proposed here reconciles these two sets of apparently contradictory data: pIIb does indeed divide prior to pIIa, while the precursor of the neurone and sheath cells, pIIIb, divides after pIIa.

In a previous study, we have described the pattern of cell divisions in this lineage. Cell divisions were detected by anti-

α-tubulin staining to reveal mitotic spindles and sense organ cells were identified using the A101 marker. The mitotic spindles of dividing pI, pIIa and pIIIb cells were detected, but we failed to observe mitotic spindles corresponding to dividing pIIb [note that the division of pIIb reported in Gho and Schweisguth (1998) corresponds to the division of the pIIIb identified here]. This was probably due to the fact that mitotic spindles oriented perpendicularly to the plane of the epithelium are difficult to recognize in optical sections that bisect the spindles. In these experiments, we often observed a small subepithelial A101-positive cell. Because only two rounds of divisions were detected, hence generating four cells, we assumed that this small cell, now identified as the sense organ glial cell, was not clonally related to sense organ cells.

Tissue-polarity and apicobasal polarity cues regulate the orientation of asymmetric divisions in this lineage

The study by Gho and Schweisguth (1998) indicated that pI and pIIa divided parallel to the AP axis, while pIIIb divided perpendicularly to the AP axis. Here, we have used centrosomal markers to measure in three dimensions the orientation of the division axis of the pI, pIIa, pIIb and pIIIb divisions.

This study confirms that pI and pIIa divide within the epithelial plane and along the AP axis. The orientation of the pI division is regulated by Fz signalling. By contrast, the orientation of the pIIa division relative to its sister cells does not require *fz* activity (Gho and Schweisguth, 1998). The positioning of the mitotic spindle in pIIa might be influenced by cell signalling from anterior pIIb and/or pIIIb cells, or by cortical marks deposited during the previous pI division. Consistently, the mitotic spindle of pIIa is often tilted basally toward pIIIb.

This study establishes that both pIIb and pIIIb divide perpendicularly to the epithelial plane. This contrasts with our previous conclusion that pIIIb divides within the plane of the epithelium and perpendicularly to the AP axis (Gho and Schweisguth, 1998). Because horizontal sections were projected along the z-axis in our previous study, only mitotic spindles tilted relative to the apicobasal axis were recognized. This led us to an erroneous conclusion. Our previous observation that Numb localized away from the midline (Gho and Schweisguth, 1998), however, is consistent with our present finding that the most basal centrosome associated with the Numb crescent often occupies a lateral position.

The regulatory mechanisms involved in positioning the centrosomes along the apicobasal axis in pIIb and pIIIb are unknown. In the *Drosophila* embryo, the activity of *inscuteable* is known to be required in neuroblasts to coordinate the basal localization of fate determinants with the apicobasal orientation of the mitotic spindle (Kraut et al., 1996). Moreover, it has been shown that the ectopic expression of *inscuteable* in ectodermal cells results in a 90° rotation of the mitotic spindle: it is normally found within the plane of the ectoderm, but, upon *inscuteable* expression, it adopts an apicobasal orientation. It is tempting to speculate that *inscuteable* might be specifically expressed in pIIb and pIIIb to regulate their orientation of division.

Our analysis highlights that the microchaete lineage in *D. melanogaster* is an appropriate model system to study genetically how planar and apicobasal polarity cues are interpreted and integrated during asymmetrical cell division in an epithelium.

The asymmetric distribution of Prospero and Numb in dividing pIIb

Our results confirm that Numb is asymmetrically distributed in dividing pI, pIIa and pIIIb cells, and is unequally inherited by the pIIb, shaft and neurone cells (Wang et al., 1997; Rhyu et al., 1994; Gho and Schweisguth, 1998). We also establish that Numb forms a basal crescent in pIIb and segregates into the sense organ glial cell.

In contrast with Numb, Prospero was not detected in dividing pI and pIIa (see also Manning and Doe, 1999; Reddy and Rodrigues, 1999). We have shown here that Prospero, like Numb, forms a basal crescent in pIIb and pIIIb, and preferentially segregates into the future glial cell and neurone. By contrast, two recent reports indicated that Prospero is uniformly localized at the cell cortex in dividing pIIb (Manning and Doe, 1999; Reddy and Rodrigues, 1999). In these studies, the distribution of Prospero was examined in confocal sections perpendicular to the apicobasal axis of dividing pIIb. Therefore, it is possible that the basal distribution of Prospero could have escaped detection. A detailed co-localization analysis of Numb and Prospero in dividing pIIb and pIIIb revealed that these two fate determinants did not strictly co-localize. In these cells, Prospero was mostly found at the basal pole, while Numb was also found to accumulate in the cortical region of cell contact between sense organ cells. It will be interesting to examine how cell-cell interactions between sense organ cells regulate the activity of the protein complexes involved in the polar distribution of both Numb and Prospero (Spana and Doe, 1995; Schuldt et al., 1998; Shen et al., 1997; Lu et al., 1998; Knoblich et al., 1995; Ikeshima-Kataoka et al., 1997).

Our analysis of the pIIb division reveals a striking analogy between the pIIb division in the notum and the neuroblast division in the embryo (for a review, see Hawkins and Garriga, 1998). First, both cells divide unequally to produce two cells of different size. Second, in both cases, the division is oriented along the apicobasal axis and the small daughter cell appears at the basal pole. Third, Numb and Prospero specifically localize at the basal pole and segregate into the small basal cell. It will thus be of interest to examine whether asymmetry is established by similar molecular mechanisms in both pIIb and neuroblast.

The basal pIIIb cell that inherits Numb and Prospero is proposed to be the neurone

As in dividing pIIb, Prospero was found to localize asymmetrically at the basal pole of pIIIb, while Numb localized in a basolateral crescent. Both proteins segregated preferentially into the basal daughter. Because Numb was found to segregate into the basal daughter, we propose that the basal pIIIb daughter cell is the neurone. The apical pIIIb daughter must therefore be the sheath cell.

Our interpretation that the neurone corresponds to the basal pIIIb daughter cell implies that accumulation of Prospero in the neurone is only transient and that the high level accumulation of Prospero in the sheath cell is due to de novo synthesis. A transient accumulation of Prospero in the neurone would also be consistent with the hypothesis formulated by Manning and Doe (1999) that Prospero functions in the neurone to regulate axonal pathfinding.

Origin and specification of sense organ glial cells

Glial cells constitute a crucial component of the nervous system. They wrap the neuronal somata and axons and play a number of roles during normal neuronal activity and development, including axonal growth. Gliogenesis in the peripheral nervous system (PNS) of the adult fly has been best described in the wing (Giangrande, 1994, 1995; Giangrande et al., 1993). In this tissue, glial cells originate from regions of the ectoderm that also give rise to sense organs. Glial cells then migrate along the nerve following the direction taken by the axons. In addition, mutations that induce ectopic sense organs also lead to the emergence of ectopic glial cells. Conversely, mutations that reduce the number of sensory bristles result in a significant reduction of the number of glial cells. These observations led to the hypothesis that gliogenesis is induced in the ectoderm by neighbouring sense organ cells (Giangrande, 1995). However, the exact origin of the glial cells is not known. Our finding that sense organ glial cells are produced by the asymmetric division of pIIb in the notum offers a novel interpretation for all these earlier observations and suggests that, in the wing, glial cells originate from sensory lineages.

The division of pIIb is intrinsically asymmetric. It produces a small subepithelial cell that will adopt a glial fate and a larger pIIIb cell. The intrinsic nature of this division suggests that expression of *gcm* in the small subepithelial is a consequence of the initial asymmetry established in pIIb. Two fate determinants, Numb and Prospero, are unequally inherited by the future glial cell. This raises the possibility that they participate in activating *gcm* expression in the small pIIb daughter and act upstream of *gcm* in establishing a glial fate. We note, however, that the function of *gcm* in the determination of the adult PNS glial cells remains to be investigated.

Revisiting sensory organ lineages in *D. melanogaster* and other insect species

The model proposed in this study (Fig. 9) applies to the lineage of microchaete sense organs located in the dorsocentral region of the notum of *D. melanogaster*. It is therefore important to consider whether this model may also apply to other sensory lineages.

The lineage generating the notal macrochaetes has been best studied by Huang et al. (1991). Around 6 hours APF, clusters

of five A101-expressing cells were seen at macrochaete positions in the notum. The authors noted that "in the case of the aPA, it seems that just after the sensory mother cell has undergone its first mitosis a nearby cell begins to express *lacZ* and joins the cluster" (Huang et al., 1991). This fifth cell most likely corresponds to the small glial cell produced by pIIb. Thus, we propose that the revised lineage described here also applies to macrochaetes on the notum.

Should other external sense organ (es) lineages also be revised in *D. melanogaster*? In the embryo, the lineages generating chemosensory and mechanosensory organs have been defined by BrdU pulse-labelling experiments (Bodmer et al., 1989). Similarly to what was found in the notum, these experiments showed that the sheath cell and the neurone were siblings, and suggested that the socket and shaft cells were derived from a common precursor. Because the authors assumed that a single precursor generated the four external sense organ cells, a model identical to the one proposed for the microchaete lineage (Fig. 1) was put forward. More recently, lineage studies have been performed in the embryo using a different methodology based on the expression of *lacZ* in flp-out clones (Brewster and Bodmer, 1995). In both studies, BrdU-labelled or β-gal-stained cells could often be observed close to es cells, suggesting the possibility that pI generates additional cells in the embryo as well. Indeed, consistent with the possibility that sense organ glial cells are produced in es lineages, small cells surrounding the neuronal cell body and/or the peripheral nerve, called either soma-sheath cells, glial, perineural or a neurilemna cells have been described associated with es organs in the embryo (Hartenstein, 1988; and references therein). This raises the possibility that the model currently proposed for the embryonic es lineage may be incomplete.

The lineages of various sensilla have been analysed in several insect species (Bate, 1978). In particular, the lineage generating the larval mechanosensory organs has been analysed in *Oncopeltus* (Lawrence, 1966). Like in *D. melanogaster*, this lineage appears to generate five cells. The division of pI was horizontal, the first division of a secondary precursor cell was vertical and the division of the other secondary precursor cell was horizontal. At this stage, a cell that appears to be similar in structure and position to the sense organ glial cell produced by pIIb has been observed. A final division was then sometimes observed. While this lineage generated five cells, differentiated sense organs only comprise four cells. Thus, the sequence and the orientation of cell divisions in the larval bristle sense organ lineage in *Oncopeltus* appears to be identical to the one described here for the microchaete lineage in *D. melanogaster*. This suggests that this sequence of oriented cell divisions has been conserved throughout insect bristle evolution.

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