A glial cell arises from an additional division within the mechanosensory lineage during development of the microchaete on the *Drosophila* notum

G. Venugopala Reddy and Veronica Rodrigues

Department of Biological Sciences, Tata Institute of Fundamental Research, Homi Bhabha Rd, Mumbai-400 005 and the National Centre for Biological Sciences, TIFR, Bangalore 560 065, India

*Author for correspondence (e-mail: veronica@tifr.res.in)

Accepted 29 July; published on WWW 27 September 1999

**SUMMARY**

We have used different cell markers to trace the development of the sensory cells of the thoracic microchaete. Our results dictate a revision in the currently accepted model for cell lineage within the mechanosensory bristle. The sensory organ progenitor divides to form two secondary progenitors: PIIa and PIIb. PIIb divides first to give rise to a tertiary progenitor-PIII and a glial cell. This is followed by division of PIIa to form the shaft and socket cells as described before. PIII expresses high levels of Elav and low levels of Prospero and divides to produce neuron and sheath. Its sibling cell expresses low Elav and high Prospero and is recognized by the glial marker, Repo. This cell migrates away from the other cells of the lineage following differentiation. The proposed modification in lineage has important implications for previous studies on sibling cell fate choice and cell fate specification in sensory systems.

Key words: Microchaete, Mechanosensory, Repo, Prospero, Sense organ precursor, Glia

**INTRODUCTION**

The mechanosensory bristles on the notum are composed of four distinct cell types: the shaft, socket, sheath and neuron (Hartenstein and Posakony, 1989; Fig. 1A). This system has been utilized extensively to understand the role of extrinsic signals and intrinsic determinants in sibling cell fate specification (Horovitz and Herskowitz, 1992; Jan and Jan, 1995, 1998). Most of the studies carried out so far have been interpreted in the light of the lineage model summarized in Fig. 1B. The cells of a bristle arise from a single sensory organ progenitor (the SOP) which divides to yield two secondary progenitors, PIIa and PIIb; PIIa gives rise to the shaft and socket and PIIb the neuron and sheath. Support for this lineage came from studies where the pattern of precursor division was monitored following incorporation of the base analogue bromodeoxyuridine (BrdU) (Hartenstein and Posakony, 1989). Interestingly, in these experiments an additional ‘fifth cell’ was often labeled together with other cells of the lineage; the origin of this cell remained obscure.

The orientation of division planes differs for each of the progenitors resulting in distinct positions of the sensory cells relative to each other. The SOP divides in an anteroposterior orientation yielding two secondary progenitors, PIIb located anteriorly and PIIa located posteriorly (Fig. 1C; Gho and Schweisguth, 1998). A large body of literature supported the view that PIIa divided prior to PIIb. This was recently contradicted by Manning and Doe (1999) and Reddy and Rodrigues (1999) who provided convincing evidence that PIIb, in fact, divides before PIIa. The orientation of PIIb division is in an apical-basal axis and yields a small cell sub-epidermally and a larger cell located on the epidermal plane. Earlier work suggested that PIIb gives rise to a neuron and sheath cell; this conclusion is being contested in this paper. The homeodomain-containing transcription factor, Prospero (Pros) is expressed in PIIb and is inherited equally by both progeny after division. Immunoreactivity decays rapidly in the larger of the two daughters and this cell is often seen undergoing an additional round of mitosis. This additional cell division could account for clusters composed of five sensory cells (Reddy and Rodrigues, 1999). PIIa divides in a planar orientation in the anterior-posterior axis yielding the shaft and socket cells, which remain in epidermal positions (Gho and Schweisguth, 1998).

The discrepancies in the extant model for SOP lineage prompted us to re-examine cell division patterns during microchaete development using several different cellular markers and antibody reagents. Our results suggest a modified lineage diagram shown in Fig. 1D,E. We show that PIIb divides to give rise to a glial cell and a tertiary progenitor referred to as PIII. The glial cell migrates away from the sensory cells and PIII divides, after PIIa, to form neuron and sheath. The finding that a glial cell shares a common origin with sensory cells is novel and needs to be investigated further to gain insights about its possible functional significance.

**MATERIALS AND METHODS**

**Fly strains**

Sensory cells were visualized during development using the enhancer
trap line neuA101. This line carries a P- (lacZ-ry+) insertion in
neutralized (neu); the reporter enzyme β-galactosidase is expressed in
progenitor cells of sense organs as well as all their progeny (Huang et
al., 1991).

For staging, white pupae (0 hours after puparium formation; APF)
were collected and allowed to develop further on moist filter paper at
25°C. This stage lasts for 1 hour; hence the error in staging is ±30
minutes.

**Immunofluorescence**

Samples were dissected and fixed as described previously (Reddy and
Rodrigues, 1999) and incubated in phosphate-buffered saline (PBS)
containing 0.1% Triton X-100 (PTX) and 1% bovine serum albumin
(BSA). 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),
rabbit anti-β-galactosidase (1:4000, Cappel), rabbit anti-phosphohistone
(1:500) and mouse anti-β-tubulin (1:4). 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 and Cy5; Jackson
ImmunoRe; or FITC, Molecular
Probes) or biotinylated (Vector
labs, UK). Where biotinylated
secondary antibodies were used,
samples were incubated in
streptavidin-RITC (Vector
labs). The fluorescently labeled
preparations were mounted in
Vectashield (Vector Labs) and
viewed on a BioRad MRC1024
confocal microscope and pictures
were processed in Adobe
Photoshop 4.0.

**RESULTS**

We have used a variety of
cellular markers and antibodies
to follow the development of
the cells within the SOP
lineage. The enhancer trap
line, A101 labels the SOPs
and their progeny throughout
development (Huang et
al., 1991). The identity of cells
within the lineage can be
inferred by their arrangement
relative to each other; hence in
a two-celled cluster PIIb is
located anterior to PIIa (Gho et
al., 1996). The pre-divisional
PIIb cell expresses Elav and
Pros (Manning and Doe, 1999).
In the fully developed cluster
Elav is a good marker for the
neuron, Suppressor of Hairless
for the socket cell and A-2nd-29
labels the shaft and socket cells (Bier et al., 1988; Hartenstein
and Jan, 1992; Gho and Schweisguth, 1998). Cell division
patterns have been visualized by antibodies against β-tubulin
and phosphohistone as well as the DNA dye propidium iodide
(Manning and Doe, 1999; Reddy and Rodrigues, 1999).

The SOP lineage includes an additional division
resulting in five cells

Staining of developing pupal nota with antibodies against
phosphohistone, or β-tubulin or the DNA dye propidium iodide
provided compelling evidence that an additional cell division
occurs within the SOP lineage. Pupal nota from the A101
strain were stained using antibodies against β-galactosidase (red in

---

**Fig. 1.** Lineage of cells within the sensory cells of the mechanosensory bristle on the notum.

(A) Schematic diagram of the mechanosensory bristle. (B) Model for development of microchaete as
proposed by Hartenstein and Posakony (1989). The SOP divides to give rise to two secondary
progenitors, PIIa and PIIb. PIIa divides first to yield shaft and socket, followed by PIIb, which gives rise
to neuron and sheath. (C) Modification of model showing that the PIIb divides ahead of PIIa (Manning
and Doe, 1999; Reddy and Rodrigues, 1999). These studies also showed that Elav (red) and Pros
(yellow) are expressed in PIIb. PIIb divides unequally in an apical-basal orientation to yield a smaller
sub-epidermal cell and a larger cell, which remains on the epidermal plane. Both progeny inherit Pros
and Elav; Pros expression is maintained in the neuron, while Elav expression is maintained in the
sheath. (D) Revised lineage diagram accounting for the additional cell division within the sensory lineage. PIIb divides to
yield a glial cell and a tertiary progenitor PIII. PIII divides, after PIIa, to form neuron and sheath cells.
(E) In the 17- to 19-hour APF interval, PIIb, which expresses both Elav and Pros divides unequally. This
is followed by division of PIIa. Repo begins to be expressed in the sub-epidermal daughter of PIIb
which expresses high Pros and low Elav. At this time PIII enters mitosis and divides. At a five-cell stage
the Repo-positive cell is located more posteriorly, near the shaft and socket cells. In a mature sensory
clusters at 28 hours APF only four sensory cells are seen. The two anterior cells become sheath (Pros
positive) and neuron (Elav positive) and the two posterior cells become shaft and socket.
Glial cell arises in the microchaete lineage

Fig. 2. An additional division is seen in the microchaete lineage. Clusters composed of four cells were analyzed. The large epidermally located cell (PIII; small arrows) and the smaller sub-epidermal cell (presumptive glial cell; arrowheads) are progeny of PIIb. The posteriorly located shaft and socket cells (larger arrows) are daughters of PIIa. Cells of the microchaete lineage are visualized by β-galactosidase activity in the A101 strain (red in A, E, H and L). (B) Expression of Pros in the sub-epidermally located daughter of PIIb. (C) Propidium iodide staining of DNA suggests division in the presumptive PIII. (D) Merged images of A-C showing Pros staining (green) in the sub-epidermal cell and dividing chromosomes (blue) in PIII. (E) Staining with antibody to β-tubulin showing mitotic spindle (arrow). (G) Merged images of E and F showing mitotic spindle (green) in the PIII. (I) Staining with antibodies to phosphohistone revealed that PIII was undergoing division. (J) This cell also expressed high levels of Elav. (K) Merged images of H-I showing that the mitotic cell (green) expressed high levels of Elav (blue). (L-N) Same sensory cluster as in H-K at a sub-epidermal plane to reveal the presumptive glial cells. This cell expresses lower levels of Elav than PIII (M). (N) Merged images of L and M. In all panels anterior is to the top.

Fig. 3. Repo is expressed in the microchaete lineage. Sensory cells are visualized by β-galactosidase expression in the A101 strain (red in A, E and H). Progeny of PIIb: the larger epidermal cell (PIII; small arrows) and the sub-epidermal cell (the presumptive glial cell; arrowheads), and progeny of PIIa: shaft and socket (larger arrows), are indicated. (A-D) In four celled clusters at 20-22 hours APF, PIII (thin arrow) can be seen undergoing mitosis, revealed by diffuse cytosolic expression of β-galactosidase compared to the non-dividing cells, which show intense nuclear β-galactosidase expression (arrowhead and larger arrows in A). (B) Repo (green) is expressed in the sub-epidermal cell while the dividing cell (PIII), expresses Elav (blue) at higher levels (C). (D) Merged image of A-C showing Elav expression in PIII and Repo staining in the presumptive glial cell. (E-G) In sensory clusters consisting of 5 cells the Repo-expressing cell (green in F and the merged image in G) moves more posteriorly than in A-D to become located near the shaft and socket nuclei. (H-J) Somewhat older five celled clusters than in E-G showing the glial cell (green in I and the merged picture in J) to have migrated away from the sensory cluster. In all panels anterior is to the top.
Fig. 2). We selected sensory clusters with four cells for further analysis; these clusters were produced by division of both secondary progenitors, PIIa and PIIb. The plane of division of PIIb resulted in a large epidermally located cell (small arrow in Fig. 2) and a small cell located below the epidermal plane (arrowheads in Fig. 2). Hence a four cell cluster would be composed of three epidermal cells and one sub-epidermal cell (Fig. 2A,E). Interestingly the intensity of the β-galactosidase reporter is significantly less in the sub-epidermal cell, leading this cell to have been missed in earlier studies.

Previous data had shown that Pros was expressed in PIIb and inherited by both the progeny following division (Manning and Doe, 1999; Reddy and Rodrigues, 1999). Shortly after division of PIIb, immunoreactivity becomes much more pronounced in the sub-epidermal cell (arrowhead in Fig. 2B,D) and is weak in the larger cell (arrow in Fig. 2B). The latter cell can be seen to be in mitosis when stained with propidium iodide (Fig. 2C) or antibodies against either β-tubulin (Fig. 2F,G) or phosphohistone (Fig. 2I,K). Since this cell was observed to undergo division in all clusters examined, we suggest that it is a tertiary progenitor which we denote PIII.

PIII can be identified by low Pros (Fig. 2B) and high Elav (Fig. 2I) immunoreactivity. Both markers become cytosolic during division and are probably inherited equally by both progeny. We examined sensory cells in fully differentiated sensory clusters, after division of PII, by staining with antibodies against Pros and Elav (data not shown). Elav was expressed strongly in the differentiated neuron while Pros is expressed in the sheath cell. This observation implies that Pros is up regulated specifically in the sheath cell as suggested before (Manning and Doe, 1999; Reddy and Rodrigues, 1999).

The PIIb lineage gives rise to a tertiary progenitor (PIII) and a glial cell

Division of PII would be expected to produce sensory clusters composed of five cells (Fig. 1D). Several such clusters were observed in nota from pupae 20-22 hours APF (75 in 4 preparations). Surprisingly, after 28 hours APF no clusters with five sensory cells were observed (data not shown). There are several possible explanations for this. (i) The β-galactosidase reporter of A101 decays rapidly from the sub-epidermal cell thus preventing its visualization. (ii) This cell could have migrated away from the sensory lineage, suggesting a glial identity. (iii) It is formally possible that this ‘fifth cell’ is removed by programmed cell death.

In order to establish the identity of the additional cell in the SOP lineage, we stained pupal nota with antibodies against Repo, a homeodomain transcription factor which is specific to differentiated glial cells (Xiong et al., 1994; Halter et al., 1995). The A101 strain was used as it allows recognition of the sensory cells by reporter β-galactosidase expression (red in Fig. 3). Repo immunoreactivity was not observed in clusters composed of two or three cells (data not shown). At the four celled stage the sub-epidermal cell (arrowheads in Fig. 3) was stained by antibodies to Repo (green in Fig. 3). A comparison of these images with those in Fig. 2A-D, suggested that this cell is one of the progeny of PIIb, which showed high Pros immunoreactivity. Its sibling cell can be recognized as it undergoes division in the four celled cluster (small arrow in Fig. 3A,D). This cell expresses high levels of Pros and Elav (Fig. 3C). The expression of Repo in the sub-epidermal cell was observed in most of the five cell clusters examined at 20-22 hours APF (71 out of 75 cases).

The time course of Repo staining suggests that its expression begins in the sub-epidermal daughter of PIIb some time after its birth, indicating differentiation to the glial fate. At this time, the sibling cell (PIII) begins to undergo mitosis to give rise to neuron and sheath cell. Examination of several sensory clusters from 20-22 hour APF nota lead us to conclude that the glial cell migrates away from the other cells of the cluster. In Fig. 3J, the Repo-positive cell (arrowhead) can be seen to have become more posteriorly located and in some cases is located some distance from the rest of the sensory cells.

These data convincingly demonstrate that the PIIb lineage undergoes two cell divisions and gives rise to three cells of different fates, a neuron, sheath and glial cell. In the adult the glial cell is not closely associated with the rest of the cells of the sense organ and apparently migrates away from these cells.

DISCUSSION

A revised model for microchaete development incorporates the additional cell division that occurs in the SOP lineage

Our data allow us to propose a modified model for sense organ development in the notum, which reconciles recent observations about birth order of cells with previous lineage descriptions for the sensory cells (Fig. 1D) (Hartenstein and Posakony, 1989; Manning and Doe, 1999; Reddy and Rodrigues, 1999). Between 17 and 19 hours APF, the secondary progenitor PIIb divides unequally to give rise to a small sub-epidermally located cell and a tertiary progenitor which we designate PIII. The sibling secondary progenitor PIIa, divides shortly after this to give rise to the shaft and socket cells. At 20 to 22 hours APF each sensory cluster is composed of four cells, three of which are located in the epidermal plane and one is sub-epidermal. The sub-epidermal cell is small and expresses lower levels of the A101 reporter enzyme β-galactosidase and hence could have been undetected in earlier analyses (Gho and Schweisguth, 1998). Subsequent division of PIII gives rise to a neuron and sheath cell and therefore as previously reported, these are the last two cells of the lineage to arise (Hartenstein and Posakony, 1989). At around the time when PII divides, the sub-epidermal cell expresses the glial marker Repo and migrates away from the rest of the cells of the sensory cluster.

This lineage of the SOP is particularly important since this system has been extensively used to study acquisition of cell fates within sensory systems. In earlier analysis the formation of the tertiary progenitor went unnoticed because of the lack of adequate markers. The sub-epidermal daughter of PIIb could only be unambiguously identified by staining with antibodies to Pros, thus revealing, contrary to existing tenets, that PIIb in fact divided before PIIa. Of the progeny of PIIb, the larger cell expressed low levels of Pros and high Elav, while the smaller sub-epidermal cell expressed high Pros and low Elav (Fig. 1E; Manning and Doe, 1999; Reddy and Rodrigues, 1999). However, these workers still interpreted their findings within the frame of existing lineage models and concluded that the sub-epidermal cell was the sheath cell while its sibling was the...
neuron. This erroneous conclusion was based on the observation of the neuronal marker Elav in PII.

The unequivocal identification of the sub-epidermal cell as a glial cell was made possible by staining with antibodies to Repo (Fig. 1E). We have also observed that this cell migrates away from the other cells of the sensory lineage and would therefore not be easily detected in the fully developed bristle cluster.

**Glial and sensory cells arise from a common lineage in the peripheral nervous system of the adult**

Our analysis has shown for the first time that a glial cell and neuron share a common lineage in peripheral nervous system development. Earlier studies have shown that glial cells are closely associated with peripheral neurons on the wing and have shown that the wing margin glial cells share a common lineage and originate from disc epithelium (Giangrande et al., 1993; Giangrande, 1994). However, it is not clear whether all glial cells on the wing margin share a common lineage. It is not uncommon to have glial cells of different origin, as shown in the case of retinal basal glia that originate from the optic stalk (Choi and Benzer, 1994). The possibility that some of the glia in fact arise from the mechanosensory bristle lineage is tantalizing in view of observations demonstrating that glial cells arose from clonal patches covering the regions of anterior wing margin, which is packed with mechanosensory bristles (Giangrande, 1994).

A close association between glial cell development and neurogenesis has been documented previously (Giangrande, 1995). Loss-of-function mutations in theachaete-scute complex genes, which act as proneural genes for the sensory bristles also affect the development of wing glia. Further, overexpression of the proneural genes lead to development of glia at ectopic sites. These observations suggest a close relationship between gliogenesis and neurogenesis and achieve a special significance in the context of our observation that glial cells arise within microchaete bristle lineage.

**Glial cell could play a role in guiding axonal projections in the sensory system**

There are several studies implicating a role for glia during the development and function of the embryonic central nervous system (CNS) and the retinal neurons in the optic lobe (Halter et al., 1995; Xiong and Montell, 1995). Mutations in repo affect the differentiation of the glia and result in reduced glial cell number as well as a disruption in axon fasciculation and the inhibition of ventral nerve cord condensation in embryonic CNS (Halter et al., 1995). Studies on optic lobe development have shown that repo function in laminar glia is essential for the survival of laminar neurons (Xiong and Montell, 1995).

In this study we have shown that among the progeny of PIIB, the cell expressing higher Pros levels assumes glial cell fate. Loss of pros function in clones results in defects in the mechanosensory neurons; neurons are either absent or show failure of axon outgrowth (Reddy and Rodrigues, 1999; Manning and Doe, 1999). Since the neurons do not express Pros it is possible that the defects in the sensory neuron are an indirect consequence of Pros lack of function in the glia. Further work aimed at understanding the development and function of the glial cell should highlight its role within a sense organ.

**Sibling cell fate specification and gliogenesis**

Our observation of an additional division and generation of a glial cell within the lineage has opened up new questions in sibling cell fate specification during microchaete development. Earlier studies have highlighted the role of extrinsic signaling mediated by the membrane bound receptor, Notch in sibling cell fate specification during microchaete development (Hartenstein and Posakony, 1990; Guo et al., 1996; Wang et al., 1997). Notch signaling has been implicated in the binary cell fate choice between PIIB-PIIIb, shaft-socket and neuron-sheath. The differential activity of Notch among siblings is determined by differential inheritance of Numb, a cytosolic protein that is known to bind Notch and inhibits its activity (Rhyu et al., 1994; Wang et al., 1997; Frise et al., 1996). It will be interesting to examine whether Notch and Numb mediated mechanisms of cell fate choice operate in choosing glial cell in PIIB lineage.

As predicted by the model proposed in this paper, we observe six sensory cells when PIIB has been converted to PIIB by misexpression of Numb in both secondary progenitors. Two of these cells express Repo (V. Reddy and V. Rodrigues, unpublished data). Conversely, misexpression of activated Notch which causes a PIIB to PIla conversion resulted in four cells within the sensory clusters, none of which expressed Repo. In addition to these predictable effects, we also observed more curious cases of three or four Repo-positive cells upon Numb misexpression within sensory clusters, suggesting a possible role for Notch signaling in the glia cell/PII sibling cell choice. A careful analysis of Numb expression pattern in PIIB and its progeny is warranted in order to gain better insights into the process.

We are grateful to Chris Doe, for his encouragement and support and Gerald Technau for anti-Repo antibodies. We are indebted to Satyajit Mayor, for continual help and interest; K. VijayRaghavan and Krishanu Ray for discussions and comments on the manuscript and Xiaohang Yang and William Chia for several important reagents. We thank the referees whose comments helped greatly in improving the previous version of the manuscript.

**Note:** While this manuscript was being reviewed, a similar study was published showing an additional asymmetric division in the microchaete lineage (Gho, M., Bellachie, Y. and Schwesiguth, F. (1999). Revisiting the Drosophila microchaete lineage: a novel intrinsically asymmetric cell division generates a glial cell. Development 126, 3573-3584). These authors also demonstrate the asymmetric localization of Pros in PIIB as well as PIII, which is inherited predominantly by the glial cell and the neuron respectively. However in our studies, we observed Pros immunoreactivity in both progeny of PIIB as well as PIII, although at different levels. This implies that even though the localization of the molecule in progenitors could be asymmetric, its inheritance is unequal but not strictly asymmetric.

**REFERENCES**


Giandrange, A. (1994). Glia in the fly wing are clonally related to epithelial cells and use the nerve as a pathway for migration. Development 120, 523-534.


