Lineage diversity in the Drosophila nervous system
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The detailed descriptions of cellular lineages in the Drosophila nervous system have provided the foundations for an in-depth genetic analysis of the mechanisms that regulate fate decisions at every cell cycle.

Introduction
An important issue in neurobiology is to understand how cell type diversity is generated in the nervous system. In invertebrates, neural precursor cells follow stereotyped sequences of cell divisions and generate complex arrays of neurons and associated cells. Each of these sequences defined a specific cellular lineage. In *Drosophila*, these precursor cells are the neuroblasts in the central nervous system (CNS) and the sensory organ precursor (SOP) cells in the PNS. Here, we review recent advances in the description of neuroblast and SOP cell lineages in *Drosophila*. These descriptions are a prerequisite for addressing the following questions: first, what are the mechanisms that confer on neural precursor cells particular identities so that they follow defined lineages? Second, within each lineage, how do daughter cells become different from each other and from their mother cell? We will not, however, cover here the mechanisms that regulate the unequal segregation of cell fate determinants that underlie the generation of cell fate diversity during asymmetric cell division [1–3].

Lineage analysis in the CNS
The formation of the CNS starts with the determination and delamination of neural precursor cells, or neuroblasts, from the single-layered neuroectoderm [4]. Each neuroblast then undergoes several rounds of asymmetric cell division to produce, in a stem cell mode, a series of ganglion mother cells (GMCs). Each GMC divides in turn asymmetrically, usually just once, to generate two distinct neuronal and/or glial siblings (Figure 1a). In each abdominal and thoracic hemisegment, 30 different neuroblasts, which can be uniquely identified based on their stereotyped position, delaminate in five successive waves, denoted S1 to S5, and generate about 400 progeny cells.

Pioneering studies by Doe and Goodman (reviewed in [4]) demonstrated that neuroblasts and GMCs have very different developmental potentials. Indeed, if GMC1-1a, the GMC born from the first neuroblast 1-1 division (Figure 1b), is laser ablated in grasshopper embryos, it is not replaced and its two progeny cells, the aCC and pCC neurons, will be missing. In contrast, if neuroblast 1-1 is killed just after it has generated GMC1-1a, a neighbouring neuroectodermal cell will replace it and will produce a second GMC1-1a, which leads to a duplication of the aCC and pCC neurons [5]. These results demonstrate that two distinct types of mitotic cells are the constitutive elements of the CNS lineages.

More recent studies have provided a detailed description of the all progeny cells produced by each of these 30 neuroblasts in the embryo [6,7••,8,9]. In these studies, individual neuroblasts were labeled prior to their delamination with a lineage tracer, Dil, and their lineage and progeny cells examined in both living and fixed embryos. These studies not only provide a framework for future experimental studies but have also established several important general conclusions.

Space and specification of distinct neuroblast lineages
First, each neuroblast appears to be unique with respect to the number and fate of its progeny cells. Second, neurons most often remain clustered close to their place of birth, indicating that they do not migrate significant distances. The final architecture of the CNS largely results, therefore, from stereotyped sequences of cell divisions. Third, a correlation exists between the dorsoventral position of the neuroblast and its type of lineage. Specifically, within each hemisegment, 18 of the 30 neuroblasts can be grouped into nine pairs on the basis of lineage similarities in terms of number of neurons and glial cells, neuronal types and initial neurite outgrowth [7••]. Within each pair, the two neuroblasts are always found at the same distance from the midline. Fourth, most motoneurons are generated by medial neuroblasts whereas many of the interneurons are produced by lateral neuroblasts [8,9]. This correlation is, however, not absolute and lateral neuroblasts do also produce motoneurons [7••]. Likewise, many of the glial cells are generated by lateral neuroblasts, but later migrate towards the midline [10]. Finally, most medial motoneurons innervate ventral muscle whereas lateral motoneurones innervate dorsal muscles [7••] (both medial and lateral motoneurons usually project ipsilaterally).

These observations indicate that positional information along the medial–lateral axis of the embryo regulates lineage type, identity of progeny cells as well as motoneuron–muscle connections. In contrast, no such correlation has been observed between the anterior–posterior position of the neuroblast within a segment and its lineage.

Abbreviations
CNS central nervous system
GMC ganglion mother cell
PNS peripheral nervous system
SOP sensory organ precursor

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Recently, patterning genes that define the three medial, intermediate and lateral neuroblast columns have been identified [11–18]. These genes are excellent candidates for regulating neuroblast medial–lateral identity, thereby specifying a stereotyped sequence of asymmetric divisions and influencing the general organization of the muscle–CNS connections. Interestingly, closely related genes have been shown to be expressed in corresponding ventral, intermediate and dorsal domains in the neural tube of vertebrates, suggesting that some of the CNS patterning mechanisms have been conserved during evolution [19].

**Time and changes in stem cell identity within a lineage**

A broad correlation has also been established between the time of birth of the neurons and their identity [7**,**8,9]. Motoneurons usually arise first, followed by intersegmental interneurons and local interneurons. Neuroblasts therefore sequentially produce GMCs with distinct differentiation potentials. The description of the expression pattern of the *hunchback* (*hb*), *castor* (*cas*) and *pdm* genes has provided one starting point to explore how a neuroblast might switch identity after a defined number of cell divisions [20–23,24•]. A subset of S1 neuroblasts appear to sequentially express four transcription factors: Hb, the redundant POU transcription factors Pdm-1 and Pdm-2 and a zinc-finger protein, Cas (also known as Ming; Figure 1B). These genes are required to regulate fate decisions at reproducible points in neuroblast lineages, and might therefore specify sublineages [20,23,25]. Switches in the expression of the *hb*, *pdm* and *cas* genes are thought to take place in the neuroblast, with the GMCs inheriting different gene products over time. These switches account for the formation of three cell layers in the CNS that are marked by the expression of *hb*, *pdm* and *cas* [23]. Interestingly, isolated neuroblasts cultured in vitro recapitulate in part the sequential accumulation of Hb, Pdm and Cas [24•]. This finding has raised the possibility that an intrinsic timing process regulates when these switches occur in the neuroblast. The initiation of this timing mechanism does not depend on the previous expression of *hb*, as the onset of *cas* expression appears unchanged in *hb* mutant embryos [23]. It might instead depend on the precise stage at which neuroblasts delaminate. Indeed, late-born neuroblasts directly express *pdm* (this is the case of the S2 neuroblast 4-2 [22]) or *cas* [23]. Note, however, that the expression of these genes was for the most part described at the level of the entire CNS. It will therefore be important to identify lineages in which these four genes are expressed sequentially.

Neuroglioblast stem cells switch from the production of neurons to the production of glial cells [26–30]. Two different types of switches have been proposed. In the 6-4T lineage, the neuroglioblast divides asymmetrically to produce two precursors with restricted developmental potential: one glioblast that generates only glial cells and one neuroblast that generates only neurons. This switch...
depends on the expression of the gial cell missing (gcm) gene in the neuroglioblast and on the unequal segregation of the gcm transcript into the future glioblast [26]. A second type of switch has been recently proposed for the 1-1A lineage, in which the neuroglioblast produces intermediate bipotential precursors that divide asymmetrically once to generate one glial cell and one neuron [30]. In this lineage, signaling by the Notch receptor acts upstream of Gcm to specify the glial fate. Interestingly, a programmed production of different types of neurons and glial cells at different times by neural stem cells is also seen in the cerebral cortex of mammals. Moreover, Notch appears to regulate the switch from neurogenesis to gliogenesis [31,32]. Last, this programmed production can be observed on isolated cortical stem cells using clonal stem cell culture [33]. This again suggests the existence of an intrinsic timing process in the mammalian cortex. It has been proposed that a signal produced by the neurons generated early feeds back to the stem cell to alter its developmental potentials [33,34]. Whether a similar feedback mechanism regulates the sequential expression of transcription factors in Drosophila neuroblasts remains to be investigated.

**Variations on a stereotyped sequence of cell divisions in the PNS**

The PNS of the larva or of the adult fly is composed of various types of external and internal sense organs. Each sense organ is usually produced by a single SOP cell (also called pI) via a stereotyped sequence of asymmetric cell divisions producing a fixed number of neurons and a unique set of accessory cells. Pioneering lineage studies in the PNS were based on retrospective clonal and clonal analyses [35–38]. More recent studies have examined the sequence of cell divisions in mono-innervated external sense (mes) organs [39•–41•]. In particular, the mes organ lineage of the adult fly was analyzed in living pupae by time-lapse imaging using a nuclear GFP protein (nlsGFP) as a lineage marker [39•].

In the pupa, each mes precursor cell undergoes a series of four asymmetric divisions to produce the four sense organ cells, which together compose one external sense organ, and a fifth cell that migrates away [39•,40•] (Figure 2). This migrating cell expresses gcm and becomes a peripheral glial cell, and may then go through additional rounds of cell division [42].

In the embryo, the lineage of the five ventral embryonic mes cells has been recently described [41•]. This lineage is identical to the one described for the adult mes organs with one difference: the migrating cell does not express gcm and differentiates into a multidendritic (md) neuron. Interestingly, the cell lineage that generates six of the cells in the internal chordotonal organ is very similar to the lineages described for the embryonic and the adult mes organs. In this view, the gcm-expressing ligament cell in the chordotonal organ is homologous to the glial/md cell [35,41•] (Figure 2). This observation led to the proposal that all mono-innervated sense organs follow the same invariant sequence of cell divisions, and may therefore represent a primordial type of sensory lineage [41•]. This further suggests that the developmental program represented by this particular sequence of cell division is a property of the pI cell.

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![Current Opinion in Genetics & Development](image)
Specification of distinct PNS lineages

Although the regulatory mechanisms that generate diversity between lineages within the PNS are poorly understood, we do know that the types of proneural genes expressed in pII cells are important for this diversity. The Achaete and Scute proteins specify an external sensory organ fate to the pII in which they accumulate, whereas accumulation of Atonal confers a chordotonal sense organ fate [43–45]. Importantly, Achaete- and Scute-expressing pII cells express the Cut homedomain protein. In cut mutant embryos, external sense organs are partially transformed into internal sense organs [46]. One function of Atonal in chordotonal pII cells is to repress the expression of Cut [47]. Pox-neuro (Poxn) is specifically expressed in the pII cells that generate the polyinnervated external sense organs in the embryo. In poxn mutant embryos, polyinnervated sense organs are transformed into mes organs, indicating that it is required for generation of polyinnervated sense organs [48,49]. Lineage diversity, therefore, appears to depend in part on specific combinations of transcription factors in the pII cell. As a result, both the stereotyped sequence of cell divisions and the variations on this sequence might be defined in the pII cell as it adopts its fate.

Regulation of cell identity in the PNS

The division of the mes pI in the pupa generates two secondorder precursor cells, pIIa and pIIb, that both differ from their mother cell. Although we do not know what makes the daughter cells different from their mother, how pIIb becomes different from pIIa is partly understood. The protein Numb is specifically inherited by one of the two pII daughter cells and acts as a cell-fate determinant to specify pIIb fate by antagonizing Notch signaling [50–54]. How Numb inhibits Notch signaling is, however, not known. Mammalian Numb has recently been shown to localize to endocytic vesicles, to bind the appendage domain of α-adaptin and to regulate the internalization of activated EGF receptors [55]. Recently, the Wiskott-Aldrich Syndrome protein (Wasp), which appears to link endocytic machinery to microfilaments [56], has been shown to be required for Notch signaling in Drosophila and to act downstream of Numb in the pIIb cell [57]. Whether Numb and Wasp regulate the endocytosis of Notch–Delta complexes and thereby modulate Notch signaling in the pIIb cell remains to be investigated. In the CNS, the Numb protein segregates specifically into GMCs. However, there is no evidence that Numb and Notch regulate the fate of the GMC [52]. Instead, the transcription factor Prospero, which co-segregates with Numb into GMCs, appears to regulate the program of gene expression and of cell proliferation in the GMCs [58,59].

The pupal pIIb cell differs from the embryonic pIIb cell by the fates of their migrating daughter cells. The fate of this cell depends on the expression of the gem gene, which itself depends on the inhibition of Notch signaling by Numb [60]. The gem gene is expressed in the migrating glial cell in the pupa but not in the embryonic md neuron [39*]. In gem mutants, peripheral glial cells adopt a neuronal fate in the pupa [42]. This difference in gem transcriptional regulation is unlikely to result from a difference in proneural gene expression. Indeed, in both lineages, SOP specification depends on Achaete and/or Scute activities. Understanding the molecular mechanisms regulating gem expression in the PNS might help us determine how diverse types of lineages have arisen during evolution.

Conclusions and future directions

The recent descriptions of cellular lineages in the CNS and in the PNS have provided the foundations for a detailed genetic analysis of the mechanisms that regulate fate decision at every cell cycle. Future studies should reveal to what extent the identity of neural precursor cell changes with each cell cycle and whether they do so in response to a cell-autonomous timekeeping process or to extracellular signals triggering temporal switches. Although the functional importance of the cell-fate determinants Numb and Prospero in alternative cell fate decisions has been established, it will be important in the future to determine the interplay between these determinants and the changes in cell identities at each cell cycle.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest

Pattern formation and developmental mechanisms


31. Anderson DJ: Revisiting the intermediate column identity. The role of Inscuteable in regulating the thoracic mono-innervated sense organs of the adult fly. Both papers identify a novel asymmetric cell division that produces a small, subependymal cell, which migrates away and does not participate to the formation of the sense organ. Instead, it becomes a PNS glial cell. A G F P time-lapse imaging approach was used in [39*] to describe a living pupa this complete lineage, as well as the migration of the glial cell.

32. Ogurozou V, Schweisguth F, Bellaiache Y: Lineage, cell polarity and InsCuteable function in the peripheral nervous system of the Drosophila embryo. Development 2001, 128:631-643. This paper describes each of the four asymmetric cell divisions that generate one mono-innervated sense organs and its associated multidendritic neuron in the fly embryo. It also reports on the role of InsCuteable in regulating the polarity of the dividing pibl cell. This analysis indicates that a similar stereotyped sequence of asymmetric division can generate various types of sensory organs in the embryo as well as in the pupa.


The authors used immunogold labeling on ultrathin cryosections to show that mammalian Numb localizes to endocytic vesicles and is co-trafficked with internalizing epidermal growth factor (EGF) receptors. Numb directly binds the α-adaptin via its appendage domain. These observations raise the possibility that Numb inhibits Notch signaling by regulating its internalization.


60. Van De Bor V, Gangananda A: Notch signaling represses the glial fate in fly PNS. Development 2001, 128:1381-1390.