

Neural Stem Cells: From Fly to Vertebrates

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ABSTRACT: Our goal in this review is to explore the relationship between *Drosophila* and vertebrate neural stem cell development by comparing progress in each system with the aim of answering several central questions in stem cell biology: (a) How are stem cells formed? (b) Do stem cells divide symmetrically or asymmetrically? (c) How is stem cell fate maintained? (d) How is stem cell differentiation initiated? (e) How are different stem cell fates determined? (f) How “plastic” are different neural stem cell fates? (g) How do neural stem cells produce different progeny? and (h) What regulates stem cell proliferation

versus quiescence? Not surprisingly, research in *Drosophila* and vertebrate systems each have their own biases, strengths, and weaknesses; we hope that by directly comparing progress in each field, new experiments and interpretations in both vertebrate and *Drosophila* research will become apparent. It has become increasingly clear that vertebrates and *Drosophila* share many fundamental mechanisms of neurogenesis, validating a comparative approach. © 1998 John Wiley & Sons, Inc. *J Neurobiol* 36: 111–127, 1998

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In discussing stem cells, it is necessary to carefully define several terms—in particular, “stem cell” and “asymmetric division.” Here, we define a stem cell as a cell that can divide to regenerate itself as well as produce a sibling with a more restricted developmental potential. The term “regenerate itself” is slightly ambiguous, because it is virtually impossible to be sure a stem cell is exactly the same following each division, and specific exceptions to this definition will be noted below. We define “asymmetric division” as any cell division that produces sibling cells with reproducibly different cell fates; unique sibling fates can be a result of intrinsic factors partitioned unequally during mitosis, extrinsic factors that differentially affect each sibling, or a combination of both mechanisms. In addition to

asymmetric cell divisions, neural stem cells may undergo proliferative divisions (to expand the stem cell population) or differentiative divisions (to reduce the stem cell population), or enter a quiescent state (Fig. 1).

We begin with a brief synopsis of vertebrate and *Drosophila* neural stem cell development. The following section lists a number of important questions in neural stem cell development, along with a summary of vertebrate and *Drosophila* results pertaining to each question; within each section, we try to identify topics where *Drosophila* research is likely to guide vertebrate research, and where vertebrate research suggests new experiments in *Drosophila*.

VERTEBRATE NEURAL STEM CELLS

This section is a substantially abridged summary of several excellent recent reviews (Fisher, 1997; McKay, 1997; Morrison et al., 1997; Temple and Qian, 1996; and reviews in this issue); in addition, the other articles in this issue provide even more up-

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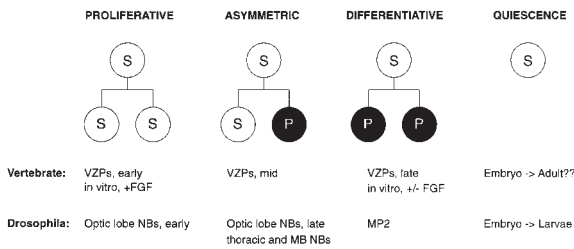


Figure 1 Modes of stem cell division. Proliferative divisions produce two multipotent stem cells (S). Asymmetric divisions regenerate a stem cell and produce a more developmentally restricted precursor (P). Differentiative divisions produce two developmentally restricted precursors or postmitotic progeny. Alternatively, a multipotent stem cell may remain quiescent through adulthood. Examples of the various modes of stem cell division in vertebrates and *Drosophila* are given below; see text for details. S = stem cell; P = more differentiated progeny; VZP = ventricular zone precursor; bFGF = basic fibroblast growth factor; NBs = neuroblasts; MB = mushroom body.

to-date coverage of vertebrate neural stem cell biology. Stem cells have been characterized in both central and peripheral nervous systems (CNS and PNS, respectively). In the CNS, stem cells develop from a dorsal neuroepithelium, the neural tube, and undergo cell division in the ventricular zone, although there are also stem cells in the subventricular zone in some brain regions (Fisher, 1997; McKay, 1997; Morrison et al., 1997; Garcia-Verdugo et al., this issue; Weiss and Van der Kooy, this issue). In the PNS, stem cells can be found in the dorsal region of the neural tube as well as among the migrating neural crest (Bronner-Fraser and Fraser, 1997, this issue).

Central and peripheral stem cells have been shown to be stem cells by virtue of their ability to renew themselves while producing more differentiated progeny; this has been most clearly shown by *in vitro* clonal culture experiments (Morrison et al., 1997), although it has also been inferred from *in vivo* lineage analysis (Reid et al., 1997). Vertebrate neural stem cells are likely to produce intermediate precursors (often called progenitor cells) which generate specific subpopulations of neurons and/or glia; stem cells or their progeny may also directly differentiate into neurons or glia.

Neural stem cells can be found in both the embryonic and adult CNS, but it is not clear if these are the same cells. Adult stem cells may be quiescent during embryogenesis and only proliferate in the adult (e.g., in response to injury); alternatively, some proliferating embryonic stem cells may become quiescent and persist as adult stem cells.

DROSOPHILA NEURAL STEM CELLS

The *Drosophila* CNS develops from a ventral neuroectoderm, similar in many ways to the dorsal neuroepithelium that produces the vertebrate CNS (Holley et al., 1995). In *Drosophila*, about one in five cells of the neuroectoderm will delaminate into the embryo to form a subepidermal array of neural stem cells (see How Are Neural Stem Cells Formed). These CNS stem cells are termed “neuroblasts” (although some are known to produce mixed lineages of neurons and glia) (Bossing and Technau, 1993; Doe and Technau, 1993). Embryonic neuroblasts are arranged in a reproducible orthogonal pattern that allows each one to be individually named, its gene expression profile characterized, and its cell lineage to be determined (see How Many Types of Neural Stem Cells Exist, and How Is Each Specified?). Neuroblasts undergo repeated asymmetric divisions to regenerate a neuroblast and bud off smaller progeny, called ganglion mother cells (GMCs). GMCs are intermediate precursors that divide just once to produce postmitotic neuronal siblings, or in some cases, neuron/glial siblings (reviewed in Doe and Technau, 1993) (Fig. 2).

In this review, we will consider neuroblasts to be stem cells because they can divide asymmetrically to regenerate themselves and produce a more differentiated daughter cell. In addition, neuroblasts can undergo proliferative divisions and differentiative divisions, and go through periods of mitotic quiescence (Fig. 1)—attributes shared with vertebrate neuronal stem cells. All neuroblasts have the following molecular and morphological features: apical location relative to the GMC, large size (8–12 μm), relatively low nuclear/cytoplasm ratio, and high proliferation. All GMCs share the following attributes: basal location relative to the neuroblast, small size (3–5 μm), limited mitotic potential, and inheritance of numerous asymmetrically localized proteins and RNAs during mitosis (reviewed in Fuerstenberg et al., 1998; Lin and Schagat, 1996).

QUESTIONS IN NEURAL STEM CELL BIOLOGY

In this section, we highlight a number of key questions in neural stem cell development, briefly summarize relevant experiments in vertebrate systems, and then discuss work in *Drosophila* pertaining to each question. In some cases, more is known about vertebrate stem cells, thus illuminating possible research approaches in *Drosophila*. In other cases, the

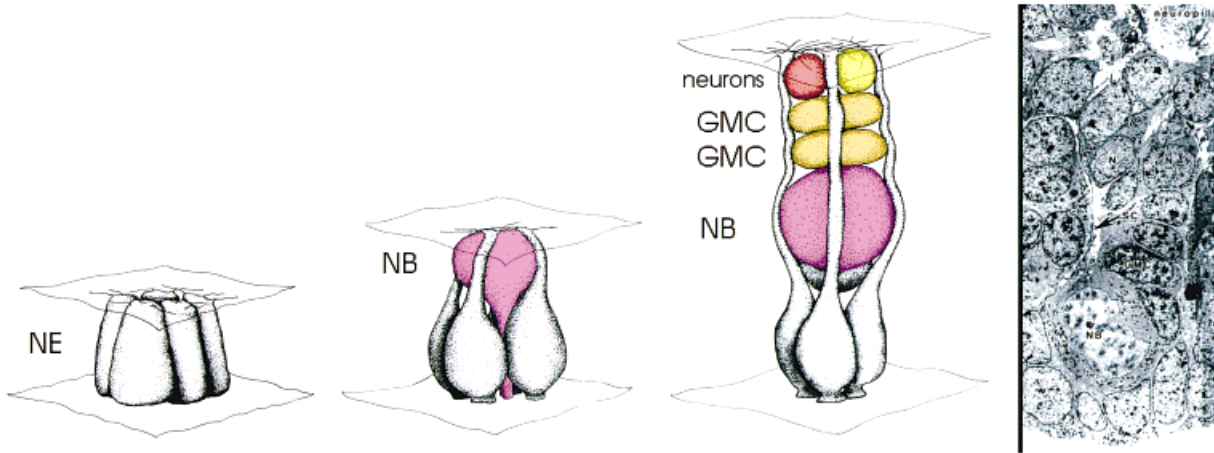


Figure 2 Neuroblast delamination and differentiation. Abdominal and thoracic neuroblasts (NB, fuschia) are formed from a cluster of neuroectodermal cells (NE) expressing one or more proneural genes (not shown); the neuroblast enlarges and delaminates toward the interior of the embryo. Successive asymmetric divisions of neuroblast stem cells give rise to ganglion mother cells (GMCs, orange), which divide once to produce sibling neurons or neuron/glia cell (red and yellow). On the far right, a transmission electron micrograph showing a neuroblast (NB), its GMC progeny, and postmitotic neurons (N); sheath cells (SC, arrow) enwrap the progeny of each neuroblast. Ventral is down. Both the camera lucida drawing and electron micrograph are of grasshopper embryos; the process is similar in *Drosophila*.

Drosophila research is further advanced and can provide molecular entry points for parallel vertebrate research. In the section below, we address the following questions:

1. How are stem cells formed?
2. Do stem cells divide symmetrically or asymmetrically?
3. How is stem cell fate maintained?
4. How is stem cell differentiation initiated?
5. How are different stem cell fates determined?
6. How “plastic” are different neural stem cell fates?
7. How do neural stem cells produce different progeny?
8. What regulates stem cell proliferation versus quiescence?

How Are Neural Stem Cells Formed?

Vertebrates. The short answer is that it is not known how vertebrate neural stem cells are formed. In *Drosophila*, proneural gene activity promotes neuroblast formation, whereas Delta/Notch (ligand/receptor)-mediated signaling limits neuroblast formation (see below); does a similar mecha-

nism control the formation of neural stem cells in vertebrates? Homologues of *Drosophila* proneural and Notch pathway genes have been cloned from *Xenopus*, chick, mouse, rat, and other vertebrates. In every case where expression of these genes has been examined, it is detected at times and places where neurogenesis is being initiated, consistent with a role in neural stem cell formation (reviewed in Robey, 1997).

In *Xenopus*, the initial wave of neurogenesis gives rise to a simple pattern of primary neurons, which can be identified by expression of neuron-specific β -tubulin. The proneural-related gene *neurogenin* (*X-ngnr-1/neuro D3*) is expressed in the neural plate prior to *N-tubulin* expression, as are the Notch pathway homologues *X-Notch-1* and *X-Delta-1*. *X-Notch-1* is broadly expressed and *X-Delta-1* is observed in scattered single cells that are likely to be the developing primary neurons (Chitnis et al., 1995; Chitnis and Kintner, 1995). Ectopic expression of the proneural *neurogenin* or *Xash-3* genes leads to the formation of additional primary neurons, whereas ectopic expression of *X-Delta-1* or a constitutively activated *X-Notch-1* reduces or eliminates primary neuron formation (reviewed in Kageyama and Nakanishi, 1997). Conversely, expression of a dominant negative form of *X-Delta-1* expands the domain of primary neuron formation

(Chitnis et al., 1995). These results suggest that proneural genes promote primary neuron formation, whereas Notch pathway genes limit primary neuron formation. Similar results are observed in mice, where proneural and Notch pathway genes are expressed early in neurogenesis (Ma et al., 1997; Sommer et al., 1996) and mutations in the Notch pathway genes *Notch-1* or *RBP-Jk* [a *Su(H)* homologue] lead to increased proneural gene expression and neurogenesis (de la Pompa et al., 1997).

In general, the vertebrate data are consistent with the known roles of proneural and Notch pathway genes in regulating neural stem cell formation in *Drosophila* (see below). It is important to note, however, that the relevance of primary neuron formation to neural stem cell formation is unclear, and that none of the vertebrate proneural or Notch pathway genes have been studied specifically in relation to promoting neural stem cell formation.

Drosophila. Approximately one in five ventral neuroectodermal cells will delaminate into the embryo and enlarge to form a neuroblast, while the remaining superficial neuroectodermal cells will differentiate into epidermis. How is the neuroblast/epidermal cell fate decision made? Neuroblast formation is regulated by the balance of proneural and neurogenic gene activity in the neuroectoderm (for a detailed review, see Campos-Ortega, 1995). Proneural genes encode basic helix–loop–helix transcription factors expressed in clusters of about five neuroectodermal cells and act to promote neuroblast formation. In embryos lacking proneural gene function, there is a decrease in the number of neuroblasts formed (Skeath and Carroll, 1994). Conversely, mutations in Notch pathway genes disrupt “lateral inhibition” within a proneural cluster, and all cells of the cluster form neuroblasts (Brand and Campos-Ortega, 1988; Cabrera, 1990; Martin-Bermudo et al., 1995; Skeath and Carroll, 1992). In most cases, functional Delta/Notch signaling also requires the nuclear proteins Mastermind (Mam), Suppressor of Hairless [Su(H)], Neuralized, and Enhancer of split [E(spl)] (reviewed in Artavanis-Tsakonas et al., 1995; Campos-Ortega, 1995).

Do Neural Stem Cells Divide Symmetrically or Asymmetrically?

Unless there is a unique neural stem cell for each type of neuron or glial cell in the CNS, at some point, stem cells must divide asymmetrically to generate multiple types of neurons and glia. In addition, stem cells may divide symmetrically to expand the

stem cell population (proliferative divisions) or to reduce the stem cell population (differentiative divisions). In this section, we give a few examples of these three stem cell division modes (asymmetric, proliferative, and differentiative) (Fig. 1) and describe where they have been observed in the vertebrate and *Drosophila* CNS.

Vertebrates. Proliferative symmetric divisions are observed in stem cells expanded *in vitro* in the presence of basic fibroblast growth factor (bFGF) (reviewed in Fisher, 1997), and are likely to occur in ventricular zone precursors (putative stem cells) during early neurogenesis (Caviness, 1982; Chenn and McConnell, 1995). Asymmetric divisions have been directly observed in ventricular zone precursors midway through neurogenesis (Chenn and McConnell, 1995), although the ultimate fate of the different siblings is not known. In addition, it is thought that oligodendrocyte-astrocyte (O2A) embryonic stem cells can divide asymmetrically to regenerate an O2A embryonic stem cell and produce an O2A adult precursor (reviewed in Fisher, 1997). Differentiative divisions may occur in ventricular zone precursors late in neurogenesis, resulting in a burst of neuronal differentiation (Caviness, 1982; Chenn and McConnell, 1995); they may also occur in cultured stem cells following withdrawal of bFGF (reviewed in Morrison et al., 1997), although these stem cells might differentiate directly into neurons without a terminal mitosis.

Drosophila. All three types of stem cell divisions are also observed during *Drosophila* neurogenesis (Fig. 1). Proliferative neuroblast divisions occur early in optic lobe development (Hartenstein and Campos-Ortega, 1984), while asymmetric divisions occur later in optic lobe development, as each neuroblast begins to bud off GMCs (White and Kankel, 1978). In addition, the majority of neuroblasts within the thoracic and abdominal CNS divide asymmetrically to bud off GMCs from the time they are formed (Doe, 1992; Poulson, 1950). Differentiative divisions occur in the neuroblast-like MP2 precursor: The MP2 delaminates from a neuroectodermal proneural cluster just like a neuroblast and has all the morphological hallmarks of a neuroblast, but divides to produce two postmitotic neurons (Doe, 1992; Spana et al., 1995). A number of proteins and RNAs are distributed unequally during asymmetric neuroblast divisions, but not during symmetric proliferative or differentiative divisions; these molecules will be discussed in the following two sections.

How Is Stem Cell Fate Maintained?

Stem cells can divide asymmetrically to regenerate themselves as well as produce a more differentiated daughter cell. How is stem cell fate maintained at each asymmetric division? Answers to this question may help (a) identify stem cell populations *in vivo* (by providing molecular markers), (b) improve methods for culturing stem cells *in vitro*, and (c) design strategies to curb unrestrained cell proliferation of neural tumors.

Vertebrates. Much of what we know about the factors required to maintain the CNS stem cell fate is from *in vitro* culture of stem cells derived from embryonic cerebral cortex, striatum, and spinal cord (Temple and Davis, 1992; Reynolds et al., 1992; Kalyani et al., 1997). These cells are defined as stem cells by several criteria: They are nestin positive (an early neural marker), they are capable of extensive self-renewal, and they produce both neurons and glia. Survival and proliferation of multipotent stem cells from the striatum depend on the presence of EGF; cortical stem cells require bFGF and glial-conditioned medium; and spinal cord stem cells are supported by bFGF, fibronectin, and embryo extract. These results suggest that local environmental cues may play a role in stem cell maintenance *in vivo*. Despite the rapid progress in understanding stem cell maintenance *in vitro*, it is unknown whether these growth factors maintain stem cell fate in stem cell populations *in vivo*.

In addition to extrinsic factors, it is conceivable that intrinsic factors also play a role in maintaining stem cell fate (although this has not yet been demonstrated). In the mouse and ferret, cortical ventricular zone precursors can divide horizontally to generate an apical daughter (presumptive stem cell) and a basal daughter which migrates from the ventricular zone (presumptive neuron). During these asymmetric divisions, m-numb protein is specifically localized into the apical presumptive stem cell in the mouse (Zhong et al., 1996), whereas the Notch-1 protein is localized specifically into the basal presumptive neuron in the ferret (Chenn and McConnell, 1995). The function of Notch and m-numb proteins in this context is unknown, but the fact that dividing ventricular zone cells have a mechanism for apical/basal protein localization raises the possibility that intrinsic factors may exist which control stem cell maintenance or stem cell differentiation—the former could be localized apically (into the stem cell) and the latter could be localized basally (into the differentiating neuron).

Drosophila. Neuroblasts divide asymmetrically to produce another neuroblast and a smaller GMC (see the earlier discussion). These cells are different in many regards, particularly in size and mitotic potential (the larger neuroblast can divide over 100 times; the smaller GMC divides just once). How does a dividing neuroblast maintain its stem cell identity? In the grasshopper embryo, cytoplasmic volume is strongly correlated with neuroblast and GMC cell fate. Neuroblast volumes normally range from 3700 to 13,800 μm^3 , whereas GMC volumes normally range from 1100 to 2000 μm^3 , but cells of intermediate size can be produced by using a microneedle to shift the position of the mitotic spindle during early anaphase (Yamashiki and Kawamura, 1986). In these experiments, cells with volumes of $\geq 2600 \mu\text{m}^3$ always developed as neuroblasts (bilobed nuclei, short cell cycle, high mitotic potential, lightly condensed DNA), whereas cells with volumes $< 2000 \mu\text{m}^3$ always developed as GMCs (spherical nucleus, long cell cycle, divides only once, highly condensed DNA). This is true even when the new GMC is forced to bud from the apical side of the neuroblast (it normally buds from the basal side). In particular, this last experiment reveals many factors that do not correlate with neuroblast/GMC identity: extrinsic cues from adjacent apical/basal tissues, or intrinsic cues such as apical/basal differences between spindle poles, chromosomes, or cortically anchored molecules. We currently do not know what aspect of cell size is important for neuroblast and identity (e.g., cytoplasm/nuclear ratio, or the specific amount of a cytoplasmic or membrane component). Moreover, the relevance of this mechanism to maintaining neural stem cell fate in *Drosophila* or vertebrates is unknown.

In *Drosophila*, there is no information on what controls the maintenance of neuroblast identity. Single neuroblasts isolated in culture will divide asymmetrically to regenerate another neuroblast and a more differentiated GMC (Broadus and Doe, 1997; Huff et al., 1989), showing that extrinsic signals are not required for maintenance of neuroblast fate, and suggesting that intrinsic factors are involved in distinguishing stem cell versus GMC fate. Consistent with this observation, *in vitro* cultured neuroblasts have a mechanism for asymmetric localization of proteins into the neuroblast or GMC at mitosis: Inscuteable protein is segregated into the neuroblast, while Prospero and Staufen proteins are segregated into the GMC (Broadus and Doe, 1997). Although Inscuteable is localized into the neuroblast at mitosis, it is not required for maintaining neuroblast identity, because neuroblasts persist in insc

mutants (despite having defects in spindle orientation and protein localization; see How Is Stem Cell Differentiation Initiated?). Nevertheless, the existence of an apical/basal protein localization mechanism raises the possibility that “stem cell determinants” could be segregated into neuroblasts or “stem cell repressors” could be partitioned into GMCs during neuroblast cell division.

How Is Stem Cell Differentiation Initiated?

Asymmetric stem cell divisions produce another stem cell and a daughter cell with a more restricted developmental fate; differentiative stem cell divisions produce two daughter cells that both have a more restricted developmental fate. In each case, how does one or both daughter cells initiate neural differentiation?

Vertebrates. Many different mechanisms have been proposed for regulating the differentiation of stem cell progeny or of stem cells themselves: cell division history, increased cell density, exposure to different levels or types of growth factors (produced from either neural or non-neural tissues), or altered substratum-linked factors (reviewed by Fisher, 1997; Morrison et al., 1997; Shen et al., this issue). The multiplicity of mechanisms may reflect stem cell heterogeneity (see the following section), with different stem cells using distinct mechanisms to trigger differentiation. For example, some *in vitro* cultured stem cells will differentiate upon withdrawal of growth factors (reviewed in Fisher, 1997), which strongly suggests extrinsic regulation of differentiation. In contrast, cultured primary stem cells from E10 rat embryos show stereotyped cell lineages in which proliferating cells are observed adjacent to lineally related differentiating cells, despite exposure to identical culture conditions (Xian et al., 1998; Shen et al., this issue). Because many of the observed cell lineages are reproducible, it is more likely that intrinsic factors rather than a purely stochastic mechanism is involved in triggering neuronal differentiation. Clearly, this is an interesting area where more research needs to be done.

In vertebrates, most data indicate extrinsic regulation of stem cell differentiation; in *Drosophila*, intrinsic factors appear to play a primary role in triggering differentiation of stem cell progeny (see below). In the future, it will be essential to define the relative roles of extrinsic and intrinsic factors in triggering differentiation of neural stem cells in both vertebrate and *Drosophila*.

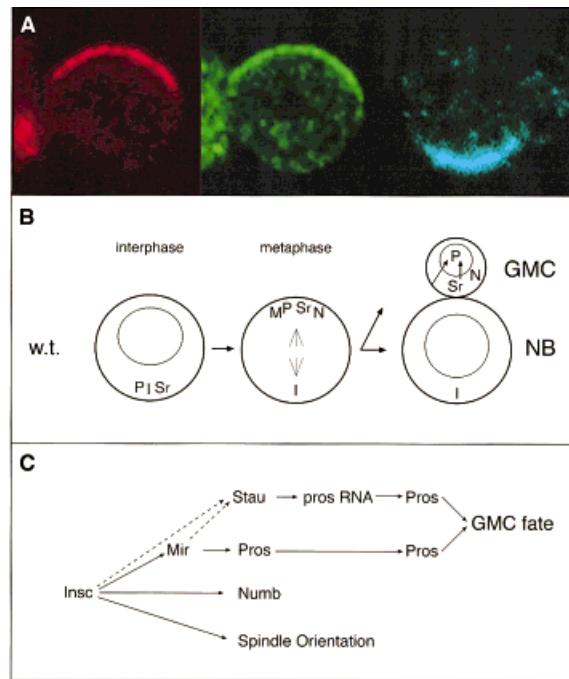


Figure 3 Asymmetric protein localization in mitotic neuroblasts. (A) Neuroblasts cultured *in vitro* show basal localization of Prospero (red) and Staufen (green), and apical localization of Iscuteable (turquoise). (B) Summary of RNA and protein localization in neuroblasts. See text for details. Prospero (P), Inscuteable (I), Staufen (S), Numb (N), Miranda (M), *prospero* RNA (r). (C) Regulatory hierarchy of RNA and protein localization in neuroblasts. See text for details. In all panels, apical is down and basal is up.

Drosophila. In *Drosophila*, neuroblasts divide asymmetrically to produce smaller GMCs which have a much more limited developmental potential (they usually generate just two neurons). There is good evidence that intrinsic factors are necessary to specify GMC fate (Fig. 3). Prospero is a divergent homeodomain transcription factor which is synthesized in the neuroblast but required in GMCs to activate GMC-specific gene expression and to repress neuroblast-specific gene expression (Chu-La-Graff et al., 1991; Vaessin et al., 1991; Matsuzaki et al., 1992; Doe et al., 1991; Vaessin et al., 1991). Interphase neuroblasts have very low levels of Prospero protein (although they have abundant *prospero* mRNA). As the neuroblast enters mitosis, the level of Prospero protein appears to increase, and the protein is transported to the basal side of the neuroblast, where it forms a tight crescent centered over the basal centrosome (Knoblich et al., 1995; Spana and Doe, 1995; Hirata et al., 1995). As the GMC buds from the neuroblast during anaphase, Prospero re-

mains tightly associated with the basal cell cortex and is ultimately segregated into the GMC. Once the GMC nuclear membrane forms, Prospero protein is translocated from the cortex into the nucleus, where it is required to establish GMC-specific gene expression.

Recently, it has been shown that both *prospero* RNA and the Staufen RNA-binding protein are also segregated into the GMC (Broadus et al., 1998; Li et al., 1997). During neuroblast mitosis, *prospero* RNA moves to the basal neuroblast cortex and is partitioned solely into the GMC, where it is released into the cytoplasm. Staufen protein is colocalized with *prospero* RNA, and *stauften* mutants fail to properly localize *prospero* RNA, although Prospero protein localization is unaffected (Broadus et al., 1998; Li et al., 1997). Loss of *prospero* RNA localization (in *stauften* mutants) does not obviously alter GMC identity; it is likely that *prospero* RNA and protein localization provide redundant functions in the GMC (Broadus et al., 1998).

How are Prospero and Staufen tethered at the basal neuroblast cortex? Both require *miranda* function for cortical localization. Miranda is a pioneer protein which was cloned from a two-hybrid screen for Prospero-binding proteins (Ikeshima-Kataoka et al., 1997; Shen et al., 1997). The 830 aa Miranda protein is predicted to contain a large “coiled coil” central domain, many consensus phosphorylation sites for protein kinase-C, and four divergent ubiquitin-dependent destruction signals (Ikeshima-Kataoka et al., 1997; Shen et al., 1997). During metaphase, Miranda forms a basal crescent at the cortex which precisely coincides with that of Prospero and Staufen (Ikeshima-Kataoka et al., 1997; S. Fuers-tenberg, P. Alvarez, C.-Y. Peng, and C. Q. Doe, unpublished data). Within the newborn GMC, *miranda* is rapidly degraded, Prospero translocates into the nucleus, and Staufen moves into the cytoplasm (Broadus et al., 1998; Ikeshima-Kataoka et al., 1997; Shen et al., 1997; Spana and Doe, 1995). In *miranda* mutant embryos, Prospero protein is uniformly distributed in the cytoplasm of dividing neuroblasts, and it enters the nucleus of both neuroblast and GMC following cytokinesis (Ikeshima-Kataoka et al., 1997; Shen et al., 1997). *miranda* mutants have GMC defects similar to hypomorphic *prospero* mutations (probably owing to the lower levels of Prospero in the GMCs); the fate of the neuroblasts in *miranda* mutants has not been assayed in detail.

How are the processes of protein/RNA localization and spindle orientation coordinated so that the GMC always inherits Miranda, Prospero, Staufen, and *prospero* RNA? The answer is *inscuteable*

(Kraut and Campos-Ortega, 1996; Kraut et al., 1996). *Inscuteable* encodes a protein containing an SH3-binding domain, divergent ankyrin repeats, and a putative WW domain (Kraut and Campos-Ortega, 1996). *Inscuteable* is localized to the apical cortex of the neuroblast from late interphase at least through metaphase. At anaphase, *Inscuteable* appears to be delocalized or degraded *in vivo* (Kraut et al., 1996), but neuroblasts cultured *in vitro* show apical *Inscuteable* localization throughout the entire cell cycle, with selective segregation into the neuroblast (Broadus and Doe, 1997). In *inscuteable* mutant embryos, localization of Miranda, Prospero, and Staufen is randomized with respect to surrounding tissue as well as with respect to the spindle (Kraut et al., 1996; Li et al., 1997). In addition, spindle orientation is also randomized with respect to the apical/basal axis. Thus, *Inscuteable* appears to independently regulate both spindle orientation and the basal localization of Miranda/Prospero/Staufen.

The fact that intrinsic factors are important for GMC determination does not rule out a role for extrinsic factors. For one thing, no known mutation produces a full transformation of GMC to neuroblast fate, suggesting other factors (intrinsic or extrinsic) required for GMC differentiation remain to be discovered. A challenge for the future is to determine the relative importance of intrinsic and extrinsic factors using a combination of genetics, cell ablations, and *in vitro* cell culture experiments. For example, can mutations be identified which switch neuroblast division from asymmetric (neuroblast/GMC) to proliferative (neuroblast/neuroblast) or to differentiative (GMC/GMC or neuron/neuron)? Can *in vitro* culture conditions be modified to support either asymmetric, proliferative, or differentiative neuroblast divisions?

How Many Types of Neural Stem Cells Exist, and How Is Each Specified?

At one extreme, there could be a multiplicity of neural stem cells, each devoted to producing a specific type of neuron or glia; at the other extreme, there may be one fundamental type of neural stem cell, with all cellular diversity generated amongst the stem cell progeny. The two questions we will address in this section are: How many types of neural stem cells exist? and, What are the mechanisms that generate different neural stem cells? We will only consider the initial specification of stem cell fate (i.e., the fate of newly formed stem cells); the

possibility that stem cell fates change over time is addressed in the following section.

Vertebrates. There is a growing body of evidence that extrinsic growth factors can directly modify neural stem cell fate, biasing their subsequent differentiation. If clonal stem cell populations derived from the rodent hippocampus are transiently exposed to ciliary neurotrophic factor (CNTF), they produce astrocytes at the expense of neurons; if they are transiently exposed to thyroid hormone (T3), they produce oligodendrocytes at the expense of neurons. The same results are observed with embryonic or adult stem cells (Johe et al., 1996). These data suggest that growth factors directly affect stem cell fate, resulting in the production of progenitor cells committed to generating neurons, astrocytes, or oligodendrocytes. Similar results have been observed using clonally cultured neural crest cells: BMP2 induces autonomic neurons, glial growth factor induces Schwann cell fate, and transforming growth factor- β (TGF- β) induces smooth-muscle differentiation (Shah et al., 1994, 1996). These data suggest that either hippocampal stem cells consist of a single primitive stem cell fate, or inherent differences in stem cell fate can be easily modified by exposure to extrinsic signals. (However, these culture conditions may preselect for survival of only one neuronal stem cell type.) Comprehensive descriptions of the role of growth factors in specifying stem cell/progenitor cell fate can be found elsewhere in this issue and in recent reviews (Fisher, 1997; McKay, 1997; Morrison et al., 1997).

The role intrinsic factors play in neural stem cell specification is not known. A rapidly growing number of transcription factors (of the homeodomain, Pax, bHLH classes) have been detected in the ventricular zone within restricted domains of the CNS, consistent with a role in regulating region-specific stem cell identity (reviewed in Boncinelli et al., 1995; Kageyama and Nakanishi, 1997; Mansouri et al., 1996), and many of these genes have *Drosophila* homologues that are required for specifying neuroblast identity (see below). Where mutations have been assayed (*Bf-1*, *Otx2*, and *Dlx-1*), there is a deficit in the neurons derived from the brain regions expressing the gene (Acampora et al., 1995; Anderson et al., 1997; Ang et al., 1996; Xuan et al., 1995); the phenotypes could be due to defects in the formation, specification, survival, cell division, or differentiation of the stem cells. For the remaining genes, their role in neurogenesis remains to be tested, although the *Drosophila* homologues of the Pax and

bHLH genes are required for establishing neuroblast cell fate (see below).

Overall, there is clear evidence that extrinsic cues can bias neural stem cell development; what remain unknown are the degree of intrinsic heterogeneity among stem cells and the degree to which neural stem cells are irreversibly committed to generating a particular type of neuronal or glial progeny. The generation of additional molecular markers that distinguish neuronal and glial subtypes—or neural stem cell types—will be necessary to answer this question.

Drosophila. One advantage of studying *Drosophila* neural stem cells, neuroblasts, is that they are each uniquely identifiable based on their morphology and position in the embryo. The earliest-forming neuroblasts are arranged in an orthogonal grid of four rows (1, 3, 5, and 7) along the anterior–posterior (AP) axis and three columns (ventral, intermediate, and dorsal) along the dorsoventral (DV) axis within each half segment of the embryo (Figs. 4 and 5). Later-forming neuroblasts occupy the even-numbered rows, fill in the odd-numbered rows, and generate two additional columns to produce the final complement of 30 neuroblasts. Nearly every neuroblast within this array is different from the others based on molecular and developmental criteria: Each forms at a precise time, expresses a unique combination of genes, produces a characteristic number of progeny (ranging from two to over 30), and, most importantly, generates a unique family of neurons and/or glia (Fig. 4) (Bossing and Technau, 1993; Broadus et al., 1995; Buenzow and Holmgren, 1995; Cayre et al., 1994; Chu-LaGraff et al., 1995; Doe, 1992; Higashijima et al., 1994; Udolph et al., 1993).

Recent work has provided a fairly clear picture of the mechanisms controlling the initial specification of neuroblast cell fate (Fig. 5). Genes expressed in orthogonal rows or columns within the neuroectoderm provide positional cues that specify the initial identity of each delaminating neuroblast. The earliest neuroblasts are specified by nuclear Pax and bHLH proteins. The tandem *gsb* genes encode Pax-type transcription factors expressed in row 5 neuroectoderm and neuroblasts (Skeath et al., 1995; Zhang et al., 1994). In *gsb* mutants, row 5 neuroblasts are transformed into row 3 neuroblasts (Skeath et al., 1995). Ectopic *gsb* expression gives the opposite phenotype: row 3 neuroblasts are transformed into row 5 neuroblasts (Skeath et al., 1995; Zhang et al., 1994). Moreover, ectopic *gsb* can only produce a row 3 to 5 transformation when expressed

prior to neuroblast delamination (Skeath et al., 1995). Similar results are observed for *achaete-scute* proneural genes, which encode bHLH proteins initially expressed in rows 3 and 7 of the neuroectoderm. In addition to their well-known function in promoting neuroblast formation, their neuroectodermal expression is necessary for correct specification of row 3 and 7 neuroblast identity (Parras et al., 1996; Skeath and Doe, 1996). These results show that neuroblast identity is specified in the neuroectoderm prior to delamination of the neuroblasts, and that neuroblast identity can be specified by regionally expressed Pax and bHLH genes within the neuroectoderm.

Later-forming neuroblasts are also uniquely specified by regionally expressed genes within the neuroectoderm, although these genes encode secreted proteins. The *wingless* (*wg*) gene is ex-

pressed in the row 5 neuroectoderm and encodes a secreted “wnt” protein that can be detected in the adjacent row 4 and 6 neuroectoderm (van den Heuvel et al., 1989). Experiments using a temperature sensitive *wg* mutation show that *wg* function in the neuroectoderm (but not in the neuroblasts themselves) is necessary and sufficient to specify the fate of row 4 and 6 neuroblasts (Chu-LaGraff and Doe, 1993). Similarly, the secreted Hedgehog (Hh) protein is produced in rows 6 and 7, and is required to specify later-forming neuroblasts in the adjacent rows 5 and 1 (McDonald and Doe, 1997).

Genes that specify neuroblast fates along the DV axis have only recently been identified. The *vnd* homeobox gene is transcribed primarily in the ventral column of the neuroectoderm and neuroblasts. Mutations in *vnd* lead to ventral column neuroblasts failing to form or assuming an intermediate column neuroblast identity; conversely, misexpression of *vnd* leads to intermediate column neuroblasts adopting ventral column fates (Jimenez and Campos-Ortega, 1990; Jimenez et al., 1995; Mellerick and Nirenberg, 1995; J. A. McDonald, S. Holbrook, C. Q.

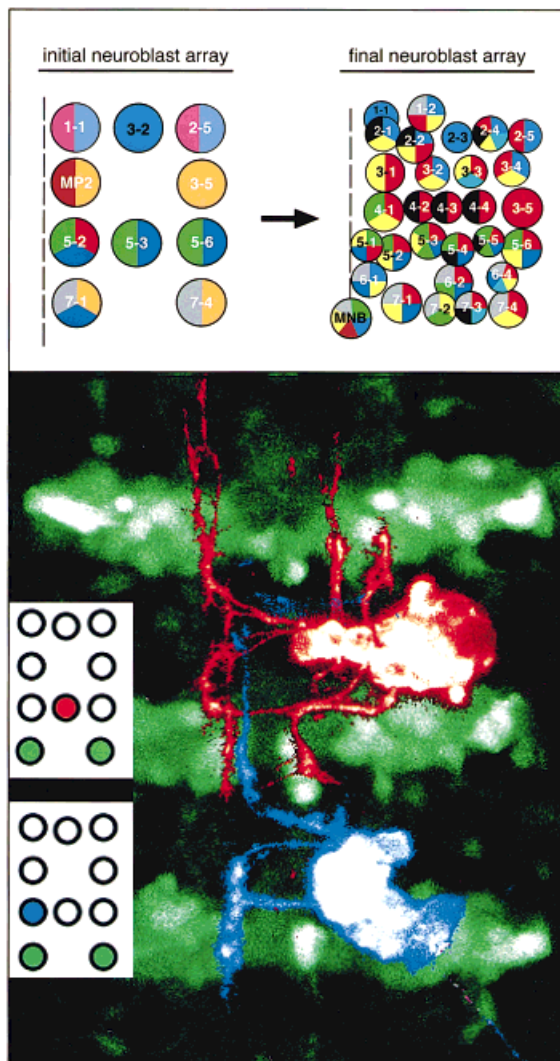


Figure 4 Neuroblasts can be uniquely identified by position, gene expression, and cell lineage. (Top) The initial array of 10 neuroblasts is arranged in an orthogonal pattern of four rows (1, 3, 5, and 7, from top to bottom) and three columns (ventral, intermediate, and dorsal from left to right). Later-forming neuroblasts lead to a roughly seven row/five column array. Each array a single hemisegment; i.e., it is bilaterally arranged and reiterated in each segment of the embryo (there are three subesophageal, three thoracic, and eight abdominal segments in *Drosophila*). Each neuroblast can be uniquely identified by its position in the array and the combination of genes it expresses. (For the gene names and color key, see Broadus et al., 1995, or visit the “hyper neuroblast map” at <http://www.life.uiuc.edu/doelab/nbmap.html>.) (Bottom) The confocal micrograph shows three segments of the CNS near the end of embryogenesis. The characteristic clone of about 30 interneurons produced from neuroblast 5-2 is shown in red, the clone of about 20 inter- and motoneurons produced by neuroblast 5-3 is shown in blue, and green fluorescent protein (GFP) expressed in the engrailed pattern is shown in green. To generate these data, neuroblast 5-2 in the middle segment was labeled with DiI (red) and neuroblast 5-3 in the bottom segment was labeled with DiD (blue) before either neuroblast had begun to produce progeny (summarized in the insets for each segment: 5-2 is in red, 5-3 is in blue, and the landmark engrailed-GFP is in green); as the neuroblasts divide, DiI or DiD is inherited by all of the progeny and reveals their axonal and glial morphology.

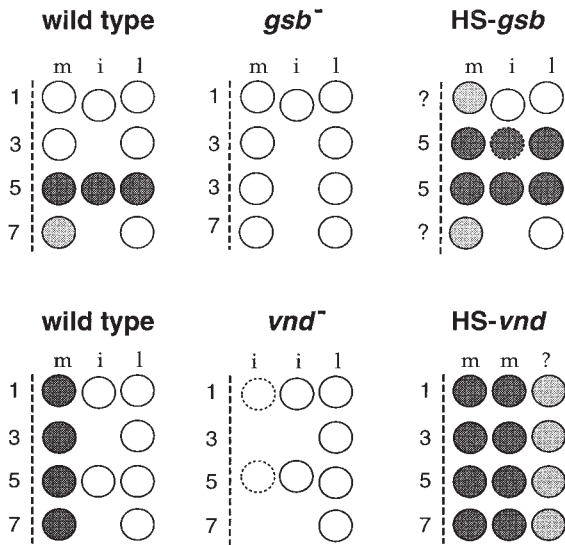


Figure 5 Positional cues in the neuroectoderm specify neuroblast identity along the anterior-posterior and dorso-ventral axes of the CNS. (Top) In wild-type embryos, the Pax-type *gooseberry* genes (*gsb*) are expressed in row 5 neuroectoderm and neuroblasts (dark fill), and transiently in the ventral row 7 neuroblast (gray); *gsb* mutants show a row 5 to row 3 transformation of neuroblast identity, whereas ubiquitous *gsb* in the neuroectoderm (HS-*gsb*) gives the opposite phenotype (dark fill reflects row 5 neuroblast fates). (Bottom) In wild-type embryos, the homeobox *vnd* gene is expressed in the medial column of neuroectoderm and neuroblasts (dark fill); *vnd* mutants show a medial to intermediate transformation of neuroblast identity, whereas ubiquitous *vnd* in the neuroectoderm (HS-*vnd*) gives an intermediate (and possibly lateral) to medial transformation of neuroblast identity. Dotted line = ventral midline; anterior is up.

Doe, and D. Mellerick, submitted). Thus, *vnd* is necessary and sufficient for ventral column neuroblast fates (within the ventral and intermediate domains). The *msh* homeobox gene is expressed in the dorsal column of neuroectoderm and neuroblasts; mutations in *msh* lead to defects in dorsal neuroblast cell fates (including possible dorsal-to-ventral transformations), whereas misexpression of *msh* in the ventral neuroectoderm produces defects in ventral neuroblast cell fates (Isshiki et al., 1997). Both *vnd* and *msh* have vertebrate homologues with similar expression patterns during early neurogenesis (e.g., ventrally expressed *Nkx2.2* and dorsally expressed *Msx* genes). Results from *Drosophila* raise the possibility that these vertebrate genes may control region-specific neural stem cell fate along the DV axis.

Taken together, the descriptive and experimental data allow us to make the following conclusions:

(a) There are about 30 different neuroblasts in each bilateral hemisegment; each reproducibly expresses a specific combination of genes and produces a stereotyped family of neurons and/or glia. (b) The *initial* identity of each neuroblast (i.e., its gene expression profile at formation and the fate of its first progeny) is determined in the neuroectoderm; this fate is shared by three to five neuroectodermal cells, but only one will delaminate to form a neuroblast. (c) Both secreted proteins (Wg and Hh) and nuclear proteins (Gsb, Vnd, and Msh) can regulate neuroblast identity; the common denominator is that all are expressed in orthogonal stripes along the AP or DV axes of the neuroectoderm, and thus can provide positional information to the delaminating neuroblasts.

There is little evidence for growth factors instructing neuroblast identity or the fate of their GMC progeny in *Drosophila*, primarily because the question has just begun to be addressed. The interplay between the TGF- β family member Decapentaplegic (Dpp) and its inhibitor Short gastrulation (Sog) establishes the neuroectodermal anlagen, similar to the role of their homologues BMP4/chordin in establishing the neural anlagen in vertebrates (Holley et al., 1995). There is increasingly good evidence that the Spitz protein (a TGF- α protein), secreted from the ventral midline, is required to specify ventral neuroectodermal cell fates via activation of the *Drosophila* EGF receptor (Gabay et al., 1996; Golembo et al., 1996; Schweitzer et al., 1995; Schweitzer and Shilo, 1997); presumably, this would have a direct effect on ventral neuroblast identity, but neuroblast fates have not been directly assayed in Spitz/EGF-receptor experiments. Taking a cue from vertebrate research, it is now technically feasible to determine the effect of growth factors on neuroblast identity during *in vitro* primary culture: A number of markers can be scored in neuroblasts cultured *in vitro* (Huff et al., 1989; Spana and Doe, 1996), but to date, these types of experiments have not been reported.

How “Plastic” Are Neural Stem Cell Fates?

In *Drosophila*, neuroblasts are molecularly distinct from the time they form and always produce a characteristic family of neurons and/or glia. However, an invariant lineage and pattern of gene expression does not mean that cell fate is cell autonomously determined—it could just as easily be due to exposure of the cell to a reproducible set of extrinsic signals. How “plastic” are neuroblast fates? In ver-

tebrates, regional differences in gene expression may lead to molecularly different populations of stem cells. Does this molecular heterogeneity lead to cell autonomous programs of stem cell differentiation, or can stem cell fate be modified by environmental signals?

Vertebrates. Clonal lines of immortalized stem cells can differentiate into a wide range of cell types *in vitro* or following *in vivo* heterotopic transplantation (reviewed in Fisher, 1997; Morrison et al., 1997; Temple and Qian, 1996). More important, clonal cultures of primary neural stem cells also can differentiate into a variety of region-specific neurons and/or glia following heterotopic transplantation (reviewed in Fisher, 1997; Morrison et al., 1997). For example, embryonic cerebellar stem cells transplanted into the hippocampus can differentiate into neurons characteristic of the hippocampus (Vicario-Abejon et al., 1995), and adult hippocampal stem cells can show migration and molecular features characteristic of olfactory bulb neurons (Suhonen et al., 1996). It is formally possible that each region of the brain contains many different types of neural stem cells, with region-specific “selective” mechanisms promoting death or proliferation of different subsets of progeny. However, the available data indicate that there are a minimal number of different neural stem cell fates, or, more likely, that stem cell fates are quite “plastic” and readily modified by a novel host environment.

Drosophila. Although there are 30 different neuroblasts based on descriptive data (gene expression and cell lineage), this does not mean that each neuroblast is autonomously committed to a specific cell fate. *In vitro* cultures of neuroblasts dissociated from embryos show that neuroblast identity can be maintained for at least one cell division (judged by the production of the appropriate first GMC) (Huff et al., 1989). Although the initial specification of neuroblasts occurs in the neuroectoderm and can be maintained for at least one cell cycle *in vitro*, it remains unknown how “plastic” neuroblast fate is—for example, whether intrinsic or extrinsic factors regulate later aspects of neuroblast fate (e.g., changing patterns of gene expression or generation of correctly specified late-born progeny). Transplantation of neuroectodermal cells shows that they can differentiate into host-specific neuroblast fates (Udolph et al., 1995), consistent with evidence described above that neuroblast identity is determined in the neuroectoderm, but the key experiment of transplanting mature neuroblasts has not been done.

Transplantation or *in vitro* culture of identified neuroblasts, followed by assaying the identity of both early- and late-born progeny, would provide insight into the degree to which neuroblasts are autonomously committed to producing their characteristic family of neurons and glia.

How Do Neural Stem Cells Produce Different Progeny?

In vertebrates, it appears that individual stem cells (immortalized or primary) can produce a complex population of different neurons and glia, often by the production of progenitor cells with a restricted developmental potential (reviewed in Fisher, 1997; McKay, 1997; Morrison et al., 1997; Temple and Qian, 1996; and other reviews in this issue). In *Drosophila*, there are 30 different neuroblasts in each segment (Broadus et al., 1995); yet, each one must still produce multiple types of neurons and/or glia, usually by production of intermediate precursors called GMCs (Doe and Technau, 1993; Goodman and Doe, 1993). In all systems, how does a single neural stem cell generate multiple types of progenitor cells or GMCs?

Vertebrates. Retroviral lineage studies show that a single ventricular zone (VZ) precursor can produce neurons in all cortical layers, and even in widely dispersed areas of the cortex (Reid et al., 1997; Walsh and Cepko, 1988, 1992). In addition, descriptive studies show that VZ precursors can express different genes as they produce neurons destined for different cortical layers (Frantz et al., 1994). There are several mechanisms that could account for these observations. First, intrinsic mechanisms within the VZ precursor could lead to temporal alterations in gene expression, induce migration, and control the specification of distinct progeny fates. Second, migratory VZ precursors may encounter different environmental cues that could result in temporal alterations in gene expression and the specification of distinct progeny fates. Finally, VZ precursors that do not migrate may be exposed to changing environmental cues that could alter VZ precursor gene expression and the specification of distinct progeny fates. Independent of whether the VZ precursor is stationary or migratory, there are two relevant questions: What is the importance of transient gene expression in VZ precursors as they produce progeny with different laminar fates? and What is the relative importance of intrinsic and extrinsic signals in regulating VZ precursor gene ex-

pression and establishing different VZ precursor progeny fates?

Until gene knockouts or misexpression studies are done, the role of any gene expressed in the ventricular zone will remain unknown. As for the relative importance of intrinsic or extrinsic cues in establishing different stem cell progeny fate, it is well known that growth factors can bias the type of progeny produced from clonal stem cell cultures *in vitro* (Johe et al., 1996), but recent experiments provide hints that intrinsic mechanisms may also be involved. Primary stem cells from rat embryos show stereotyped cell lineages when cultured *in vitro*, with some progeny differentiating and other maintaining a high mitotic potential (Xian et al., 1998; Shen et al., this issue). Interestingly, the cell lineages observed are complex, but many are reproducible. If we assume that a specific lineage history is one aspect of cell fate, then these cultures show single cells that can produce multiple cell fates despite homogenous culture conditions. Obviously, the use of molecular markers for distinguishing different neuronal and glial fates in these cultures would strengthen these conclusions.

An attractive hypothesis for the sequential specification of stem cell progeny fate is that stem cells change their pattern of gene expression over time in response to extrinsic and/or intrinsic cues, resulting in early-born and late-born progeny inheriting different combinations of gene products, and that these gene products contribute to specifying the distinct identity of each daughter cell.

Drosophila. All neuroblasts express different genes as they produce early-, mid-, and late-born GMCs, resulting in each GMC inheriting a different combination of gene products (Broadus et al., 1995; Cui and Doe, 1992; Kambadur et al., 1998). This is remarkably similar to the observation that mammalian VZ precursors express different genes as they produce neurons destined for different cortical layers (see the previous section). As with VZ precursors described above, these observations raise two questions: How is the timing of neuroblast gene expression regulated? and Do genes expressed at different times during a neuroblast lineage lead to different GMC fates?

The timing of neuroblast gene expression is under complex regulation (Cui and Doe, 1992). In some neuroblasts, blocking the cell cycle will prevent late gene expression; this suggests, but does not prove, that a cell-cycle-linked intrinsic mechanism regulates activation of late gene expression. In other neuroblasts, blocking the cell cycle does not affect

late gene expression; this result is consistent with extrinsic regulation of neuroblast gene expression. Finally, in some neuroblasts, blocking cytokinesis but not the nuclear cell cycle prevents late gene expression; this result is also consistent with extrinsic regulation of neuroblast gene expression. Nevertheless, the precise mechanism and molecules involved in regulating neuroblast gene expression remain unknown. It remains unknown whether neuroblast gene expression is controlled by continuously changing extrinsic cues (from the adjacent neuroectoderm or early-born GMCs) or whether each neuroblast embarks on an autonomous program of gene expression that is specified at the time of neuroblast formation. It would be useful to examine the temporal pattern of gene expression in neuroblasts isolated *in vitro*, or the pattern of gene expression in neuroblasts following ablation of early-born GMCs. These are difficult experiments, but they are necessary to determine whether intrinsic or extrinsic signals trigger the observed temporal changes in neuroblast gene expression.

As mentioned above, neuroblasts go through a stereotyped sequence of gene expression, resulting in each GMC inheriting a different combination of gene products. Does differential inheritance of these gene products lead to different GMC cell fates? The answer is yes, at least for the first two genes assayed. *klumpfuss* (*klu*) encodes a predicted zinc finger protein detected in the second and subsequent GMCs produced by NB 4-2; it is not detected in the first-born GMC (Yang et al., 1997). Mutations in *klu* produce a duplication of the first GMC at the expense of the second GMC; conversely, misexpression of *klu* in the first GMC prevents its normal differentiation (there are no markers for the second GMC, so it is unknown if the Klu-positive first GMC is transformed into a second GMC). Thus, the lineage-specific expression of *klu* is required to distinguish the second GMC fate (Klu positive) from the first GMC fate (Klu negative). *klu* is expressed in a similar manner in most neuroblast lineages, so it may have a widespread function in establishing later-born GMC fates. The *castor* gene (also called *ming*) encodes a predicted zinc finger protein that, in many neuroblasts, is detected only after the first or second GMC is born (Cui and Doe, 1992; Kambadur et al., 1998; Mellerick et al., 1992). Mutations in *castor* lead to altered neuronal cell fate (Cui and Doe, 1992; Kambadur et al., 1998; Mellerick et al., 1992), and recent lineage analysis indicates that *castor* mutations may transform late-born GMCs into early born GMCs (A. Schmid and C. Q. Doe, unpublished data). Thus, two genes, *klu*

and *castor*, are expressed in neuroblasts after production of the first GMCs, are detected in late-born but not early-born GMCs, and appear necessary for distinguishing late-born GMC fates from early-born GMC fates. The isolation of vertebrate homologues of *klu* and *castor* may shed light on the differential specification of stem cell progeny within the CNS.

What Regulates Stem Cell Quiescence Versus Proliferation?

Neural stem cells persist into adulthood in both mammals and insects (reviewed in Fisher, 1997; Shepherd, 1994), but it is unknown whether embryonic and adult stem cells represent the same cells at different stages of development, or two independent populations of cells. In either case, how is adult stem cell fate maintained during periods of mitotic quiescence?

Vertebrates. Quiescent stem cells exist in several regions of the mammalian adult CNS. For example, primary cultures from the rodent subependyma, hippocampus, striatum, or septum contain cells that show self-renewal ability, are capable of proliferation in response to EGF or bFGF, and generate multiple neural and glia cell types following growth factor withdrawal (Reynolds and Weiss, 1992; Morshead et al., 1994; Palmer et al., 1995, 1997; Gritti et al., 1996). The number of stem cells in the striatal subependyma is not effected when constitutively proliferating cells are killed by high doses of [³H]thymidine, suggesting that these stem cells are either quiescent or slowly dividing (Morshead, 1994). In addition, there is evidence for quiescent oligodendrocyte precursor cells: demyelination leads to precursor cell proliferation and the differentiation of new oligodendrocytes (Gensert and Goldman, 1997).

In vitro studies show that growth factors are an important regulator of stem cell proliferation. Stem cells from a number of CNS and PNS regions can be maintained as a proliferating population *in vitro* by addition of growth factors (reviewed in Fisher, 1997; Morrison et al., 1997; reviews in this issue). In many cases, withdrawal of growth factors from *in vitro* stem cell cultures will trigger exit from the cell cycle and differentiation, implicating growth factors in positively regulating the transition from quiescence to proliferation and negatively regulating the transition from proliferation to differentiation (Fisher, 1997; Morrison et al., 1997). Similar effects have been shown *in vivo*, as intracerebroven-

tricular infusion of EGF can stimulate subependymal stem cell proliferation (Craig et al., 1996).

Drosophila. There are three populations of *Drosophila* neuroblasts that become quiescent between embryogenesis and larval development, and one population that is continuously proliferative. The optic lobe, central brain, and thoracic neuroblasts proliferate during embryogenesis, become quiescent near the end of embryogenesis, and resume dividing during larval development (Truman and Bate, 1988; White and Kankel, 1978). In contrast, mushroom body neuroblasts divide continuously throughout embryogenesis and larval development (Ito and Hotta, 1992).

Two genes have been identified that regulate the timing of the quiescence-to-proliferation transition of the optic lobe, central brain, and thoracic neuroblasts. The *anachronism* (*ana*) gene encodes a secreted glycoprotein expressed in the glial cells adjacent to quiescent neuroblasts, but not in the neuroblasts themselves (Ebens et al., 1993). A hypomorphic *ana* mutation leads to premature activation of proliferation (i.e., the quiescent phase is shortened), and consequently, more neurons are observed in the adult brain (Ebens et al., 1993). Thus, *ana* encodes a novel secreted protein that is required to maintain neuroblast quiescence. A second gene, *terribly reduced optic lobes* (*trol*), has the opposite phenotype. Hypomorphic mutations of *trol* result in optic lobe, central brain, and thoracic neuroblasts remaining quiescent longer than normal, and consequently, there is a decrease in the number of neurons in the adult brain (Datta, 1995). Thus, *trol* is required for the timely reactivation of neuroblast proliferation. Interestingly, mushroom body neuroblast proliferation is not affected in *trol* mutants, suggesting that *trol* is required for the reactivation, but not the maintenance, of cell proliferation and that *trol* does not encode a general factor required for cell cycle progression. *trol, ana* double-mutant larvae exhibit the *ana* phenotype—premature activation of neuroblast proliferation (Datta, 1995)—supporting the conclusion that *trol* does not affect the cell cycle machinery but may play a specific role in releasing neuroblasts from *ana*-mediated quiescence.

In addition to *ana* and *trol* genes, recent work has shown that quiescent neuroblasts are mitotically reactivated in response to larval feeding (Britton and Edgar, 1998). Normally fed larvae incorporate BrdU into all larval populations of neuroblasts as they reenter mitosis; in contrast, nutrient-deprived larvae show BrdU incorporation only in the continu-

ously replicating mushroom body neuroblasts. However, when isolated nervous systems from starved larvae are cocultured with an isolated fat body from fed larvae, both brain and thoracic neuroblast cell cycles are reactivated, providing evidence that reactivation is not tissue autonomous, and that a secreted factor from fat body is sufficient to induce the cell cycle in quiescent CNS neuroblasts. Nutrient-deprived *ana* (or *trol*) mutant larvae fail to reactivate neuroblast proliferation, suggesting that nutritional dependence acts upstream of *ana/trol* regulation. Further molecular and genetic analysis of the *ana* and *trol* genes, as well as the fat body-derived proliferation signal, will provide insight into the mechanisms regulating neuroblast quiescence and proliferation in *Drosophila*, and may ultimately lead to insights into the regulation of vertebrate neural stem cell quiescence.

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