Perspectives

Maintaining Youth in Drosophila Neural Progenitors

Takako Isshiki1,*
Chris Q. Doe2

1Genetic Strains Research Center; National Institute of Genetics; Mishima, Shizuoka, Japan
2Institutes of Neuroscience and Molecular Biology; Howard Hughes Medical Institute; University of Oregon; Eugene, Oregon USA

*Correspondence to: Takako Isshiki; Genetic Strains Research Center; National Institute of Genetics; 1111 Yata; Mishima, Shizuoka 411-8540 Japan; Tel.: +81.55.981.5825; Fax: +81.55.981.5826; Email: tisshiki@lab.nig.ac.jp

Received 12/14/03; Accepted 12/17/03
Previously published online as a Cell Cycle E-publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=718

KEY WORDS

neuroblast, competence, Hunchback, lineage restriction

ABSTRACT

An important question is how dividing stem cells maintain competence to generate multiple cell types, whereas most other cells become progressively restricted during development. The molecular basis for progenitor competence—or how competence is progressively restricted—has remained mysterious. Recent work has shown that Drosophila neuroblasts and mammalian neural progenitors are more similar than previously appreciated, and provide an excellent model system for using Drosophila genetics to unravel the molecular nature of progenitor competence and how it becomes progressively restricted during development.

INTRODUCTION

An important question in developmental and stem cell biology is how cells maintain (or lose) competence to generate multiple cell types as they go through multiple rounds of cell division. For example, mammalian cerebral cortical progenitors are initially multipotent, but lose competence to make early-born cell types over time.1 Similarly, retinal progenitors are thought to progress through a series of competence states that leads to the orderly production of each retinal cell type.2-3 However, the molecular basis for progenitor competence—or how competence is progressively restricted—has remained mysterious. Recent work has shown that Hunchback, an Ikaros family zinc finger transcription factor, is transiently expressed in young neuroblasts, where it is necessary and sufficient for specifying first-born temporal identity in most neuroblast lineages.4 New work has shown that neuroblasts become progressively restricted in their ability to respond to Hunchback, and that a transient pulse of Hunchback in mitotic progenitors, but not post-mitotic neurons, can establish long-lasting first-born temporal identity.5 These results show that Drosophila neuroblasts and mammalian neural progenitors are more similar than previously appreciated, and provide an excellent model system for using Drosophila genetics to unravel the molecular nature of progenitor competence and how it becomes progressively restricted during development.

The central nervous system (CNS) comprises a highly organized architecture composed of diverse cell types. Its construction requires precise spatial and temporal fate specification of each component cell. Neural progenitors change their properties over time, generating diverse cell types in a reproducible order in retina,6-8 cerebral cortex,9,10 and probably in the spinal cord.11 However, it is poorly known how they change over time. Drosophila neuroblasts are an ideal model for studying how neural progenitors generate diverse progeny over time, i.e., how temporal identity is regulated. All Drosophila neuroblasts can be individually identified based on their position and molecular markers,12 and each neuroblast generates a unique and invariant lineage.13,14 Neuroblasts undergo repeated asymmetric divisions to regenerate themselves and to bud off a series of daughter cells, called ganglion mother cells (GMCs). GMCs typically divide just once to produce two differentiated neurons or glia. Each GMC has a unique temporal identity based on its birth order within neuroblast lineage. We have previously found that neuroblasts sequentially express the transcription factors Hunchback, Kriippe (Kr), Pdm and Castor (Cas) over time, while GMCs and their progeny maintain the expression profile of the transcription factor present at their birth (Fig. 1).4 These transcription factors are good candidates for specifying temporal identity of the sequentially born GMCs, and so far this has been confirmed for both Hunchback and Kr, which are necessary and sufficient to specify temporal fates of early-born and middle-born GMC/neurons respectively.4 Yet many important questions...
remain. Where do Hunchback and Kr act—in the neuroblast, GMC, or neuron? Does a neuroblast undergo irreversible changes with every division (as perhaps suggested by its changing gene expression profile) or does a neuroblast maintain competence to generate all cell types at all times?

In vertebrates, it has been proposed that retinal and cerebral cortical progenitors change their competence over time, leading to the sequential production of distinct neural cell types.1-3 The mammalian cerebral cortex is a laminated structure, which has six distinct layers. Neurons contained in each layer share similar morphological features. Single progenitors located in the ventricular zone divide asymmetrically to generate neurons that are dispersed into all cortical layers: neurons produced later pass through deeper layers consisting of neurons born earlier, and move outward to generate upper layers. Consequently, the laminar fate of a neuron has a tight correlation to its birth date: The earlier it is born, the deeper it is located. Furthermore, it has been reported that the laminar fate of cortical neurons is fixed in the S/G2 phase just prior to its final cell division.15 Transplantation studies in ferrets have revealed that cortical progenitors are multipotent at first, but that their potential is gradually restricted.1,15,16 Early progenitors transplanted into the brains of older hosts can generate either deep layer neurons when transplanted just prior to mitosis, or upper layer neurons when transplanted during S phase.15 Mid-stage progenitors transplanted into older brains can adapt to host environment and generate upper layer neurons.1 In contrast, when the progenitors are transplanted into younger brains, they generate middle layer neurons, appropriate for their origin, and also slightly deeper layer neurons, that are appropriate for neither the host nor the donor environment.1 However, they never give rise to the deepest layer neurons,1 which the endogenous progenitors are generating. These indicate that mid-stage progenitors are partially restricted in their potential. Finally, late progenitors are committed to producing only upper layer neurons in any environment.16 Thus, progenitor competence has been shown to be restricted in at least two distinct ways within the cortex or retina. However, the molecular basis for cortical and retinal progenitor competence—or how competence is progressively restricted—remains completely unknown.

**PROGRESSIVE RESTRICTION IN DROSOPHILA NEUROBLASTS**

Recent work has shown that *Drosophila* neuroblasts become progressively restricted,5 similar to vertebrate neural progenitors. The early-born determinant Hunchback was used as a tool to probe the competence of progressively older neuroblasts, using a specific neuroblast called NB7-1 due to its well-described, diverse, and lengthy cell lineage.12-14,17,18 In this lineage, the first five GMCs (GMC1-GMC5) each generate a single U motor neuron (U1-U5) and a U sibling cell (U1sib-U5sib ) that lacks a prominent axon,13,14,17,18 Hunchback is expressed GMC1 and GMC2 and their U1 and U2 neuronal progeny.4 To ask whether neuroblasts undergo progressive restriction, fly embryos were genetically manipulated to express Hunchback at various levels and time points during the NB7-1 lineage. Pulses of Hunchback during the normal window of Hunchback expression can extend the Hunchback expression window and lead to extra U1 neurons.6,5 But what about pulses of Hunchback expression after downregulation of endogenous Hunchback? Is the neuroblast equally competent to respond to Hunchback throughout its lineage? When Hunchback is continuously expressed at high level after GMC3 is born, the neuroblast can generate many extra U1 neurons, revealing the important finding that loss of endogenous Hunchback does not limit competence of the neuroblast to respond to Hunchback. Is competence ever lost? When transient pulses of Hunchback were given at various points throughout the lineage, it was observed that the production of early-born neurons gradually declines as the Hunchback pulse is given progressively later, and following the birth of GMC5 there is no longer any detectable response to Hunchback. Thus, NB7-1 appears to undergo a progressive loss of competence to respond to Hunchback, similar to progressive restriction in potential of cortical progenitors (Fig. 1).

Interestingly, the neuroblast entirely loses competence to respond to Hunchback at about the time Castor is first expressed.5 Hunchback and Cas are both C1H2 zinc finger proteins, and share similar DNA-binding consensus sequences, but their structure outside of the DNA binding domains is quite different each other.19 These evoke an idea that Cas binds to the same targets where Hunchback bound earlier, resulting in an irreversible conformational change to chromatin configuration, which prevent access or effect of Hunchback on the targets, perhaps even after Cas disappears. Such memorable marks on chromatin can be due to various kinds of histone modifications, called “histone codes,” that cause either gene silencing or activation20 (discussed further below).

**TRANSIENT HUNCHBACK IN MITOTIC PROGENITORS INDUCES STABLE EARLY-BORN NEURONAL IDENTITY**

To determine whether only mitotic neuroblasts and GMCs have competence to respond to Hunchback, or whether Hunchback can also act in mature differentiated neurons, ectopic Hunchback was provided to either neuroblast/GMCs or post-mitotic neurons.5 Misexpression of Hunchback in mitotic neuroblast and GMCs was sufficient to induce extra early-born U1 and U2 neuronal fates; interestingly, a transient burst of Hunchback in neuroblasts/GMCs was sufficient to confer long-lasting early-born U1 cell fates (until at least the end of embryogenesis). These ectopic U1 neurons did not express Hunchback protein, but they maintained U1 molecular marker expression (Zfh1+ Kr+ Zfh2: Runt+ Cas+ ) and projected to the U1 dorsal muscle field.5 Thus, transient Hunchback in mitotic progenitors leads to heritable early-born fate. It is unknown whether

---

Figure 1. Competence to respond to Hunchback in the early lineage of NB7-1 (modified from ref. 5). (A) NB7-1 gradually loses the competence to generate early-born neurons [i.e., U1 and U2] in response to Hunchback. MN: motor neuron, IN: interneuron. (B) The competence to generate early-born neurons in response to Hunchback is lost in mature post-mitotic neurons.
both neuroblasts and GMCs are competent. Conversely, persistent high level expression of Hunchback specifically in post-mitotic neurons had no effect: the U1-U5 neurons expressed their normal molecular markers despite high Hunchback levels. Thus, the responsiveness of the Hunchback-regulated genes (Zfh14, Kr+ Zfh2, Runt-Cas+) changes during the process of neuronal differentiation: from Hunchback-responsive in mitotic neuroblasts/GMCs to Hunchback-independent in post-mitotic neurons. It is worth noting that, as described above, the laminar fate of cortical neurons is also established in mitotic progenitors and can’t be altered in post-mitotic neurons. In the future it will be important to determine the molecular basis for this switch in competence as a cell becomes post-mitotic.

How might Hunchback act transiently in mitotic progenitors to specify early-born neuronal fates? During the early stages of Drosophila embryonic segmentation, Hunchback directly represses and activates the Hox, pair-rule, and gap genes, including hunchback and Kr (reviewed in ref. 21). Subsequently, during the process of segmental identity specification, transient Hunchback leads to permanent silencing of anterior Hox gene expression by epigenetic mechanisms involving Polycomb group proteins.22,23 The Hunchback-related Ikaros protein functions from the early stage of hematopoiesis in lymphocyte lineage development24 (reviewed in ref. 25). In both systems, Hunchback/Ikaros proteins associate with Mi-2, an ATP-dependent chromatin remodeler.25-27 Mi-2 as well as histone deacetylases are components of NuRD complex that functions in both chromatin remodeling and histone deacetylation.26,27 Hunchback/Ikaros recruits the NuRD complex to its binding sites. At first, the NuRD complex might provide “chromatin fluidity” — an equilibrium between open and closed chromatin states—near their binding sites by its chromatin remodeling activity.25 This might enable the young Hunchback+ neuroblasts to establish a new transcriptional state by allowing the access of transcription factors present at the time of neuroblast formation (when neuroblast identity is assigned). This may be part of the molecular basis of neuroblast competence. Subsequently, perhaps in the GMC or young neuron, the Hunchback/NuRD complex may confer long-lasting stable transcriptional states to their target genes by recruiting Polycomb group proteins.23,28 In this way, the role of Hunchback in segmentation and CNS development may share a common parallel: an initial role in direct transcriptional regulation followed by a subsequent role in establishing stable chromatin states. This hypothesis remains to be tested experimentally. In addition, many other open questions remain. If Hunchback functions with the NuRD complex in the CNS, what cells and phases of cell cycle does the Hunchback/NuRD complex function? Hunchback mediated recruitment of the active NuRD complex on chromatin may occur only at particular phase of cell cycle, such as S phase25 or G1/M phase. Does the Hunchback recruited NuRD complex always lead to Polycomb-mediated gene silencing? Are the loci of Hunchback/NuRD targets the same or different in neuroblasts and GMCs? What are the targets and function of Hunchback in post-mitotic neurons?

CONCLUSIONS

It appears that both mammalian and Drosophila neural progenitors share several important features. Individual progenitors are multipotent, generate different neural types in a predictable sequence, become progressively restricted as they go through their cell lineage, and their post-mitotic progeny are unresponsive to intrinsic (fly) or extrinsic (mammal) temporal identity cues. In Drosophila, the zinc finger Hunchback protein regulates neuronal competence by an unknown mechanism, providing the first molecular entry point for studying neuronal competence. In C. elegans, the hbl-1 gene (a hunchback homolog) has been also reported to play a role in temporal fate specification in certain cell lineages.29,30 It will be interesting to know whether hbl-1/hunchback homologs also regulate competence of precursors and progenitors in other organisms. Above all, we are waiting to know whether mammalian cortical or retinal progenitors utilize this molecular mechanism for regulating cell competence.

References


