

# Turning back the clock on neural progenitors

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## Summary

*Drosophila* neural progenitor cells, or neuroblasts, alter their transcriptional profile over time to produce different neural cell types. A recent paper by Pearson and Doe shows that older neuroblasts can be reprogrammed to behave like young neuroblasts, and to produce early neural cell types, simply by expressing the transcription factor, Hunchback.<sup>(1)</sup> The authors show that competence to respond to Hunchback diminishes over time. Manipulating neural progenitors in this way may have important implications for therapeutic uses of neural stem cells. *BioEssays* 26:711–714, 2004.

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## The developing invertebrate and vertebrate nervous system

Nervous system development requires the generation of a great diversity of cell types in a highly specific spatial and temporal arrangement. In both vertebrates and invertebrates, neural progenitor cells generate different neuronal subtypes in a stereotypical sequence. Initially, neural progenitors have the potential to produce a wide variety of neural cell types. However, as time passes, the range of cell types that can be produced is gradually reduced. This progressive restriction of neural progenitors is seen in the mammalian neocortex, the mouse and *Xenopus* retina, and the *Drosophila* embryonic central nervous system (CNS).<sup>(2–6)</sup>

The *Drosophila* CNS is generated from a region of ventral ectoderm called the ventral neurogenic region (vNR). From this flat sheet of neuroectodermal cells, an array of 30 neuroblasts is determined in each hemisegment. Neuroblasts delaminate from the neuroectoderm into the embryo in a stereotyped spatiotemporal pattern. Each neuroblast has a specific identity and produces a characteristic and invariant cell lineage.<sup>(7–9)</sup> Neuroblasts divide asymmetrically, in a stem-cell-like fashion, to produce a series of smaller cells, called ganglion mother cells (GMCs). GMCs then divide only once to generate two postmitotic neurons or glia.<sup>(10)</sup>

Recent studies have shown that many *Drosophila* neuroblasts go through a characteristic sequence of temporally ordered changes in gene expression.<sup>(11–14)</sup> Neuroblasts express four transcription factors in succession: first Hunchback (Hb), then Krüppel (Kr), POU Domain (Pdm) and Castor (Cas). Each GMC, and their differentiated progeny, expresses the transcription factor that its mother neuroblast expressed at the time of its birth (Fig. 1). The transcription factor confers a temporal identity to the lineages and specifies the particular neurons/glia produced.

In the vertebrate CNS, neural progenitor cells also divide asymmetrically, generating a population of cells that differentiate into neurons and glia. The mammalian neocortex is made up of six layers of neurons and glia. Each layer is composed of neurons with distinct connections, morphologies and physiological properties.<sup>(15)</sup> Birthdating studies using [<sup>3</sup>H]thymidine show that the deepest layers are formed first, and the more superficial layers are formed sequentially.<sup>(5,16,17)</sup> Clonal analysis using retroviruses indicates that neural progenitor cells generate cells for all six layers of the neocortex.<sup>(17–19)</sup> Therefore, an individual progenitor cell can produce a number of different cell types in a distinct temporal order, as opposed to having different progenitor populations for each cell type or layer. This connection between birth order and neuronal fate is also seen in the vertebrate retina and elsewhere in the vertebrate CNS.<sup>(3,4,6)</sup> There are therefore striking similarities between neurogenesis in the mammalian cortex and the *Drosophila* CNS.

## Misexpression of Hunchback in *Drosophila* neuroblasts

Neuroblast 7-1 (NB7-1), like most neuroblasts, produces a stereotyped constellation of neural cell types in a precisely defined birth order (Fig. 1). The first five divisions generate GMCs that each divide to produce a single motor neuron and another cell. The later divisions of the neuroblast generate approximately 30 interneurons. The motor neurons generated by the first five divisions are named sequentially, based on birth order, as U1–U5. Each neuron can be identified by its position, the muscle that it innervates and its combinatorial expression of transcription factors. All five U neurons express the transcription factor Even-skipped (Eve).

What is required to specify an early neuroblast fate? Pearson and Doe have partially addressed this question by

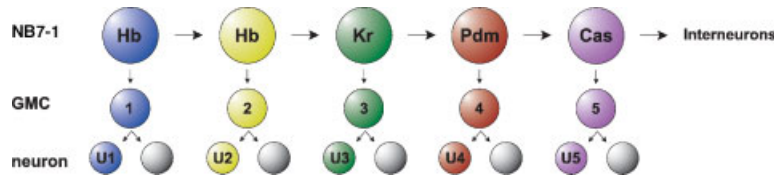
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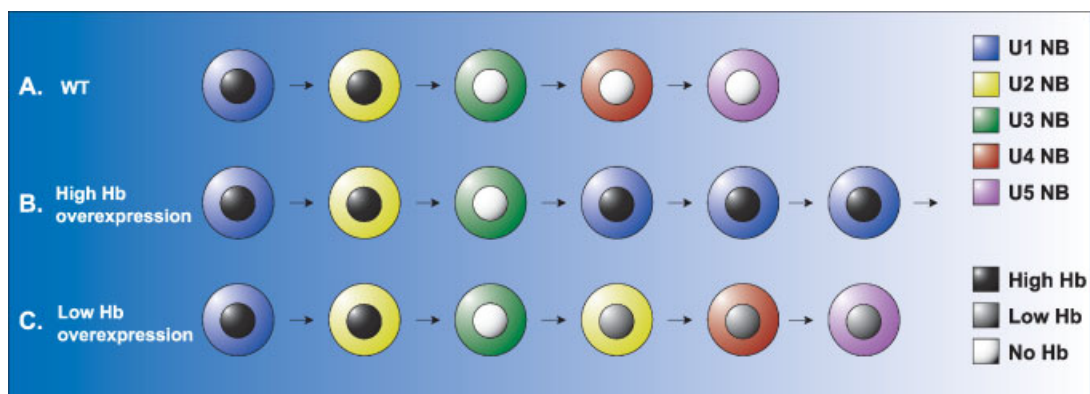


**Figure 1.** The lineage of neuroblast 7-1. Neuroblasts divide repeatedly in a stem-cell-like manner, renewing the neuroblast and budding off a smaller daughter cell called a ganglion mother cell, or GMC. GMCs divide only once to give rise to two postmitotic neurons or glia. The GMCs that arise from the first five divisions of neuroblast 7-1 (NB7-1) produce five Eve-positive motor neurons (U1–U5) and five Eve-negative cells (grey). NB7-1 expresses a stereotyped sequence of transcription factors during development: first Hb, then Kr, Pdm and Cas. Each GMC expresses the transcription factor expressed by NB7-1 at the time the GMC was born: GMC-1 expresses Hb (blue), as does its daughter, U1; GMC-2 and its daughter, U2, express Hb (yellow); GMC-3 expresses Kr (green), as does its daughter, U3; GMC-4 and its daughter, U4, express Pdm (red), and GMC-5 expresses Cas (purple), as does its daughter, U5 (purple). NB7-1 then continues to divide, producing ~30 interneurons. Adapted from Pearson and Doe (2003).

investigating the effect of misexpressing the transcription factor Hunchback on a single neural progenitor cell.<sup>(1)</sup> Hunchback is expressed in NB7-1 during its earliest two divisions, which generate GMC-1 and GMC-2. The protein continues to be present both in GMC-1 and GMC-2 and their motor neuron progeny (U1 and U2), but is not present in GMC-3 or in later GMCs and their progeny. Previous work by the Doe laboratory had indicated that Hb was both necessary and sufficient to specify first born fates in many neuroblasts lineages.<sup>(13)</sup> However, it was not clear when during their lineage neuroblasts were able to respond to Hunchback expression, and if their competence to respond changed over time. To address this question, Pearson and Doe used the GAL4 system to overexpress Hb in NB7-1 at different times during its development. They assayed changes in cell fate in the U neurons, and the generation of supernumerary U motor neurons.

In one experiment, the authors misexpressed Hb in NB7-1 after the birth of GMC-3, which does not normally express Hb (Fig. 2). Consistent with a cell intrinsic model of cell fate determination, the first three motor neurons (U1–U3) appear to be completely normal. However, markers normally expressed by the U4 and U5 motor neurons were absent. Furthermore, six extra Eve-positive cells were generated, in addition to the first three Eve-positive motor neurons. The additional cells appear to be U1 motor neurons, based on the marker genes that they express and the muscles that they innervate. The results suggest that NB7-1 is still competent to respond to Hb at a stage when endogenous Hb is normally switched off. Furthermore, NB7-1 can be reprogrammed to produce early cell fates in response to Hb expression.

When this experiment is repeated using lower levels of Hb protein expression, only a single additional U2 motor



**Figure 2.** The effects of ectopic Hb on the lineage of NB7-1. **A:** Hunchback (black nuclei) is normally expressed early in the NB7-1 lineage, when the NB gives rise to U1 (blue) and U2 (yellow) motor neurons. **B:** Ectopic expression of high levels of Hb can convert a U4 NB (red) back to a U1 NB (blue). **C:** Ectopic expression of low levels of Hb (grey nuclei) can convert a U4 NB (red) back to a U2 NB (yellow), after which the sequence resumes by producing U4 (red) and U5 (purple) motor neurons. This ability to respond to Hb diminishes over time — expressing Hb in NB7-1 after 10 hours of development has no noticeable effect on the lineage.

neuron is produced (Fig. 2C). The ability of Hb to reprogram neuroblast fate appears, therefore, to depend on the concentration of Hb.

From these experiments, it was clear that expression of Hb relatively early in the NB7-1 lineage can generate early cell fates. However, it was not clear if the competence to respond to Hb persists in older neuroblasts. Therefore the authors used a heatshock-inducible transgene to express Hb at low levels during four progressively later time windows. Early expression in NB7-1 leads to additional U1 and U2 motor neurons. At later time points, only additional U2 motor neurons are generated, and the number of additional motor neurons decreases. After about 10 hours of development, at the time when the last motor neurons are being generated, the NB is no longer competent to generate early born fates. These data lead the authors to conclude that neuroblasts remain plastic and can be reprogrammed by misexpression of Hb. However, as the neuroblasts get older their competence to respond to this transcription factor diminishes.

### Progressive restriction of competence

The gradual reduction in competence of neuroblasts to respond to Hb is similar to the progressive restriction in potential of neural progenitors during mammalian cortical development. During the initial stages of cortical development, neural progenitor cells are multipotent and can generate all neural cell types in the cortex. As time passes the number of cell types that can be generated by a neural progenitor is reduced. This was elegantly demonstrated in experiments carried out on the ferret neocortex.<sup>(20)</sup> Younger neural progenitor cells were transplanted into older brains and, conversely, older progenitors were transplanted into younger brains. These heterochronic transplants showed a striking result: younger progenitors responded to their new environment and generated neurons of an appropriate laminar fate, while older progenitors could not respond appropriately to the younger environment, and continued to produce later neural cell types. It was therefore proposed that neural progenitors exhibit a progressive restriction in the neural cell types that they can generate.

It is attractive to speculate that the progressive restriction seen in vertebrate neural development and the reduction of competence seen in *Drosophila* neuroblast is due to a conserved molecular mechanism. However, the evidence is limited and there are significant differences in the nature of the progressive restriction seen in vertebrates and in *Drosophila*. In the vertebrate heterochronic transplants, it is a loss of competence to respond to an extrinsic signal whereas, in *Drosophila* neuroblasts, it is the loss of ability to respond to an intrinsic signal. It remains to be seen if both processes are truly homologous and if they are regulated by similar molecular mechanisms.

### Future directions

There are many questions that remain to be answered. Does Hunchback really confer youth to neuroblasts, resetting the clock, or does it just confer a Hb-specific cell state? Are there timing mechanisms that keep on 'counting' even though Hunchback is expressed? If Hunchback is really 'resetting' an intrinsic clock then one would predict that downregulation of Hb would allow the temporal sequence to proceed as normal. Results in the paper hint that this may indeed be occurring, at least to some degree, but further experiments are needed to be sure.

Another question that persists is whether Hb homologues function in a similar fashion in other organisms. Hunchback-like proteins are expressed in the nervous systems of animals throughout the animal kingdom, potentially indicating that they have a conserved role in neurogenesis. Hunchback homologues have been described in the CNS of *C. elegans* as well as in the leech.<sup>(21–23)</sup> To date there is no published data to suggest that the function of any hunchback-like genes in mammals parallels that of *Drosophila* Hunchback in neuroblasts. However, it is known that, in mammals, the Ikaros family of Hunchback-related transcription factors are expressed predominantly in embryonic CNS.<sup>(24)</sup>

The ability to reset a neural progenitor's 'intrinsic clock' to an early state may have important implications for therapeutic uses of human neural stem cells. As neural progenitors age, it appears that they become progressively restricted in the cell types that they can produce. Multipotency could be restored by resetting the 'clock' of these cells, potentially increasing the efficacy of any neural stem-cell-based treatment by enabling the full range of neural cell types to be generated in vivo or in vitro. This may be important for a range of neurological illnesses, including Huntington disease (HD), amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD), in which specific neural cell types degenerate.<sup>(25)</sup>

It is not clear what the mechanism is by which Hunchback confers early temporal identity. As Hb is a transcription factor, the obvious mechanism is the direct activation or repression of target genes. However, data from both *Drosophila* and mammals show that Hb can also lead to site-specific chromatin modification. Hb and its mammalian homologue, Ikaros, both bind to Mi-2-Polycomb complex proteins, leading to histone deacetylase-mediated gene silencing and chromatin remodelling.<sup>(26–28)</sup> Doe and Pearson suggest a model whereby Hb recruits Mi-2-Polycomb, leading to the repression of genes conferring later-born temporal identities. If this is the case, then Polycomb-mediated chromatin remodelling may provide a mechanism by which the long-term Hunchback expression seen in GMCs and neurons is maintained.

### Conclusions

Pearson and Doe's work has provided exciting insights into the mechanisms that control the generation of neural diversity in

*Drosophila*. Elucidating the mechanisms that regulate the temporal identity of neural progenitors will not only provide a mechanistic understanding of neurogenesis but may also have important medical implications. The ability to manipulate or reset a neural progenitor's 'intrinsic clock' would clearly be beneficial in therapeutic uses of neural progenitor cells. Ease of genetic manipulation and close similarities between the vertebrate and invertebrate nervous systems make *Drosophila* the ideal organism to further study this process.

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