Stem Cell Transcriptional Loops Generate Precise Temporal Identity

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Neuronal diversity is generated from small pools of progenitors whose fate potential changes over time. Recently in *Cell*, Baumgardt et al. (2009) showed that multiple, simultaneously activated transcriptional cascades regulate the timing and specification of distinct neurons from the lineage of a single embryonic *Drosophila* neural stem cell.

Before stem cell researchers can hope to generate specific neurons to replace those that are lost or damaged, it is essential to understand how each neuronal subtype is normally generated. In many organisms, from *Drosophila* to humans, neural stem cells are topographically organized in response to the expression of distinct combinations of Hox genes, which also determine their capacity to give rise to specific cell types (Rubenstein et al., 1998). In addition, many, if not all, neural stem cells change their potential to generate specific progeny over time, greatly expanding the diversity of neuronal cell types produced during development (Guillemot, 2007). Despite its importance, the mechanism underlying temporal specification of neurons is still largely unknown. In a recent issue of *Cell*, a study by Baumgardt et al. (2009) reveals a complex interplay between three transcriptional cascades simultaneously activated by the transcription factor, Castor, that control the precise timing with which a single neural stem cell specifies distinct neuronal subtypes over time.

The molecular mechanism regulating temporal specification of neurons is best understood in *Drosophila*, where neuroblasts (neural stem cells) sequentially express the transcription factors Hunchback → Krüppel → Pdm → Castor (Cas) → Grainyhead (Grh) as they undergo a series of asymmetric cell divisions. The neural progeny generated from each division acquires an identity specified by the temporal identity factor expressed by the parental neuroblast. As a result, each neuroblast can give rise to a lineage of distinct progeny over multiple cell divisions (Isshiki et al., 2001; Maurange et al., 2008). Little is known, however, regarding what regulatory factors operate downstream of the temporal identity factors to confer a particular cell fate. Furthermore, some temporal identity factors, like Cas, are expressed over multiple neuroblast divisions, during which distinct progeny are generated. How such broad temporal windows are further subdivided so that distinct cell types are specified is not well understood.

The work by Baumgardt et al. (2009) addresses these questions by focusing on the four Apterous (Ap) neurons generated at the end of a 20-neuron lineage of a single neuroblast, NB5-6. These neurons express the LIM homeodomain transcription factors Ap and the cofactor Eyes Absent (Eya). They are further separated into three distinct subtypes: Ap1 and Ap4, which express the neuropeptides Nplp1 and FMRFamide (FMRFa), respectively, and two generic Ap neurons, termed Ap2 and Ap3. The three subtypes of Ap/Eya neurons are born sequentially (Ap1, Ap2/Ap3, then Ap4) at the end of the temporal window during which the neuroblast expresses Cas. Cas is required to generate all Ap neurons, but how are the three subtypes sequentially specified? The authors take advantage of their ability to follow the fate of the progeny of NB5-6 over time using neuronal subtype specific markers to address this question.

In a series of elegant genetic experiments, Baumgardt et al. find multiple, simultaneously activated transcriptional cascades—all set in motion by Cas—that subdivide the Cas temporal identity window to generate three distinct Ap neuron subtypes. Cas initially upregulates the Collier (Col) transcription factor so that all four newly born neurons adopt a generic Ap neuron identity by expressing Ap and Eya. Ap/Eya and Col cooperate in the postmitotic neuron to activate the neuropeptide Nplp1^{+}, thereby specifying the Ap1 subtype identity through a "feed-forward" loop (Figure 1, left pathway). Cas simultaneously initiates a different feed-forward loop that upregulates Squeeze (Sqz) and Nab, which together downregulates Col after Ap/Ey activation. The delay in Nab activation allows Col expression to be maintained only in Ap1, and consequently, Ap2, Ap3, and Ap4 adopt other fates (Figure 1, middle pathway). In yet a third transcriptional cascade, Cas expression gradually upregulates the transcription factor Grainyhead (Grh), culminating in the expression of the neuropeptide FMRFa in the last-born Ap neuron, thereby specifying the Ap4 identity (Figure 1, right pathway). In addition, Grh represses *cas*, limiting the production of Ap2/3 neurons. Thus, three transcriptional cascades lead to the staggered production of Ap1, Ap2/3, and Ap4 neurons.

Like most good papers, this one raises more questions than it answers. Why does Cas activate the *col*, *sqz*, and *grh* only in the latter half of the Cas expression window? Does Cas directly activate *col*, *sqz*, and *grh* transcription, or does it act indirectly by terminating Pdm expression

(Tran and Doe, 2008)? Testing for Cas binding to each target gene or assaying *pdm cas* double mutants for *col*, *sqz*, and *grh* expression would help address these issues. Importantly, it is unclear which of the Cas-initiated transcriptional cascades occurs in the neuroblast and which in the postmitotic progeny. Data suggest at least some of these cascades can function in the postmitotic neurons, as targeted misexpression of Grh in neurons can respecify all Ap neurons to the FMRFa$^+$/Ap4 identity. However, how is the Cas → Sqz → Nab feed-forward loop kept from propagating into the Ap1 neuron and inappropriately turning off Col? Neuroblast- and neuron-specific manipulation of Cas, Sqz, and Nab expression would help determine which cells are competent to respond to these transcriptional cascades.

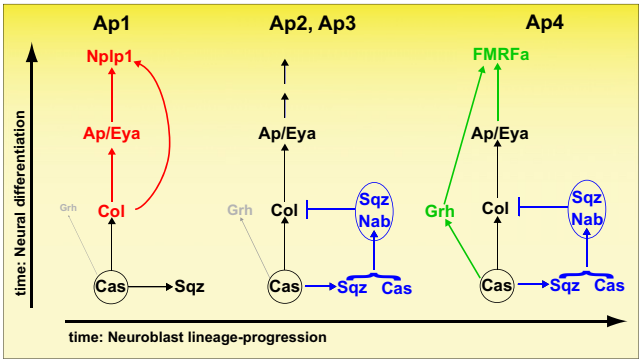

Figure 1. Three Cas-Induced Transcriptional Cascades Generate Neuronal Subtype Identity within a Single Progenitor Lineage

Left: a Cas → Col → Ap-Eya cascade (red) results in transcriptional activation of Nplp1 to specify the Ap1 neuronal identity. Middle: a Cas → Sqz feed-forward loop activates Nab (blue); the delay in Nab expression permits Ap1 to be specified, but then Sqz/Nab repress Col, thus allowing Ap2/3/4 neuronal identities to be specified. Right: prolonged Cas expression transcriptionally activates sufficient Grh to promote expression of the neuropeptide FMRFa (green), thereby specifying the Ap4 identity.

It is becoming clearer that "temporal specification" involves an intricate network of transcriptional regulation that occurs at multiple levels of neurogenesis. Do these same mechanisms operate in other *Drosophila* neuroblast lineages? What about in neural stem cells of other organisms? There is mounting evidence that temporal specification of neurons is a widespread phenomenon in many regions of the developing mammalian central nervous system such as the spinal cord, cortex, and retina (reviewed in Guillemot, 2007; Pearson and Doe, 2004). Interestingly, recent studies suggest conservation of function in orthologs of temporal identity factors in mouse (Elliott et al., 2008), underscoring the importance of these factors in brain development. In addition, investigating the crosstalk between spatial and temporal cues will help explain how distinct populations of neural stem cells can utilize common temporal cues to generate unique progeny. Finally, it would be fascinating to find how temporal regulatory mechanisms discussed above are used in other, non-neuronal systems. For example, in *C. elegans*, Hunchback-like is a key player in the timing of sequential larval developmental stages (Abrahante et al., 2003). Perhaps we will continue to find multiple examples of temporal factors regulating key aspects of development and aging. These findings will inevitably be crucial in allowing us to harness stem cell potential for tissue therapies.

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