Chinmo and Neuroblast Temporal Identity

Chris Q. Doe[1,*]
[1]Institutes of Neuroscience and Molecular Biology, Howard Hughes Medical Institute,University of Oregon, Eugene, OR 97403, USA
*Contact: cdoe@uoneuro.uoregon.edu
DOI 10.1016/j.cell.2006.10.008

Although spatial patterning during embryonic development is well characterized, a corresponding framework for temporal patterning has not been established. In this issue, Zhu et al. (2006) identify the Chinmo protein as conferring temporal identity on the neural progeny of *Drosophila* neuroblasts, revealing appealing parallels with spatial patterning.

The neuroblasts of developing fly larvae divide asymmetrically to bud off a series of smaller ganglion mother cells, which then generate two postmitotic neurons. In the abdomen of developing fly larvae, neuroblasts produce few progeny, and temporal identity is regulated in part by the sequential expression of the transcription factors Hunchback, Krüppel, Pdm, and Castor (Grosskortenhaus et al., 2006; Isshiki et al., 2001; Novotny et al., 2002). In contrast, neuroblasts of the larval brain typically generate a far greater number of progeny (>100 neurons), all of which have a similar morphology. For example, neuroblasts of the fly brain mushroom body sequentially produce the γ, α′β′, pioneer αβ (pαβ), and αβ neurons (Lee et al., 1999) (Figure 1, top). Thus, there must be a mechanism to specify temporal identity in this neuroblast lineage. The work of Zhu et al. (2006) reported in this issue provides a satisfying solution with the identification of the *chinmo* gene that specifies temporal identity in both the mushroom body and anterodorsal neuroblast lineages of developing fly larvae.

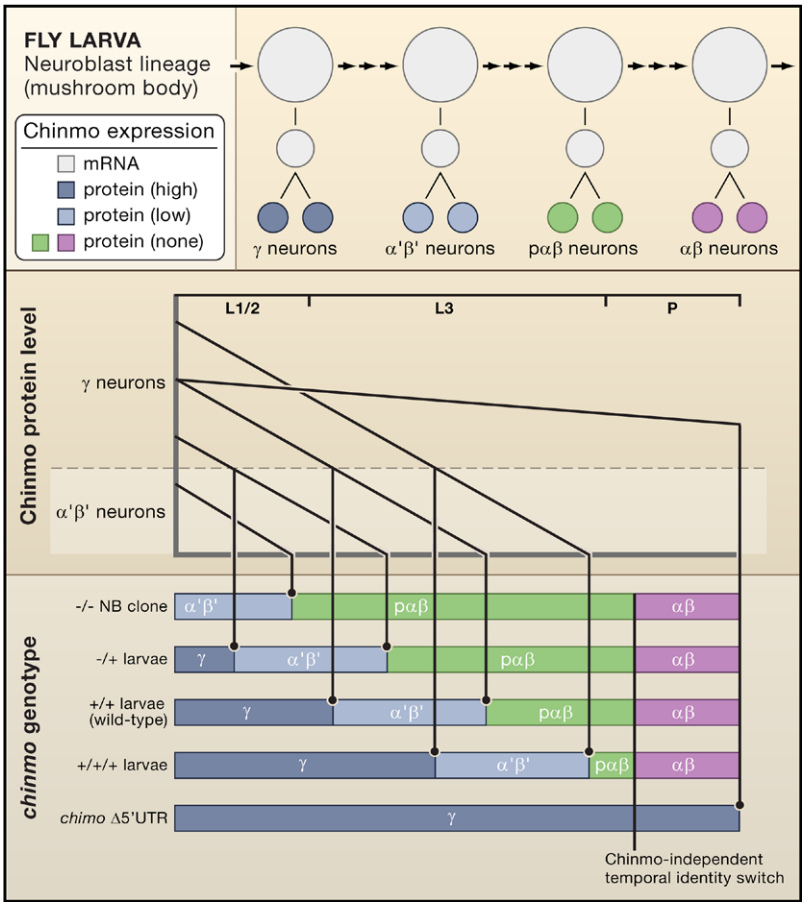

Figure 1. The Chinmo Protein Specifies Temporal Identity of Fly Neuroblast Progeny
(Top) Shown is expression of *chinmo* mRNA (gray) and protein (blue) in the neuroblast lineage of the mushroom body in the larval fly brain.
(Bottom) Chinmo specifies temporal identity of neural progeny in a dose-dependent manner. Five different genotypes are represented. The diagonal lines depict the gradient of Chinmo protein expression over time in each genotype, with the vertical lines being connected to the corresponding neural identities: γ, α′β′, pαβ, and αβ neurons.

Zhu et al. (2006) screened for mutations that alter temporal identity within the neuroblast lineage of the fly brain mushroom body using a technique called MARCM (Mosaic Analysis with a Repressible Cell Marker). This technique generates positively-marked mutant clones in flies with an otherwise wild-type genetic background (Lee and Luo, 1999). When wild-type neuroblast clones from the mushroom body are induced to divide at early stages of larval development, they contain all four known neuronal subtypes: γ, α′β′, pαβ, and αβ. Each neuronal subtype has a unique axon or dendrite morphology, and several subtypes can be distinguished with molecular markers. The authors identified a single mutant in their screen that had fewer early-born γ and α′β′ neurons and more

late-born pαβ and αβ neurons (in the absence of a dramatic alteration in clone size, consistent with a transformation of early-born to late-born neurons). Three additional experiments supported their conclusion: (1) single neuron mutant clones generated neuronal fates that matched those normally produced 1 day later, (2) heterozygous larvae generated neurons at each time point that matched those normally made 0.25 days later, and (3) early- to late-born projection neuron transformations were observed in the anterodorsal neuroblast lineage. Important controls included precise larval staging to eliminate differences in the rate of development and the use of both molecular markers and axon projections to score neuronal identity (therefore excluding the possibility that the mutation merely affects axon outgrowth). Thus, four different loss-of-function assays revealed a mutant phenotype in which there is an early-born to late-born change in temporal identity (Figure 1, bottom). This led Zhu et al. (2006) to name their mutant gene *chinmo* (*ch*ronologically *in*appropriate *mo*rphogenesis).

The *chinmo* mutation was mapped to the previously uncharacterized *CG31666* gene encoding a putative transcriptional repressor containing an amino-terminal BTB domain (*b*road complex, *t*ramtrack, *b*ric a brac) and a carboxyl-terminal pair of C_2H_2 zinc fingers. Antibody staining revealed that the Chinmo protein has a nuclear location, with highest expression in early-born γ neurons, lower expression in the next-born α′β′ neurons, undetectable expression in the latest-born pαβ and αβ neurons, and no expression in neuroblasts (Figure 1, top). This corresponds well with the mutant phenotype, in which the early-born γ and α′β′ neurons are transformed to the later-born pαβ and αβ neurons.

How is the "temporal gradient" of the Chinmo protein generated? Interestingly, *chinmo* mRNA is ubiquitously expressed throughout the entire neuroblast lineage, indicating that posttranscriptional regulation is required to form the observed temporal protein gradient. Zhu et al. (2006) generated *chinmo* transgenes lacking either the 5′ untranslated region (UTR) (*UAS-chinmoΔ5′UTR*) or the 3′ UTR (*UAS-chinmoΔ3′UTR*). They then expressed the transgenes in the neuroblast lineage of the mushroom body in the developing fly. The *UAS-chinmoΔ3′UTR* transgene could partially rescue the *chinmo* mutant phenotype and showed a relatively normal gradient of Chinmo protein. In contrast, the *UAS-chinmoΔ5′UTR* transgene resulted in uniformly high levels of Chinmo protein that did not decline in the late-born neurons. Thus, translational repression via the *chinmo* 5′ UTR is essential for establishing the temporal gradient of the Chinmo protein.

The fact that the *UAS-chinmoΔ5′UTR* transgene was not translationally regulated allowed Zhu et al. (2006) to determine the effect of misexpressing high levels of Chinmo protein. When the construct was expressed throughout the neuroblast lineage of the mushroom body, there was a clear transformation of late-born pαβ and αβ neurons to an early-born γ neuron fate (Figure 1, bottom). Interestingly, when the construct was expressed only in mature neurons, there was little or no effect. This is similar to the finding that embryonic fly neurons lose their competence to respond to the temporal identity factors Hunchback, Krüppel, and Pdm (Cleary and Doe, 2006; Pearson and Doe, 2003). Given that Chinmo is not detected in neuroblasts and that mature neurons are not competent to respond to Chinmo, this protein seems to act in ganglion mother cells or young neurons to specify temporal identity. Both loss-of-function and misexpression studies show that Chinmo is necessary and sufficient for the temporal identity of early-born neurons of the neuroblast lineage in the fly larval mushroom body (Figure 1, bottom).

The discovery and characterization of *chinmo* is a major step forward in understanding the specification of temporal identity in *Drosophila*. It is the first gene known to specify temporal identity in multiple lineages of fly larval neuroblasts. Chinmo appears to act as a "temporal morphogen" that specifies at least three cell fates in the neuroblast lineage of the mushroom body (high protein = γ neurons; low protein = α′β′ neurons; no protein = pαβ neurons). This has appealing parallels with spatial patterning, where morphogen gradients often provide a gross level of patterning that is refined by transcription factor combinatorial codes. It remains to be determined whether there are finer gradations of neuronal diversity within each major subtype of Chinmo-dependent cell fates.

The work of Zhu et al. (2006) raises many interesting questions. First, is Chinmo part of an evolutionarily-conserved mechanism for specifying temporal identity? Fly larval neuroblasts appear similar to mammalian neural progenitors in their ability to produce many closely related neurons. Thus, Chinmo and its mechanism of action may be more relevant to mammalian neurogenesis than the rapid cycles of expression of Hunchback/Krüppel/Pdm/Castor in embryonic neuroblasts. Second, what is the translational control mechanism? It is tempting to speculate that miRNAs regulate *chinmo* translation, thereby connecting the new gene for temporal identity in flies (*chinmo*) with the first mechanism known to regulate temporal identity in the nematode (*the* heterochronic miRNAs). However, there is currently little evidence that miRNAs can act through 5′ UTR sequences. Third, what factors act downstream of Chinmo? Based on its nuclear localization and BTB/zinc finger domains, Chinmo is likely to be a transcriptional regulator (Albagli et al., 1995). Identification of its target genes will be a necessary first step in determining how Chinmo acts in a concentration-dependent manner to specify different temporal identities. Finally, Chinmo does not regulate all transitions in temporal identity in the mushroom body neuroblast lineage. For example, loss or reduction of Chinmo does not change the timing of the pαβ/αβ transition (Figure

1, bottom); the *chinmo*-independent mechanism regulating this transition is unknown.

The work of Zhu et al. (2006) provides important new insights into the specification of temporal identity in the *Drosophila* neuroblast lineage. But there are still many questions that need to be answered before we fully understand the mechanisms of temporal identity in fly development.

REFERENCES

Albagli, O., Dhordain, P., Deweindt, C., Lecocq, G., and Leprince, D. (1995). Cell Growth Differ. *6*, 1193–1198.

Cleary,M.D., and Doe, C.Q. (2006). Genes Dev. *20*, 429–434.

Grosskortenhaus, R., Robinson, K.J., and Doe, C.Q. (2006). Genes Dev. *20*, 2618–2627.

Isshiki, T., Pearson, B., Holbrook, S., and Doe, C.Q. (2001). Cell *106*, 511–521.

Lee, T., and Luo, L. (1999). Neuron *22*, 451–461.

Lee, T., Lee, A., and Luo, L. (1999). Development *126*, 4065–4076.

Novotny, T., Eiselt, R., and Urban, J. (2002). Development *129*, 1027–1036.

Pearson, B.J., and Doe, C.Q. (2003). Nature *425*, 624–628.

Zhu, S., Lin, S., Kao, C.-F., Awasaki, T., Chiang, A.-S., and Lee, T. (2006). Cell, this issue.

Do Sigma Factors Need Help with a Meltdown?

Ruth M. Saecker,[1,*] Caroline A. Davis,[2] and M.T. Record, Jr.[1,2,*]
[1]Department of Chemistry
[2]Department of Biochemistry
University of Wisconsin-Madison, Madison, WI 53706 USA
*Contact: rmsaecker@wisc.edu, record@biochem.wisc.edu

DOI 10.1016/j.cell.2006.10.009

In this issue of *Cell*, Hsu et al. (2006) report on the binding activity of a variant of the bacterial transcriptional specificity factor sigma (σ) to promoter DNA. This study demonstrates that the σ variant induces a large distortion in the transcriptional start site in the absence of core RNA polymerase, raising intriguing new questions about the roles of σ and core RNA polymerase in transcription initiation.

Transcription begins when RNA polymerases (RNAP) recognize specific promoter sequences in double-stranded (ds) DNA. In bacteria, binding of the RNAP holoenzyme (holoE)—which consists of the core subunits $\alpha_2\beta\beta'\omega$ and a σ factor—to promoter DNA triggers a series of conformational changes that destabilize the DNA duplex. As a result, the template and nontemplate strands unwind and separate from the –10 region to just beyond the start site at +1 (Helmann and deHaseth, 1999; Kontur et al., 2006). Although core RNAP has a high affinity for single-stranded (ss) DNA and progressively separates the DNA strands in front of the active site during elongation, this subunit assembly cannot open DNA and initiate transcription from intact duplex DNA. The ability to "melt" DNA during transcription initiation is conferred by "housekeeping" σ specificity subunits (σ^{70} in *Escherichia coli*, σ^A in *Bacillus subtilis*), which by themselves cannot bind to either ds or ssDNA due to the presence of a negatively charged autoinhibitory domain (region 1.1; Helmann and deHaseth, 1999). The interaction between core RNAP and σ allows promoter recognition, opening of the start site, and subsequent initiation of transcription. In this issue of *Cell*, Hsu et al. (2006) add a twist to our current understanding of transcription initiation by demonstrating that a truncated σ^A variant can recognize and alter the conformation of the start site region of promoter DNA in the absence of core RNAP.

Hsu et al. (2006) report that when region 1.1 of *B. subtilis* σ^A is deleted, this σ variant (termed SND100-σ^A) binds both specifically and nonspecifically to a DNA fragment containing a promoter sequence. To detect DNA opening in these complexes, the authors exploit the use of potassium permanganate ($KMnO_4$), which preferentially oxidizes exposed, unstacked thymines. The specific binding between SND100-σ^A and promoter DNA gives rise to marked $KMnO_4$ reactivity even on linear DNA fragments. Unlike a similarly truncated *E. coli* σ^{70} variant (Young et al., 2004), the SND100-σ^A bacterial mutant does not require association with core RNAP or negatively supercoiled, underwound DNA to achieve this effect (Hsu et al., 2006).