Otx2's Incredible Journey

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A surprising new mechanism that regulates the plasticity of postnatal neurons is reported in this issue by Sugiyama et al. (2008). These authors show in mice that visual experience triggers cell-to-cell transfer of the homeoprotein Otx2 to cortical interneurons, where it promotes maturation of inhibitory neural circuitry and opens the critical period for plasticity in the visual cortex.

Since the Nobel Prize-winning studies of David Hubel and Torsten Wiesel, the visual cortex has been held up as the premier model of brain plasticity. Inputs from each eye, wired through the thalamus, drive sets of neurons in the visual cortex organized into so-called "ocular dominance" columns. If one eye is deprived of visual inputs during a critical period of postnatal development, that eye's inputs to the visual cortex are weakened, and inputs from the nondeprived eye are strengthened and take over areas innervated by the deprived eye (Wiesel and Hubel, 1963). Such changes occur in humans in amblyopia or "lazy eye," a loss of visual acuity due to unilateral visual deprivation during childhood. In this issue, Sugiyama et al. (2008) provide evidence for a surprising new mechanism for ocular dominance plasticity involving the maturation of inhibitory cortical circuitry by the cell-to-cell transfer of the homeoprotein Otx2.

The Hensch lab, one of the two major contributors to the present paper, has focused on the role of inhibitory intracortical circuits in ocular dominance plasticity. In previous work, they showed that cortical neurons in mice deficient in GAD65, one of the enzymes that synthesizes the inhibitory neurotransmitter GABA, display weak release of synaptic GABA. In these mice, ocular dominance plasticity fails to occur after monocular deprivation (Hensch et al., 1998). Interestingly, normal plasticity can be restored in *GAD65* knockout mice at any age through the use of benzodiazepines, which enhance GABA signaling; benzodiazepines can also trigger an earlier onset of plasticity in wild-type mice (Fagiolini and Hensch, 2000). The inhibitory circuit includes the large-basket cells that express the calcium-binding protein parvalbumin, also called PV cells (Hensch, 2005). Large-basket cells notably send axons across multiple ocular dominance columns and embrace pyramidal cell bodies with their GABA-containing synaptic terminals (Huang et al., 2007). The maturation of the GABAergic synapse and the presence of $GABA_A$-α1 receptors on pyramidal cells are crucial for defining the period of ocular dominance plasticity in postnatal development (Hensch, 2005).

The new work by Sugiyama and colleagues reveals the importance of the homeodomain transcription factor Otx2 in the development of this inhibitory circuit in mice. They show that the Otx2 homeoprotein is found in PV cells in the mouse visual cortex, concomitant with the time course of parvalbumin expression. Otx2 is weakly expressed before the critical period and strongly expressed in PV cells during the critical period and into adulthood. Otx2 expression in the mouse visual cortex is also dependent on activity, because dark-rearing or surgical removal of the eye reduces both parvalbumin and Otx2 signals, impli-

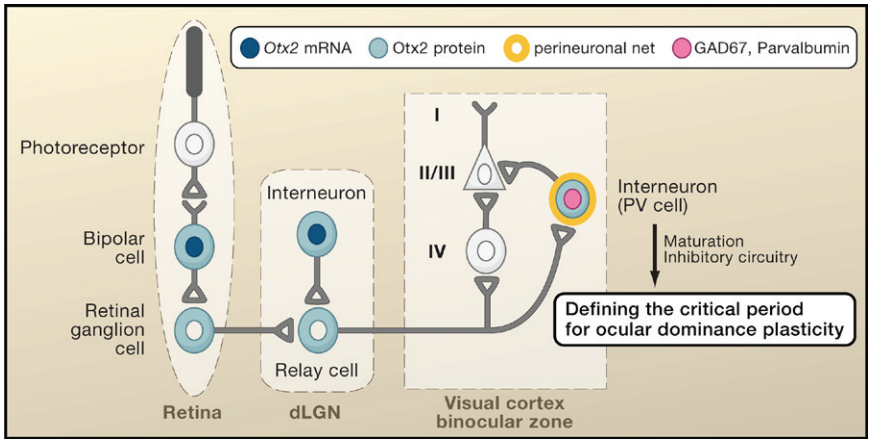

Figure 1. Otx2 Expression in the Visual Pathway
Otx2 mRNA and protein expression is crucial for inducing plasticity in the visual pathway during postnatal development. In the mouse retina, Otx2 protein is present in bipolar cells and retinal ganglion cells, whereas *Otx2* mRNA is present only in bipolar cells. Similarly, in the dorsal lateral geniculate nucleus (dLGN) of the thalamus, GABAergic interneurons express *Otx2* mRNA, whereas Otx2 protein is present in some relay cells and interneurons. In the visual cortex, *Otx2* mRNA is not found in any cell type. However, Otx2 protein accumulates in interneurons, coinciding with the expression of parvalbumin and GAD67 during the critical period for ocular dominance plasticity. Otx2 protein induces the maturation of PV cells, resulting in increased parvalbumin expression. A possible source of cortical Otx2 protein is the retina, through connections with dLGN. Otx2 may be relayed by thalamocortical axons that form synapses on inhibitory interneurons during development. The specificity of Otx2 internalization may be linked to the perineuronal nets surrounding PV cells in the visual cortex.

cating a link between Otx2 and PV-cell maturation. Supporting these findings, infusion of Otx2 into animals reared in the dark (which normally have delayed ocular dominance plasticity) rescues the maturation of the PV cells. Along the same lines, the loss of Otx2 signal in the visual cortex in *Otx2* conditional knockout mice blocks PV-cell maturation.

The first intriguing aspect of this story is that Otx2, like GABA manipulation, modifies ocular dominance plasticity. When Otx2 is infused into the mouse visual cortex, it can prematurely open the critical period for plasticity. Furthermore, *Otx2* conditional knockout mice, which lack cortical Otx2, do not display ocular dominance plasticity. However, plasticity can be rescued in the latter mutant mice by treatment with benzodiazepines, suggesting that Otx2 is a crucial signal for the development of the inhibitory circuitry.

Perhaps the most remarkable twist is that the effect of Otx2 on PV-cell maturation and ocular dominance plasticity is non-cell autonomous, that is, *Otx2* mRNA is not synthesized in the visual cortex but rather by cells outside of the visual cortex. In addition, the conditional knockout mouse used by the authors does not target the *Otx2* gene in the inhibitory interneurons, yet the Otx2 signal in the cortex still disappears. So where does Otx2 come from? The authors argue that Otx2 in the visual cortex is transported from the retina to the dorsal lateral geniculate nucleus (dLGN), and then from there to the visual cortex. If this is the case, then Otx2 would have to "jump" at least two synapses (and likely more), because the protein is present in projecting neurons, but *Otx2* mRNA is found only in interneurons in the retina and dLGN (Figure 1).

Homeoproteins are transcriptional regulators of body and tissue patterning (Brunet et al., 2007). The Prochiantz lab, the other major contributor to this study, has previously shown that proteins containing a homeodomain motif can be released by one cell and taken up by another, giving rise to the notion of homeoprotein transfer (Brunet et al., 2007). This finding was initially met with some resistance but has prevailed with the development of a now widely used artificial "carrier" peptide (called penetratin) that is based on the homeodomain motif of the homeoprotein Antennapedia (Joliot and Prochiantz, 2004). Prochiantz's group has also teamed up with Christine Holt and colleagues to demonstrate another unorthodox action of released homeoproteins: exogenous application of the homeoprotein Engrailed-2 can differentially guide temporal versus nasal retinal growth cones (Brunet et al., 2005). This guidance effect requires the internalization of Engrailed-2 in growth cones and subsequent local protein synthesis (Brunet et al., 2005).

The real proof for Otx2 transfer from the retina to the cortex comes from an experiment in which biotinylated Otx2 is injected into the mouse eye. Subsequently, the biotinylated Otx2 is found in the visual cortex specifically in PV cells. This result is surprising, considering the many synapses biotinylated Otx2 must cross and the precision of this transfer. Which part of the long voyage of Otx2 from retina to cortex is experience dependent? It is most likely to be the transport of Otx2 somewhere between the retina and the cortex, because Otx2 expression in the retina is perfectly normal in dark-reared mice even though Otx2 signal is absent in the visual cortex.

Still problematic is whether Otx2 transport from the retina to the cortex is crucial for ocular dominance plasticity. Using an Otx2-blocking antibody in the cortex or intraocular injection of a short interfering RNA against Otx2, the authors prevented plasticity, but these results do not necessarily show that the transfer of Otx2 from the retina is itself required. It will be a challenge to prove this theory, especially because the sequences important for Otx2 transfer are in the homeodomain, and thus any mutation to block the transport would also affect the function of the homeoprotein (Brunet et al., 2007).

The answer to the issue of specific uptake and transfer of Otx2 may lie in the web of extracellular matrix molecules surrounding the PV cells. These "perineuronal nets" have been in the limelight in studies of brain plasticity (Hensch, 2005; Pizzorusso et al., 2002). The nets harbor extracellular matrix components, such as hyaluronic acid and chondroitin sulfate proteoglycans. Remarkably, if these nets are dissolved with chondroitinase, the critical period of plasticity can be reopened in adults (Pizzorusso et al., 2002). One possible clue to the specificity of Otx2 uptake by PV cells could be specific sugar codes on the extracellular matrix of the perineuronal nets that trap Otx2 and may aid in its internalization.

As with most breakthrough findings, more questions than answers surround Otx2's fantastic journey. How do release and uptake of homeodomain proteins proceed at each step from eye to cortex? How does Otx2 induce the maturation of PV cells? What triggers the series of transfers of Otx2 upon visual experience, and what are the targets of Otx2? Like other homeoproteins, Otx2 could act as a translational regulator by interaction with the translation initiation factor eIF4E (Brunet et al., 2007), adding even more complexity to the noncanonical cell-to-cell transfer of homeoproteins. Nevertheless, the Sugiyama et al. study opens up an entirely new field of research. What are the non-cell-autonomous roles of homeoproteins? And are these roles a widespread yet unseen phenomenon conserved among species?

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