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The Metamorphosis Antidote

What if you woke up one morning and your eyes were on the top of your head? You would be confused to see the sky in front of you and would worry that if you took a step to the rear you would smash into the ground. This could never happen unless you lived in a Kafkaesque story and had been transformed into a giant clawed frog. *Xenopus* really do experience such a change in the position of their eyes during their *Metamorphosis*, but they have an antidote to this disorienting morphogenetic movement.

In *Xenopus*, the laterally positioned eyes of the filter-feeding tadpole move dorsally and frontally during metamorphosis, which results in the predatory frog's binocular overlapping visual field (Grobstein and Comer, 1977; Grant and Keating, 1986). Unlike the retina in mammals or birds, the frog retina continues to grow throughout life by adding newly born cells to the ciliary marginal zone (CMZ) at the periphery (Glücksmann, 1940; Perron et al., 1998). It has long been known that retinal growth in the ventral CMZ is much greater than in the dorsal CMZ during and after metamorphosis (Hollyfield, 1971; Straznicky and Tay, 1977; Beach and Jacobson, 1979). This asymmetrical growth pattern displaces the "old" retina dorsally and compensates for the dorsal movement of the eyes (see figure). Thus, a particular locus in the "old" retina continues to represent a similar location in the space in relation to the body, reducing the mismatch between the "interocular" and "visuosomatic" map (Grant and Keating, 1986). Thyroid hormone is a key molecule during metamorphosis and is involved in a variety of processes, including the generation of an ipsilateral retinal projection that serves binocular vision (Hoskins, 1986). Thyroxine can cause the asymmetrical growth pattern precociously in premetamorphic tadpoles, and an inhibitor of thyroid hormone synthesis can block the differential growth pattern, suggesting that thyroid hormone is sufficient and necessary for the changes in retinal growth during metamorphosis (Hoskins, 1986). However, the molecular mechanism by which the hormone elicits differential growth in the dorsal and ventral CMZ has been an open question, since the receptors for thyroid hormone are expressed ubiquitously in the nervous system and equally in the dorsal and ventral CMZ (Kawahara et al., 1991). In this issue of *Neuron*, Marsh-Armstrong et al. (1999) give a final answer to this long unsolved problem by analyzing the function of D3, an enzyme that inactivates thyroid hormone, in the metamorphosing retina.

Earlier work from the same lab (Wang and Brown, 1993) characterized a number of genes that are induced in response to thyroid hormone. In the present paper, by screening hormone-responsive genes in the metamorphosing retina, Marsh-Armstrong et al. found that D3 was expressed highly in the dorsal CMZ but not in the ventral CMZ. D3 is a member of a class of enzymes called deiodinases, which inactivate thyroid hormone by catalyzing the removal of iodine from the inner ring. When the function of D3 was inactivated by iopanoic acid (IOPA), a specific inhibitor of the enzyme, the asymmetric growth pattern in response to thyroid hormone was lost, and both dorsal and ventral CMZ grew equally. In complementary experiments, they introduced a D3 transgene under the control of a ubiquitous or neuron-specific promotor, which resulted in the inhibition of the growth burst in the ventral retina during metamorphosis. These results clearly show that the expression of D3 in the dorsal CMZ inactivates thyroid hormone locally, which prevents the cells from responding to the high level of circulating thyroid hormone during the metamorphic climax.

The steroid hormones are known to regulate various aspects of nervous system development, and there are several cellular mechanisms to regulate the region-specific responses. The first mechanism, commonly found in other endocrine systems, is the expression of the receptor only in target tissues. The second is the expression of different receptor subtypes in each target organ. A striking example of this strategy is found in insect metamorphosis. In *Drosophila*, it has been established that the differential responses to ecdysone, a steroid hormone that induces metamorphosis in insects, are regulated by the discrete expression of specific receptor subtypes (Talbot et al., 1993). The tissues that follow the same fate (proliferation, differentiation, cell death) during metamorphosis have the same expression patterns of ecdysone receptor A and ecdysone receptor B. The third mechanism, as shown by Marsh-Armstrong et al. in this issue, is the inactivation of the ligand in a region-specific manner. It would be intriguing to know if similar regulatory mechanisms are involved in other regions of the brain or in other animals.

In addition to the effect on the retinal growth, Marsh-Armstrong et al. showed a reduction in the number of

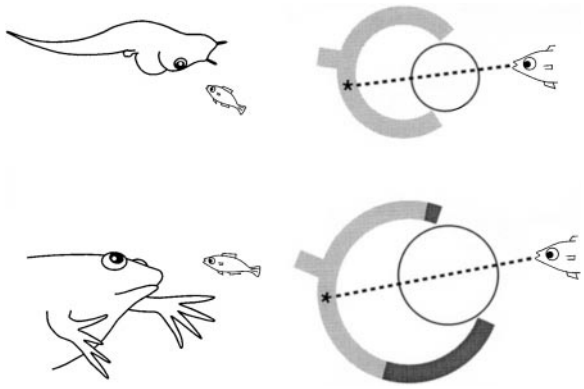

Image Constancy in a Metamorphosing Frog

During metamorphosis, the tadpole retina moves dorsally to the top of the frog's head. The ventral growth of the retina (dark shadow) exceeds dorsal growth and compensates the movement of the eyes. Consequently, the same region in the retina (asterisk) can receive visual information from an object (fish) that is located "lateral" to the body.

ipsilaterally projecting retinal ganglion cells in the D3 transgenic frog. Since D3 reduces the proliferation in the CMZ, it is not clear if D3 overexpression directly affects the fate of the ipsilaterally projecting cells or just decreases the number of ventral ganglion cells generated during metamorphosis. Whichever the case, further study of frog metamorphosis will possibly open a door to another long-unanswered question, the differential decussation of retinal axons at the chiasm.

Shinichi Nakagawa and William A. Harris
University of Cambridge
Department of Anatomy
Downing Street
Cambridge CB2 3DY
United Kingdom

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Transcriptional Control of Cortical Connectivity

The development of connections in many regions of the mammalian brain is characterized by the formation of widespread axon projections, followed by elimination of inappropriate axon terminals. For example, layer 5 neurons in the visual cortex initially extend axons into the pons, superior and inferior colliculus, and spinal cord. Later in development, the axonal branches that project to the inferior colliculus and spinal cord are eliminated, leaving behind the mature projection pattern (Stanfield et al., 1982; Stanfield and O'Leary, 1985). While such large-scale remodeling of axon terminal fields is important for the establishment of functional connections, the molecular mechanisms that regulate this process are not well understood. In this issue of *Neuron*, Weimann and colleagues (1999) provide evidence that the transcription factor Otx1 is required for the elimination of inappropriate subcortical axon projections.

Otx1 is a mammalian homolog of the *Drosophila* gene *orthodenticle*, a homeodomain transcription factor (Simeone et al., 1992). Otx1 is expressed in progenitor cells in the cortical ventricular zone and in deep layers of the cortex (Frantz et al., 1994). To determine the function of Otx1 in cortical development, Weimann et al. first examined the pattern of Otx1 protein localization in the developing cerebral wall. They found that Otx1 was excluded from the nucleus in ventricular zone cells, but was predominantly nuclear in a subset of cortical layer 5 neurons, suggesting that there is a regulated translocation of Otx1 into the nucleus during development. By combining retrograde labeling from target structures with Otx1 immunofluorescence, they also found that the Otx1 protein is present in subcortical, but not callosal, projection neurons.

To determine the functional significance of Otx1 expression in cortical neurons, Weimann et al. analyzed the consequences of a targeted disruption of Otx1 on the development of cortical efferent projections. The *Otx1* mutant mice were generated by targeting the first and second exons of the *Otx1* gene (which include most of the homeodomain) (Acampora et al., 1996). These mice express a truncated Otx1 protein. While it is difficult to know whether the truncated protein retains any Otx1 function, the protein is likely to be functionally compromised since the homeodomain is disrupted, and Otx1 immunoreactivity is lost (Weimann et al., 1999). Somewhat surprisingly, in situ hybridizations using probes to the third exon revealed that transcripts containing that exon are expressed in all layers of the cortical plate in the mutant mice. This is in contrast to normal mice, in which expression is restricted to the deep cortical layers, and suggests that the targeted region of *Otx1* might contain DNA sequences that normally restrict Otx1 expression to deep cortical layers.

Analysis of the *Otx1* mutant mice revealed striking defects in the patterning of subcortical projections. Efferent projections from the mature visual cortex are normally restricted primarily to the thalamus, superior colliculus, and pons. In *Otx1* mutant mice, these projections appear normal, but in addition there is extensive innervation of the inferior colliculus and the spinal cord. Since innervation of the inferior colliculus and spinal cord from visual cortex is normally seen early in development but later is eliminated, the phenotype of the *Otx1* mutant mice suggests that Otx1 is required for the elimination of exuberant axon projections from inappropriate targets.

It is not clear why visual cortical projections to the inferior colliculus and spinal cord are maintained in *Otx1* mutant mice. One possibility is that Otx1 function is required for the specification of regional identity in the cortex. Presumptive visual cortical neurons in *Otx1* mutant mice might retain their projections to inferior colliculus or spinal cord if their identities were switched to those of auditory or motor cortex. Regional identity, however, appears to be unaffected in the *Otx1* mutants as projections from visual cortex to the lateral geniculate nucleus develop normally in these mice (Weimann et al., 1999). It would be useful to know if other aspects of regional identity (such as innervation from specific thalamic nuclei) are also normal in *Otx1* mutants.