Target practice: Zic2 hits the bullseye!

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A major challenge in biology is to unravel how the billions of neurons of the nervous system make appropriate functional connections. This is known to be a multistep developmental process that includes guiding, targeting and refinement of axons. In this issue of *The EMBO Journal*, García-Frigola and Herrera show compelling evidence that, within the visual system, the same transcription factor (Zic2) that governs axon guidance at the midline has an independent role for the subsequent axonal refinement at targets in the brain. This latter property is controlled by the direct regulation of the neurotransmitter serotonin uptake modulator, *SERT*. This supports the commonly held notion that neural activity sculpts the crude circuit map initially laid down by molecular guidance. Overall, this study reveals new mechanistic insights into how transcriptional instructions mediate a neuron's unique response to its external environment, shaping the development of highly ordered neural circuits.

Within the visual system, much progress has been made in understanding how the circuits that underlie depth perception/binocular vision are established. These circuits are relatively simple in design yet bear all the classical hallmarks of the development of a typical circuit. Binocular vision is produced by retinal neurons (retinal ganglion cells, RGCs) that transmit visual information either to the same side (ipsilateral neurons) or cross the midline to the opposite side of the brain (commissural neurons). Commissural neurons from one eye, therefore target the same brain hemisphere as ipsilateral neurons from the other eye. Generally, this balance of commissural and ipsilateral neurons permits integration of neural information between both sides of the body and within the visual system results in binocular vision.

During eye development, ipsilateral and commissural RGCs are distinguished by their retinal cell body position and the selective expression of the transcription factor Zic2 in ipsilateral neurons. It has been shown previously that Zic2 prevents midline crossing of ipsilateral neurons by regulating the expression of the axon guidance receptor EphB1 (Herrera *et al*, 2003; Williams *et al*, 2003). RGC axons then innervate their brain targets topographically initially in a crude and exuberant manner reaching far beyond their final positions. The mature targeting pattern is sculpted by the selective refinement and segregation of axons (Figure 1). Given its importance in function, there has been much interest in understanding how axons become spatially restricted following this initial exuberance. Within the visual system, sponta-

neous neural activity, which requires no external sensory input, is a critical part of this refinement process and is thought to be controlled, at least in part, by the actions of

Figure 1 Zic2 regulates two independent components of ipsilateral RGC development. Schematic representation of the developmental progression of RGC axons at three key stages. Commissural (c) and ipsilateral (i) neurons are shown in red and green, respectively. (A) Early in development, ipsilateral RGC neurons selectively express the transcription factor Zic2. This results in the direct expression of the axon guidance molecule EphB1 in ipsilateral neurons but not in commissural neurons. EphB1 interacts with the ligand EphrinB2 at the optic midline resulting in chemotactic repulsion and avoidance of the midline by ipsilateral neurons. (B) RGC axons then head to their central brain targets, lateral geniculate nucleus (LGN) and superior colliculus (SC). Initially, these axons extend exuberantly, projecting beyond their final positions. At the same time (late gestation), spontaneous neural activity begins. Zic2 regulate the expression of SERT, which modulate spontaneous serotonergic activity in ipsilateral neurons. (C) By P15, RGC axons have been refined and elaborate arbors within their target zone.

cholinergic and serotonergic activity (Penn *et al*, 1998; Bastos *et al*, 1999; Upton *et al*, 2002; Rebsam *et al*, 2009).

García-Frigola and Herrera show here that a molecule that modulates serotonin levels, *SERT*, is a direct transcriptional target of *Zic2* and is selectively expressed in RGC ipsilateral neurons. When *Zic2* is ectopically expressed in RGC commissural neurons three main findings are observed: (i) a proportion of the neurons misproject ipsilaterally, (ii) the neurons target correctly in the brain albeit on the wrong side and (iii) RGC axons fail to refine, a phenotype that is partially rescued by inhibition of *SERT*.

These experiments are consistent with previous findings that midline crossing and targeting of ipsilateral and commissural neurons are independent events (Wolf and Chiba, 2000; García-Frigola and Herrera, 2010). Importantly, García-Frigola and Herrera show here a new role for *Zic2* in modulating axonal refinement of ipsilateral but not commissural neurons. They suggest a model in which *Zic2* regulates *SERT* expression that ultimately controls serotonin levels locally at ipsilateral neuronal terminals. As all RGC neurons express the serotonin receptor 5-HT_{1B} presumably this leads to local changes of serotonergic activity in ipsilateral neurons resulting in axonal refinement. Finally, they clearly demonstrate that unlike *Zic2*, *SERT* does not control midline crossing of RGC axons, implying that *Zic2* is regulating these features independently.

Lingering questions remain regarding how modulation of serotonin directs axonal refinements. *Zic2* and *SERT* appear to modulate the refinement of only a subset of RGC neurons, suggesting that other players may be involved. Moreover, in

an apparent dichotomy, either upregulation, inhibition or ablation of *SERT* produces a similar effect on axonal refinement (Bastos *et al*, 1999; Upton *et al*, 2002; García-Frigola and Herrera, 2010). Further studies into how relative levels of activity between individual neurons influences refinement will give a clearer understanding of how visual circuits are assembled.

Taken together, these experiments suggest that binocular vision is established in a series of steps directed in part by *Zic2*'s control of ipsilateral properties. First by the governance of midline crossing then independently by controlling axonal refinements. Transcription factors have been shown to coordinate neural circuit formation in many contexts including examples of midline crossing and axonal refinements but unlike the García-Frigola and Herrera paper, few studies have linked transcriptional regulation to both downstream effectors and biological function (Weimann *et al*, 1999; Wilson *et al*, 2008). Overall, this infers that understanding how *Zic2* encodes ipsilateral RGC traits will give key insights into how visual circuits are sculpted.

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Conflict of interest

The author declares that she has no conflict of interest.

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