D-Eph-ective endocytosis disrupts topographic mapping

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Since the discovery of the ephrin/Eph family of axon guidance molecules in the early '90s, research has often uncovered rather surprising aspects of their biology (Pasquale, 2008). A paper in this issue of *The EMBO Journal* provides further, novel insights into ephrin/Eph function. Here, the Park lab demonstrates the importance of Eph/ephrin endocytosis for retinotectal mapping, an interesting—though not unexpected—finding in itself. However, the surprise comes with their demonstration that endocytosis of the Eph/ephrin complex into neurons of the target structure, rather than into the retinal axons themselves, is of critical importance.

Endocytosis has been considered to be important for Eph family function for some time now to resolve the following paradox: the high-affinity interaction between Eph and ephrin-expressing cells is normally repellent, but both the Ephs and the ephrins are membrane-bound. This implies that the interaction between Ephs and ephrins must somehow be 'broken' to enable a separation of the cells bearing these two membrane proteins. In fact, recent *in vitro* work has shown that cell contact is terminated by induced shedding of ephrins (Hattori *et al*, 2000; Janes *et al*, 2005) or by endocytosis of the entire Eph/ephrin complex (Marston *et al*, 2003; Zimmer *et al*, 2003; for a recent review, see Pitulescu and Adams, 2010). *In vivo* experiments from the Park lab now provide intriguing new insights into the role of endocytosis in the development of the retinocollicular map in mice.

In this model system, topographic mapping of retinal axons to their correct termination zones in the superior colliculus (SC) is controlled by multiple gradients of Ephs and ephrins expressed in both the retina and the SC

(Huberman *et al*, 2008). The Park lab has investigated the targeting of nasal RGC axons to the posterior SC, which depends predominantly on the repulsion of nasal axons expressing ephrinAs from the anterior SC expressing numerous different EphAs (Rashid *et al*, 2005; Lim *et al*, 2008) (Figure 1A).

Initially the authors found that overexpression of EphA8 in the anterior SC (via bacterial artificial chromosomes) pushes

Figure 1 The key players in retinocollicular map development are members of the ephrin/Eph family. A characteristic of this family is their capacity to signal bi-directionally—that is, both Ephs and ephrins can function in a context-dependent manner as either receptors or ligands. Overall, multiple complementary EphA/ephrinA gradients control the steering of retinal axons to their correct location in the superior colliculus (SC) along the antero-posterior axis. (A) Here only the gradients of ephrinA 'receptors' in the retina (nasal>temporal) and EphA 'ligands' in the tectum (anterior>posterior) are shown, which are involved in guiding nasal retinal axons to the posterior SC based on a repulsion from the anterior SC. (B) Overexpression of EphA8 in the SC leads to a shift of nasal axons to more posterior positions. (C, D) Overexpression of endocytosis-deficient EphA8 or of dominant-negative Rac (D) leads to a shift to more anterior positions.

the termination zones of nasal axons even more posteriorly within the SC (Figure 1B). This is expected, as this manipulation renders the anterior SC more repellent for nasal axons due to the higher expression of EphAs. However, their intriguing finding was that expression of an endocytosis-deficient EphA8 in the anterior SC results in the opposite phenotype; nasal axons are now terminated not only in the posterior but also in the anterior SC (Figure 1C). This finding is surprising, since if EphAs in the SC functioned only to activate axonal ephrinAs, then the expression of either normal or endocytosis-deficient EphA8 in the anterior SC should lead to the same—rather than opposite—phenotype.

The most parsimonious explanation for these observations is that the anterior SC is now less repellent for nasal axons, allowing the formation of additional termination zones. This is supported by experiments by the Park lab showing that *in vitro* nasal RGC axons are repelled from normal EphA8-expressing cells but grow well on cells expressing endocytosis-deficient EphA8. Endocytosis-deficient EphA8 was also found to form heteromeric complexes with other EphAs, preventing their endocytosis too. This suggests that endocytosis-deficient EphAs can exert a dominant-negative effect, suppressing the effects of other EphAs expressed in the anterior SC.

As much of the evidence for EphA endocytosis was based on *in vitro* approaches, Park *et al* sought to demonstrate the validity of this model by homing in on the cytoskeleton. Reorganisation of the local actin cytoskeleton is necessary for endocytosis of Ephs, which requires the small GTPase Rac (Marston *et al*, 2003) and upstream regulators, the RacGEFs (Cowan *et al*, 2005). Indeed, one explanation for the lack of internalisation of the endocytosis-deficient EphA8 is that part of the deletion encompasses a juxtamembrane tyrosine phosphorylation site that is a target for RacGEFs. If Rac is indeed required for EphA endocytosis, then Rac inactivation in the anterior SC should produce similar topographic targeting defects to the expression of the endocytosis-deficient EphA8. When the authors tested this prediction, they found that expression of a dominant-negative Rac in the anterior SC produced a mapping shift of nasal axons towards the anterior SC (Figure 1D), consistent with an important role of endocytosis in the SC.

Thus, the authors propose a scenario in which during initial retinal axon ingrowth in the SC high-affinity interactions are formed between ephrinAs on axons and EphAs on SC cells. This interaction triggers repellent signalling and collapse of the cytoskeleton in the axons, followed by its disengagement from SC cells. However, this disengagement critically depends on the endocytosis of EphAs. In this process, it is likely that ephrinAs are ruptured out of the axonal membrane and 'trans-endocytosed' together with EphA receptors into the SC cells.

Thus, the consequence of endocytosis-defective EphA expression is that of preventing the separation of retinal axons and SC cells, leading to an effectively more adhesive anterior SC. Thus, the region with the highest concentration of EphA molecules exerting the strongest repulsion on nasal axons morphs into the region with the strongest adhesion, and hence ectopic TZs would be expected to form (also) in the anterior SC, which indeed is what has been found (Figure 1C).

Many questions still remain. A second possibility to account for the disruption of cell–cell interactions would be that EphA binding induces shedding of ephrinAs by metalloproteases and γ -secretases.

Insights from cell biological studies suggest that the direction of endocytosis is determined by Eph versus ephrin-mediated signal transduction. For example, in case of signalling-deficient EphB receptors lacking the cytoplasmic domain, internalisation of the EphB/ephrinB complex proceeds into ephrinB-expressing cells, while the reverse scenario, binding of full-length EphB and truncated ephrinB, leads to trans-endocytosis in the EphB-expressing cells (Marston *et al*, 2003; Zimmer *et al*, 2003). As in the SC ephrinAs signal into the axons, presumably the ephrinA/EphA complex is endocytosed not only into SC cells but also into the axonal compartment. The repulsion of ephrinA-expressing nasal axons from growing on EphA-Fc in stripe assay experiments (Rashid *et al*, 2005) suggests that endocytosis can indeed occur in ephrinA-expressing cells.

Finally, a puzzling aspect is how endocytosis of guidance molecules expressed in smooth gradients and topographic mapping go together. It can be assumed that endocytosis itself will quite substantially affect local protein concentrations on the membranes of axons and SC cells, and disrupts the shape of the Eph and ephrin gradients. Some compensatory or homeostatic mechanisms might re-establish these gradients; however, recycling might not be a possibility given the trans-endocytosis mechanism of internalisation of EphA and ephrinAs.

Overall, this work by Yoo *et al* (2011) represents a good step forward towards understanding topographic mapping in the visual system.

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Conflict of interest

The author declares that he has no conflict of interest.

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