Regulation of axial patterning of the retina and its topographic mapping in the brain

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Topographic maps are a fundamental organizational feature of axonal connections in the brain. A prominent model for studying axial polarity and topographic map development is the vertebrate retina and its projection to the optic tectum (or superior colliculus). Linked processes are controlled by molecules that are graded along the axes of the retina and its target fields. Recent studies indicate that ephrin-As control the temporal–nasal mapping of the retina in the optic tectum/superior colliculus by regulating the topographically-specific interstitial branching of retinal axons along the anterior–posterior tectal axis. This branching is mediated by relative levels of EphA receptor repellent signaling. A major recent advance is the demonstration that EphB receptor forward signaling and ephrin-B reverse signaling mediate axon attraction to control dorsal–ventral retinal mapping along the lateral–medial tectal axis. In addition, several classes of regulatory proteins have been implicated in the control of the axial patterning of the retina, and its ultimate readout of topographic mapping.

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Abbreviations

A–P	anterior–posterior
BF	brain factor
BMPs	bone morphogenetic proteins
CALI	chromophore assisted laser inactivation
D–V	dorsal–ventral
En	engrailed
GH6	gallus gallus homeobox 6
GPI	glycosylphosphatidylinositol
L–M	lateral–medial
OT	optic tectum
RGCs	retinal ganglion cells
RGM	repulsive guidance molecule
RPCs	retinal progenitor cells
SC	superior colliculus
Sema	semaphorin
Shh	sonic hedgehog
SOHo	sensory organ homeobox
T–N	temporal–nasal
TZ	termination zone
V–N	ventral–nasal
V–T	ventral–temporal

Introduction

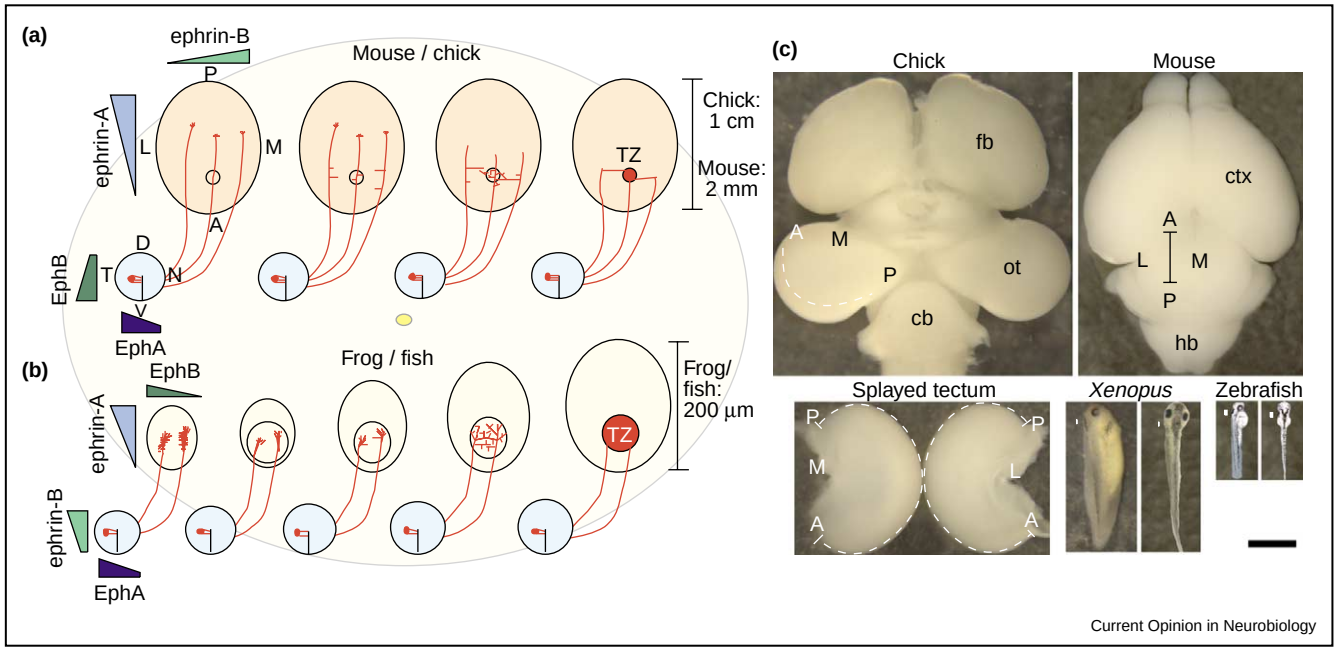
Development of the appropriate functional connections of the nervous system requires a series of steps ranging from the specification of neurons and their target cells to the formation of axonal connections between them. Many axonal projections within the brain establish an orderly arrangement of connections in their target field, termed a topographic map. These maps are organized such that the spatial arrangement of the cells of origin is reflected in the order of their axon terminations, hence, neighbouring cells project to neighbouring parts of the target to form a continuous map. Topographic projections are especially evident in sensory systems. The dominant model system for studying map development is the projection from the retina to its major midbrain target, namely the optic tectum (OT) of frogs, fish and chicks or its mammalian homologue, the superior colliculus (SC) of rodents. The spatial ordering of axonal arborizations of retinal ganglion cells (RGCs) within the OT/SC maps the retina, and therefore visual space, along two sets of orthogonally oriented axes. These are the temporal–nasal (T–N) axis of the retina along the anterior–posterior (A–P) axis of the OT/SC, and the dorsal–ventral (D–V) retinal axis along the lateral–medial (L–M) OT/SC axis.

Here, we consider two crucial issues for the development of retinotopic maps in the brain: one, the regulatory mechanisms that control the axial patterning of the retina, including the expression of topographic guidance receptors by RGCs; and the other, the molecular control of the topographic mapping of RGC axons within the brain. As retinotopic mapping is the ultimate readout of retinal polarity, and often used as an assay for studying the regulation of axial patterning, we first consider recent advances in our understanding of the molecular control of mapping and then progress in research on the axial patterning of the retina.

Molecular control of topographic mapping

The currently held concepts of the molecular control of topographic mapping are based on the chemoaffinity hypothesis of Sperry [1]. Sperry suggested that each point in the optic tectum has a unique molecular address determined by the graded distribution of topographic guidance molecules along the two tectal axes. Each RGC has a unique profile of receptors for those molecules, that results in a position-dependent, differential response to the guidance molecules by RGC axons. Although the control of topographic mapping has been intensively studied for decades, the first identification of topographic guidance molecules came with the cloning of

Figure 1



Development of the retinotopic projection in the primary model animals. **(a)** In the mouse and the chick, the overall distribution of Ephs in the retina and ephrins in the OT/SC is depicted. Initially, RGC axons enter the OT/SC and extend posterior to the location of their future TZ (circle). The low-to-high A–P gradient of ephrin-As stops the posterior extension of growth cones at various positions depending on RGC EphA level. Interstitial branches form along the axon shaft in a distribution biased for the A–P location of the TZ. Interstitial branches extend laterally or medially towards their future TZ. EphBs and ephrin-B guide interstitial branches appropriately. Upon reaching their TZ, branches elaborate complex arbors and the initial axon overshoot begins to decrease. The TZ becomes dense with arbors, and inappropriate axon segments are eliminated. **(b)** In the frog and the zebrafish, the overall distributions of Ephs and ephrins are similar to those in the chick and the mouse (see above). Depicted here are the gradients of ephrin-Bs in the retina and EphBs in the tectum (also present in the chick and the mouse). The tectum and the retina expand throughout the development of the retinal projection. During retinotopic map development, the tectum is much smaller in relation to a typical growth cone in *Xenopus* and zebrafish than in chicks and mice. RGC axons extend into the tectum and elaborate many small branches from the base of the growth cone. Arbors elaborate from these backbranches. The TZ becomes dense and refines as the tectum enlarges. The two ovals in the background represent the relative sizes of the chick tectum (large oval) and *Xenopus* or zebrafish tectum (small oval). **(c)** The photographs are all at the same scale. The chick OT rotates during development such that the posterior pole (P) is near the midline. The OT is cut along its A–P axis at its L–M midline (dashed line) and splayed. The distance from the anterior to the posterior pole along the cut edge is 1cm (dashed lines in the splayed tectum). The mouse SC is about 2 mm in length along the A–P axis (bar). For *Xenopus* and zebrafish, the entire animal is shown in lateral and dorsal views. The white bar on the left of each panel represents the approximate A–P position and size of the tectum. The tecta for these organisms are approximately 200 μm along the A–P axis. cb, cerebellum; ctx, cortex; fb, forebrain; hb, hindbrain. Scale bar = 2 mm.

ephrin-A2 [2] and ephrin-A5 [3]. These proteins, like all members of the ephrin-A family, are anchored to the cell membrane by a GPI-linkage, and bind with similar affinities and activate the same members of the EphA subfamily of receptor tyrosine kinases [4]. Several functional *in vitro* and genetic *in vivo* studies have shown that ephrin-As, acting through their EphA receptors, partially control the topographic mapping of the T–N retinal axis along the A–P OT/SC axis. In addition, the EphB subfamily of receptor tyrosine kinases and the ephrin-Bs control the topographic mapping of the D–V retinal axis along the L–M OT/SC axis.

Mechanisms of map formation

The predominant model animals for the development of retinotopic connections have been frogs, fish, chicks and rodents. These species have substantial differences in the absolute size of their OT/SC, with a near 50-fold difference in the length of the A–P axis of the OT of chick compared to that of frogs and fish. These species also have important differences in the development of the visual system (Figure 1). However, each of these species has unique features that makes it appealing as a model. For example, mice and zebrafish benefit from their well-developed genetics, whereas chicks and frogs are readily manipulated genetically. Importantly, of the species represented here, mice are the most closely related to humans, both phylogenetically and in the organization of their visual system. Chicks appear to have the most in common with mice in terms of their mapping mechanisms.

It was recognized about a decade ago that the development of retinotectal topography in chicks [5] and rodents [6,7,8] is a multistep process that involves axon overshoot

and interstitial branching. Recent detailed quantitative analyses have indicated, however, that axon overshoot and interstitial branching is the exclusive mechanism for map development in these animals. These analyses are beginning to define the relative importance of directed axon extension and branching, and the role of guidance molecules in controlling these processes [9••,10••]. Initially, the primary growth cones of RGC axons enter the OT/SC and extend posteriorly past the location of their future termination zone (TZ) (Figure 1). RGC axons from a given D–V location have a broad distribution along the L–M tectal axis, with a peak centred on the location of the future TZ [8,10••]. The first indication of appropriate topography is the formation of interstitial branches from the primary axon shaft. Branches form *de novo* from the axon shaft hundreds of microns or even millimetres behind the growth cone. Interstitial branching exhibits a significant degree of topographic specificity along the A–P axis, with the highest percentage of branches found at the A–P location of the future TZ [9••]. Interstitial branches form roughly perpendicular to the primary axon, and preferentially extend along the L–M axis towards their future TZ [5,10••]. The branches arborize at the appropriate L–M and A–P location of their TZ, and this appears to be the exclusive means by which RGCs form permanent, topographically ordered, synaptic connections [9••]. Although RGC axons are broadly distributed across the L–M axis of the OT/SC, their distribution does not change even though their numbers decline and the map undergoes considerable refinement coincident with the death of a substantial proportion of RGCs [8,10••]. Therefore, the position of an RGC axon along the L–M axis relative to its TZ does not bias its ability to make a connection to the TZ, and maintain it.

In frogs and fish, the initial D–V mapping along the L–M axis is much more accurate than it is in chicks and rodents. The RGC axons extend directly to the correct location of their TZ in these animals (Figure 1). As the growth cone of the primary RGC axon reaches the location of its future TZ, it stops and exhibits a phenomenon termed 'backbranching'. Backbranching is characterized by the formation of short terminal branches at or near the base of the growth cone, which itself often acquires a branch-like morphology, and together they elaborate a local terminal arborization of the distal part of the primary axon [11–13]. The processes of backbranching and terminal arborization are believed to be in part attributable to a neuropilin-1-mediated response of the growth cone to Sema3A, which is expressed in the OT [14•]. Thus, backbranching — as originally defined — is a phenomenon that is distinct in scale, location, and purpose from the interstitial branching in chicks and rodents described above.

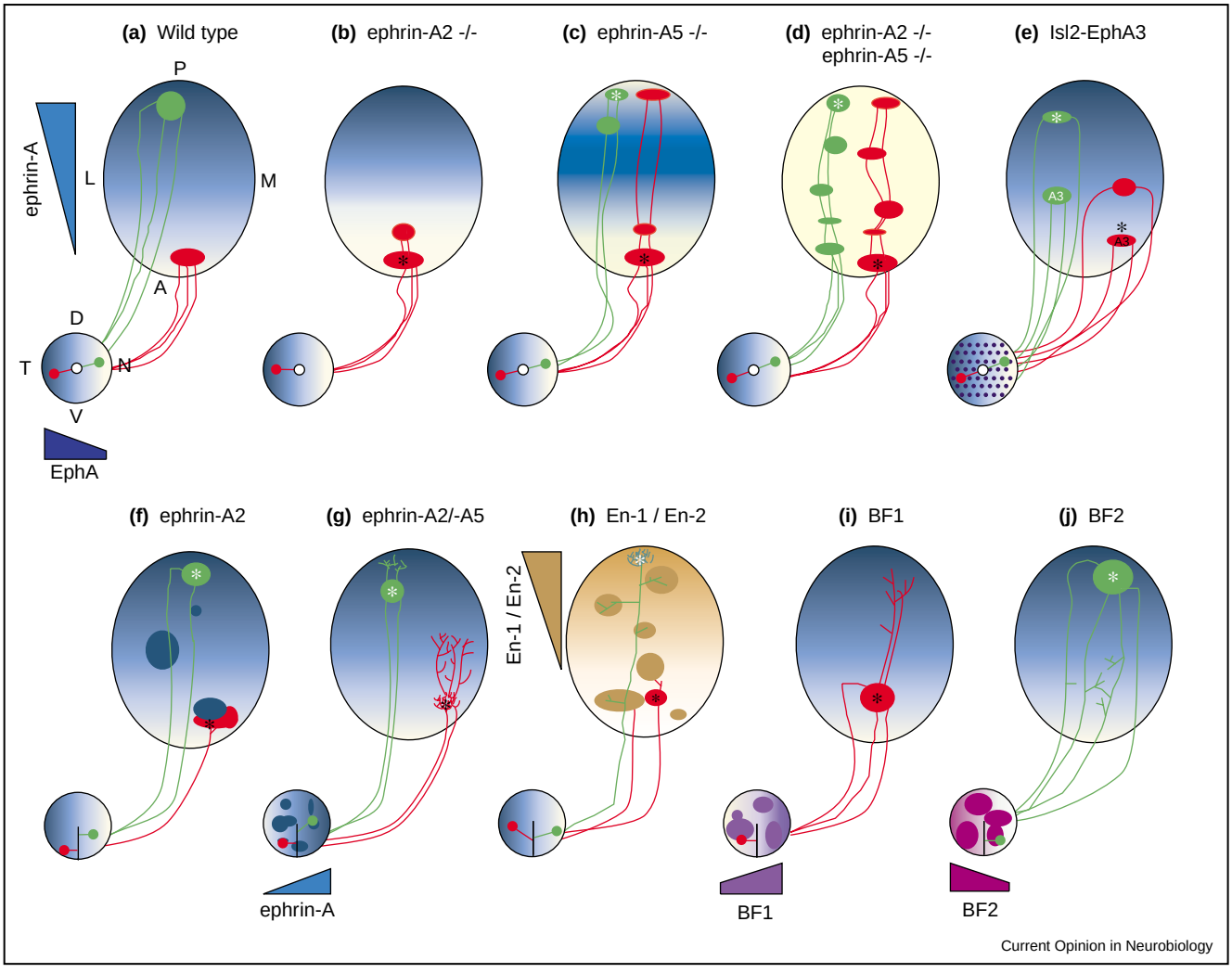
The sizes of individual arbors along the A–P axis are relatively larger in the OT of frogs and fish than in the OT of chicks and the SC of mice. This is partly due to the fact that the RGC axons of frogs and fish reach the tectum and arborize at an early stage of tectal neurogenesis when the tectum is very small and only rostral tectum has been generated. Therefore, although RGC axons are not described to overshoot their TZ in frogs or fish, their RGC axonal arbors are disproportionately large compared to the tectum during the early stages of tectal neurogenesis, and cover a greater percentage of its surface area than at later stages. In frogs and fish, arbors cover progressively less of the A–P axis as map development advances because the tectum expands substantially more than the arbors and some arbor refinement occurs [15]. In contrast, the OT/SC of chicks and rodents expand relatively little over the period of map development (Figure 1).

Molecular control of anterior–posterior topographic mapping

The largest family of receptor tyrosine kinases, the Eph family, and their ligands, the ephrins, are expressed prominently in the visual system and are major players in retinotopic mapping. In particular, ephrin-A2 and ephrin-A5, and a subset of their EphA receptors, are expressed in complementary graded patterns in the OT/SC and retina. Several lines of *in vitro* and *in vivo* evidence indicate that ephrin-As are growth cone repellents [16,17,18] and interstitial branch inhibitors [9••,19] that preferentially affect temporal RGC axons. The overall low-to-high A–P gradient of ephrin-As is likely to be controlled by the engrailed homeodomain proteins En-1 and En-2 [20,21]. Each engrailed gene is also expressed in a low-to-high A–P gradient, and their ectopic expression results in T–N mapping defects [22,23]. Figure 2 summarizes these expression patterns and most of the phenotypes caused by altering the expression of ephrin-As, EphAs, or regulatory genes that influence T–N mapping. Although species-specific differences are apparent in the particular EphAs and ephrin-As that are expressed, or in their patterns of expression, the basic theme is constant across vertebrates.

Analyses of mice with targeted deletions of ephrin-A5 [24], ephrin-A2, or both [25••], show that ephrin-As are required for proper T–N mapping along the A–P axis. Each of these mutants has aberrant maps with ectopic TZs that relate to the expression patterns of the deleted and remaining ephrin-As. As expected, the magnitude of the aberrancies is most substantial in the ephrin-A2/ephrin-A5 double mutant. Surprisingly, however, each mutant has a TZ at the appropriate A–P location in addition to the ectopic TZs. This is true even in the double mutant that lacks all ephrin-A expression in the SC [25••]. The formation of a TZ in the appropriate location in ephrin-A2 and ephrin-A5 mutant mice clearly shows that additional signals are required for proper A–P mapping. This could include the recently cloned axon repellent, RGM (repulsive guidance molecule) [26•]. Before the discovery of the ephrins, the most compelling

Figure 2



Phenotypic defects in temporal–nasal to anterior–posterior topographic mapping. **(a)** Wild type mapping function and the distributions of EphAs in the retina and ephrin-As in the OT/SC. **(b-d)** Temporal RGC axons in mice lacking ephrin-A2 [25••], ephrin-A5 [24], or both [25••] have, in addition to a normally positioned TZ, ectopic TZs in posterior locations. The penetrance and severity of this phenotype varies with genotype. The mildest defects occur in ephrin-A2 mutants and the most severe in ephrin-A2/ephrin-A5 double mutants. Furthermore, nasal RGC axons in ephrin-A5 and ephrin-A2/ephrin-A5 mutant mice have, in addition to a normal-appearing TZ, ectopic TZs in anterior locations. **(e)** In a knock-in study in which EphA3 was expressed in a subset of RGCs on top of the existing EphA gradient in the retina, essentially two maps develop [32••]. The RGCs expressing EphA3 form a map that is compressed into anterior SC, whereas the wild type RGCs form a map that is compressed into posterior SC. However, neither map forms at the expected location. **(f)** Retroviral overexpression of ephrin-A2 in chick tectum creates multiple domains of high levels of ephrin-A2 [16]. Whereas nasal RGC axons are not grossly influenced, temporal RGC axons halt their extension upon reaching domains of ectopic ephrin-A2 and do not arborize in them. **(g)** Retroviral overexpression of ephrin-A2 [29] or ephrin-A5 [57] in the chick retina on top of the normal high-to-low N–T gradient of these molecules results in mapping defects. Temporal RGC axons do not form dense TZs, and maintain and elaborate axonal extensions and arborizations in posterior positions. **(h)** In tecta in which the homeodomain transcription factors En-1 or En-2 were ectopically expressed, temporal RGC axons and their branches avoid domains of ectopic expression, whereas nasal axons branch into them, even in topographically incorrect areas [22,23]. The domains of en-1 or en-2 expression exhibit properties of the posterior tectum, including ephrin-A expression [20]. **(i,j)** The winged-helix transcription factors BF1 and BF2 are expressed in opposite N–T gradients in the retina. **(i)** Retroviral overexpression of BF1 affects the correct mapping behaviour of temporal RGC axons. In addition to a correctly located TZ in the anterior tectum, some axons project into posterior tectum [50]. **(j)** Overexpression of BF2 results in nasal RGC axons forming, in addition to a normally located TZ in posterior tectum, ectopic branches and arbors anterior to the TZ [50]. **(b-j)** Asterisks indicate the correct TZ location as determined by retinal location.

evidence for topographic guidance molecules came from the membrane stripe assay. This assay showed that temporal RGC axons exhibit a strong preference to grow on their topographically appropriate anterior tectal membranes. The growth preference of temporal axons is due to a repellent activity that is associated with posterior tectal membranes and biochemically isolated to a 33-kDa GPI-anchored protein referred to as RGM [27]. RGM is

expressed in a similar A–P graded pattern in the OT as ephrin-As, and *in vitro* RGM has inhibitory properties that are also similar to those of ephrin-As; therefore, it is likely that RGM plays a role in retinotopic mapping [27,28].

During map development in chicks and rodents, RGC axons initially overshoot their correct TZ along the A–P axis, but later they exhibit a topographic bias in interstitial branching. This suggests that a primary role for ephrin-As is to inhibit branching along RGC axons posterior to the correct A–P location of their TZ [9••]. This conclusion is supported by work in which a modified membrane stripe assay was used to show the role of ephrin-A-mediated RGC branch inhibition *in vitro* [9••] and the chromophore assisted laser inactivation (CALI) technique that confirmed this role *in vivo* [19]. Modeling indicates that to account for the topographic specificity in interstitial branching observed *in vivo*, a second activity must limit interstitial branching anterior to the TZ [9••]. This could be either a branch inhibiting activity in an opposing gradient to ephrin-As or a branch-promoting activity that parallels that of ephrin-A inhibition. In addition to the high-to-low T–N gradient of EphAs in the retina, RGCs express ephrin-As in a low-to-high T–N gradient, and ephrin-As are present on chick RGC axons [29]. Experimental evidence suggests that this countergradient of ephrin-As in the retina sharpens the gradient of functional EphA receptors [29]. However, EphAs are also expressed in the tectum in a high-to-low A–P gradient. Thus, if EphA-ephrin-A interactions between RGC axons and tectal cells result in a bidirectional repellent signal, this interaction could function as a graded counter-repellent and inhibit interstitial branching along axons both anterior and posterior to the A-P location of their TZ, thereby accounting for the observed topographic specificity in interstitial branching. EphB-ephrin-B interactions are known to result in bidirectional signaling, characterized by signaling into cells expressing EphB receptors (i.e. forward signaling) and into cells expressing ephrin-Bs (i.e. reverse signaling) [30]. Also consistent with this speculation ephrin-As may be capable of acting as a receptor for EphAs and, by complexing with other proteins, signal into ephrin-A expressing cells [31].

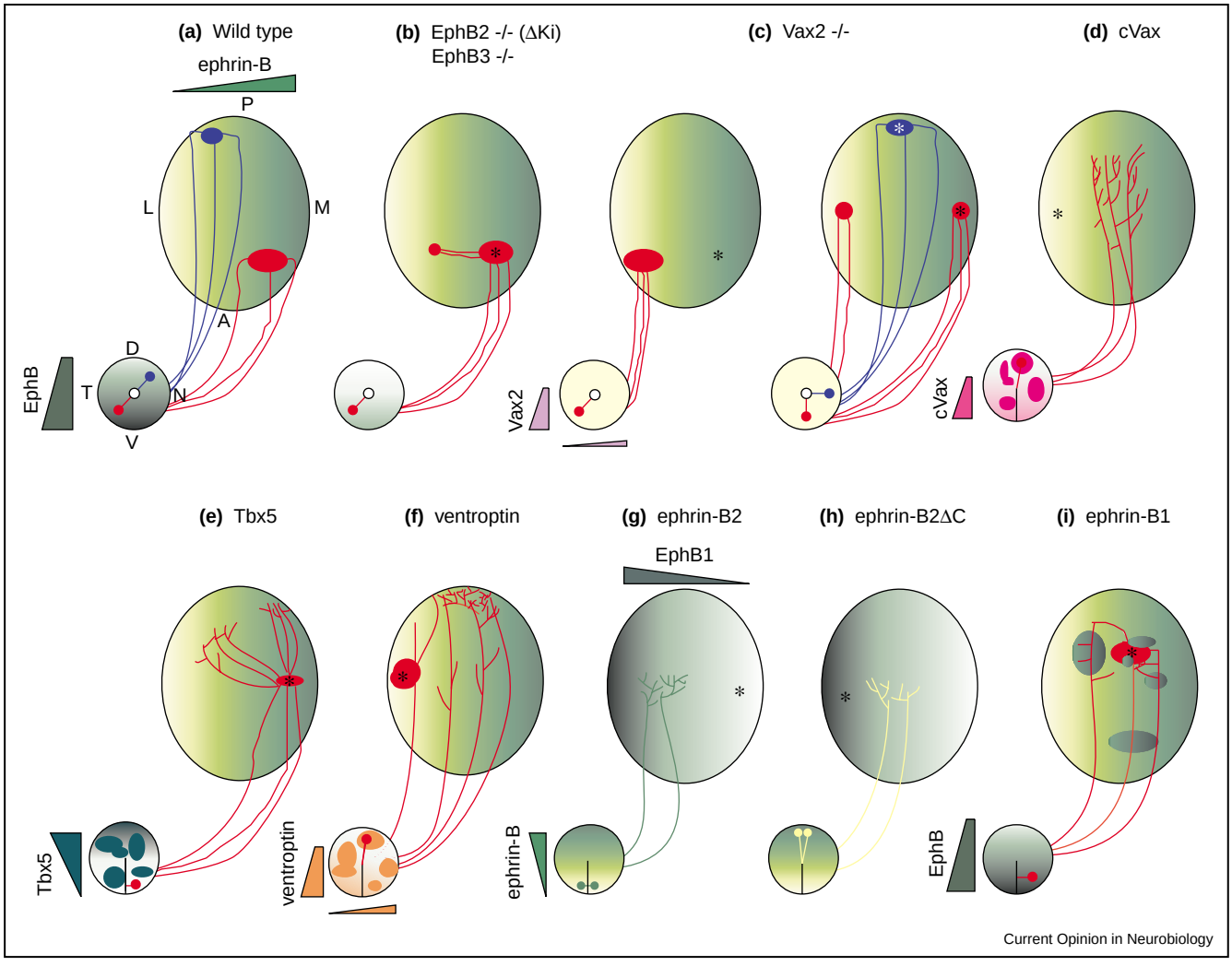
The only study to date of the role of EphA receptors in retinotopic mapping used a gene knock-in (for a definition of knock-in see annotation [32••]) strategy that took advantage of the facts that ephrin-As bind and activate most EphA receptors with similar efficacy, and that EphA3 is not expressed by RGCs in mice. Mice were generated in which EphA3 was ectopically expressed in about half of all RGCs scattered across the retina (those that expressed the homeodomain protein Isl2). This produced two subpopulations of RGCs, one with the wildtype gradient of EphA receptors (EphA5 and EphA6), and one with an elevated gradient of overall EphA expression caused by the added expression of EphA3 [32••]. In these mice, the projection of the EphA3 RGCs is compressed to the anterior half of the SC, indicating that the level of EphA receptor dictates the degree to which an RGC axon is repelled by ephrin-As. Unexpectedly, the projections of the wildtype RGCs are compressed to the posterior half of the SC, and are likely to be excluded from the anterior SC by competitive interactions with the EphA3 expressing RGCs. Thus, T–N topographic mapping is controlled by relative rather than absolute levels of EphA signaling between RGC axons. These findings also reveal a hierarchy in the mechanisms for generating topographic maps, showing that molecular axon guidance information, such as that mediated by ephrin-As and EphAs, dominates over activity-dependent patterning mechanisms that are based on neighbor relationships of RGCs and correlated patterns of neural activity.

Molecular control of lateral–medial topographic mapping

Recently, two studies identified axon guidance molecules that control mapping of the D–V retinal axis along the L–M OT/SC axis [10••,33••]. Both studies show that EphB–ephrin-B interactions are responsible, in part, for D–V retinotopic mapping. Ephrin-B reverse signaling plays a dominant role in retinotopic mapping in *Xenopus*, [33••], whereas EphB forward signaling is dominant in mice [10••]. Although the two studies were performed in different systems and analyse different features of axon mapping, both groups found strong evidence for an attractant effect of EphB–ephrin-B interactions in D–V mapping. Figure 3 summarizes the relevant expression patterns and most of the phenotypes that were caused by altering the expression of ephrin-Bs, EphBs, or regulatory genes that influence D–V mapping.

In *Xenopus*, the ectopic expression of wild type or dominant negative forms of ephrin-Bs in the retina indicates that during normal development dorsal RGC axons that express ephrin-Bs are attracted to the lateral OT that expresses EphBs, where they form terminal arbors [33••]. As described above for the chick and mouse models, RGC axons form interstitial branches that are biased for the A–P location of their TZ. These branches also correct inaccuracies in the initial axon position along the L–M axis by preferentially extending towards the TZ. This process of directional branch extension is controlled in part by EphB–ephrin-B1 interactions. EphBs are expressed in a low-to-high D–V gradient by RGCs, and ephrin-B1 is expressed in a low-to-high L–M gradient in the SC [10••,34,35]. Evidence from EphB2 and EphB3 mutant mice suggests that forward signaling through these receptors is responsible for medially directed branch extension towards increasing levels of ephrin-B1. This indicates that ephrin-B1 can act as an attractant for interstitial branches [10••]. Modeling suggests that proper mapping of the D–V axis requires that the attractant activity of

Figure 3



Phenotypic defects in dorsal–ventral to lateral–medial topographic mapping. **(a)** Wild type mapping function and the distributions of EphBs and ephrin-Bs in the retina and OT/SC. **(b)** Deletion of EphB2 and EphB3 leads to defects in topographic mapping of ventral RGC axons. In addition to a correctly positioned main TZ (*), an ectopic TZ is present in lateral SC. A similar phenotype is observed in mice in which the intracellular domain of EphB2 has been deleted (EphB2 ΔK_i) and which are null for EphB3 [10••]. **(c)** Deletion of the homeobox transcription factor Vax2 leads to distinct errors in L–M topographic mapping that depend on retinal position [45•,46••]. Vax2 is normally expressed in a high-to-low V–N to D–T gradient. Ventral axons from more temporal locations in the retina map exclusively to the lateral side of the SC (left). This defect gradually lessens, shifting to more nasal positions in ventral retina. Ventral DiI injections reveal a normally located TZ and an ectopic TZ in the lateral SC (right, red axons). Nasal RGC axons appear to map normally (right, blue axons) [46••]. **(d)** Overexpression of the cVax homeobox transcription factor in chick retina leads to L–M mapping defects. Dorsal RGC axons have aberrantly medial trajectories and do not form a dense TZ in the correct lateral location [56]. **(e)** Overexpression of the dorsal marker Tbx5 in chick retina leads to mapping defects. Ventral RGC axons, in addition to a normally positioned TZ in medial tectum, aberrantly extend processes medially [54••]. **(f)** Ventroptin is expressed in chick retina in a high-to-low V–N to D–T gradient. Overexpression of ventroptin leads to mapping defects for dorsal RGC axons. Although a normal appearing TZ is seemingly present, some dorsal RGC axons have aberrantly medial trajectories. In addition, dorsal RGC axons have ectopic arborizations posterior and medial to the location of their TZ [53••]. **(g,h)** Ephrin-Bs are expressed in the retina in a high-to-low D–V gradient, and EphB1 is present in the tectum in a high-to-low L–M gradient. **(g)** In *Xenopus*, misexpression of ephrin-B2 in the ventral retina results in ventral RGC axons with aberrantly lateral trajectories and arborizations [33••]. **(h)** Expression of a dominant negative ephrin-B2 construct (ephrin-B2ΔC) in *Xenopus* dorsal retina results in dorsal RGC axons with aberrantly medial trajectories and arborizations [33••]. **(i)** Retroviral misexpression of ephrin-B1 in chick tectum affects the branching behavior of RGC axons but not axonal trajectory. In regions with high levels of ectopic ephrin-B1, branches are preferentially oriented laterally. At later stages, regions of ectopic ephrin-B1 are relatively devoid of dense arborizations (McLaughlin T, Hindges R, O'Leary DDM, personal communication). **(b-i)** Asterisks indicate the correct TZ location as determined by retinal location.

ephrin-B1 cooperates with a repellent activity in a gradient that resembles that of ephrin-B1 [10••].

Interestingly, ephrin-B1 acts as an axon repellent in other systems [36]. EphBs and ephrin-Bs have similar expression patterns in chicks [37,38,39] and mice [10••,34,35]. It is possible, therefore, that the repellent activity suggested by modeling is, in fact, ephrin-B1. In this scenario, ephrin-B1 acts as both an attractant and a repellent to control, in a context-dependent manner, the directional extension of interstitial branches by RGC axons that arise from the same D–V position and presumably express the same level of EphB receptors. Branches that arise from axons positioned lateral to the correct TZ are attracted medially up the gradient of ephrin-B1 toward the TZ; whereas branches that arise from axons positioned medial to the same TZ are repelled laterally down the ephrin-B1 gradient toward the TZ (T McLaughlin, R Hindges, P Yates, D O'Leary, unpublished data). In principle, this proposed bi-functional action of ephrin-B1 is sufficient to account for D–V retinotopic mapping. Even if this scenario acts *in vivo*, however, it does not preclude other guidance molecules or reverse signaling from having a role in D–V mapping, particularly for the dorsal retina.

Retinal polarity

Axial patterning of the retina is a hierarchical process that begins at the earliest stages of retinal development. It includes the transcriptional control of the graded expression by RGCs of guidance molecules that are required for topographic mapping. Signaling molecules that are secreted from patterning centers and several transcription factors have recently been implicated in both establishing the polarity of the T–N and D–V retinal axes and regulating the molecules that control topographic mapping. Vertebrate eye development consists of several different processes, from the initiation of its development in the anterior neural plate to the final shaping of the eye. In this review, we do not discuss vertebrate eye formation and the key regulators (for review see [40]) but focus on the initiation and subsequent formation of retinal polarity and its consequences for topographic mapping.

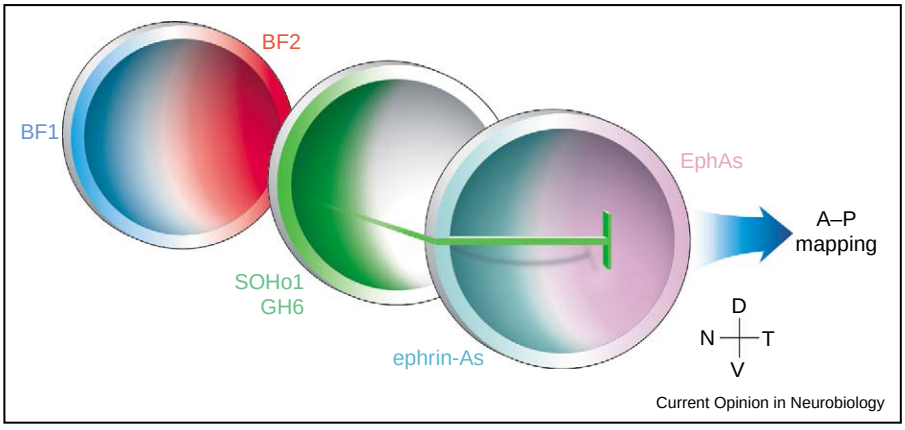
The anlage of the early developing retina, the optic vesicle, which forms as an evagination of the ventral diencephalon, is likely to be patterned by secreted morphogens such as Shh and BMPs [41]. During this process, the complementary expression of the paired box homeodomain proteins Pax2 and Pax6, whose expression domains initially overlap, becomes distinct through mutual repression. This forms a sharp border at the boundary between the optic stalk (a Pax2 domain) and the neural retina (initially a Pax6 domain) [42]. This distinction between the optic stalk and retina may also involve the homeodomain proteins Vax1 and Vax2, as is suggested by their expression domains and mutant phenotypes [43,44,45•,46••]. Furthermore, in Pax6 mutant mice (i.e. the small eye mutant), Vax1 is ectopically expressed throughout the early optic vesicle and may account in part for the defective eye development that is characteristic of the small eye mutant [47]. In the early optic vesicle, no genes are differentially expressed within the retinal progenitor cells (RPCs) in a pattern that suggests axial polarity. However, initial expression patterns in Pax6-knockout mice suggest that Pax6 is a critical regulator of not only the ventral retinal marker Vax2 but also the nasal and temporal markers BF1 and BF2 [48••]. Furthermore, when analysing a conditional mutant of Pax6, in which Pax6 expression is selectively lost in more nasal and temporal parts of the retina after the optic vesicle stage, it appears that BF1 and BF2 expression is not maintained and expression of the dorsal marker Tbx5 is not induced after the loss of Pax6 expression [48••]. Therefore, the early expression of Pax6 plays a role in the development of polarity along both retinal axes.

The combined expression of a set of transcription factors (Pax6, Six3, Rx1, Chx10 and Hes1) initially marks the RPCs. The subsequent segregation of these transcription factors into distinct expression domains is crucial to the differentiation of the RPCs into the seven main retinal cell subtypes [49]. RGCs are the first post-mitotic retinal neuron type generated. Positional information that is inherited by RGCs is maintained as they differentiate their cell-type specific features and connections. Therefore, the acquisition of positional information by RPCs is an important step in defining the retinal axes in parting positional information to RGCs, and their subsequent development of retinotopic projections.

Patterning of the temporal–nasal retinal axis

The first indications of the axial polarity of the retina are found along the T–N axis, and D–V axial polarity becomes evident soon thereafter. Figure 4 summarizes a regulatory scheme for T–N patterning. Along the T–N axis, the polarized expression of the winged-helix transcription factors BF1 and BF2 is apparent in the neural retina at the optic cup stage (reviewed in [41]). BF1 is expressed in the nasal part of the optic cup and the optic stalk, whereas BF2 is restricted to the temporal part. During the optic cup stage and thereafter, the expression domains of BF1 or BF2 do not overlap or abut but are separated by a distinct gap. Ectopic expression of BF1 in the temporal retina of an embryonic chick, using a recombinant retrovirus, results in some temporal RGC axons aberrantly maintaining their normally transient projection to posterior tectum and occasionally forming ectopic arbors (Figure 2; [50]). Similarly, ectopic expression of BF2 in nasal retina results in some nasal RGC axons maintaining branches and arborizing at sites anterior to their appropriate TZ [50]. The extent to which transcription factors or axon guidance molecules are changed by ectopic expression of BF1 or BF2 in chick retina remains to be determined [50,51•]. Therefore, BF1

Figure 4



Regulation of temporal–nasal retinal polarity. The schematic represents the expression patterns and interactions of most genes shown to be involved in establishing retinal polarity along the T–N axis from early (left) to late (right) time points. Early in retinal development, BF1 and BF2 mark nasal and temporal retina. Subsequently, two nasal markers (SOHo1 and GH6) are expressed in nasal retina. There is no evidence of any interactions between BF1, BF2 and SOHo1 and GH6. Both SOHo1 and GH6 can repress the expression of EphA3. At later stages, ephrin-As and EphAs (known axon guidance molecules) are expressed by RGCs and control A–P mapping in the midbrain. The compass indicates the retinal axes.

and BF2 may regulate the expression of the recently cloned ephrin-A6 [52] or unknown axon guidance molecules, for example a receptor for RGM. Alternatively, they may control the expression of molecules that are involved in arbor formation and/or map refinement.

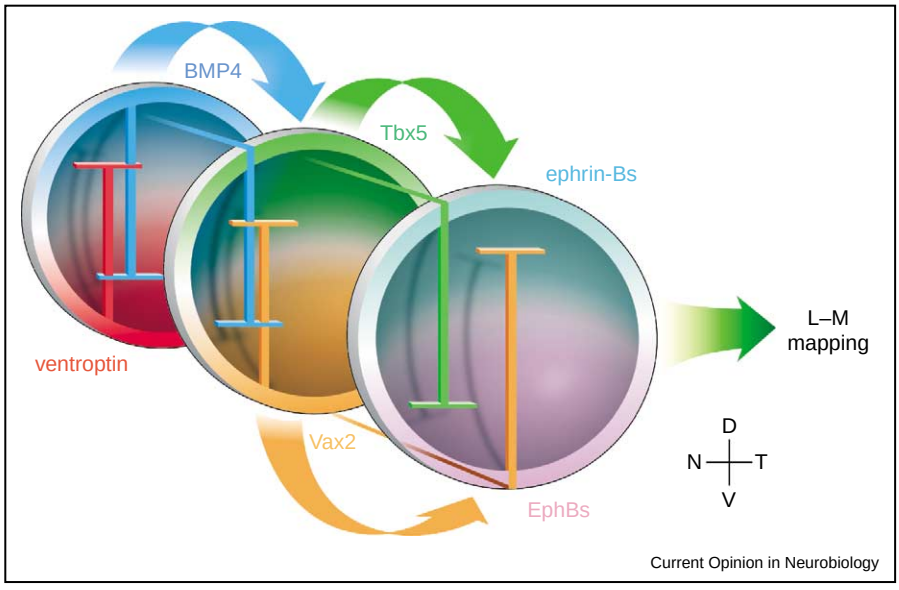
Two other regulatory genes, SOHo1 and GH6, are expressed in the nasal retina [51•]. Although SOHo1 and GH6 expression becomes apparent after the expression of BF1 and BF2, neither is up- or downregulated by the ectopic expression of BF1 or BF2 in chick retina [51•]. Ectopic expression of SOHo1 or GH6 in the temporal retina results in temporal defects in RGC axon mapping, similar to those observed when BF1 is ectopically expressed (Figure 2). However, both SOHo1 and GH6 repress EphA3 expression in retina, thereby providing a mechanism to account for the retinotectal mapping defects [51•]. In summary, BF1, BF2, SOHo1, and GH6 appear to define at least three domains along the T–N axis and contribute to T–N axial polarity, although their contributions (especially those of BF1 and BF2) are presently vague.

Patterning of the dorsal–ventral retinal axis

Soon after T–N polarity is determined, initial D–V polarity develops through the actions of Shh, retinoic acid, and BMPs [40,41]. Figure 5 summarizes a regulatory scheme for D–V patterning. The dorsalizing effect of BMP4 appears to be counteracted by ventroptin, a BMP4 antagonist that is expressed in ventral retina [53••]. Although the direct regulatory interactions are largely unknown, ectopic expression of ventroptin in dorsal retina represses the expression of BMP4 and Tbx5, both of which are normally expressed in dorsal retina, and promotes expression of cVax, which is normally expressed in ventral retina. Conversely, ectopic expression of BMP4 induces Tbx5 expression in ventral retina and represses the expression of ventral markers such as cVax, Pax2, and ventroptin [53••,54••].

A study combining clonal analysis of retinal cells and their distributions relative to the expression of D–V markers suggests that the D–V axis is subdivided into at least four domains that may be developmental compartments [55•]. This model emphasizes that D–V patterning is a complex process, and current findings indicate that it will not be a straightforward task to dissect it. Many of the effects reported after altering the expression of regulatory genes are likely to be caused indirectly and reflect a hierarchical system. For example, the ectopic expression of cVax represses Tbx5 expression and vice versa [54••,56]. However, although Tbx5 is repressed by ectopic expression of cVax in dorsal chick retina [56], it is not upregulated in the ventral retina of Vax2 mouse mutants [46••]. Taken together, the data suggest that ventroptin may indirectly repress Tbx5 by repressing BMP4, whereas BMP4 may indirectly repress cVax by repressing ventroptin. Furthermore, although ectopic expression of cVax in the dorsal retina of chick results in Pax2 expression [56], Pax2 expression is unchanged in Vax2 mouse mutants [46••]. These differing results from gain-of-function studies in chick and loss-of-function analyses in mice suggest that these regulatory loops might not be linear, but instead could have parallel (possibly redundant) pathways or even species-specific differences. In any case, these regulatory cascades control retinal polarity and the expression of topographic axon guidance molecules, which are ultimately manifested in retinotopic mapping (Figure 5).

Figure 5



Regulation of dorsal–ventral retinal polarity. The schematic represents the expression patterns and interactions of most genes shown to be involved in establishing retinal polarity along the D–V axis from early (left) to late (right) time points. At early stages, ventroptin and BMP4 are established in the ventral and dorsal retina and can repress each other. BMP4 activates Tbx5 and represses Vax2 (cVax). Tbx5 activates ephrin-Bs and represses EphBs. Vax2 enhances the transcription of EphBs and represses ephrin-Bs. Although Tbx5 and cVax are able to repress each other when ectopically expressed in chick retina, their precise distributions suggest that they do not directly interact [55•]. EphBs and ephrin-Bs control L–M retinotopic mapping. Arrows represent transcriptional activation and T-bars transcriptional repression. The events depicted should not be assumed to be complete or represent direct interactions. The compass indicates the retinal axes.

The ectopic expression of several of the genes that regulate D–V patterning in chick retina and/or their knockout in mice cause D–V mapping defects that are consistent with their regulation of EphBs and ephrin-Bs (Figure 3). For example, ectopic expression of cVax in chick dorsal retina results in the expression of EphB3 and loss of ephrin-B1 and ephrin-B2 expression; coincident with this change in expression, some dorsal RGC axons aberrantly project more medially in the tectum [56]. Mice with a targeted deletion of Vax2 have a dorsalized retina [45•,46••]. This phenotype is characterized by the most severe D–V mapping defects reported so far [46••]. In Vax2 mouse mutants, the low-to-high D–V gradient of EphB2 and EphB3 expression flattens because of their decreased expression in the ventral retina. The high-to-low D–V graded expression of ephrin-B1 and ephrin-B2 flattens because of their increased expression in ventral retina [46••]. Thus, in many respects, the ventral retina is dorsalized in Vax2 mutants. Consistent with these findings, ventro-temporal RGC axons, which normally project to medio-anterior SC, show a complete lateral shift in their terminations in Vax2 mutants and have abberant projections to the latero-anterior SC [46••]. The defects in ventral–temporal (V–T) retinal mapping in Vax2 mutants [46••] are more severe than in EphB2/3 double mutant mice [10••], supporting models indicating that molecular activities in addition to EphB2/EphB3-mediated attraction are required to account for D–V mapping along the L–M axis [10••].

Ectopic expression of Tbx5 in chick ventral retina enhances the expression of the dorsal markers ephrin-B1 and ephrin-B2, and represses the expression of the ventral markers EphB2, EphB3, cVax and Pax2 [54••]. However, even with these broad changes in gene expression that are indicative of a dorsalization of ventral retina, the ectopic expression of Tbx5 caused ventral RGC axons to exhibit defects in topographic mapping in a subset of cases, and in addition an appropriately positioned TZ was apparent in these chicks [54••]. Conversely, the ectopic expression of ventroptin in chick dorsal retina represses BMP4 and Tbx5 expression and enhances cVax expression [53••]. A subset of dorsal RGC axons from domains of ectopic expression of ventroptin do not terminate in the normally positioned TZ but rather maintain a projection posterior to it. Additionally, some dorsal RGC axons have aberrantly medial trajectories. These aberrancies may be caused in part by cVax regulating the expression of EphBs and ephrin-Bs.

It appears as though the Vax genes have a more prominent role in D–V polarity than do the Tbx genes and ventroptin. The defects in the Vax2 mutant are the most severe D–V mapping defects yet reported, and

the ectopic expression of cVax in the chick results in more severe aberrancies than those in chicks in which Tbx5 or ventroptin is expressed ectopically. This evidence suggests that Vax may be involved in earlier stages of polarity determination, and therefore may have broader effects, including being more prominently involved in regulating crucial guidance molecules. Furthermore, other genes might not compensate for the loss of Vax2.

Bi-axial patterning of the retina

Several of the regulatory genes that control retinal polarity have differential expression patterns that are not limited to a single axis. Instead, they have graded expression patterns that are distinct from a pure D–V or T–N axial pattern, being more complex or oblique to the primary retinal axes. For example, Vax2 and ventroptin have a steep low-to-high D–V expression gradient and a shallow low-to-high T–N expression gradient [46••,53••]. The graded protein concentrations appear to have roles in regulating not only the D–V axis (as detailed above) but also the T–N axis as they affect the expression of EphAs and ephrin-As. Vax2 mutants have altered EphA5 expression [46••]; and ectopic expression of ventroptin enhances ephrin-A2 expression but, surprisingly, does not affect expression of ephrin-A5 or EphA3 [53••]. Furthermore, ectopic expression of ventroptin [53••] or Tbx5 [54••] in the chick retina results not only in D-V mapping defects but also in T-N mapping defects. In contrast, ectopic expression of cVax in chick [56] or deletion of Vax2 in mice [46••] reportedly results in only D–V mapping defects (Figure 3). Even more unexpectedly, in Vax2 mutants, the V–T retina exhibits a strong mapping defect whereas the mapping of the ventral–nasal (V–N) retina is fairly normal [46••]. This phenotype is the opposite of that predicted on the basis of Vax2 expression being stronger in V–N retina than in V–T retina. This therefore underscores the hypothesis that a set of transcription factors, including Vax2, cooperate in redundant and complementary ways to regulate the expression of D–V guidance molecules by RGCs.

Conclusions

Considerable progress has been made in the past few years in clarifying the mechanisms used by RGC axons to develop topographic maps in the OT/SC. This work has highlighted the crucial roles of interstitial branching along the A–P axis and directed branch extension along the L–M axis in establishing topographically organized arborizations. Recent findings have defined novel roles for EphAs and ephrin-As, including the regulation of topographic branching along the A–P axis. The long-awaited cloning of RGM has been achieved. In the past few months, the first reports of the molecular control of D–V RGC mapping along the L–M axis of the OT/SC have implicated ephrin-B reverse signaling and EphB forward signaling in mediating RGC axon or interstitial branch attraction, respectively. Other graded molecular activities that are required to cooperate with the Ephs and ephrins to generate topographic order along each set of axes remain unidentified. Recent gain-of-function studies in chicks and loss-of-function studies in mice have implicated several families of regulatory proteins in controlling the axial patterning of the retina. The most progress has been made in understanding the patterning of the D–V axis, and in particular a prominent role for the Vax homeodomain proteins has emerged. However, the current outline of the regulatory pathways is sketchy and much work remains to be done. Further analyses of these regulatory pathways and target genes will not only define the mechanisms of axial patterning in the retina, but will also identify candidate topographic guidance molecules that may cooperate with the Ephs and ephrins to control retinotopic mapping.

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The authors show the time course of development of the chick retinotectal map by injecting small amounts of DiI into the retina and describing in quantitative detail the initial posterior RGC axon overshoot of the TZ. In addition, the authors show that interstitial branches form most often in the A–P location of their future TZ, and arbors in the TZ are exclusively

elaborated by interstitial branches. *In vitro* experiments show that branch formation is inhibited by ephrin-As. Modeling indicates that the repellent action of ephrin-As on growth cones and interstitial branches cannot be the only activity that controls A–P retinotectal mapping. Ephrin-As are likely to cooperate with an attractant that is expressed in a similar pattern to that of ephrin-As or with a repellent that is expressed in a gradient opposite to that of ephrin-As in the tectum.

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The authors analyse EphB2/EphB3 double mutant mice and show that these receptors are required for the appropriate mapping of the D–V retinal axis. In EphB null mice, V–T RGC axons form both ectopic TZs in the lateral SC and normal TZs in the medial SC. Furthermore, the authors show that the crucial determinant of D–V retinal mapping is the appropriate guidance of interstitial branches along the L–M SC axis. In addition, small DiI injections in V–T retina and subsequent scoring of interstitial branch guidance shows that the medial guidance of interstitial branches, along the low-to-high L–M gradient of ephrin-B1, is disrupted in mutant mice. The defects observed are equivalent or more severe in mice that have a form of EphB2 (EphB2-LacZ) that does not have forward signaling but retains reverse signaling, thereby indicating that the defects observed are caused by forward signaling. Modeling of the defects indicates that ephrin-B1 acts as an attractant to guide interstitial branches medially. Modeling also suggests that a repellent activity cooperates with ephrin-B1 to guide branches laterally, and that this second activity is distributed with the same gradient as ephrin-B1.

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The authors examine the role of Sema3a in the guidance of *Xenopus* RGC axons. The response to Sema3a is stage-dependent and results in the collapse or turning of the growth cone. Sema3a-mediated growth cone collapse results in terminal branching that is highly reminiscent of backbranching, a process normally observed in retinotopic map formation. The stage-dependent actions of Sema3a, and the elicited branching, suggest multiple roles for this guidance molecule in topographic map development. Sema3a may contribute to the guidance of axons posteriorly from the contralateral telencephalon, the collapse of their growth cones in the tectum, and the induction of backbranching.

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This study extends the work by Frisen *et al.* [24] that first reported an ephrin-A knockout mouse, and demonstrated the required role for ephrin-A5 in mapping the T–N retinal axis along the A–P SC axis. The authors show that ephrin-A2 is required for A–P mapping, and also that mapping defects are exaggerated in ephrin-A2/ephrin-A5 double-knockout mice. Both temporal and nasal RGCs exhibit mapping defects in ephrin-A knockout mice, and the ectopic terminations of nasal RGCs are found anterior to their correct A–P sites, supporting a role for competitive interactions in mapping (also see [32••]).

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The authors report the long-anticipated cloning of chick RGM. Initially, RGM activity was the first to differentially affect nasal and temporal RGC axons. The authors describe the sequence of RGM, which has no homology to any known guidance molecule. RGM is expressed in a low-to-high A–P gradient in the tectum. The authors also confirm that RGM is a topographic guidance molecule that preferentially causes the growth cones of temporal but not nasal RGC axons to collapse. Furthermore, RGC specifically repels temporal RGC axons in the stripe assay.

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The authors use a gain-of-function genetic strategy in mice to address the assumption that the T–N graded repellent response of RGC axons to ephrin-As during mapping is mediated by graded EphA receptors. In the chick, RGCs express EphA3 in a high-to-low T–N gradient. In contrast, the authors show that RGCs in mice do not express EphA3 but do express EphA5 and EphA6 in a high-to-low T–N gradient. They generated mice in which an IRES-EphA3 cDNA construct was 'knocked-in' to the 3′ UTR of the Isl2 gene, leaving intact the function of Isl2, a LIM homeodomain protein expressed by a subset of RGCs scattered across the retina. In the knock-in mice, the RGCs that express Isl2 also express EphA3, resulting in a unique RGC population with an elevated level of overall EphA receptor expression intermingled with the wild type population of RGCs

expressing the normal gradient of EphA receptors. The two RGC populations formed independent orderly maps in the SC, with the Isl2–EphA3 RGC map compressed to anterior SC, and the wild type RGC map compressed to posterior SC. These findings indicate that EphAs do indeed mediate RGC axon repulsion, but that relative, rather than absolute, levels of EphA repellent signaling between RGC axons control their A–P mapping. The posterior shift in the projections of wild type RGCs is likely to have been caused by competitive interactions with the anteriorly shifted Isl2–EphA3 RGCs. When generating topographic maps, EphA repellent signaling dominates over activity-dependent mechanisms that are based on neighbor relationships.

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This paper describes defects in Vax2 mutant mice. The authors report an early D–V marker in retina (Tbx5) is unchanged, whereas late markers (EphB2, EphB3, ephrin-B1, ephrin-B2) have dorsalized expression patterns. In contrast to Barbieri *et al.* [45•] these investigators find no incidence of coloboma, apparently normal ipsilateral projection, and a flattened gradient of EphA5 expression along the T–N retinal axis. This latter phenotype is unexpected, but is consistent with in situ analyses revealing that Vax2 is expressed in a shallow high to low T–N gradient in addition to its strong D–V gradient. Finally, a careful point-to-point mapping of the retina to the SC shows that V–T RGC axons aberrantly map exclusively to lateral SC, ventral RGC axons map to both medial and lateral SC, and N–V RGC axons map essentially normally. This unexpected phenotype led the authors to propose a model in which Vax2 regulates D–V retinal polarity in cooperation with one or more regulatory genes to control the expression of axon guidance molecules along both the D–V and T–N retinal axes.

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The authors utilise several genetic manipulations of the Pax6 gene to dissect its functional modalities. The α enhancer controls expression of Pax6 in temporal and nasal retina, whereas other regulatory elements control expression in central retina, lending insight into the dynamic expression of Pax6 throughout retinal development. In addition, $Pax6^{-/-}$ mice have altered retinal expression patterns for genes that are expressed along both retinal axes. In Pax6 knockout mice, the expression pattern of Vax2 is expanded and the expression of Tbx5 is reduced. Furthermore, both the onset and maintenance of expression of BF1 and BF2 requires Pax6.

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The authors examine the functions of two homeobox genes, SOHo1 and GH6, expressed in a low to high T–N gradient in the retina. Retroviral misexpression of these genes reveals that the expression of EphA3 is specifically repressed in domains of ectopic expression in the retina. Surprisingly, the expression of EphA3 is not affected in ectopic domains of SOHo1 or GH6 in the brain. The expression of many other Eph receptors and ephrins is not affected by misexpression of SOHo1 or GH6. In addition, BF1 and BF2 do not appear to genetically interact with SOHo1 or GH6, indicating that key players in the establishment of T–N retinal polarity are unknown. Misexpression of SOHo1 or GH6 results in topographic map defects consistent with a reduction in EphA3 expression.

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•• The authors report the cloning of a BMP antagonist expressed in a high-to-low N–V to D–T gradient. They show, with retroviral overexpression, that ventroptin can repress the expression of the dorsal genes BMP4 and Tbx5 and enhance the expression of the ventral gene cVax. Retroviral overexpression of ventroptin also results in retinotectal mapping defects. The ventral retina of chicks that have been electroporated with ventroptin shows defects in mapping along the A–P and L–M tectal axes. In each case, a normal appearing TZ is evident in addition to posterior arborizations and possibly aberrantly lateral RGC axonal pathways. The expression of the nasal marker ephrin-A2 is enhanced in temporal retina by ventroptin overexpression.

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•• The authors use retroviral misexpression studies to analyze the Tbx5 gene in chick retinal development. Tbx5 is expressed in dorsal retina, and when misexpressed throughout retina it represses ventral markers such as cVax, Pax2, EphB2 and EphB3. In addition, misexpression induces the expression of the dorsal markers ephrin-B1 and ephrin-B2. Tbx5 misexpression also results in retinotectal mapping defects consistent with a dorsalization of retina. Furthermore, misexpression of BMP4 also dorsalizes the expression of several genes, perhaps by enhancing Tbx5 expression.

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• The authors show the precise distributions, and the relationships between the distributions, of multiple genes expressed along the D–V retinal axis. Using very high quality double *in situs* on flat-mounted chick retina, the authors demonstrate the relationships among known D–V markers, such as cVax, BMP4, Tbx5, EphB2, EphB3, ephrin-B1, and ephrin-B2. Importantly, the authors demonstrate a gap between the ventral expression of cVax and the dorsal expression of Tbx5, which would strongly suggest that these genes do not directly co-regulate each other. These markers can be divided into at least four zones along the D–V axis. In addition, the authors perform a clonal analysis of retinal cells that uses a replication incompetent retrovirus that expresses alkaline phosphatase to show that retinal cells rarely cross the expression boundaries of these markers. The authors suggest that EphBs and ephrin-Bs may mediate these affects.

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