The Multiple Decisions Made by Growth Cones of RGCs as They Navigate from the Retina to the Tectum in Xenopus Embryos

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ABSTRACT: Retinal ganglion cells (RGCs) of Xenopus laevis send axons along a stereospecific pathway from the retina to their target the optic tectum. Viewed from the point of the growth cone, this journey is reflected by discrete processes of axon initiation, axon outgrowth, navigation, target recognition, and innervation. These processes are characterised by distinct signalling mechanisms that trigger dynamic changes in growth cone morphology and behavior. Here we review work primarily from our laboratory, examining these events from a cellular and molecular perspective, focusing on the roles of FGFs, netrins, receptors, and intracellular effectors.

The growth of retinal ganglion cell (RGC) axons to their tectal targets is perhaps one of the best understood cases of axon navigation over an entire pathway. Decades of work from many laboratories have yielded insights into both the cellular and molecular mechanisms that regulate growth cone guidance in the retinotectal system, from the initiation of axonogenesis, to axon outgrowth and pathfinding, to target recognition, and finally to topographical map formation (Hynes and Lander, 1992; Holt and Harris, 1993; Chien and Harris, 1994; Tessier-Lavigne and Goodman, 1996). We discuss this journey from the viewpoint of the RGC growth cone: what it senses and how it responds. In the retinotectal system we have additional information about synapse formation and activity-dependent plasticity of connections in the tectum (see review by Holt and Harris, 1993; Zhang et al., 1998), but because these processes are not within the realm of the growth cone, they are not part of this review.

AXONOGENESIS AND GROWTH CONE EXTENSION

RGCs in the dorsocentral Xenopus retina are the first to extend axons beginning around stage 28, and axonogenesis continues in a dorsal–ventral gradient (Holt, 1984, 1989). Axonogenesis is first evident as a polarized thickening of the plasma membrane close to or at the vitreal surface of RGCs [Holt, 1989; Fig. 1(A)]. In some cases axons appear from other parts of the cell or from a primary dendrite shaft. Once axons reach the vitreal surface, they immediately orient and extend along the ganglion cell fiber layer toward the optic nerve head [Fig. 1(B)], where they leave the eye and join the optic nerve [Fig. 1(C)].
Figure 1  Development of the *Xenopus* visual system. (A) Transverse sections show the position and growth cone dynamics of pioneering axons from the right eye. RGCs from the dorsocentral retina are the first to extend axons beginning at stage 28. (B) These pioneering axons grow ventrally close to the vitreal surface of the eye toward the ONH. (C) At the ONH, growth cones become highly complex as they dive down into the ON. (D) Growth cones appear torpedo shaped as they grow along the ON. (E) Growth cones then adopt a complex morphology when they reach the ventral diencephalon, at the brain entry point. At stage 32, growth cones cross over to the contralateral side of the brain at the optic chiasm (F) and then navigate in the neuroepithelium close to the pial surface. Axons first reach the mid–optic tract by stage 35/36, where they make a caudal turn that reorients them with the tectum (G). The first growth cones reach the anterior tectum by stage 39 and undergo dramatic morphological changes (H). Within the tectum, terminal arbors develop that form a topographical map (I). ONH, optic nerve head; ON, optic nerve; vs, vitreal surface; ps, pial surface.
Integrin Signaling

The integrin complex, a heterodimer of α and β subunits, is a structural component of focal adhesions and acts as a link between the plasma membrane and the extracellular matrix (ECM; Hynes, 1992; Aota and Yamada, 1997). Expression of a variety of chimeric integrins, which are either impaired in β1 subunit binding to the RGD site in fibronectin or fail to localize to focal adhesions, severely compromise the initiation of RGC axons and their extension within the retina (Lilienbaum et al., 1995). These data suggest that cell adhesion to the ECM via β1 integrins is required for normal axon outgrowth. However, although substrate-adhesion is essential for generating the forces required for axon outgrowth, simple cell–cell or cell–ECM adhesion may not be sufficient for promoting axonogenesis. Adhesion is poorly correlated with stimulating neurite growth in culture (Gunderson, 1987), and numerous CAMs can promote adhesion but do not stimulate growth (Gunderson, 1987; Hall et al., 1987; Lemmon et al., 1992). Together, these results suggest that there are additional signaling events involved in CAM-mediated axonogenesis.

Focal adhesions are not only sites that couple the plasma membrane with the cytoskeleton, but are also macromolecular signaling complexes (Giancotti and Ruoslahti, 1999; Fig. 3). Composed of both structural (e.g., cytoskeleton-binding) and signaling proteins, focal adhesions play important roles in a variety of cellular processes (e.g., cell cycle, cell migration, and anoikis; Aota and Yamada, 1997; Giancotti and Ruoslahti, 1999). Key components of the focal adhesion signaling complex are the cytoplasmic tyrosine kinases (TKs), pp60c-src and pp125FAK (focal adhesion kinase). Both TKs play important roles in regulating the array of extracellular signals elicited by integrins, neuropeptides, and growth factor receptors. They are therefore prime candidates for mediating either integrin- or CAM-dependent axonogenesis.

FAK is strongly expressed in RGC neurites, growth cones, and filopodia in a punctate pattern that colocalizes with phosphotyrosine immunolabelling (Worley and Holt, 1996). Herbimycin A (HA), a TK inhibitor, reversibly inhibits axon extension from RGC in culture and in vivo (Worley and Holt, 1996a,b). In addition, HA treatment results in the redistribution of FAK from filopodia into a more diffuse pattern within the center of the growth cone (Worley and Holt, 1996b). Likewise, src is highly expressed in RGCs and within the optic nerve, and its function is correlated with neurite outgrowth (Maness et al., 1988, 1990; Cox and Maness, 1991; Bixby and Jhabvala, 1993). A constitutively active form of src induces neurite outgrowth when expressed in PC12 cells (Cox and Maness, 1991). In cerebellar neurons derived from either src−/− or fyn−/− mice, neurite outgrowth is severely impaired when plated on certain CAMs, suggesting that src acts downstream of CAM binding (Beggs et al., 1994; Ignelzi et al., 1994).

In the Xenopus visual system, expression of constitutively active (ca) forms of either c-src or its neuronal splice variant n-src severely impairs axonogenesis and subsequent axon outgrowth (Worley et al., 1997). For ca c-src, up to 85% of the RGC axons terminate at the optic nerve head (ONH). A direct link between src and FAK function in mediating CAM-dependent axon outgrowth has yet to be shown, although recent evidence suggests that src-dependent phosphorylation of cellular substrates depends on FAK activity (Schaller et al., 1999). Therefore, FAK activation following integrin binding in RGCs may recruit src TKs to focal adhesions, and thereby regulate phosphorylation of cytoskeletal proteins required for initiating and maintaining axon outgrowth (Fig. 3).

CAM Signaling

Cadherin-dependent axon outgrowth appears to involve a signaling mechanism in addition to cell adhesion. Neural or N-cadherin is a 130-kD, type 1 membrane glycoprotein that mediates Ca2+-dependent cell–cell adhesion (reviewed in Shapiro and Colman, 1998) and promotes neurite outgrowth from chick ciliary ganglion neurons and Xenopus RGCs (Tomasselli et al., 1988; Riehl et al., 1996). The cytoplasmic domain of N-cadherin is coupled to the actin cytoskeleton via interactions with catenins. However, the outgrowth-promoting property of N-cadherin in vivo does not require the catenin binding domain, suggesting that a separate region of the c-terminus regulates axon outgrowth (Riehl et al., 1996). Expression of the juxtamembrane region of N-cadherin, which lacks the
Figure 2  Some of the molecules that regulate RGC growth cones dynamics during the development of the Xenopus visual system. Axonogenesis occurs at the vitreal surface of RGCs. Outgrowth of axons is then stimulated by a variety of molecules within the retina and optic tract. At key points along the visual pathway, growth cones make specific navigational choices leading to a change in their direction of growth. Once growth cones have reached their target, they undergo a series of dynamic morphological changes including the loss of the primary growth cone, and the development of backbranches along the axon shaft. Within the target, terminal arbor formation begins in which appropriate topographical choices are made.
The catenin binding domain, is a potent inhibitor of axon outgrowth, yet has little or no effect on epithelial cell–cell adhesion (Riehl et al., 1996). This indicates that there may be protein–protein interactions with the juxtamembrane region of N-cadherin and other cytoplasmic proteins. The juxtamembrane region of N-cadherin binds to the cytosolic protein p120ctn, a newly discovered member of the catenin family. The function of p120ctn remains to be elucidated although in epithelial cells it may play a role in regulating the strength of cadherin-based adhesion (reviewed in Provost and Rimm, 1999). Overexpression of p120ctn in fibroblasts results in a remarkable phenotype characterized by the induction of long filopodial-like processes (Reynolds et al., 1996). Therefore, it is tempting to speculate that p120ctn (originally identified as a substrate for c-src) may be an important component of the N-cadherin–mediated axon-outgrowth signal (Fig. 3).

**Growth Factors**

In addition to CAMs and ECM, axon initiation and outgrowth in the visual system depends on growth factor receptors, such as the fibroblast growth factor receptor [FGFR; Fig. 2(A,B)]. RGC growth cones express FGFRs, and axon outgrowth is strongly stimulated by FGFR2 (McFarlane et al., 1995). FGFRs are also abundantly expressed along the length of the optic pathway (McFarlane et al., 1995), suggesting that FGF and the FGFR are involved in stimulating axon outgrowth. When a dominant negative form of the FGFR, XFD, is expressed in RGCs, axon outgrowth is significantly impaired, such that axons advance at only 60% of the normal speed (McFarlane et al., 1996). XFD, however, has no effect on axonogenesis. In contrast, expression of the FGFR mutant HAVΔφ, which is unable to bind FGF and therefore cannot act as a dominant negative (Amaya et al., 1993), inhibits axonogenesis in vivo, suggesting that a non-FGF ligand is involved. The identity of the non-FGF ligand(s) remains to be identified.

CAMs may represent a family of non-FGF FGFR ligands involved in promoting axon outgrowth [Fig. 2(B)]. In culture, the CAMs L1, N-CAM, and N-cadherin, exert potent axon outgrowth-promoting activity in an FGFR-dependent manner (Doherty and Walsh, 1996; Lom et al., 1998; Fig. 3). Although a physical association of the FGFR and CAMs has yet to be shown, the CAM homology domains (CHD) may be involved in heterophilic protein–protein interactions between the FGFR and CAMs. Peptides corresponding the CHD domains inhibit FGFR-dependent CAM-stimulated axon outgrowth (Williams et al., 1994).

Thus, at least three FGFR-dependent signals are elicited in the RGC growth cone. First, non-FGF ligand activation is required for axonogenesis, which appears to be distinct from the second CAM-mediated, FGFR-dependent stimulation of axon outgrowth. Finally, there is third, FGF-dependent FGFR activation of axon extension. How can three FGFR-dependent signals elicit several different growth cone responses? First, cellular responses to FGF signaling vary, depending on the spatial and temporal expression of both ligand and receptor (Klint and Claesson-Welsh, 1999). Three different FGFRs are expressed in the developing retina at a time when axonogenesis is occurring (McFarlane et al., 1998) and therefore could...
Rho Family of GTPases

Stimulation of axonogenesis and growth cone advance, whether through CAMs, the ECM, or growth factor receptors, must at some point act through cytoplasmic effectors in order to modify the actin cytoskeleton (Figs. 2 and 3). The prime candidates are the Rho family of small GTPases. The Rho-dependent control of the actin cytoskeleton has been well characterized in fibroblasts and is now being investigated in neurons (Luo et al., 1997). Axonogenesis in the Xenopus visual system is particularly sensitive to the unregulated activity of the Rho GTPases. Expression of constitutively active forms of RhoA, Rac1, and Cdc42 essentially eliminates RGC axons (Ruchhoeft et al., 1999). However, in RGCs only Rac1 function appears to be necessary for axonogenesis, because only expression of a dominant negative Rac1 is able to inhibit axon initiation (Ruchhoeft et al., 1999). This is similar to observations made in Drosophila (Luo et al., 1994). A possible effector of Rac1 in regulating axonogenesis is the complex of cyclin-dependent kinase 5 (cdk5) and its regulatory subunit p35 (Nikolic et al., 1998). Cdk5, originally isolated through its structural homology to human Cdc2, is abundantly expressed in the nervous system, with a staining pattern similar to actin filaments (Nikolic et al., 1996). In addition, the cdk5/p35 complex colocalizes with Rac1 in growth cones (Nikolic et al., 1996). The dn cdk5 is able to partially rescue axonogenesis in ca Rac1 expressing RGCs (Ruchhoeft et al., 1999). Because growth cones appear to be sensitive to perturbations of Rho-family GTPase activity, the dn Cdk5 likely compensates for the ca Rac1, thus bringing Rac1 activity to a level amenable to the formation and extension of axons.

Rac, and possibly Cdc42, antagonize Rho activity by promoting the phosphorylation of myosin-II heavy chain (MLC; van Leeuwen et al., 1999). Non–muscle myosin-II is found in an actinomyosin cytoskeletal complex that is rapidly remodeled during growth cone advance and retraction. Activation of myosin-II by Rho-dependent kinases leads to actinomyosin contraction and growth cone retraction (Burridge and Chrzanowska-Wodnicka, 1996). Rac-mediated phosphorylation of MHC causes the disassembly of myosin-II from the actinomyosin complexes and cell spreading (van Leeuwen et al., 1999). In contrast, Rho promotes myosin-II activity by stimulating the phosphorylation of myosin-II regulatory light chain (MLC; Amano et al., 1996; Kimura et al., 1996). In Xenopus axons, disruption of myosin–actin interactions or inhibition of MLC kinase (MLCK), causes growth cone collapse and stalling, suggesting that myosin-II activity is required for growth cone motility (Ruchhoeft and Harris, 1997). Therefore, actinomyosin dynamics may be finely regulated by a series of phosphorylation events of MLC and MHC by the Rho GTPases.

Overexpression of Rho GTPases in RGC axons also induces a series of dramatic changes in growth cone morphologies (Ruchhoeft et al., 1999). Expression of and RhoA induces the formation of abnormal, thickened filopodia with a balled appearance, and reduces the area of the growth cone. Consistent with its role in antagonizing Rho activity (reviewed in Lim et al., 1996), expression of wt Cdc42 increases the size and complexity of growth cones and induces the formation of backbranches along the axon shaft. There is also a decrease in the rate of axon extension. In contrast, expression of a dn form of Cdc42 reduces the overall complexity resulting in growth cones with smaller areas and decreases in the number of filopodia, suggesting that RhoA activity may be increased in these growth cones.
target derived cues. Demonstrate that isolated growth cones possess the branches [Fig. 1(H); see below]. Together these data including a decrease in speed and the initiation of back-ward reorientation of the growth cone. One of the most dramatic changes in the morphology of RGC growth cones occurs when axons leave the ganglion cell fiber layer and enter the optic nerve head [ONH; Fig. 1(C)], to join the optic nerve. A prime candidate for promoting this turn toward the ONH is the soluble chemoattractant netrin-1 (de la Torre et al., 1997; Deiner et al., 1997; Hopker et al., 1999). Netrin-1 is expressed in the optic disk of a variety of vertebrates, including fish, rodent, and chick (Kennedy et al., 1994; Deiner et al., 1997; Lauderdale et al., 1997; Livesey and Hunt, 1997; Strahle et al., 1997). Likewise, in Xenopus, RNA in situ hybridiza-

Role of Filopodia

Filopodia are thought to play numerous roles during growth cone guidance ranging from motor, steering, and sensory functions (Kater and Rehder, 1995). Therefore, the increased complexity of RGC growth cones at choice points may reflect the importance of filopodia in growth cone guidance. To address this question, the ability of RGC growth cones lacking filopodia to make correct pathway decisions has been tested (Chien et al., 1993). Using an exposed brain preparation, it is possible to treat the developing optic tract with cytochalasin B (CB) at a time when growth cones have crossed the chiasm and have entered the contralateral ventral optic tract. DII-labeled growth cones treated with CB lack filopodia, yet have active lamellipodia. CB-treated axons have a decrease in the rate of growth. In addition, there is a general disorganization of the optic tract in CB-exposed brains. Normally, when growth cones reach the mid-diencephalon, they make a 50° turn posteriorly, which realigns them with the optic tectum [Harris et al., 1987; Fig. 1(G)]. The CB-treated growth cones, however, fail to reorient and instead continue to grow dorsally toward the pineal.

The ability of growth cones to detect and respond to their environment is an integral trait of growth cones themselves. In Xenopus, RGC axons severed from their soma are fully capable of growing and responding appropriately to cues along the optic tract (Harris et al., 1987). HRP-labeled axons isolated from their soma by removing the retina continue to grow and navigate appropriately for up to 4 h. Severed axons do not stray from their normal trajectory, and if growth cones are close enough to their target, they undergo the characteristic morphological and behavioral changes associated with target recognition, including a decrease in speed and the initiation of back-branches [Fig. 1(H); see below]. Together these data demonstrate that isolated growth cones possess the means necessary to detect and respond to pathway and target derived cues.

Exiting the Eye

One of the most dramatic changes in the morphology of RGC growth cones occurs when axons leave the ganglion cell fiber layer and enter the optic nerve head [ONH; Fig. 1(C)], to join the optic nerve. A prime candidate for promoting this turn toward the ONH is the soluble chemoattractant netrin-1 (de la Torre et al., 1997; Deiner et al., 1997; Hopker et al., 1999). Netrin-1 is expressed in the optic disk of a variety of vertebrates, including fish, rodent, and chick (Kennedy et al., 1994; Deiner et al., 1997; Lauderdale et al., 1997; Livesey and Hunt, 1997; Strahle et al., 1997). Likewise, in Xenopus, RNA in situ hybridiza-
tion experiments show that netrin-1 is expressed in the ONH and optic nerve (ON; de la Torre et al., 1997; Fig. 4). The receptor for netrin-1, Deleted in colorectal cancer (DCC), is also expressed in RGC axons (de la Torre et al., 1997), consistent with a role for netrin-1 in mediating the exit of growth cones from the eye. To address whether netrin-1 alone could cause growth cone turning, the effect of soluble netrin-1 gradients on RGC growth cone behavior have been examined in culture using the growth cone turning assay (Lohof et al., 1992). In this assay, growth cones are subjected to a stable gradient of soluble chemoattractant produced following its pulsatile release from a small glass pipette. This rapidly establishes a gradient in the order of a 1000-fold between the pipette tip and the growth cone. When subjected to a netrin-1 gradient, RGC growth cones specifically orient in a DCC-dependent manner toward the source of netrin-1 (de la Torre et al., 1997). In addition, application of netrin-1 to cultures produces a dramatic change in growth cone morphology, reminiscent of the complex growth cones observed in vivo at the ONH [de la Torre et al., 1997; Holt, 1989; Fig. 1(C)]. The turning response by the growth cone toward a netrin-1 gradient is converted to a repulsive response by the depletion of intracellular cAMP or the inhibition of protein kinase A (PKA; Ming et al., 1997). These findings place netrin-1 within the group I of soluble chemoattractant molecules (Song and Poo, 1999). Netrin-1, brain-derived neurotropic factor (BDNF), acetylcholine (ACh), and myelin-associated glycoprotein (MAG) are included in group I because the direction of their induced turns depends on the level of cytosolic cAMP, the activity of PKA, and the presence of extracellular Ca$^{2+}$. Growth cone responses to group II members, which include semaphorin III (SemaIII) and NT-3, on the other hand, are independent of Ca$^{2+}$ and are regulated by cytosolic cGMP and PKG.

**ECM Modulation of Netrin Response**

The conversion of growth cone attraction to repulsion may play a significant role during the exit of growth cones through the ONH [Hopker et al., 1999; Figs. 1(C) and 4]. As described above, growth cones in culture turn toward a gradient of netrin-1; however, if the same growth cones are cultured on the ECM substrate laminin, the turning response is converted to repulsion (Hopker et al., 1999). Repulsion to a netrin-1 gradient can be inhibited if growth cones are treated with function-blocking antibodies against $\beta_1$ integrins, a component of the laminin receptor $\alpha_x\beta_1$ integrin complex. This strongly suggests that $\beta_1$ integrins are involved in changing the growth cone response to netrin gradients. A soluble peptide fragment of the laminin-1 B1 chain YIGSR, which mediates cell attachment but has no neurite outgrowth promoting activity, mimics the laminin-induced switch from attraction to repulsion. Surprisingly, however, a 19-amino acid peptide containing the IKVAV sequence from the laminin-1 A1 chain, which promotes neurite formation and outgrowth (Sephel et al., 1989), has no effect on the turning response. Function-blocking antibodies to the 67-kD laminin receptor, which binds to the YIGSR peptide and may function to stabilize laminin binding to integrin receptors, are able to block both laminin and YIGSR effects on growth cone turning. Application of Sp-cAMPS (a cAMP analogue
that activates PKA) abolishes the YIGSR effect on growth cone turning. Likewise, YIGSR-treated growth cones show a net decrease in cAMP levels compared to growth cones treated with netrin alone. Therefore, the B1 chain of laminin through the YIGSR peptide may be responsible for mediating the conversion in growth cone responses.

To address whether laminin and specifically the YIGSR peptide could affect growth cone dynamics in vivo, developing retinas have been treated with YIGSR peptides (Hopker et al., 1999) at the time when RGC are first extending axons across the vitreal surface. In contrast to control-treated eyes in which axons converge at the ONH into a single large fascicle that exits the eye [Fig. 1(C)], RGC axons in YIGSR-treated eyes form disorganized fascicles that in many cases fail to exit the eye. Together, these data suggest that laminin and its receptors play a critical role in regulating the orderly arrangement and navigation of RGC axons at the ONH.

A question that has remained unanswered for some time is how are axons able to turn away from the vitreal surface and enter the ONH (Fig. 1). These results suggest that growth cone responses to a netrin gradient may be involved in mediating this event. As discussed previously, the response of RGC axons to a netrin gradient can be either attractive or repulsive, depending on the level of intracellular cAMP. In the case of group I chemotropic agents such as netrin, high cAMP levels lead to actin polymerization, whereas low levels of cAMP trigger actin depolymerization. By manipulating the levels of cAMP across a growth cone, it is then possible to control the level of actin polymerization/depolymerization and cause turning toward or away from a guidance cue. In fact, an increase in the concentration of intracellular cAMP of only 10% across a growth cone is sufficient to induce attractive turning (Lohof et al., 1992). At the ONH, laminin-1 is localized to the vitreous surface (Hopker et al., 1999), whereas netrin-1 is expressed abundantly throughout the ONH (de la Torre et al., 1997; Fig. 4). Thus, RGC growth cones reaching the ONH are confronted with two opposing signals that could establish an intracellular cAMP gradient. Low levels of cAMP could be established in the growth cone at the vitreal surface by netrin/laminin responses, whereas high concentrations of cAMP could develop on the ONH side in the presence of netrin only. Together, the difference in cAMP concentrations across the growth cone could cause an increase in actin polymerization on the ONH side and depolymerization close to the vitreal surface, thus promoting a turn away from the fiber layer and growth into the ONH (Fig. 4).

### Ephrin-B–Dependent Turning at the Optic Chiasm

In the premetamorphic tadpole, all RGC axons cross at the optic chiasm and project to the contralateral side of the brain [Fig. 1(F)]. However, during metamorphosis in which the tadpole undergoes extensive external changes, an ipsilateral retinothalamic projection arises from a subpopulation of newly born cells in the temporal and ventral retina (Hoskins and Grobstein, 1985a,b). This ipsilateral projection does not arise if development is stalled at a premetamorphic stage (Hoskins and Grobstein, 1984, 1985c). Moreover, transplants of postmetamorphic ventral retina, which would normally project ipsilaterally, into retinas of young embryos do not develop an ipsilateral projection (Nakagawa et al., 2000). Together, these data suggest that early tadpoles do not express the appropriate ipsilateral guidance cue(s) at the chiasm.

The Eph receptors and their ligands, the ephrins, are required for a variety of developmental processes including the appropriate topographic mapping of RGC axons to the optic tectum (Cheng et al., 1995; Drescher et al., 1995). Because Eph receptors are expressed at high levels in the ventrotemporal retina (Cheng et al., 1995; Drescher et al., 1995), Eph–ephrin interactions at the chiasm could account for the ipsilateral projection. Indeed, there is a stage-dependent expression of ephrin-B at the chiasm. Ephrin-B expression rises sharply following metamorphosis (stage 60) and is not detected in the chiasm of premetamorphic tadpoles (stage 54; Nakagawa et al., 2000). Targeted expression of ephrin-B2 in the chiasm of premetamorphic tadpoles is sufficient to induce an ectopic ipsilateral retinal projection that is restricted to the EphB-expressing population of RGCs growth cones from the ventral retina (Nakagawa et al., 2000). Thus, the stage-dependent EphB and ephrin-B interaction at the chiasm can generate an ipsilateral projection that is essential for establishing binocular vision in the adult frog.

### Growth Cone Turning in the Optic Tract

The development of the visual projection depends on an orderly advance RGC growth cones. This is achieved by an integrated response of the growth cone to a variety environmental cues acting either from a distance through diffusion gradients or by local short-range effects that include cell–cell interactions [Tessier-Lavigne and Goodman, 1996; Fig. 2(C)]. In the Xenopus visual system, both types of cues appear to be important for growth cone turning at choice points. The turn at the mid–optic tract, where growth cones turn caudally to reorient themselves with their
Changes in Growth Cone Dynamics

During axon growth along the optic tract, growth cones advance at a constant rate of approximately 50 μm h⁻¹ (Harris et al., 1987). However, when they reach the border of their target, the optic tectum, growth cones undergo a rapid and remarkable change in behavior [Harris et al., 1987; Fig. 1(H,I)]. Their growth rate decreases significantly to about 15 μm h⁻¹, and they lose their characteristic morphology, becoming highly complex and elongated with lamellipoedia and filopodia extending in all directions, essentially adopting a “hairy” appearance (Harris et al., 1987; Holt, 1989). Growth cones also begin to meander through the tectum with sharp turns, in marked contrast to the smooth trajectories observed while axons are growing across the forebrain (Harris et al., 1987). Target innervation also triggers the development of small side (terminal) branches along the axon shaft, leading to the development of a complex network of terminal arbors [Sakaguchi and Murphy, 1985; Harris et al., 1987; Holt, 1989; Fig. 1(I)]. The primary axon always advances by way of a single growth cone, although growth cones are rarely observed on the secondary branches (Harris et al., 1987). However, once terminal branching begins, the appearance of the primary growth cone changes to resemble a side branch (Harris et al., 1987).

FGF

What triggers these dramatic changes in behavior? One possibility is a switch in the extracellular environment to one that does not favor axon growth. Laminin, for example, is expressed only along the optic tract during the time that RGCs are extending toward the tectum (Cohen et al., 1987). At later stages, when cultured in vitro, these axons lose their response to laminin in a target-dependent manner (Cohen et al., 1986, 1989). Thus, a change in laminin expression and/or growth cone response to laminin at the tectal border may trigger a change in axon growth rate.

In the *Xenopus* visual system, the growth factor FGF2 together with FGF2-binding heparan sulfate proteoglycan sidechains (HS) are potent stimulators of RGC axon outgrowth [McFarlane et al., 1995; Walz et al., 1997; Fig. 2(B)]. Both molecules are highly expressed along the optic tract, but their levels drop off significantly within the anterior tectum (McFarlane et al., 1995; Walz et al., 1997). Exogenous application of either FGF2 or HS to the developing optic tract causes a severe mistargeting of retinal growth cones in which axons grow dorsally or ventrally around their target (McFarlane et al., 1995; Walz et al., 1997). These data are consistent with a model in which a change in the rate of axon outgrowth at the target border is critical for target recognition and innervation. Because FGF2 is a potent stimulator of axon outgrowth, the continued stimulation by FGF2 may make the growth cones insensitive to cues within their target. However, experiments in which RGC axons express a dominant negative FGFR, XFD, argue against this hypothesis. XFD-expressing axons are significantly impaired in their ability to grow, yet they are nevertheless able to navigate appropriately along the optic pathway (McFarlane et al., 1996). However, when reaching the tectal border, many of these axons veer around rather than entering their target. In contrast, growth cones expressing nonfunctional FGFRs (i.e., that cannot act as dominant negatives nor stimulate FGFR activity), enter the tectum normally (McFarlane et al., 1996). This clearly shows that FGFR signaling is required for target recognition, yet the evidence appears paradoxical, because either sustained increases or decreases in FGFR signaling...
result in similar targeting defects. One simple model to explain these observations is that during target recognition, growth cones sense a change in FGFR signaling, likely from high to low, which then triggers their morphological and behavioral changes. Such growth cones switch from active growth to arborization [Figs. 1(H) and 3].

**FGFR Signalling**

FGFRs signal through a multitude of secondary pathways (e.g., src, PLCγ, ras/raf/MAPK; reviewed in Klint and Claesson-Welsh, 1999). During growth cone extension, FGFR signaling whether stimulated via FGF2 or CAMs appears to be mediated by the activation of the phospholipase C-γ (PLCγ) pathway [Doherty and Walsh, 1996; Lom et al., 1998; Figs. 2(B,D) and 3]. Pharmacological inhibition of the PLCγ pathway significantly reduces FGFR-dependent neurite outgrowth in culture (Saffell et al., 1997). Likewise, inhibition of PLCγ activity in *Xenopus* embryos using the exposed brain preparation reversibly impairs RGC axon outgrowth; yet, significantly, there is no effect on target recognition (Lom et al., 1998). Therefore, target recognition and innervation probably involve an FGFR-dependent signaling cascade that does not include PLCγ. Activated FGFRs in *Xenopus* associate with a number of adapter/effector molecules (SH2/SH3 domain proteins) that link activated receptors to downstream second-messenger cascades (Anderson et al., 1990; Klint and Claesson-Welsh, 1999: Fig. 3). Adapters that are known to associate with the FGFR in *Xenopus* include Grb2 and Nck (Ryan and Gillespie, 1994). Nck may be a candidate component of the FGFR-dependent target recognition signal. In *Drosophila*, DOCK (the homologue to vertebrate Nck) mutants are characterized by visual system pathfinding errors in which R cell axons exhibit pronounced targeting defects (Garrity et al., 1996).

**SemaiIIIA and cGMP**

The group II guidance protein, semaiIIIA, induces an age-dependent response in *Xenopus* RGC growth cones (D. Campbell, J. Lopez, T. Odagiri, personal communication). At early stages (before stage 32), growth cones are not repulsed by a gradient of semaiIIIA nor collapse when semaiIIIA is added to the culture medium. However, axons from retinal explants older than stage 32 respond robustly to semaiIIIA, and these responses are converted by the activation of PKG. In the *Xenopus* visual system the semaiIIIA receptor neuropilin-1 is not expressed until after stage 32 (Fujsawa et al., 1995), which would explain the age-dependent responses. In addition, semaiIIIA in *Xenopus* is predominantly expressed within the caudal third of the tectum (Regan and Tannahill, personal communication). Therefore, semaiIIIA may play a role in visual system development following target innervation, such as preventing axons from overshooting their target and entering the hindbrain. Alternatively, semaiIIIA may act to promote branching and arborization of axons within their target [Fig. 1(H,I)]. In culture, *Xenopus* growth cones recovering from semaiIIA-induced collapse are more likely to form branches than control growth cones (J. Lopez, D. Campbell, personal communication).

**TOPOGRAPHICAL MAP FORMATION**

Once within their target, growth cones form a topographical map corresponding to the position of their cell bodies within the retina [reviewed in Holt and Harris, 1993; Figs. 1(I) and 2(E)]. Axons from the anterior retina project to the posterior tectum, posterior retina to the anterior tectum, dorsal retina to the ventral tectum, and ventral retina to the dorsal tectum. Considerable effort has been made in identifying and characterizing what Sperry (1963) referred to as “cytochemical tags.” It is now thought that Eph receptor tyrosine kinases and their ligands, the ephrins, expressed in gradients within the retina and tectum, respectively, are partially responsible for appropriate topographical mapping (Cheng et al., 1995; Drescher et al., 1995). We will not review the evidence for this here, because this work has largely been done in other systems, and it is reviewed elsewhere (Drescher et al., 1997 and references therein). In *Xenopus*, Eph receptors and ephrins are expressed in appropriate gradients in the retina and tectum (S. Nakagawa and F. Mann, personal communication) and are likely to play a similar role in this system.

**CONCLUSION**

The retinotectal system in *Xenopus* is an excellent model system for studying the mechanisms that control growth cone dynamics along an entire neural pathway. This review describes a wide breadth of research from our laboratories aimed at understanding the molecular and cellular mechanisms involved in this process. And, although our efforts may have addressed many questions, there are still many more to be answered. The main challenges ahead will be to find the molecular mechanisms that integrate the various guidance cues and then drive growth cone dynamics during particular pathfinding events.
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REFERENCES


