The Trio family of guanine-nucleotide-exchange factors: regulators of axon guidance

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Summary

Axon guidance requires the integration of diverse guidance signals presented by numerous extracellular cues and cell-cell interactions. The molecular mechanisms that interpret these signals involve networks of intracellular signaling proteins that coordinate a variety of responses to the environment, including remodeling and assembly of the actin cytoskeleton. Although it has been clear for some time that Rho family GTPases play a central role in the orchestration of cytoskeletal assembly, our understanding of the components that regulate these important molecules is far more primitive. Recent functional studies of the Trio family of guanine-nucleotide-exchange factors reveal that Trio proteins play a vital role in neuronal cell migration and axon guidance. Although the molecular analysis of Trio proteins is still in its infancy, accumulated evidence suggests that Trio proteins function as integrators of multiple upstream inputs and as activators of multiple downstream pathways. Future studies of these mechanisms promise to yield insights not only into neural development but also into the ongoing function and remodeling of the adult nervous system.

Key words: GTPase, Rho family, Actin, Rac, Pak, Dock, Kalirin

Introduction

The development of the embryonic nervous system requires that nascent axons extend across a complex extracellular terrain in order to reach their specific synaptic targets. As an axon grows, the growth cone at its advancing edge encounters specific 'choice points' at which extracellular guidance cues steer specific axons towards their appropriate destinations (reviewed by Tessier-Lavigne and Goodman, 1996). Such cues may attract a subset of axons towards a given domain, repel axons from inappropriate target regions or simply provide a permissive substrate for axonal outgrowth. In each of these cases, guidance signals must be relayed through the growth cone to the actin cytoskeleton, a dynamic network of filaments and associated proteins that is largely responsible for motility and growth cone steering (reviewed by Korey and Van Vactor, 2000). Our understanding of how nascent axons interpret guidance information relies on identifying cellular factors that influence actin and its associated proteins.

Members of the Rho subfamily of small GTPases, including Rho, Rac and Cdc42, play pivotal roles in conveying signals to the cytoskeleton (reviewed by Symons and Settleman, 2000). In a simple interpretation, these molecules act as molecular switches that cycle between an active GTP-bound state and an inactive GDP-bound state brought on by intrinsic hydrolytic activity. When active, Rho family members bind to downstream effectors that ultimately alter actin dynamics and/or localization. In many cases, these effectors are specific to particular Rho family members; for example, the serine-threonine kinase ROK (Rho kinase) is specifically activated by GTP-bound Rho, but not Rac or Cdc42, and phosphorylates myosin light chain phosphatase to increase actin-myosin contractility (reviewed by Amano et al., 2000). In contrast, GTP-bound Cdc42 specifically binds to the WASP (Wiscott-Aldrich Syndrome Protein) family, inducing localized actin polymerization by the Arp2/3 complex (reviewed by Welch, 1999; Takenawa and Miki, 2001). Thus, specificity in effector activation allows different Rho family members to mediate distinct effects on the actin cytoskeleton.

Following inactivation by GTP hydrolysis, Rho family members must release GDP and bind free cytoplasmic GTP in order to be reactivated. This nucleotide replacement is accelerated by guanine-nucleotide-exchange factors (GEFs), a family of molecules that bind to inactive GTPases and induce a conformational change favoring GDP release. GEFs that are specific for the Rho family have in common the Dbl homology (DH) domain, a conserved amino acid sequence that is the main site of exchange activity (Hart et al., 1994). Many Dbl family GEFs also contain other protein-protein interaction domains, which allow GTPases to be activated in specific signal transduction pathways. For example, the Dbl family member Vav contains an SH2 domain that binds to the tyrosine kinase Syk, which phosphorylates and activates the GEF in response to integrin engagement (Deckert et al, 1996; Miranti et al, 1998). The coupling of DH domains to various protein-protein interaction domains represents a means of integrating Rho GTPase activity in response to signals from upstream activators.

Trio, a novel member of the Dbl family, encodes a large protein that has numerous catalytic domains and putative signaling domains and might therefore function in multiple signaling pathways. Indeed, genetic analyses in model systems have exposed roles for Trio homologs in several actin-dependent processes, such as cell migration and neuronal extension. Here, we focus on recent advances in our understanding of the roles of Trio family members in orchestrating cellular responses to developmental cues through activation of Rho family GTPases and their effectors.

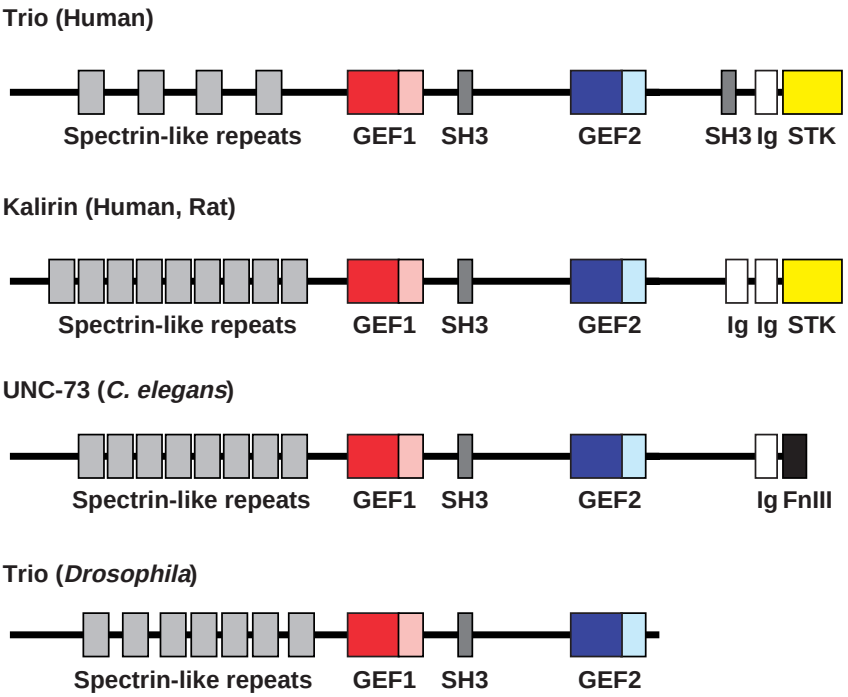

Fig. 1. The Trio family. Members identified in vertebrates (Trio and Kalirin), *C. elegans* (UNC-73) and *Drosophila* share a series of N-terminal spectrin-like repeats, two GEF domains consisting of a DH domain coupled to a pleckstrin homology (PH) domain, and an SH3 domain located between the two GEF domains. The C-terminal of the molecule differs in all members identified thus far; human Trio encodes a second SH3 domain, an Ig-like domain and a serine/threonine kinase (STK) domain, kalirin encodes two Ig-like repeats and an STK domain, UNC-73 possesses an Ig-like repeat and a fibronectin-like repeat (FNIII), and *Drosophila* Trio terminates immediately following the C-terminal PH domain. In addition to the molecules depicted, several alternatively spliced variants have been described for kalirin and UNC-73.

The Trio family

Human Trio was first identified in a yeast interaction screen using the intracellular domain of the receptor-like tyrosine phosphatase (RPTP) LAR (leukocyte-antigen-related protein) as bait (Debant et al., 1996). A large multidomain protein, human Trio has a series of spectrin-like repeats, two DH domains, two SH3 domains, an immunoglobulin-like (Ig) domain and a C-terminal serine-threonine kinase (Debant et al., 1996; Seipel et al., 1999; Fig. 1). Accumulated evidence suggests that the LAR family of RPTPs act as receptors that convey extracellular cues to the actin cytoskeleton during development. For example, a homolog of LAR is required for axon guidance during embryonic development in *Drosophila*; mutations in *Dlar* cause axonal defects that are reproduced by loss of Rac function or by direct perturbation of actin by cytochalasin D (Kreuger et al., 1996; Kaufmann et al., 1998). Furthermore, the LAR homolog Crypα is required for maintenance of actin-rich lamellipodia in cultured chick retinal ganglial cells (Mueller et al., 2000). Despite known developmental roles for RPTPs, little is known about the cellular components that are required for their function. The identification of Trio as a LAR interactor provides a promising link between RPTPs and the actin cytoskeleton through the activation of Rho family GTPases.

In vitro guanine-nucleotide-exchange assays show that the N-terminal GEF domain (GEF1) of human Trio activates Rac1, but not RhoA or Cdc42Hs, whereas the C-terminal GEF domain (GEF2) specifically activates RhoA (Debant et al., 1996; Fig. 2). These biochemical assays are supported by microinjection studies in cultured fibroblasts, in which injection of GEF1 or the constitutively active mutant $Rac1^{V12}$ leads to increased cell spreading and lamellipodia formation, while injection of GEF2 or $RhoA^{V14}$ causes an increase in stress fibre formation (Ridley and Hall, 1992; Ridley et al., 1992; Bellanger et al., 1998a). However, subtle differences in the phenotypes induced by Trio GEF domains and activated GTPases suggest that other molecules are involved in Trio signaling through its DH domains. For example, in addition to stress fibre formation, expression of GEF2 also induces localized actin-rich 'mini-ruffles' at the cell membrane, which are not observed when $RhoA^{V14}$ is injected. In the case of GEF1, recent experiments have shown that the Rac-like GTPase RhoG is probably the relevant in vivo target, as a dominant negative RhoG construct abolishes the formation of actin structures induced by Trio GEF1 expression (Blangy et al., 2000). GTP-bound RhoG subsequently activates both Cdc42Hs and Rac1 by an unknown mechanism, leading to the formation of both lamellipodia and filopodia through Rac1- and Cdc42Hs-specific effectors (Gauthiere-Rouviere et al., 1998). Thus, through its two GEF domains, human Trio regulates the activity of at least four different GTPases.

A second Trio-like molecule was identified in two independent yeast interaction screens, which used the Huntingtin-associated protein HAP1 and the cytoplasmic domain of peptidylglycine α-amidating monooxygenase (PAM) as baits (Alam et al., 1996; Colomer et al., 1997). Called kalirin, the full-length molecule has a domain structure nearly identical to that of Trio (Fig. 1) but also encodes a variety of alternative transcripts in which various domains and potential regulatory elements are not present (Penzes et al., 2000; Johnson et al., 2000). The different kalirin isoforms have distinct subcellular localizations; for example, the full-length kalirin-12 is restricted to the soma of primary cortical neurons, whereas an isoform lacking the second GEF and kinase domains (kalirin-7, also known as Duo) is observed in a punctate pattern in neuronal processes (Johnson et al., 2000). This suggests potential functional differences between isoforms that require localization to specified cellular compartments. Although potential functions for kalirin have been suggested, including PAM internalization from the cell membrane (Alam et al., 1996) and inhibition of inducible nitric-oxide synthase (Ratovitski et al., 1999), it is not yet clear

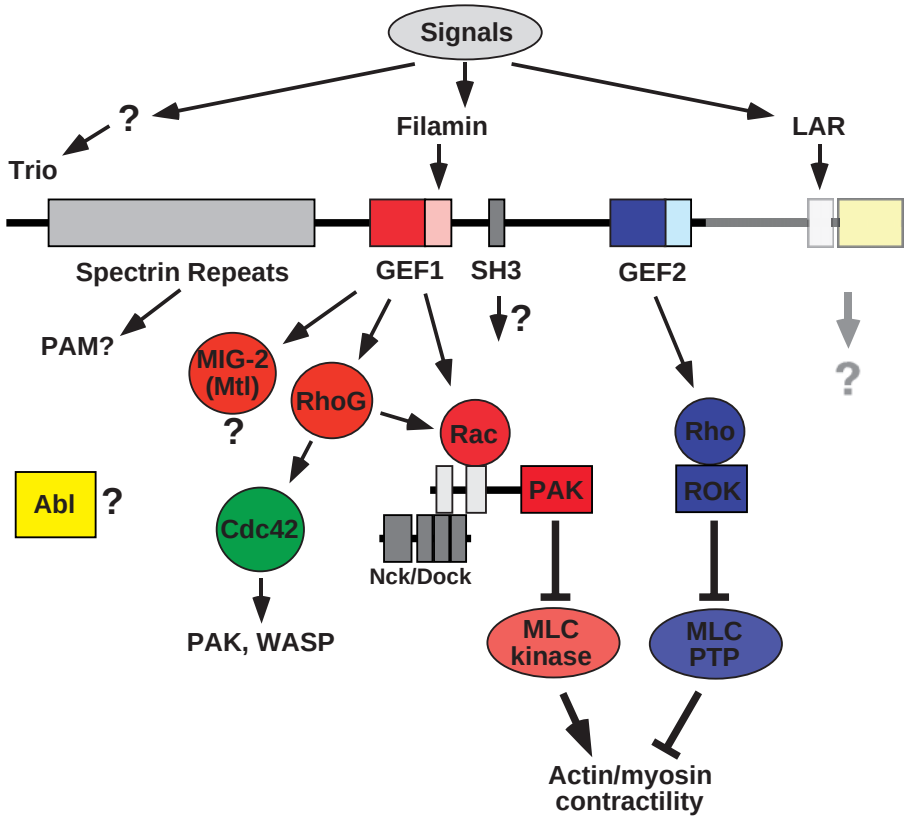

Fig. 2. Potential interactions in Trio signal transduction. The figure represents a generic Trio family member and incorporates biochemical and genetic data from different organisms. In response to upstream cues (possibly through localization by filamin or LAR-family RPTPs), Trio activates the Rac pathway (red) and/or the Rho pathway (blue) through its two GEF domains. GTP-bound Rac, in cooperation with membrane-localized Nck/Dock, can bind and activate PAK, leading to phosphorylation of substrates that affect the actin cytoskeleton, including inhibitory phosphorylation of myosin light chain kinase (MLC kinase). Theoretically, GTP-bound Rho might bind to and activate Rho-associated kinase (ROK), causing inhibitory phosphorylation and inactivation of MLC phosphatase (MLC PTPase) and subsequent changes in cytoskeletal dynamics. GEF1 also activates RhoG in vertebrates, which subsequently activates Rac and Cdc42 signaling pathways. In invertebrates, GEF1 activates the Rac-like GTPase MIG-2, which signals through unknown effectors. A region of the spectrin repeats of kalirin interacts with PAM and may regulate receptor internalization. Genetic interactions from *Drosophila* suggest that Trio functions with components of the Abl pathway (including Fax and Ena), but the biochemical nature of this interaction is unclear. Putative binding partners for the SH3 domain and regions within the C-terminal variable region (shown as an Ig/kinase as in human Trio) are as yet poorly understood.

how these putative duties might be distributed among different kalirin splice variants.

Whereas Trio is expressed at moderate levels in all tissues, kalirin expression is specific to the central nervous system in the adult rat (Debant et al., 1996; Alam et al., 1997). Although they possess slight structural differences, it is plausible that kalirin and Trio function in a similar manner in their different tissues. The observations that the N-terminal GEF domain of kalirin is capable of activating Rac1 in vitro and that expression of the domain in cultured fibroblasts results in cell spreading and lamellipodia formation are consistent with this idea (Alam et al., 1997; Mains et al., 1999; Penzes et al, 2000). However, the functions of other Trio/kalirin domains are as yet poorly understood; for example, putative binding partners for the SH3 domains have not yet been reported. Furthermore, it is unclear whether the kinase domains are active or display substrate specificity, although a highly related kinase named Duet is autophosphorylated in vitro (Kawai et al., 1999). Further biochemical studies should identify other signaling partners, shedding light on potential functions of Trio family members.

Trio function during development

The above biochemical studies of Trio and kalirin have initiated our understanding of Trio signaling mechanisms in a cellular context, and data from model genetic systems have begun to shed light on potential roles of Trio signaling during development. For example, mice lacking Trio die during the late stages of embryogenesis, demonstrating that Trio is essential for viability (O'Brien et al., 2000). Analysis of *trio*$^{-/-}$ embryos has shown a defect in late stages of myogenesis that produces misshapen myofibres in skeletal muscles. It is not known whether this defect is directly tied to GTPase activation, but studies of *Drosophila* have shown a role for Rac activation in myoblast fusion (Luo et al., 1994). In addition to skeletal muscle defects, loss of Trio causes subsets of cells within the hippocampus and olfactory bulb to become disorganized, although the overall structure of the brain does not appear to be affected (O'Brien et al., 2000). Thus, Trio appears to mediate fine-scale mapping of neuronal cell positions once the global arrangement of the brain has been established. Further analysis will determine whether other aspects of neuronal development, such as axon guidance, are also affected in mutant mice.

The *C. elegans* mutant *unc-73*, which displays an 'uncoordinated' motility phenotype in adults, encodes a multidomain protein highly related to Trio (Steven et al., 1998; Fig. 1). As in the vertebrate, UNC-73 possesses an N-terminal GEF domain that activates Rac-like molecules, and a C-terminal GEF specific for Rho (Steven et al., 1998; Merz and Culotti, 2000). In an interesting twist of evolution, UNC-73 differs from vertebrate Trio and kalirin at its C-terminus, encoding a fibronectin-III-like domain rather than a serine/threonine kinase. The functional significance of this difference is dubious because it is not yet clear whether Trio/kalirin kinases are active or what their substrates might be. The fibronectin III domain could potentially interact with other cellular factors to facilitate or influence UNC-73 function, but

it remains to be determined what these factors may be and how this might differ from the function of Trio molecules in other species.

Antibody staining and GFP fusions show that UNC-73 is expressed generally in the developing embryo, with highest levels being observed in the nervous system (Steven et al., 1998). *unc-73* mutants show extensive abnormalities in neural development, including axonal extension, fasciculation and guidance defects (Hedgecock et al., 1987; Desai et al., 1988; McIntire et al., 1992). Defects are also observed in cell migrations and asymmetric cell divisions, which may be analogous to the brain abnormalities observed in *trio*$^{-/-}$ mice (Hedgecock et al., 1987; Desai et al., 1988; Way et al., 1992). The upstream signals that utilize UNC-73 signaling in these diverse contexts are as yet unreported; however, Rac activation appears to be critical for UNC-73 function, given that a point mutation abolishing exchange activity of the N-terminal GEF domain produces a strong loss-of-function mutant (Steven et al., 1998). The role of Rho activation is less clear; although a minigene lacking the sequence that encodes the C-terminal GEF domain rescues several *unc-73* mutants, it appears that these alleles still express C-terminal portions of the gene from alternative start sites and thus retain Rho exchange activity (Steven et al., 1998; R. Steven and T. Pawson, personal communication).

Adding a new chapter to the story, several research groups have recently described a *Drosophila* Trio homolog. As in the worm, *Drosophila trio* is expressed throughout the developing embryo, and high levels are observed in nervous tissues (Awasaki et al., 2000; Bateman et al., 2000; Liebl et al., 2000). Mutations in *trio* produce pleiotropic axonal phenotypes in both the embryonic nervous system and retinal axon projections, including stalling, missed targets and inappropriate fasciculation (Awasaki et al., 2000; Bateman et al., 2000; Liebl et al., 2000; Newsome et al., 2000). These defects are similar to those observed in *unc-73* mutants, which suggests some conservation of cellular functions across evolutionary lines.

As in other species, the N-terminal GEF domain of *Drosophila* Trio has specific exchange activity for Rac family GTPases in vitro (Newsome et al., 2000). However, no in vitro exchange activity has been observed for GEF2 on several GTPases, including DRhoA. Consistent with this, in vivo rescue experiments show that GEF1, but not GEF2, activity is required for correct pathfinding of retinal axons (Newsome et al., 2000). Furthermore, a partial loss of *trio* function suppresses a gain-of-function retinal morphology phenotype of Drac1 but not DrhoA (Bateman et al., 2000). Thus, the Rac-specific GEF1 appears to be the major signaling domain for retinal developmental processes that require *Drosophila* Trio, highlighting the importance of a signaling pathway involving Rac.

The retinal axon defects observed in *Drosophila trio* mutants are similar to those of the previously identified mutant *Pak-kinase* (*Pak*), which encodes the *Drosophila* homolog of the p21-activated kinase PAK (Hing et al., 1999; Newsome et al., 2000). In both *trio* and *Pak* mutants, retinal axons fail to recognize their appropriate synaptic targets, instead improperly fasciculating and extending into deeper regions of the brain. Dosage-sensitive genetic interactions between *trio* and *Pak* suggest that they may operate in a common pathway to regulate retinal axon guidance (Newsome et al., 2000). Indeed, previous studies of the vertebrate homolog PAK have shown that it is a downstream effector of Rho family GTPases; activation of PAK kinase activity requires prior binding of its p21-binding domain by GTP-bound Rac or Cdc42, which unleashes the kinase domain from a sterically hindered conformation (Daniels and Bokoch, 1999; Hoffman and Cerione, 2000). Activated PAK then phosphorylates substrates that affect the actin cytoskeleton. For example, PAK phosphorylation of myosin light chain kinase (MLCK) decreases phosphorylation of the regulatory subunit of myosin, altering actin-myosin contractility (Sanders et al., 1999). Such changes in cytoskeletal dynamics might then aid in guiding a growth cone towards its appropriate target region (Lin et al., 1996; reviewed in Jay, 2000). Although genetic evidence in the *Drosophila* retina is consistent with a Pak pathway for Trio signaling, it is also possible that Pak activation occurs independently of Trio through activation of Rac by other GEFs such as PIX (Manser et al., 1998). Future experiments should address the GEF specificity of Pak activation in different developmental events.

PAK activation also involves the SH2-SH3 adapter protein Nck (Bokoch et al., 1996; Lu et al., 1997). In response to extracellular cues, Nck binds to activated receptors at the cell surface through its SH2 domain, while simulaneously binding PAK through one of its three SH3 domains (reviewed in McCarty, 1998). The subsequent recruitment to the membrane is important for PAK activation; artificial targeting of PAK to the membrane by myristilation can lead to activation of its kinase activity (Lu et al., 1997). This relationship holds true in *Drosophila*, in which the Nck homolog Dreadlocks (*dock*) is required for proper development of retinal axons (Garrity et al., 1996). As is the case for *Pak* and *trio*, mutations in *dock* cause axons to improperly fasciculate and extend past their appropriate targets. Again, the primary function of Dock is presumably to bring Pak to the membrane, because axonal defects observed in *dock* mutants can be rescued by myristilated PAK (Hing et al., 1999). In theory, Pak localization to the membrane then facilitates its activation by GTPases through local GEFs such as Trio.

Although the interaction between Pak and Dock is conserved in the *Drosophila* retina, the upstream receptors and signals that localize and activate Trio and Dock, and thus ultimately guide neurons, are as yet unknown. In vertebrates, the Dock homolog Nck binds to receptor tyrosine kinases such as EphB1 and the epidermal growth factor receptor (EGF-R; Park and Rhee, 1992; Li et al., 1992; Stein et al., 1998), but those receptors have not been implicated in retinal axon guidance in *Drosophila*. However, the cell-surface protein Dscam has recently been shown to bind to Dock in *Drosophila*, and mutations in *Dscam*, *dock* and *Pak* cause identical defects in the guidance of Bolwig's nerve in the *Drosophila* embryo (Schmucker et al., 2000). It will be interesting to determine whether Trio also functions in this particular guidance system. Similarly, mutants of the LAR family phosphatase *Dptp69D* have an axonal phenotype resembling that of *trio* and *dock* in loss-of-function mutant retinas, which suggests that the phosphatase participates in similar signaling events (Garrity et al., 1999). The physical interaction of LAR and Trio in humans provides an attractive model for RPTP signaling, but it is not clear whether this relationship exists in *Drosophila*, because the C-terminal portions of Trio that are responsible for

phosphatase binding are not conserved in the fly (Debant et al., 1996; Fig. 1). However, embryonic phenotypes caused by mutations in the LAR homolog *Dlar* are exacerbated by loss of either *trio* or *Drac1*, which indicates that the Trio/Rac pathway is important for RPTP signaling (Kaufmann et al., 1998; Bateman et al., 2000). Perhaps *Drosophila* RPTPs signal through Trio via other cellular factors (e.g. Abl, see below), rather than through a direct physical interaction.

Other signaling partners

Although PAK is a strong candidate for transduction of Trio signals, several lines of evidence suggest that Trio must also operate through alternative pathways. For example, retinal axon phenotypes in *trio* and *Pak* mutants are similar but not identical. In *trio* mutants, a subset of axons stop short of their target regions rather than projecting past them; this defect is not observed in retinas lacking *Pak* or *dock* (Garrity et al., 1996; Hing et al., 1999; Newsome et al., 2000). Thus, PAK must convey only targeting information, whereas other molecules must be responsible for axonal extension directed by Trio. This is consistent with embryonic analyses, in which mutations in *trio* cause an extension defect in the motor nerve ISNb (Awasaki et al., 2000; Bateman et al., 2000). *Pak*-mutant embryos have yet to be described, but mutations in *dock* cause a much milder ISNb phenotype and therefore cannot entirely account for Trio signaling (Desai et al., 1999). However, mutations in the tyrosine kinase Abl cause an ISNb defect that is indistinguishable from that of *trio* mutants (Wills et al., 1999). Furthermore, altering the genetic dose of *trio* or *Abl* enhances CNS axonal defects caused by loss of either gene alone (Liebl et al., 2000). Although there is currently no biochemical evidence to support a direct interaction between Abl and Trio, the Abl pathway is a possible alternative to PAK activation in Trio signal transduction.

The activity of the Trio Rac-specific GEF itself suggests that PAK-independent signaling must occur. In *Drosophila*, GEF1 not only activates the Rac homologs Rac1 and Rac2 but also activates a close relative of the Rac genes, MIG-2-like (Mtl; Newsome et al., 2000). This novel GTPase is the *Drosophila* homolog of MIG-2, which is required for several cell migrations in *C. elegans* (Hedgecock et al., 1987; Zipkin et al., 1997). MIG-2 seems to be a significant in vivo target for UNC-73 signaling, because defects observed in weak loss-of-function *unc-73* mutants are enhanced by mutations in *mig-2* and suppressed by increasing the genetic dose of *mig-2* (Zipkin et al., 1997; Honigberg and Kenyon, 2000). Despite significant sequence similarity between Rac and MIG-2 homologs, Mtl does not bind to Pak (Newsome et al., 2000), and therefore must signal through other downstream effectors that could be unique to MIG-2/Mtl or shared by activated Rac. Alternatively, MIG-2/Mtl may activate other GTPases, as observed for RhoG in vertebrates, although it is currently unclear whether these molecules are analogous in function.

Genetic data from the *Drosophila* retina seem to preclude a role for Rho activation in *Drosophila trio* signaling, but other observations suggest that Rho-like proteins are involved. First, expression of *Drosophila* Trio GEF2 in rat embryo fibroblast (REF) cells causes formation of stress fibres, which implies that the ability to activate Rho-like GTPases is conserved (Newsome et al., 2000). Furthermore, loss-of-function mutations in *trio* cause a dramatic overextension of neurites in the mushroom bodies of the developing brain, a phenotype observed in loss-of-function clones of *rhoA* (Awasaki et al., 2000; Lee et al., 2000). Perhaps *Drosophila* Trio is an activator of RhoA only in a particular cellular context that is irrelevant in the retina but required in other tissues. Alternatively, Trio might display exchange activity for other uncharacterized Rho-like proteins but not RhoA itself. Further biochemical and genetic experiments must be undertaken if we are to distinguish between these possibilities.

Two-hybrid screens using the GEF1 domain of human Trio as bait have identified two actin-binding proteins (ABPs) that could mediate aspects of Trio signaling. In one study, the PH domain of GEF1 was shown to bind filamin (Bellanger et al., 1998b; Bellanger et al., 2000), a large ABP that mediates actin crosslinking (Hartwig and Stossel, 1975) and interacts with several signaling proteins (Sharma et al., 1995; Marti et al., 1997). Genetic analysis has shown that filamin is necessary for cell migrations in *Dictyostelium* (Cox et al., 1992) and the vertebrate cortex (Fox et al., 1998) and for proper formation of actin structures during *Drosophila* oogenesis (Li et al., 1999; Sokol and Cooley, 1999). A functional link between filamin and human Trio was established by expression of Trio GEF1 in cells lacking filamin; in this case, the normal formation of actin-rich lamellipodia is abolished, which demonstrates that filamin is required for Trio GEF1 effects on the actin cytoskeleton (Bellanger et al., 2000). However, in vitro exchange assays show that filamin does not directly affect GEF1 exchange activity on RhoG. Filamin might play a role in Trio signaling by localizing it to actin-rich sites of the cell in response to guidance cues or through local recruitment of other cytoskeletal factors.

In another two-hybrid screen and subsequent coimmunoprecipitation studies, the novel actin-binding protein Tara was shown to interact with the GEF1 domain of human Trio (Seipel et al., 2001). Tara contains a PH domain and a coiled-coil domain, either of which might mediate interactions with other cellular factors. Ectopic expression of Tara in Hela cells results in an increase in cell spreading, which may be mediated in part by stabilizing latrunculin-B-sensitive actin structures (Seipel et al., 2001). However, as is the case with filamin, Tara does not affect Trio exchange activity in vitro. Future experiments aimed at the functional relationship between Tara and Trio should improve our understanding of Trio signaling mechanisms and its influence on the actin cytoskeleton.

Conclusions and perspectives

The above genetic and biochemical analyses have provided insight into developmental processes requiring Trio activity, highlighting a role in axon guidance. But what exactly does Trio signaling accomplish? Presumably the activation of Rho family GTPases ultimately signals to the actin cytoskeleton of a pathfinding growth cone, influencing axon guidance and outgrowth. Both *unc-73* and *Drosophila trio* are required autonomously within developing neurons rather than in surrounding tissues, which is consistent with a role in growth cone signaling (Steven et al., 1998; Awasaki et al., 2000; Bateman et al., 2000; Newsome et al., 2000). However, given the widespread defects observed in *unc-73* and

trio mutants, it seems that Trio molecules are not part of a specific axon guidance pathway; rather, Trio must be part of a common machinery used by multiple guidance cues during different signaling events. Furthermore, none of the phenotypes observed in *unc-73* or *trio* mutants is completely penetrant, even in alleles that appear to remove the gene completely. Thus, Trio function must overlap with that of other cytoskeletal modulators, acting as a branch in a network of multiple signaling cascades. Such functional overlap has become a theme as more genetic analyses of signaling molecules are reported, which emphasizes the importance of partial redundancy in ensuring proper execution of a developmental program.

Much attention has been given to the role of Trio in neural development, but evidence for a function in the adult nervous system also exists. For example, kalirin is abundant in the adult rat brain, and the isoform kalirin-7 localizes to postsynaptic densities (Alam et al., 1997; Penzes et al., 2000). Similarly, *Drosophila* Trio is present in the mushroom bodies of the adult brain and localizes to dendritic terminals of the lamina (Awasaki et al., 2000). This expression pattern is consistent with a role in synaptic function, perhaps in actin-mediated processes such as receptor clustering (Dai et al., 2000) or in Rho-family-dependent control of dendritic arborization (Nakayama et al., 2000; Li et al, 2000). Indeed, recent work has shown that overexpression of kalirin-7 in primary cortical neurons causes an increase in spine-like structures that is dependent upon GEF1 activity (Penzes et al., 2001). Furthermore, kalirin 7 contains a C-terminal motif that mediates interactions with PDZ-domain proteins that are involved in receptor clustering (Sheng and Pak, 1999; Penzes et al., 2001). However, the PDZ-binding motif does not appear to be conserved in other Trio family members, and thus this function may not be universal across species. Alternatively, Trio might function at the synapse in receptor internalization. Consistent with this is the finding that kalirin binds to an 18-residue region of PAM that is necessary and sufficient for recycling from the cell surface to the trans Golgi network (Alam et al., 1997). Internalization of cell surface components by Trio could again be mediated by GTPase activation, given that both Rac and Rho have been implicated in endocytic pathways (reviewed by Ellis and Mellor, 2000).

Roles for Trio in the adult nervous system highlight a multiplicity of functions observed in many molecules implicated in signaling to the actin cytoskeleton. Although actin dynamics must be regulated during development in order to achieve a specified cell architecture, the cytoskeleton must then be continually remodeled post-development in response to signals from neighbors in the adult cellular community. Our understanding of Trio and other actin modulators is aided by the combined exploration of these different avenues of study, providing insight into various biological processes required for normal cell biology and homeostasis.

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