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Previews

Trio Quartet in *D. (melanogaster)*

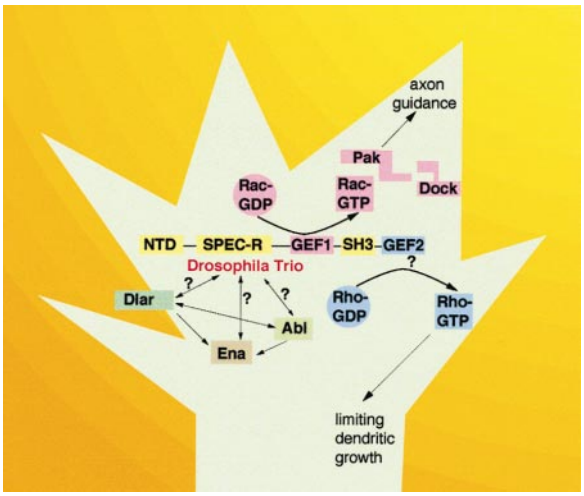
As a growth cone navigates along its path, it constantly interprets extracellular cues to decide its next move. Extracellular cues—attractants and repellents—must eventually instruct the local reorganization of the actin cytoskeleton in the filopodia and lamellipodia of the growth cone, such that growth cones are steered toward sources of attractants and away from sources of repellents. How such localized reorganizations lead to directed turning remains an open and important question in the field of axon guidance. Despite an explosion of knowledge on the molecular mechanisms of axon guidance in the last decade (for a recent review, see Mueller, 1999), our understanding of the intracellular signal transduction pathways that link the reception of guidance cues to the regulation of the actin cytoskeleton remains sketchy. Three papers in this issue of *Neuron* (Awasaki et al., 2000; Bateman et al., 2000; Liebl et al., 2000) and another paper in the April 28 issue of *Cell* (Newsome et al., 2000) report the identification of *Drosophila* Trio, a guanine nucleotide exchange factor (GEF) for the Rho subfamily of small GTPases. This Trio quartet identifies crucial roles for Trio in regulating different axon guidance pathways in *Drosophila* and thus confirms the importance of previously suspected signaling pathways involving the Rho family of small GTPases (in particular Rac) in the regulation of growth cone guidance (reviewed by Luo et al., 1997).

Rac1, RhoA, and Cdc42 are the three best-studied Rho family small GTPases (reviewed by Hall, 1998) and have been shown to be key regulators of actin polymerization. For instance, in mammalian fibroblasts, RhoA regulates the formation of specific actin-based structures, called stress fibers, whereas Rac and Cdc42 regulate the formation of lamellipodia and filopodia, respectively (Hall, 1998). Given the importance of the actin cytoskeleton in growth cone steering, a number of studies, both in vitro and in vivo, have examined the role of the Rho family in neuronal development and have shown that perturbation of Rho GTPase activity can lead to a variety of defects in neuronal migration, axon growth, guidance, and dendritic morphogenesis (reviewed by Luo et al., 1997; Mueller, 1999). Rho, Rac, and Cdc42 function as molecular switches in intracellular signaling pathways. These GTPases exist in two distinct forms—a GDP-bound inactive form and a GTP-bound active form, which binds to specific effectors to elicit their biological functions. Two classes of cellular enzymes catalyze the conversion between the active and inactive forms. The guanine nucleotide exchange factors (GEFs) help convert the GDP-bound form to the GTP-bound form, thus switching on these proteins, whereas the GTPase-activating proteins (GAPs) facilitate the intrinsic GTPase activity, thus switching off these proteins.

One way to link the reception of guidance cues to the regulation of the actin cytoskeleton by Rho GTPases is through the local activation or recruitment of Rho GEFs. And thus Trio enters the picture. Originally identified as a binding partner for a receptor tyrosine phosphotase Lar (Debant et al., 1996), human Trio is a large protein possessing three (hence the name) distinct enzymatic activities: two GEFs and a C-terminal serine/threonine kinase domain. GEF1 and GEF2 of human Trio possess specific exchange activity for Rac1 and RhoA, respectively. In addition to the three enzymatic domains, human Trio also contains spectrin repeats, two SH3 domains, and one Ig domain (Debant et al., 1996). The *C. elegans* protein UNC-73 is very similar to human Trio in overall structure and has been shown to activate Rac and be required for cell and growth cone migration (Steven et al., 1998). However, neither *Drosophila* Trio nor *C. elegans* UNC-73 contain the C-terminal kinase domain found in human Trio or the LAR binding domain located in the extreme C terminus in human Trio (see figure).

Newsome et al. (2000) identified Trio through an elegant genetic screen for *Drosophila* photoreceptor axon guidance mutants, in which only photoreceptor axons, but not the target tissues or the rest of the organism, are mutant for the candidate genes. In the same screen, they also identified new mutants in Dock, an adaptor protein containing one SH2 and three SH3 domains, and Pak, a protein kinase that is activated by Rac and Cdc42 and thus serves as a downstream effector for Rac/Cdc42. Both Dock and Pak have previously been shown to be required for photoreceptor axon guidance, with Dock acting upstream of Pak by targeting Pak to plasma membrane (Hing et al., 1999). The fact that *trio* mutants exhibited similar photoreceptor-autonomous axon guidance phenotypes to those of *dock* and *pak* suggests that these gene products may be components of the same signaling pathways. Structure–function analyses indicated that the first GEF domain, which possesses exchange activity for Rac, but not for RhoA or Cdc42 in vitro, is both necessary and sufficient for Trio activity in photoreceptor axon guidance. Thus, a mechanistic picture emerges in which Trio working through Rac activates Pak, which may have been targeted to the membrane by Dock prior to the activation by Rac via a potentially separate input pathway (see Figure 7 of Newsome et al., 2000).

Liebl et al. (2000) identified Trio through an entirely different strategy: as a genetic modifier of tyrosine kinase Abl in *Drosophila*. Abl and its other genetic modifiers (notably *enabled*, or *ena*) have previously been implicated in regulating axon guidance in *Drosophila* and mammals (reviewed by Hu and Reichardt, 1999). Using embryonic CNS axon development and organismal viability as assays, Liebl et al. (2000) showed that a point mutation in a conserved region of the first GEF domain of Trio (again pointing out the importance of Rac exchange) can enhance phenotypes of *abl* mutants in a dosage-sensitive way, thus also implicating Trio in the regulation of axon guidance. Bateman et al. (2000) also showed that Trio is required for the formation of correct embryonic CNS and motor axon pathways, and that it genetically interacts specifically with Rac but not with Cdc42. Interestingly, although *Drosophila* Trio lacks the Dlar



Structure and Potential Functions of *Drosophila* Trio

Abbreviations: NTD, N-terminal domain; SPEC-R, spectrin repeats; GEF1 and GEF2, guanine nucleotide exchange domains; Pak, p21-activated kinase; Dock, Dreadlock; Abl, *Drosophila* Abelson kinase homolog; Ena, Enabled; Dlar, *Drosophila* Lar homolog. A question mark indicates that biochemical evidence is lacking.

binding domain found in human Trio, Bateman et al. showed that *trio* and *Dlar* interact genetically, since reducing *trio* dose enhances axonal phenotypes exhibited by *Dlar* mutations (Bateman et al., 2000). The studies of Liebl et al. and Bateman et al. provide potential links that Trio may have with other signaling proteins previously implicated in growth cone signaling, including the receptor tyrosine phosphotase (Dlar), a tyrosine kinase (Abl), and a regulator of actin cytoskeleton (Ena) (see figure). It is conceivable that the regulation of tyrosine phosphorylation of Dlar or Abl could signal through adaptor proteins such as Dock, thus feeding into the pathway of Trio–Rac to regulate Pak in a coordinated way. On the other hand, Trio–Rac and Dlar–Abl could represent entirely parallel pathways with the convergence occurring much later, at the level of regulating actin polymerization. Genetic interaction studies alone do not allow us to distinguish between these possibilities.

Awasaki et al. (2000), the fourth paper of the Trio quartet, corroborated the findings by Bateman et al. and Liebl et al. that Trio is important for embryonic CNS and motor axon development. In addition, they showed that Trio expression is highly enriched in neurons that compose the mushroom bodies (MBs), the insect center for learning and memory, and that *trio* mutants have severe defects in MB axon development. Furthermore, Awasaki et al. showed using mosaic analysis that the observed axonal defects are due to Trio's function in MB neurons. Moreover, *trio* mutant neurons extend processes in the dendritic region beyond their wild-type confines, a phenotype very similar to that observed in *RhoA* loss-of-function mutants (Lee et al., 2000). In this regard, it is of interest that although *Drosophila* Trio appears to have no exchange activity for *Drosophila* RhoA in vitro, microinjection of GEF2 of *Drosophila* Trio does appear to be capable of stimulating stress fiber formation in a manner analogous to what is observed when an activated form of RhoA is microinjected (Newsome et al., 2000). Taken together, these experiments raise the possibility that, in addition to its function in regulating Rac activity in axon guidance, Trio may also regulate an additional RhoA-like activity in limiting dendritic growth.

Now that the important roles of Trio in axonal (and perhaps dendritic) development have been established, what's next? Clearly, how guidance cues activate Trio to provide localized activation of Rac (and perhaps Rho) remains a key question. One can envision at least two different scenarios. On the one hand, upon binding to extracellular cues, activated receptors could recruit Trio to their vicinity to activate Rac locally. Alternatively, Trio may have already been targeted to the vicinity of receptors, and what activated receptors do is to expose the GEF domain(s), thus activating Trio locally. Imaging Trio localization during growth cone turning may help distinguish between these possibilities. These studies also raised several additional interesting questions. How do other proteins that genetically interact with Trio feed into these pathways? Given the size and complex domain structure of Trio, might there be other outputs from this protein besides activating GTPases? Understanding the functions of the other domains in Trio should help address some of these questions and should begin to bridge our knowledge gap between the reception of guidance cues and changes in growth cone behavior.

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Selected Reading

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