Rac GTPases and their effectors control cellular morphogenesis in a wide range of developmental contexts by regulating the structure and dynamics of the actin cytoskeleton. Although much is known about the biochemistry of Racs and Rac regulators, less is known about how Racs control cellular morphogenesis, including axon development, in vivo. Recent loss-of-function genetic studies using model organisms have shown that Racs and their effectors are required for multiple aspects of axon development, including axon outgrowth, axon guidance and axon branching. Interestingly, these studies have also revealed that Rac activity is required to prune spurious axons and branches. Analyses of Racs and their upstream and downstream effectors suggest that Rac signaling is complex. Different neurons utilize distinct combinations of upstream Rac regulators during axon development, possibly reflecting responses to different axon path-finding signals, and Racs use distinct downstream effectors to mediate different aspects of axon development, possibly reflecting differential regulation of the lamellipodial and filopodial actin-cytoskeleton morphology and dynamics that mediate growth-cone outgrowth and steering in lamellipodia and filopodia, respectively [2,3].

Rac small GTPases of the Rho subfamily, which also includes Rho and Cdc42, control the structure and dynamics of the actin cytoskeleton [4]. Like other Ras-superfamily GTPases, Racs cycle between a GTP-bound active state and a GDP-bound inactive state. GTP-bound Rac small GTPases of the Rho subfamily, which also includes Rho and Cdc42, control the structure and dynamics of the actin cytoskeleton [4]. Like other Ras-superfamily GTPases, Racs cycle between a GTP-bound active state and a GDP-bound inactive state. GTP-bound Rac hydrolyze GTP to GDP, rendering themselves inactive [5]. Rac activity is regulated by GTP exchange factors (GEFs), molecules that catalyze the exchange of GDP for GTP and activate Racs, and GTPase-activating proteins (GAPs), molecules that stimulate Rac GTPase activity and inactivate Racs [6].

Previous work has shown that gain-of-function constitutively active and dominant-negative Rac molecules perturb axon pathfinding and neuronal morphogenesis in vivo [7]. This review will focus on recent studies of loss of Rac activity that demonstrate that Racs and their upstream regulators and downstream effectors are normally required for multiple aspects of axon development in vivo.

Racs mediate axon guidance, outgrowth and branching as well as suppression of ectopic axon formation

The genomes of multicellular animals contain multiple Rac-like genes that function redundantly in many morphogenetic events including axon development [8**–10**,11**,12**]. Rac-like molecules can be grouped into two families: the canonical Rac GTPases and the MIG-2-like (Mtl) GTPases. Mtls are similar to both Rac and Cdc42 and might define a new Rho GTPase family [13]. However, observations that Mtls have functional overlap with canonical Racs and that canonical Racs and Mtls share common regulators (see below) suggest that Mtls can be considered to be members of the Rac family [8**–10**,11**,12**]. An overview of Rac nomenclature in Caenorhabditis elegans and Drosophila is given in Table 1.

Loss-of-function genetic studies in C. elegans and Drosophila demonstrate that Racs are required for multiple
aspects of axon development including axon outgrowth, axon guidance and axon branching, as well as for the suppression of spurious axon branching (Figure 1). In *C. elegans*, two canonical Racs, CED-10 and RAC-2/3, and one Mtl, MIG-2, have overlapping roles in axon development: reduction of function of any one gene results in few axon defects whereas each pairwise double mutant combination displays synthetistic axon defects [8**,14**]. *Rac* double mutants display defects in axon pathfinding, including premature axon termination and axon misguidance [14*]. In *Drosophila*, two canonical Racs, Rac1 and Rac2, and one Mtl also have partially overlapping roles in axon guidance and outgrowth [9**,*10**] and are also required for normal axon branch formation [9**]. Branch formation defects are also seen in *C. elegans* Rac double mutants (EA Lundquist, unpublished data). These studies of loss of Rac function indicate that Racs are required for axon guidance, outgrowth and branch formation. *C. elegans* Rac double mutants also display ectopic axons and axon branches [8**,14**], suggesting that Racs are normally involved in the suppression of superfluous axon and branches (Figure 1).

**Racs independently control multiple aspects of axon development**

In *C. elegans*, each Rac double mutant displays the entire spectrum of axon defects described above [14**]. By contrast, Rac activity in *Drosophila* appears to be dose-sensitive, as sequential removal of Rac function reveals progressively different defects in axon development: branch formation is the first to be disrupted, followed by guidance and finally outgrowth [9**]. These Rac mutant defects reflect distinct Rac-controlled processes rather than sequentially more severe defects in the same process. The different Rac mutant defects appear independently.

**Table 1**

Rac nomenclature in *C. elegans* and *Drosophila*.

<table>
<thead>
<tr>
<th>C. elegans Racs</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CED-10</td>
<td>Similar to canonical Racs</td>
</tr>
<tr>
<td>RAC-2/3</td>
<td>Similar to canonical Racs</td>
</tr>
<tr>
<td>MIG-2</td>
<td>Member of the MIG-2-like Rac-related family</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drosophila Racs</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rac1</td>
<td>Similar to canonical Racs</td>
</tr>
<tr>
<td>Rac2</td>
<td>Similar to canonical Racs</td>
</tr>
<tr>
<td>Mtl</td>
<td>MIG-2-like, member of the MIG-2-like Rac-related family</td>
</tr>
</tbody>
</table>

*The rac-2/3 locus is a direct duplication of a rac gene resulting in two coding regions that can give rise to the nearly identical proteins RAC-2 and RAC-3 [8**].

(PDE), (b) In the wild type, the PDE axon grows ventrally to the ventral nerve cord (VNC), where the axon branches and extends anteriorly and posteriorly in the VNC. (c) Foreshortened axons indicate axon outgrowth defects. (d) Axons that retain the ability to extend but that are misguided (i.e. fail to reach the VNC and wander laterally) indicate axon-guidance defects. (e) Axons that fail to branch at the VNC indicate axon-branching defects. (f) The presence of extra axons and axon branches in Rac mutants indicates that Racs are required to suppress the formation of spurious axons and branches. (g–i) The micrographs show *C. elegans* PDE neurons visualized with green fluorescent protein. (g) A wild-type PDE neuron. A single unbranched axon extends ventrally to the VNC, where it branches and extends anteriorly and posteriorly. (h) A PDE neuron from a *ced-10*(n1993); *mig-2*(mu28) rac loss-of-function double mutant. The axon is misguided and extends posteriorly instead of ventrally to the VNC. (i) A PDE from an animal with neuron-specific expression of constitutively active RAC-2 harboring the G12V mutation. The neuron displays an ectopic axon (arrow) as well as ectopic lamellipodial and filopodial structures (arrowheads). Scale bars in (g–i) represent 10 micrometres.
of one another in both *Drosophila* and *C. elegans* [9**,14**]. For example, in *C. elegans*, misguided and terminated axons are apparent both with and without ectopic axon branches, and in *Drosophila*, branch formation defects occur in the absence of outgrowth or guidance defects. More convincing is the observation that Racs utilize distinct downstream effectors to mediate different axon development events. In *Drosophila*, CRIB-domain-containing Rac downstream effectors (where CRIB stands for 'Cde42, Rac interactive binding') such as p21-activated kinase (Pak) are involved in axon guidance and branch formation but not axon outgrowth [9**]. Thus, Rac activity is apparently involved in multiple, separate processes during axon development, including axon outgrowth, axon guidance, and axon branch formation as well as in the suppression of ectopic axon branches.

The conclusions drawn from the Rac loss-of-function studies are supported by analyses of dominant, constitutively active Rac mutants. The glycine-12-to-valine (G12V) mutation, which is canonical for constitutive activation of all Ras-superfamily GTPases, results in a molecule that cannot hydrolyze GTP and is therefore constitutively active [5]. In *C. elegans*, neuron-specific expression of mutant Rac(G12V) molecules results in extensive axon branching and the formation of ectopic lamellipodia and filopodia [14**], possibly reflecting the role of Rac activity in the formation of structures that are normally involved in axon guidance, outgrowth and branching. Furthermore, Rac(G12V) activity causes weak axon outgrowth and guidance defects [14**], possibly reflecting the normal role of Rac in the suppression of growth-cone structures. Thus, apparent loss-of-function defects induced by constitutively active Racs might reflect the role of Racs in both the formation and the suppression of actin-based morphogenetic structures.

**Racs act downstream of multiple guidance receptors**

Axon guidance signals are detected by transmembrane receptors on the growth-cone plasma membrane. Many guidance receptors and their ligands have been identified [3], two of which have recently been shown to act upstream of Rac signaling in axon development. The cytoplasmic tail of the semaphorin receptor Plexin B binds directly to Rac–GTP and inhibits Rac activity by sequestering Rac–GTP away from its effector Pak, contributing to Plexin-B-mediated axon repulsion [15,16]. Furthermore, in *C. elegans*, mutation of the Rac gene *ced-10* suppresses the effects of an activated form of the netrin receptor UNC-40 on axon development, indicating that CED-10 Rac acts downstream of UNC-40 [17**]. Interestingly, CED-10 Rac but not MIG-2 acts downstream of UNC-40, and CED-10 Rac acts in parallel to a second redundant pathway downstream of UNC-40 involving the cytoskeletal effector molecule UNC-34 Enabled [17**]. Thus, Racs can have redundant functions with one another as well as with other cytoskeletal signaling pathways that regulate axon development.

**The UNC-73 Trio GTP-exchange factor is a key regulator of Rac activity in axon development**

Although Racs can interact directly with the cytoplasmic tail of the Plexin B guidance receptor, other receptors undoubtedly utilize intermediate adaptor molecules to control Rac activity. Candidates for such adaptor molecules include the GEFs of the disabled-homology family (DH-GEFs). Recent studies implicate the UNC-73/Trio DH-GEF as a key regulator of Rac activity during axon development. In *Drosophila* and *C. elegans*, mutations in *unc-73/trio* lead to axon defects that resemble Rac loss-of-function [8**,14**,18–22]. *Racs* and *unc-73* interact genetically in *C. elegans*: weak *unc-73* mutations are enhanced by mutations in each of the three *racs*, indicating that UNC-73 acts with all three Racs in axon development [8**,14**]. Furthermore, *unc-73* mutations are suppressed by Rac overactivation, indicating that Racs act downstream of *unc-73* [12**,14**]. Moreover, overactivation of the Rac–GEF domain of *Drosophila Trio* is suppressed by Rac loss-of-function [10**]. Additionally, biochemical evidence from vertebrates, *C. elegans* and *Drosophila* indicates that UNC-73 Trio acts as a GEF for both canonical Racs and Mts [12**,21,22**]. Together, these results clearly define UNC-73 Trio as a key regulator of Rac activity in axon development. UNC-73/Trio probably controls the Racs in many aspects of axon development, as *C. elegans* *unc-73* mutants display the full range of axon development defects (outgrowth, guidance, branch formation and branch suppression defects), and each of these defects is enhanced by *rac* loss of function [14**].

Human Trio interacts with the cytoplasmic tail of a LAR receptor tyrosine phosphatase (RPTP) [23]. Mutations in *Drosophila* Dlar, which encodes a LAR RPTP, cause axon path-finding defects and enhance Trio mutations [18,24], suggesting that Trio acts downstream of Dlar RPTP in axon path-finding. Whether UNC-73 Trio acts downstream of other guidance receptors remains unclear. Unlike *ced-10* mutations, *unc-73* mutations do not suppress the effects of an activated UNC-40 guidance receptor [17**], suggesting that UNC-73 does regulate CED-10 Rac downstream of the netrin receptor UNC-40 in *C. elegans* ventral axon path-finding. However, this experiment is complicated by the fact that the *unc-73* mutations used were not null, leaving open the possibility that UNC-73 might participate with CED-10 downstream of UNC-40.

**A combinatorial model of Rac regulation during axon development**

A complex model of Rac function in axon development is emerging. First, although UNC-73/Trio probably controls multiple Racs in axon development, other upstream regulators control specific Racs. For example, CED-5, a
CDM family member that is thought to modulate Rac GTP exchange, acts in the MIG-2 pathway but not in the CED-10 or RAC-2/3 pathways in C. elegans axon development [8**,12**,25]. Second, Racs might use different regulators in response to different axon-guidance cues and receptors. The CDM protein CED-5 and the CrkII-like SH2–SH3 domain containing the receptor-adaptor protein CED-2 act with MIG-2 in motor-neuron axon development but not in amphid sensory-neuron axon development in C. elegans [12**]. Thus, Rac activity in different axon path-finding events is controlled by distinct combinations of guidance receptors and Rac regulators. An important future task will be to decipher the combinations of molecules that regulate Racs during axon development as well as to determine how these signaling pathways relate to the different guidance-receptor systems that mediate axon development.

Racs might control distinct growth-cone actin-cytoskeleton domains during axon development

The morphogenetic effects of Rac molecules are mediated in part by their regulation of the structure and dynamics of the actin cytoskeleton [4]. The growth-cone actin cytoskeleton (Figure 2) consists of multiple domains that mediate different aspects of growth-cone outgrowth (see Figure 2; [2]). The lamellipodial actin cytoskeleton consists of a network of branched and cross-linked actin filaments that is thought to mediate growth-cone motility and outgrowth, and the filopodial actin cytoskeleton consists of bundled actin filaments that protrude into the filopodial extensions and is thought to mediate growth cone steering in response to guidance signals [2,26]. Additionally, axon-shaft microtubules extend into the growth cone and interact with filopodial actin bundles in a process that is thought to stabilize filopodia and consolidate outgrowth decisions [27,28].

Rac mutations affect both axon outgrowth and axon guidance, suggesting that Racs control both the lamellipodial and filopodial actin cytoskeleton of the growth cone. Racs are also required for suppression of ectopic axons and branches. Studies in C. elegans indicate that a migrating growth cone at a guidance choice-point extends multiple filopodia, all but one of which is retracted before outgrowth proceeds along the remaining filopodium [29]. When filopodial retraction is blocked by mutation of the unc-119 gene, ectopic axon branching results [30]. Ectopic axons observed in Rac mutants could be the result of a failure in filopodial retraction, which might indicate that Rac normally plays a role in the destabilization of filopodia. Racs might control the formation or collapse of axons via the Rac–GTP/GDP cycle: Rac–GTP might mediate the formation of axons whereas Rac–GDP might control their collapse. However, constitutively active Racs cause defects in axon guidance and outgrowth, suggesting that Rac–GTP activity controls both axon formation and collapse.

Figure 2

Figure 2

Racs might influence multiple cytoskeletal domains of the growth cone during growth cone outgrowth, guidance and branching. Actin filaments are red and microtubules are green. The growth cone lamellipodium, which contains a branched network of actin filaments, mediates growth cone outgrowth. Growth cone filopodia, which contain bundles of actin filaments, mediate growth cone steering. Axon shaft microtubules interact with and stabilize specific filopodia, which consolidates guidance decisions and mediates axon branching. Microtubules are also involved in the formation of secondary growth cones from existing axons. Rac molecules might control these different cytoskeletal domains during different aspects of axon development.

Rac control of the CRIB-domain effector Pak might mediate axon guidance

Genetic studies suggest that distinct downstream Rac effectors are used to regulate different axon development events. Drosophila Rac1 carrying the Y40C mutation, which blocks Rac interaction with CRIB-domain-containing effectors such as Pak, can rescue axon outgrowth defects in Rac mutants but not guidance or branch formation defects [9**], indicating that Rac interaction with this type of effector is not required for axon outgrowth but is required for axon guidance and branch formation (Figure 3). Pak, a serine/threonine kinase, is activated by Rac [31,32] and is involved in Drosophila axon development [33]. Pak activity stimulates LIM kinase (LIMK), which in turn phosphorylates and inhibits the actin-filament-severing and depolymerizing protein coflin [34–36]. Cofilin activity has been shown to stimulate neurite extension [37]. Together, these results indicate that Rac might utilize Pak to influence the growth-cone actin cytoskeleton during axon guidance and branch formation.
branch suppression in acts downstream of Rac signaling during axon guidance, outgrowth and complex during axon path-finding. The F-actin binding protein UNC-115 suggesting that Rac activity might also regulate SCAR and the Arp2/3 complex in vitro circuitry, as Rac activity might influence the actin cytoskeleton by multiple pathways. UNC-73/Trio, a Rac-specific DH-GEF, controls Rac activity, including both canonical Rac and Mtl pathways, during axon development. *Drosophila* Rac1 might utilize the CRIB-domain-containing effector Pak to control axon guidance and branch formation. Pak activates LIMK, which phosphorylates and inhibits the actin-severing and depolymerizing protein cofillin. The Arp2/3 complex and one of its activators, SCAR, are involved in *Drosophila* axon development. The Arp2/3 complex nucleates actin filament branches from preexisting actin filaments and is involved in formation of the lamellipodial cytoskeleton, suggesting that Arp2/3 activity might be involved in axon outgrowth. To date, no interaction of Rac activity with SCAR or the Arp2/3 complex during axon path-finding has been found. However, Rac activates SCAR in vitro, suggesting that Rac activity might also regulate SCAR and the Arp2/3 complex during axon path-finding. The F-actin binding protein UNC-115 acts downstream of Rac signaling during axon guidance, outgrowth and branch suppression in *C. elegans*. Interestingly, UNC-115 might act downstream of the canonical Racs CED-10 and RAC-2/3 and not MIG-2, the Mtl Rac. Although the activity of UNC-115 on actin dynamics is not well understood, it is intriguing to speculate that UNC-115 modulates lamellipodial and filopodial dynamics in response to Rac signaling during axon guidance, outgrowth and branch suppression.

**Rac regulation of the actin-related protein 2/3 complex might mediate axon outgrowth**

A distinct downstream cytoskeletal effector of Rac is the actin-related protein 2/3 (Arp2/3) complex, a seven-molecule conglomerate that nucleates actin filaments from the sides of preexisting actin filaments [39,40]. Arp2/3 activity is thought to contribute to the branched actin network of lamellipodia [41,42]. Several molecules that activate Arp2/3-dependent actin nucleation have been identified, and canonical Rac activity stimulates at least two of these, Suppressor of cAMP receptor (SCAR) and cortactin, *in vitro* [42,43]. SCAR is a member of the WASP-homology-2/catalytic/acidic-region family of Arp2/3 activators (where WASP stands for Wiscott–Aldrich syndrome protein). Mutations in *Drosophila* Scar and in Arp3 and Arp1, which encode components of the Arp2/3 complex, cause axon defects in the CNS, including axon displacement and failure to form axon commissures [44*], indicating that SCAR and the Arp2/3 complex are involved in axon development. Although a direct functional link between Racs and the Arp2/3 complex has not been made *in vivo*, Scar, Arp3 and Arp1 defects resemble those caused by Rac mutation in the CNS [10**,44**]. It might be that the Arp2/3 complex modulates lamellipodial actin dynamics and growth-cone outgrowth in response to Rac signaling (Figure 3).

**Do Racs regulate microtubule dynamics in axon development?**

Some aspects of axon development that are dependent on Rac also involve microtubules. As diagrammed in Figure 2, axon-shaft microtubules extend into the growth cone where they are thought to interact with and stabilize filopodial actin bundles. This interaction might consolidate guidance and outgrowth decisions and mediate growth-cone branching [27,28]. Furthermore, microtubules are involved in secondary growth-cone initiation, which leads to axon branching [47,48]. A direct link between Rac signaling and microtubules is suggested by the IQGAP protein, a Rac GAP with an IQ domain (IQxGxxxxR)
that also interacts with CLIP-170 and stimulates CLIP-170-mediated microtubule-end-capturing activity [49,50]. Furthermore, Pak phosphorylates and inhibits the microtubule catastrophe factor statmin in response to Rac signaling [51]. The roles of IQGAP and statmin in axon development remain to be determined, but it might be that IQGAP and statmin influence growth-cone microtubules in response to Rac signaling during axon outgrowth, guidance and branch formation.

Conclusions

Rac GTPases have for some time been known to regulate the actin-cytoskeleton dynamics underlying cellular morphogenesis. The work summarized here represents the early stages in the dissection of the roles of Racs and their upstream regulators and downstream effectors in axon development. The emerging picture is complex. Multiple Rac molecules have functional overlap with one another and with other cytoskeletal signaling pathways during axon development. Furthermore, distinct combinations of upstream guidance receptors and cytoplasmic Rac regulators control the Racs in different axon path-finding events. Finally, Racs might utilize different downstream cytoskeletal effectors to influence the distinct filopodial and lamellipodial actin-cytoskeleton domains involved in axon development. An important future endeavor will be to identify and characterize the different combinations of guidance receptors and Rac regulators involved in specific guidance decisions and to understand the molecular mechanisms by which Racs mediate growth-cone actin-cytoskeleton change in response to guidance signals. Also, it will be important to understand how Rac interact with other cytoskeletal signaling pathways, particularly the other Rho GTPases Rho and Cdc42, during growth-cone path-finding in the developing nervous system.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

** of outstanding interest


This study of Rac loss-of-function mutations in C. elegans is the first report that Rac proteins are required for axon development. It shows that three Rac proteins, CED-10, RAC-2 and MIG-2, have redundant roles in axon development, cell migration and cell corpse phagocytosis and that the DH-GEF UNC-73 interacts with all three Racs in axon development and cell migration. Furthermore, it is the first report that CED-5, a CDC Rac regulator, is involved in axon development and acts in the MIG-2 but not the CED-10 or RAC-2/3 pathways.


A study of loss of Rac family members in Drosophila that demonstrates that different axon path-finding events are sensitive to the dose of three Rac proteins with redundant functions. Also, this study demonstrates that CRIB-domain effectors of Rac (e.g. Pak) are not required for axon outgrowth but are required for axon guidance and branch formation.


This study shows that three Drosophila Rac proteins have overlapping roles in numerous morphogenetic events including axon development and shows that the DH-GEF Trio acts with the Racs in axon development but not in epithelial morphogenesis or myoblast fusion.


A study of the redundancy of three C. elegans Racs and UNC-73 in the morphogenesis of the epithelial vulva.


This study expands upon the requirements of three C. elegans Racs and UNC-73 DH-GEF in axon development. Importantly, the study shows that the Rac regulators CED-2 CRKI and CED-5 CDM act with the Racs in some axons and not in others. Furthermore, it demonstrates that UNC-73 acts as a GEF on the C. elegans Racs (CED-10 and MIG-2) and that Rac constitutive activation can partially rescue unc-73 mutations, demonstrating that the Rac acts downstream of UNC-73 in axon development.


This work demonstrates that Rac and UNC-73 are required for the suppression of ectopic axons in addition to axon outgrowth and guidance. Furthermore, the work identifies the actin-binding protein UNC-115 as a downstream Rac effector that is necessary for Rac-induced lamellipodia and filopodia formation. Importantly, this work shows that Rac downstream effectors can be identified by suppression of constitutively active Rac.


This work elegantly demonstrates that CED-10 Rac acts downstream of the UNC-40 guidance receptor. Importantly, this work provides proof of principle that axon-development signaling pathways can be dissected using suppression of activated guidance receptors.

Signalling mechanisms


