Control of developmental networks by Rac/Rho small GTPases: How cytoskeletal changes during embryogenesis are orchestrated

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Small GTPases in the Rho family act as major nodes with functions beyond cytoskeletal rearrangements shaping the *Caenorhabditis elegans* embryo during development. These small GTPases are key signal transducers that integrate diverse developmental signals to produce a coordinated response in the cell. In *C. elegans,* the best studied members of these highly conserved Rho family small GTPases, RHO-1/RhoA, CED-10/Rac, and CDC-42, are crucial in several cellular processes dealing with cytoskeletal reorganization. In this review, we update the functions described for the Rho family small GTPases in spindle orientation and cell division, engulfment, and cellular movements during *C. elegans* embryogenesis, focusing on the Rho subfamily Rac.

Keywords:
Caenorhabditis elegans; cytoskeletal rearrangements; embryonic development; Rac; Rho; small GTPases

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Abbreviations:
GAP, GTPase activating protein; **GDI,** guanosine nucleotide dissociation inhibitor; **GEF,** guanine nucleotide exchange factor.

Introduction

Metazoan embryonic development requires a tight coordination of processes to reach a correct body plan. Embryogenesis roughly consists of cell proliferation, migration, differentiation, and removal of unwanted cells by apoptosis and engulfment to produce organs with specific shape and functionality (Supplementary Movie S1). Small GTPases are emerging as nodes to integrate developmental signals into a dynamic cellular response. They act as key signal transducers that regulate a wide variety of processes in the cell, such as actin dynamics, cell proliferation and differentiation, vesicle trafficking, and nuclear transport [1, 2]. Small GTPases are found in all eukaryotic organisms, and have been classified into at least five groups (Ras, Rho/Rac, Rab, Arf, and Ran) based on their sequence and functional similarities [1, 2]. They are enzymes that can bind and hydrolyze GTP in their active form [3]; and GTP hydrolysis, which results in the inactivation of the GTPase, is promoted by the action of GTPase activating proteins (GAPs) that stimulate their hydrolase capacity resulting in GDP bound proteins. The activation of guanine nucleotide exchange factors (GEFs) facilitates the release of GDP from the inactive small GTPases, and subsequent binding to GTP, resulting in the active GTP-bound state [4]. Subsequently the active small GTPases interact with their downstream effectors resulting in either their relocalization or their enzymatic activation, hence eliciting a cellular response. In addition, Rho and Rab small GTPases can be further stabilized by binding to guanosine nucleotide dissociation inhibitors (GDIs), which maintain the GTPases in their inactive state, extracting them from membranes and sequestering them in the cytosol by forming a high affinity complex with their prenylated tails and protecting them from proteolysis [5, 6].

Here, we highlight new functions and relationships in the Rho family GTPases focusing on CED-10/Rac. Work on

Caenorhabditis elegans has shown that CED-10/Rac, together with RHO-1/RhoA and CDC-42, are required in distinct cellular events controlling cytoskeletal rearrangements. Additionally, recent work has linked human Rac with different pathological conditions, such as cardiovascular diseases, tumorigenesis, or different stress responses [7–13]. Thus, understanding the molecular pathways mediating the activation of CED-10/Rac and their subsequent physiological outcome is important for defining new therapeutic strategies.

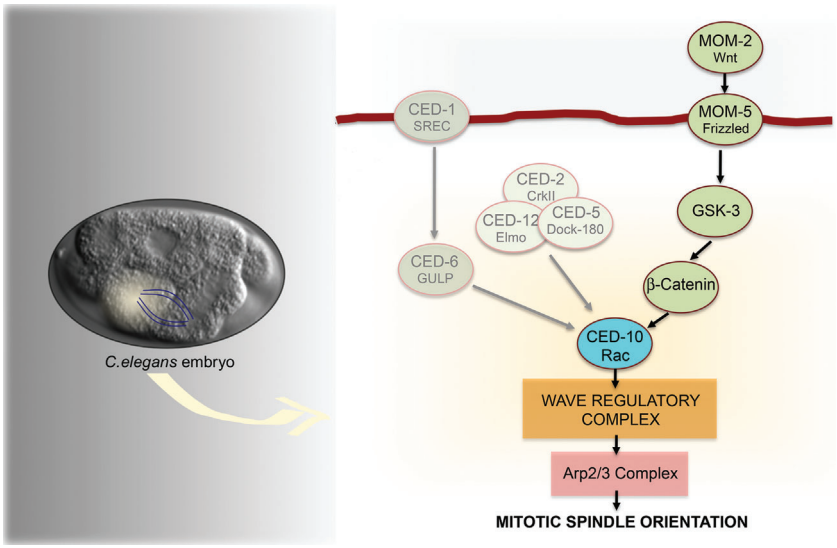

Figure 1. Mitotic spindle orientation. This figure shows the main Wnt to CED-10/Rac pathway acting on the cytoskeleton to orient properly the mitotic spindle when the embryo cells are dividing. Other signaling cascades such as CED-1/SREC-CED-6/GULP pathway and CED-2/CrkII-CED-5/Dock180-CED-12/ELMO pathway that provide a minor contribution are represented with a blurred background. Small GTPase CED-10 is shown in cyan, activators in green.

Rac/Rho small GTPases mediate spindle orientation and cell division

The development of a multicellular organism implies an accurate regulation of cell division in terms of time, position, and orientation, in order to establish its final shape. To do so, the cytoskeleton undergoes a set of striking rearrangements in each mitosis cycle to ensure the proper building and location of the spindle and the physical separation of a single cell into two daughter cells. These processes are regulated by Rho GTPases in a wide variety of species [14].

Early *C. elegans* embryos are very useful for understanding the mechanics of metazoan cell division. Its simplicity, the stereotyped cell lineage, and cell movements pattern, together with its transparency, make this animal an ideal model for studying development. Genetic studies, in combination with 4D microscopy analyses, have shown that CED-10/Rac is required for proper rotation of the mitotic spindle of some early blastomeres in the *C. elegans* embryo, named EMS – the endoderm and mesoderm precursor – and ABar – whose descendants form part of the pharynx – [15, 16]. These mitotic spindle orientations are induced by cell signaling, from blastomeres derived from the posterior cell at the two-cell stage embryo – termed P1 –, that triggers actin cytoskeletal reorganizations in the target cells. In *C. elegans*, these rearrangements are controlled by a polarized Wnt signaling [17] and are part of the process that diversify the fate of the daughter cells after division. Upon local activation of the MOM-5/Frizzled receptor by a Wnt ligand, at the cellular membrane, the signal is transduced through a signaling cascade until it reaches β-catenin. Three *C. elegans* β-catenins (WRM-1, BAR-1, and HMP-2) act redundantly upstream of CED-10/Rac to promote the mitotic spindle reorientation toward the cell pole that received the Wnt induction (Fig. 1). In addition to Wnt signaling, other pathways acting upstream of CED-10/Rac, such as those described in the engulfment of the apoptotic corpses, may provide a minor contribution to embryonic cell spindle orientation [16, 18] (Fig. 1).

Once the cell has been polarized and the mitotic spindle orientation has been defined, the mitotic spindle segregates the chromosomes to the two daughter cells that are initiating cytokinesis. In order to split the two cells, an actomyosin contractile ring is formed at the division plane. The best Rho GTPases characterized to function in *C. elegans* actomyosin constriction are RHO-1/Rho-A, CED-10/Rac, and CDC-42. Activated RHO-1/RhoA (GTP-bound RHO-1/RhoA) localizes at the cell division site, stimulating both filamentous actin assembly and myosin II-motor activation to trigger the furrow ingression [14, 19]. A key factor responsible for RHO-1/RhoA activation at the cleavage site is Centralspindlin, an evolutionarily conserved protein complex that is essential for central spindle assembly and cytokinesis. In *C. elegans*, the Centralspindlin complex is a heterotetramer composed of a dimeric kinesin, ZEN-4/MKLP1 and a dimeric Rho GAP, CYK-4/MgcRacGAP [20]. Centralspindlin targets ECT-2 (which has a C-terminal Rho GEF domain) to the central spindle, which requires regulated binding between ECT-2 and the Rho GAP CYK-4 [21, 22]. In addition, *C. elegans* has an independent mechanism to activate RHO-1/RhoA at the central spindle, through NOP-1, a nematode specific protein. Although nonessential for *C. elegans* viability, in the absence of NOP-1, cytokinesis is completed but furrow initiation is slightly delayed, and RHO-1/RhoA accumulates at a lower concentration at the central spindle [23].

In contrast to RHO-1/RhoA's role, CED-10/Rac might be a negative regulator of cytokinesis [24]. In *C. elegans* it has been shown that CED-10/Rac repression by the GAP activity of CYK-4 is necessary for downregulating Rac GTPases at the cleavage site in order to drive cytokinesis. Inhibition of CED-10/Rac at the division plane is essential in order to prevent Arp2/3 complex activation, (which induces branched actin polymerization), thus reducing cell adhesions and permitting contractile ring constriction. This inactivation of CED-10/Rac by Centralspindlin functions in parallel with RHO-1/RhoA activation in the same location to drive

cytokinesis [24]. However, the function of the CYK-4 RhoGAP domain seems to depend on the biological system and the CYK-4 mutations used in the experiments [25].

Finally, CDC-42 also plays a role in cytokinesis. Though the precise mechanism of CDC-42 is not fully understood, in most animal systems the depletion or disruption of CDC-42 activity does not block cytokinesis [23, 26]. However, constitutive activation of CDC-42 results in cytokinesis failure [27, 28]. In *C. elegans*, CDC-42 RNAi causes defects in embryonic cytokinesis, early cell polarity, and mitotic spindle orientation, though further studies are needed to understand the function of CDC-42 in cytokinesis [23, 29].

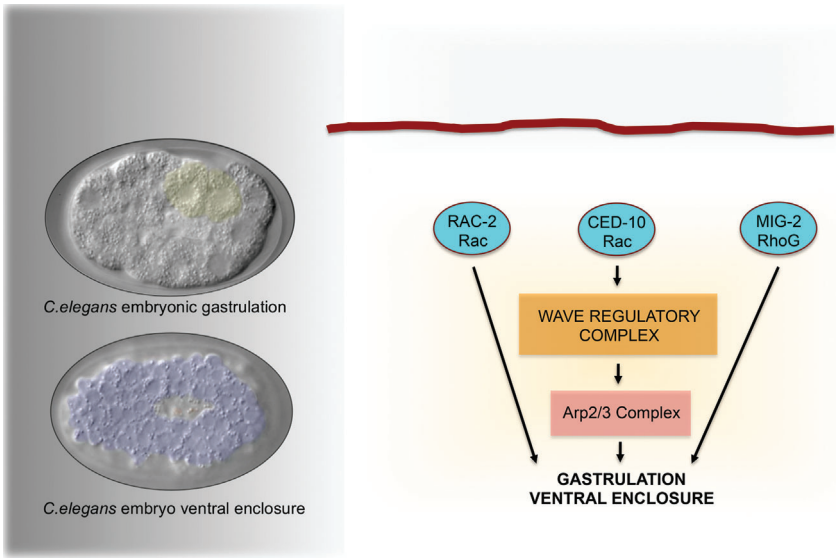

Figure 2. Embryonic cellular movements. This figure indicates that CED-10/Rac acts beyond two main processes during *C. elegans* morphogenesis: gastrulation and ventral enclosure. Both of them need cytoskeletal rearrangements that are launched by CED-10/Rac. The other rac-like genes, RAC-2/Rac and MIG-2/RhoG provide a minor contribution. Yellow cells mark endoderm precursors and blue cells mark ectodermal cells. Small GTPases: RAC-2, CED-10, and MIG-2 are shown in cyan.

Embryonic cell movements and neuronal development are regulated by small GTPases

During embryonic and postembryonic development of *C. elegans*, cell movements define different processes, such as gastrulation, gonadal development, or the establishment of nervous system topology. The well-known invariant pattern of *C. elegans* development and its transparent body make this nematode an ideal in vivo model system in which to study the molecular basis of cell migration and other cellular movements. Additionally, in mammals cell migration is essential for immune system function and wound healing. It is even important in pathological processes such as metastasis. In all these cases, the molecular mechanisms underlying cellular movements are conserved throughout evolution [30].

One of the major processes of cellular reorganization during *C. elegans* embryogenesis is gastrulation, by which endodermal and mesodermal precursors become internalized in the embryo at the 28–30 cell-stage [31]. Actomyosin contractility is essential for the ingression of cells during gastrulation [32]. Gastrulation in *C. elegans* is not as spectacular as in many other animal embryos, because cells move only over short distances, and the blastocoel space is small [33] as can be observed in Supplementary Movie S1. CED-10/Rac was shown to play an essential role during early *C. elegans* embryogenesis because *C. elegans* null homozygous *ced-10*/Rac mutant embryos arrest, manifesting defects in cell migration and gastrulation. Most of these null *ced-10*/Rac mutant embryos exhibit a terminal phenotype in which the gut is present on the exterior of the embryo termed the Gex phenotype "gut on the exterior"; where endoderm cell types that are normally internal are located on the exterior of the embryo [33].

Downstream of *ced-10*/Rac acts the Arp2/3 complex activator WAVE/SCAR, which forms a complex with GEX-2/Sra-1, GEX-3/Kette, ABI-1, and NUO-3A (Fig. 2) [33, 34]. Consequently, in the absence of CED-10/Rac, WAVE does not localize properly. It is thought that the WAVE/SCAR complex is activated through membrane recruitment by CED-10/Rac. Moreover, the three receptors that, so far, have been described as involved in cell guidance UNC-40/DCC, SAX-3/Robo, and VAB-1/Eph regulate the abundance and subcellular localization of CED-10/Rac, the WAVE/SCAR actin nucleation complex and F-actin in the embryonic epidermis [35].

In addition to this pathway, gastrulation is regulated by the *unc-34* gene, the worm homolog of the human Enabled gene (Ena), which belongs to the Ena/VASP family, formed by evolutionarily conserved actin-modulating proteins [36]. In fact, CED-10/*Rac* and UNC-34/Ena might have partly overlapping roles in this event. Null *unc-34* mutants are viable and fertile, but embryos of double mutants *unc-34(lq17); ced-10 (n1993)* (both hypomorphic alleles) arrest with the Gex phenotype, suggesting that UNC-34/Ena and CED-10/Rac have overlapping roles in gastrulation [36]. Additionally, interactions between *unc-34/Ena* and other rac genes were checked: *ced-10(n1993)* and *rac-2(RNAi)* synergized with *unc-34* in gastrulation and embryonic elongation. However, *unc-34/Ena; mig-2/RhoG* double mutants were viable and fertile, suggesting that *unc-34/Ena* and *mig-2/RhoG* might act in a common pathway in parallel to *ced-10/Rac* and *rac-2/Rac* in gastrulation [36].

Later on, at the 350 cell-stage, epidermal cells undergo ventral enclosure, a process by which epidermal cells located on the dorsal side of the embryo go through an epiboly movement to the ventral midline to cover the embryo [36] (Supplementary Movie S1). Simultaneously, on the dorsal side of the embryo, epidermal cells undergo cell intercalation and the embryo elongates [33, 37, 38].

The migration of ventral epidermal cells toward the ventral midline requires the formation of actin-rich protrusions at the

leading edge of the cells (filopodia and lamellipodia). When the epidermal cells from opposite sides meet, these actin-rich protrusions overlap to seal the epithelial monolayer [39, 40]. Genes required to form the filopodia/lamellipodia protrusions encode the GTPase CED-10/Rac, the WAVE/SCAR complex, and the Arp2/3 complex. CED-10/Rac activates the WAVE/SCAR complex, which serves as an actin-nucleation promoting factor to activate the Arp2/3 complex for the polymerization of short, branched F-actin (Fig. 2) [33, 40, 41]. Loss of CED-10/Rac, any WAVE/SCAR component, or the Arp2/3 complex, blocks cell migrations of the embryonic epidermis, whereas cell differentiation remains unaffected, in all these cases the ventral epidermal cells remain unattached on both sides of the embryo. As a result, internal cells are not fully covered by epidermis, and extrude out during elongation of the embryo, causing a Gex phenotype [33, 40]. CED-10/Rac and MIG-2/RhoG are also known to redundantly control the basolateral protrusive activity involved in cell rearrangements of dorsal epidermal cell intercalation [42]. A tight signaling control of cytoskeleton dynamics is necessary for these cellular movements that provide a correct *C. elegans* embryonic development.

In addition, Rho GTPases are required for major changes in cell shape, such as those occurring during neuronal development. The majority of *C. elegans* neurons are born during embryogenesis, close to their final positions, but the ability of cells to move is an essential step in the establishment of the nervous system, because neurons must form axons, which are guided to their targets by the growth cone, a sensory-motile structure at the distal tip of extending axons. Growth cones are composed of lamellipodia and filopodia, actin-based plasma membrane extensions where transmembrane receptors detect guidance cues. These receptors are members of the netrin, slit, semaphorin, and ephrin families [43]. As expected, Rac-small GTPases (CED-10/Rac, MIG-2/RhoG, and RAC-2/Rac), key regulators of actin dynamics, are involved in these cytoskeletal rearrangements. Single mutants in any of these genes lead to axon pathfinding defects whereas a double mutant shows stronger defects.

Axon pathfinding and cell migration are, to some extent, similar mechanisms. Both need actin cytoskeleton rearrangements in the growth cone or at the leading edges of the plasma membrane of migrating cells [36, 44, 45]. In *C. elegans*, in response to UNC-6/Netrin signaling, Rho GTPase CDC-42 activates the *C. elegans* GEF TIAM (T-cell lymphoma Invasion and Metastasis Factor 1), which is a GTP exchange factor specific for Rac and which activates Rac signaling, in lamellipodia and filopodia formation and axon guidance [46, 47]. Recently, Zheng et al. showed that the Rac GEFs UNC-73/Trio and TIAM-1 promote neuronal extension toward opposite directions: UNC-73/Trio promotes anterior extension whereas TIAM-1 causes a posterior neurite extension [48]. Also recently, Norris et al. showed that the Rac GEFs UNC-73/Trio and TIAM-1 have opposite roles in growth cone protrusion: TIAM-1 activates the Arp2/3 complex to stimulate protrusion whereas UNC-73/Trio acts through the cytoskeletal regulators UNC-44/Ankyrin and UNC-33/CRMP to inhibit protrusion (Fig. 3) [2, 49].

Downstream of Racs at least two pathways act in parallel to positively control actin dynamics and lamellipodia and filopodia formation (Fig. 3): WASP–WAVE and UNC-115/abLIM [2, 40].

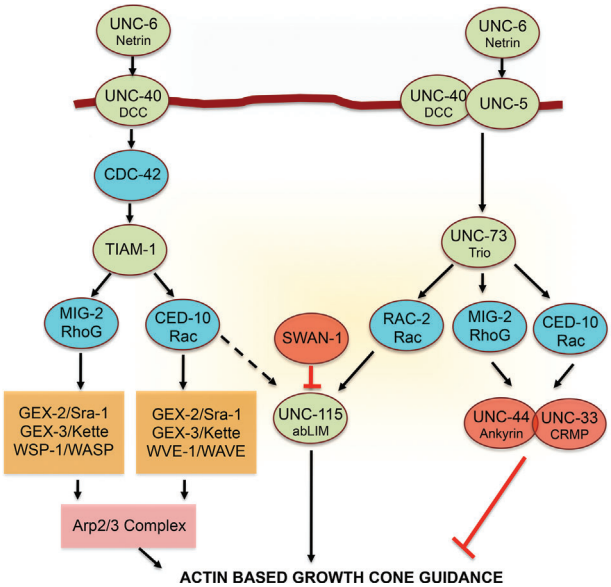

Figure 3. Neuronal development. This figure shows the Rho GTPases that act to restructure the cytoskeleton in order to achieve a functional *C. elegans* nervous system. By extending axons, *C. elegans* neurons arrive at their targets. The main pathways that control lamellipodia and filopodia formation depend on the activity of small GTPases CDC-42, MIG-2/RhoG, CED-10/Rac, and RAC-2/Rac. Small GTPases: MIG-2, CED-10, RAC-2, and CDC-42 are shown in cyan, activators in green, and repressors in red.

(1) WASP-WAVE: Genetic studies revealed overlapping roles of Rac-like downstream effectors wve-1/WAVE (a member of the WRC) and wsp-1/WASP (Wiskott Aldrich syndrome protein) during axon guidance. *wve-1*/WAVE and *wsp-1*/WASP single mutants had mild effects on axon guidance, whereas *wve-1; wsp-1* double mutants displayed severe defects [41]. Both proteins have C-terminal WH2 domains known to function as Arp2/3 activators in other systems. Genetic experiments suggest that WVE-1/WAVE acts in the CED-10/Rac pathway and WSP-1/WASP acts downstream MIG-2/RhoG in two parallel pathways, since double mutants *ced-10; wve-1* or *mig-2; wsp-1* showed few defects, whereas *mig-2; wve-1* or *ced-10; wsp-1* mutants had much more severe axon guidance defects [41].

(2) UNC-115/abLIM: Defects in axon pathfinding have also been described in *unc-115* mutants. UNC-115 is a putative actin-binding protein similar to human abLIM (actin-binding LIM) protein. When an *unc-115*/abLIM mutant is combined with a *ced-10*/Rac, *mig-2*/RhoG, or *unc-73*/Trio mutant, the double mutant shows stronger pathfinding defects than the single mutants, suggesting that *unc-115*/abLIM acts in parallel to *ced-10*/Rac and *mig-2*/RhoG. However, there is no synergistic effect in an *unc-115*/abLIM; *rac-2*/Rac double mutant. Indeed, *unc-115*/abLIM loss-of-function partially suppresses the ectopic lamellipodia and filopodia induced by activated *rac-2*/Rac suggesting that *unc-115*/abLIM might act downstream

rac-2/Rac [50]. In addition, u*nc-115*/abLIM interacts in a yeast two-hybrid screening with a conserved molecule named SWAN-1 (seven WD repeat protein of the AN11 family-1). This protein also interacts with Rac GTPases, raising the possibility that it is a molecular linker between UNC-115/abLIM and Racs. Functional studies in *C. elegans*, mammalian fibroblasts, and yeast suggest that SWAN-1/DCAF7 is an inhibitor of Rac GTPase activity. Thus, in *C. elegans*, the *swan-1(ok267)* loss-of-function mutation suppresses defects caused by the hypomorphic *ced-10 (n1993)* allele and in neurons, it enhances ectopic lamellipodia and filopodia formation induced by constitutively active Rac [51].

Furthermore, other Rac genetic interactors have been described for their role in neuronal development, although their interaction with the above pathways is only partially characterized. This is the case of MIG-15, the *C. elegans* homolog of the vertebrate NIK (Nck-interacting kinase) which affects axon pathfinding and might act in each *Rac* pathway [36, 52]. Racs control multiple aspects of axon pathfinding. The fact that distinct signaling pathways promote neurite extension in different directions, or promote or inhibit growth cone protrusion, suggest that all might be necessary to achieve a coordinated and balanced growth of axons. In addition, the redundancy of the signaling processes reflects the importance of these networks during development.

CED-10/Rac is at the core of the phagocytosis of apoptotic corpses

Building an animal body requires cell proliferation and correct cell allocation in the embryo, features that go hand in hand with the death and the removal of specific cells, leading to the formation of fully functional organs. Programmed cell death, or apoptosis, is defined in three stages: "specification," "killing," and "execution." In this last stage, the dying cells are recognized, up-taken, and degraded by engulfing cells [48, 53–55]. This process takes place with cross-communication between dying and engulfing cells. Activation of engulfment genes in the engulfing cell promotes apoptotic progression in injured cells. Clearance of apoptotic cells is carried out extremely rapidly in order to protect adjacent tissues from the release of harmful contents, which would promote inflammation and autoimmunity [56–58].

Apoptotic corpse engulfment is highly conserved throughout the animal kingdom. In mammals, this task is carried out by macrophages. In *C. elegans*, cell death take place in response to developmental cues during embryogenesis, whereas in adult worms cells die, only in the germ line, in response to DNA damage, genotoxic stress or bacterial infection. Thus, phagocytosis of cell corpses is performed by gonadal sheath cells in adults or by any cell in the embryo. The fact that apoptosis is not essential for *C. elegans* viability, as it is in mammals (for the correct establishment of organ architecture and functionality), and the easily identifiable lentil shape of the corpses in vivo, under the Nomarski optics, has made this organism an excellent model to study apoptosis and dead cell clearance.

The engulfing cell needs to enlarge its cytoskeleton in order to extend the surface of its membrane around the apoptotic cell. Currently, three signaling pathways are known to positively regulate this process, converging at CED-10/Rac, which acts as a major node to integrate the different signals in a balanced cytoskeletal rearrangement (Fig. 4).

In the first signaling cascade (Fig. 4A), the phagocytic transmembrane receptor CED-1/SREC (Scavenger Receptor from Endothelial Cells) recognizes the apoptotic cells and transduces the engulfment signal into the phagocyte [59]. The means whereby CED-1/SREC or any of its homologs in mammals (SREC, LRP/CD91, or MEGF10 – multiple EGF-like-domains 10 –), or in *Drosophila* (Draper and SIMU – Six-microns-under –) recognize apoptotic cells has largely remained elusive. A conserved "eat me" signal exposed on the surface of apoptotic cells is phosphatidylserine (PS). The extracellular domain of CED-1/SREC was shown to directly interact with the PS binding protein TTR-52/TTR, a transthyretin homolog secreted from *C. elegans* endoderm that clusters around apoptotic cells. TTR-52 may act as an extracellular bridging molecule that mediates the binding and recognition of apoptotic cells [60]. In addition, TTR-52, together with CED-7/ABCA1 (a plasma membrane ABC transporter that also colocalizes around apoptotic corpses), regulates exoplasmic PS expression in apoptotic cells and promotes the generation of extracellular PS vesicles that will lead to the appearance of exoplasmic PS on phagocytes, a process that facilitate cell corpse clearance [61].

After apoptotic cell recognition by the engulfing cell, CED-1/SREC directly interacts with the adaptor protein CED-6/GULP, leading to downstream CED-10/Rac activation [62]. CED-6/GULP will also transduce the signal from CED-1/SREC to activate other engulfment tools such as DYN-1/Dynamin, which has been suggested to promote vesicle recruitment and fusion to maturing phagosomes [63]. Epistatic analysis has revealed that DYN-1/Dynamin acts in this engulfment pathway upstream of EPN-1/Epsin and CHC-1/Clathrin, both of which are essential for the assembly and stability of F-actin underneath pseudopod membranes [64] (Fig. 4).

The second signaling pathway (Fig. 4B) is defined by the GEF complex composed of CED-5/Dock180, CED-12/Elmo, and the protein adaptor CED-2/Crk-II, which switches on CED-10/Rac to promote actin polymerization. Four different signaling pathways act upstream of this heterotrimeric CED-5/Dock180, CED-12/Elmo, CED-2/Crk-II GEF complex to activate it in the cell (Fig. 4B):

(B.1) One goes through the GEF UNC-73/Trio, which activates the small GTPase MIG-2 /RhoG and hence regulates the CED-5/Dock180-CED-12/Elmo GEF complex [65] and recruits it to the membrane.

(B.2) Wnt pathway components, between the frizzled receptor and APR1/APC, act genetically upstream of CED-10/Rac during cell corpse engulfment. In *C. elegans*, stimulation of the Wnt pathway leads to activation of the glycogen synthase kinase GSK-3, which acts in a complex with APR-1/APC. In fact, phosphorylated APR-1/APC has the

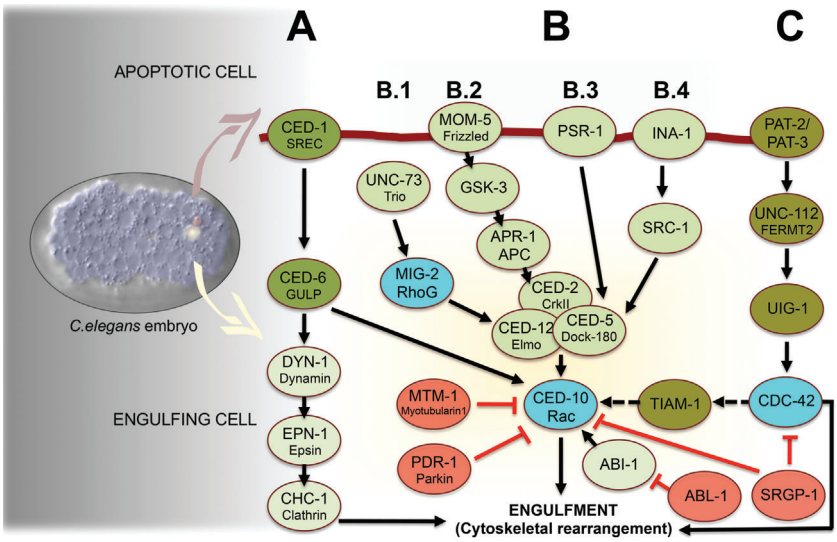

Figure 4. Phagocytosis of apoptotic corpses. This figure shows an embryo apoptotic cell corpse (marked in red) that is being engulfed by a neighbour cell (marked in yellow). Three signaling pathways act redundantly to control cytosketal rearrangements in order to drive phagocytosis of cell corpses converging in CED-10/Rac: **A:** CED-1/SREC-CED-6/GULP pathway; **B:** CED-2/CrkII-CED-5/Dock180-CED-12/ELMO pathway; **C:** CDC-42 pathway. In addtion, MTM-1, PDR-1, and SRGP-1 act as negative regulators of CED-10/Rac. In the engulfing cell pathways, Small GTPases: MIG-2, CED-10, and CDC-42 are shown in cyan, activators in green, and repressors in red.

ability to bind CED-2/CrkII in a Yeast-Two-Hybrid assay. Although it is still unclear how apoptotic corpses signal the frizzled receptor, the physical interaction between APR-1/APC and CED-2/CrkII sheds light on how the cell directs GSK-3 signaling toward the CED-10/Rac pathway to promote cell corpse engulfment [16].

(B.3) Another pathway upstream of the heterotrimeric CED-5/Dock180, CED-12/Elmo, CED-2/Crk-II GEF complex is mediated by the phosphatidylserine receptor, PSR-1, which might act in this pathway through direct interaction with CED-5/Dock180 and CED-12/Elmo. However, since *psr-1* null mutants have a weaker engulfment defect than any of the *ced-2*, *ced-5*, or *ced-12* mutants, the contribution of this receptor to apoptotic corpse removal is thought to be minor [16, 66].

(B.4) Recent studies have shown that integrins (transmembrane receptors for cell–cell or cell–extracellular matrix interactions) could function as engulfment receptors [67]. In *C. elegans*, there are two integrin α subunits, INA-1 and PAT-2, and one β subunit, PAT-3. The integrin INA-1 recognizes cell-corpses and acts through the *ced-2/-5/-12* pathway in epidermal engulfing cells. Moreover, the nonreceptor tyrosine kinase SRC-1 co-localizes with INA-1 in phagocytic cups and is thought to link the signal from INA-1 to CED-2/CrkII [66].

In addition to their function upstream of the CED-5/Dock180, CED-12/Elmo, CED-2/Crk-II GEF complex, integrins participate in the third parallel signaling pathway (Fig. 4C) that activates CDC-42 and in parallel to CED-10/Rac but also potentially through CED-10/Rac in the engulfment of apoptotic corpses. Anderson et al. proposed that there might be other yet unidentified molecular pathways that activate the Wave Regulatory Complex (WRC) in parallel to *ced-10* [68]. In fact, integrins PAT-2/PAT-3 function in muscle cells, recognizing exposed PS and signaling through UNC-112/FERMT2 and UIG-1, a GEF specific for the small GTPase CDC-42 (cell division control protein-42) [69]. Activated CDC-42 is then recruited to the plasma membrane surrounding apoptotic corpses [70]. Therefore, it seems that different integrins could work in a cell type-specific manner to promote engulfment in different tissues. Moreover, it has been suggested that CDC-42 also promotes engulfment through CED-10/Rac. In the absence of the GEF TIAM-1, CDC-42 overexpression fails to suppress the engulfment defects observed in *ced-10/RAC* mutants. This also revealed that different GEFs act in cell corpse engulfment [70] (Fig. 4).

Removal of apoptotic corpses must be tightly regulated by activators and repressors to avoid pathway over-activation. Identification of pathways involved in positive regulation of cell-corpse removal was relatively easy. Genetic screenings were designed to identify mutants that, after disruption of any of the above-mentioned pathways, accumulated persistent corpses in the head or the gonad of the worm. Only in the last years, the combination of suppressor screenings and time-lapse microscopy analyses has unmasked the existence of negative regulators of Rac dependent engulfment pathways. There is expanding research in the field of the negative regulators of cell clearance aimed at understanding the fundamental mechanisms involved in processes such as neurodegeneration [71–73]. Four independent pathways have been identified as negative regulators of CED-10/Rac during cell corpse engulfment (Fig. 4).

(1) The first negative regulator is ABL-1/Abl kinase, which blocks cell-corpse engulfment and cell migration. Although the mammalian Abl inhibits cell migrations via phosphorylation of CrkII, in *C. elegans* the mechanism is different: ABL-1 inhibits ABI-1 (both interact physically in vitro), which acts to promote engulfment. ABI-1 might act either independently of CED-10 or through CED-10 but in parallel to the ced-1 or ced-5/-12 engulfment pathways [74].

(2) The second negative regulator of apoptotic cell clearance is MTM-1 (Myotubularin 1), a phosphatase that removes phosphate groups from membrane lipids. Myotubularin family phosphatases have been found in almost all eukaryotes, but their cellular functions are not completely clear. Mutations in human MTM genes are associated with various diseases. For example, MTM-1 causes X-linked myotubular myopathy, a severe congenital muscular disorder. In *C. elegans*, MTM-1 acts through the *ced-5/-12/-10* pathway and hence its inactivation causes significant reduction in cell corpses in strong loss-of-

function mutants of *ced-1*, *ced-6*, *ced-7*, and *ced-2*, but not in *ced-5*, *ced-12*, or *ced-10* null mutant worms [75].

(3) In parallel to ABL-1 and MTM-1 a third negative regulator is acting: SRGP-1 (Slit/Robo GTPase activating protein 1), a GAP for CED-10/Rac. The GAP domain of SRGP-1 binds active-CED-10 in vitro. As a result of this activity, loss of SRGP-1 enhances engulfment signaling, removing dead cells and promoting the clearance of injured cells [57]. In addition, the GAP domain of SRGP-1 also interacts with the active form of CDC-42. Therefore, SRGP-1 might regulate engulfment signaling through both CED-10/Rac and CDC-42 GTPases [70].

(4) A fourth negative regulator, PDR-1/Parkin (a component of an E3 ubiquitin ligase complex) has been shown to directly ubiquitinate CED-10/Rac for degradation. PDR-1/Parkin maintains CED-10/Rac levels to prevent over-activated apoptotic cell engulfment or abnormal distal tip cell (DTC) migration [72]. In humans, autosomal recessive juvenile Parkinson's disease (AR-JP) is characterized by parkin mutations [76], a possible mechanism underlying this or other neurodegenerative disease characterized by abnormal CED-10/Rac degradation could be an acceleration of neurons phagocytosis. Further studies are still needed to test the relevance of Rac-1-Parkin interaction in diseases.

The wide range of signals, either promoting or inhibiting cell corpse clearance, converging on CED-10/Rac, or CDC-42, reveals their key position in the engulfment process. However, this is still an open research field, and evidence indicates that other proteins and other pathways might play a role in apoptotic corpse clearance in different cellular contexts. One example is the glycoprotein Progranulin (PGNR-1) which affects the kinetics of engulfment in *C. elegans* embryo. Lack of Progranulin causes apoptotic cells to be cleared more rapidly. Although its mechanism of action is not yet known, Progranulin needs the full engulfment machinery to affect cell corpse engulfment. This suggests a possible role of human Progranulin in injured neuron removal in frontotemporal lobar degeneration [71]. Other regulators such as SLI-1/Cbl, an E3 ubiquitin ligase and adaptor protein, might signal engulfment and cell migration. SLI-1/Cbl inhibits engulfment and cell migration, but SLI-1 does not target proteins for degradation or sequestration as most known Cbl proteins do; therefore, SLI-I/Cbl might signal through a previously unidentified pathway [68].

Conclusions and outlook

Rac/Rho GTPases are master regulators of cytoskeletal dynamics. Whenever a cell needs to change its shape, Rac/Rho GTPases signal to control the actin rearrangements that are required. Therefore, Rac/Rho GTPases are essential for different processes in embryo modeling, such as cell migration or engulfment where membrane protrusions are needed. They are especially important during the development of an organism when cells are proliferating, and either they or neuronal axons need to migrate to reach their correct position. These small GTPases respond to distinct cues in different cells or situations. Genetic evidence has shown that they have redundant roles in many biological processes: axon guidance is controlled by *ced-10*/Rac, *mig-2*/RhoG, and *rac-2*/Rac in an overlapping manner, or *mig-2*/RhoG and *ced-10*/Rac function redundantly during vulval morphogenesis. In addition, they could be required in concert, as both *ced-10*/Rac and *mig-2*/RhoG are needed in pathfinding of the Distal Tip Cells of the gonad or, conversely, *ced-10/Rac* is uniquely required for phagocytosis.

Rac small GTPases seems to act as central nodes for cytoskeletal rearrangements, receiving positive, or negative regulation from distinct pathways. All these signals are integrated to produce a coordinated response in space and time, using relatively simple molecular machinery. It would be better to discuss biological networks instead of single, linear pathways in the developing embryo. The future understanding of these *C. elegans* genetic networks will be of great interest. The Rac family is conserved from worms to mammals, and thus functions of Rac and genetic interactions might also be conserved. For this reason, studies in *C. elegans* are extremely useful, not only for the purpose of increasing knowledge but also for their possible utility in biomedical research and future medical applications in the field of neurodegenerative diseases.

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