Crosstalk between Rac1-mediated actin regulation and ROS production

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ABSTRACT

The small RhoGTPase Rac1 is implicated in a variety of events related to actin cytoskeleton rearrangement. Remarkably, another event that is completely different from those related to actin regulation has the same relevance; the Rac1-mediated production of reactive oxygen species (ROS) through NADPH oxidases (NOX). Each outcome involves different Rac1 downstream effectors; on one hand, events related to the actin cytoskeleton require Rac1 to bind to WAVEs proteins and PAKs that ultimately promote actin branching and turnover, on the other, NOX-derived ROS production demands active Rac1 to be bound to a cytosolic activator of NOX. How Rac1-mediated signaling ends up promoting actin-related events, NOX-derived ROS, or both is poorly understood. Rac1 regulators, including scaffold proteins, are known to exert tight control over its functions. Hence, evidence of Rac1 regulatory events leading to both actin remodeling and NOX-mediated ROS generation are discussed. Moreover, cellular functions linked to physiological and pathological conditions that exhibit crosstalk between Rac1 outcomes are analyzed, while plausible roles in neuronal functions (and dysfunctions) are highlighted. Together, discussed evidence shed light on cellular mechanisms which requires Rac1 to direct either actin- and/or ROS-related events, helping to understand crucial roles of Rac1 dual functionality.

1. Introduction

Small RhoGTPases are single-domain nucleotide-dependent binary switches that act as highly-tuned regulators in signal transduction [1]. The cycling between active GTP-bound and inactive GDP-bound forms allows RhoGTPases to bind to or to dissociate from downstream effectors, respectively [2]. Guanine nucleotide exchange factors (GEFs) that catalyze the exchange of GDP for GTP, and GTPase-activating proteins (GAPs) that increase intrinsic GTP hydrolysis are respectively responsible for RhoGTPases switching between their active and inactive form [3]. Furthermore, switching between GDP and GTP may involve cytosol-membrane translocation, as farnesyl or geranylgeranyl-carrying RhoGTPases can form soluble complexes with guanine dissociation inhibitors (GDIs), thus preventing RhoGTPases from membrane-targeting and GEFs-mediated activation [4]. A remarkable feature of RhoGTPases-based signaling networks is that specific interacting patterns between GEFs and RhoGTPases, coupled with post-translational modifications and scaffolding molecules lead to spatiotemporal promotion of determined outcomes [5]. Ras-related C3 botulinum toxin substrate 1 (Rac1), a member of the RhoGTPases family, has a pivotal role in the regulation of actin polymerization during cytoskeletal rearrangement events [6]. Rac1-mediated actin regulation takes place through binding of Rac1 to the scaffolding molecule known as insulin receptor tyrosine kinase substrate p53 (IRSp53), thus leading to Rac1 binding to WASP-family verprolin-homologous (WAVE) proteins [7]. As a result, WAVEs bind to and activate the actin-nucleating protein, actin-related protein 2/3 (Arp2/3) complex, which initiates growth of new branched filaments [8]. Another way in which Rac1 can regulate the actin cytoskeleton is by binding to p21-activated kinases (PAKs) which in turn conduct cytoskeletal rearrangement via phosphorylating Lim kinases (LIMKs) [9]. LIMKs phosphorylate cofilin subsequently, thereby releasing it from actin filaments and thus suppressing actin-severing activity [10]. In this way, the Rac1/Pak1/LIMK1/cofilin axis may modulate the turnover of actin filaments at the lamellipodium [11]. Rac1-mediated actin regulation has important roles in cell-cell adhesion [12], cell-extracellular matrix (ECM) early interaction [13], cell polarization [14] and cell mobility [15]. These events are widely regarded as actin-related outcomes of the Rac1 signaling axis. Additionally, Rac1 is indispensable for the assembly of the membrane-located superoxide-producing NADPH oxidase (NOX) complexes, where it is required for the electron transfer from NADPH to oxygen [16]. NOX isoforms I and II (NOX1 and NOX2) are activated via Rac1 having relevant roles in physiology and in several human diseases including neurodegenerative pathologies [17]. Besides Rac1, the assembly of the NOX complex
requires the binding of at least two cytoplasmic subunits (an activator and an organizer) to the membrane-located catalytic subunit, which in turn must be bound to a membrane-located anchorage subunit [18]. The cytoplasmic activators p67phox and NOX1A are the NOX2 and NOX1 components serving as targets for Rac1, respectively [19]. In the cytosol, prenylated Rac1 is inactive and bound to RhoGDIs [20]. Through various receptor-mediated signaling cascades that involve Rac1 GEFs, the Rac1-RhoGDI complex is translocated to the membrane [21,22]. NOXs enzymes play significant roles in endothelial functions [23], cellular proliferation [24], cancer [25], establishment of neuronal polarity [26] and neurodegeneration [27]. Taken together, it is evident that Rac1 presents two different downstream outcomes; actin- and NOX-related events (Fig. 1). How Rac1 can promote each outcome in a coordinated manner is intriguing. An example of Rac1-mediated signaling bifurcation is seen in the context of MAP kinases function. In this way, Wu et al. [28] showed that downstream from Tat (Human immunodeficiency virus type 1 transactivator of transcription) signaling, two independent Rac1-mediated outcomes take place; activation of RhoA-Nox4-dependent Ras/ERK which favors proliferation, and activation of PAK1-Nox2-dependent JNK that promotes cytoskeletal re-arrangement. It has been suggested that RhoA may favor Nox4 activity via up-regulating its expression levels during fibroblast differentiation [29]. However, the correlation between RhoA and Nox4 is not resolved and appears to be context-dependent as recent evidences show that loss of Nox4 increases levels of RhoA in Huh7 and PLC/PRF/5 cells, while overexpression of Nox4 in SNU449 cells increases RhoA levels [30]. In the present review, we are interested in Rac1-mediated signaling bifurcation regarding ROS production and actin regulation outcomes, which have been studied mostly as separate events. Here, we bring together evidence of co-occurrence and crosstalk between both functions. One layer of modulation for such outcomes is provided by Rac1 regulators, namely GEFs, GAPs and GDIs. Then Rac1 regulators driving both actin- and NOX-related outcomes are discussed. Moreover, crosstalk between Rac1 axes involving redox signaling are also addressed along with their physiological and pathological roles highlighting possible roles in neuronal functions.

2. Linking H2O2-dependent redox signaling to Rac1 activity

2.1. H2O2-induced protein modifications and redox signaling

Once produced, the anion superoxide is dismutated into hydrogen peroxide either by superoxide dismutases or spontaneously [31]. Hydrogen peroxide in turn can pass through membranes and oxidize protein thiol groups [32]. Though NOX-mediated ROS production occurs on the outer side of the plasma membrane, significant amounts of NOX-derived ROS diffuse into the cytoplasm triggering locally restricted redox events [33]. Particularly, H2O2 generates reversible oxidation of cysteine residues [34] yielding either disulfide bonds (RSSR), glutathione disulfide (GSSG) or S-glutathionylated proteins (RSSG) [35], as well as S-nitrosylation if nitric oxide, nitroxyl or peroxynitrite are involved [36]. Such events modulate signaling networks thus making hydrogen peroxide a relevant second messenger [37,38].

2.2. H2O2-mediated signaling confinement by antioxidant systems

As for case of other second messengers, H2O2-mediated signaling is spatiotemporally constrained. In this sense, compartmentalized events such as H2O2 microdomains and gradients have been implicated in several cellular functions [39,40]. How H2O2 locally exerts its role is still a matter of debate; nonetheless, the action of the antioxidant systems provide hints on how this might occur. Breakdown of H2O2 is conducted by peroxidases such as catalase, glutathione peroxidase and peroxiredoxins. Importantly, cysteine oxidations can be reversed via the activity of the latter antioxidant enzymes [41]. An excellent review by Ren et al. [42] highlighted that thioredoxin (Trx) and glutathione (GSH) systems regulate redox signaling involved in various biological events in the CNS. Peroxiredoxins have been widely regarded as H2O2 scavengers that partially impede H2O2-mediated thiol oxidation [43]. However, given some contexts peroxiredoxins have been postulated as enablers of protein thiol oxidation [44]. Which of these mechanisms prevails, or has the most significant impact on redox-signaling, is still an open question [45]. Intriguingly, Kwon et al. [46] found that peroxiredoxin6 (Prdx6) binds to NOX1 and assembles with the Nox1 complex, supporting Nox1-mediated migration of colon epithelial cells. Over the last years, peroxiredoxins have been increasingly suggested as key players in H2O2-mediated signaling [45,47]. As for the case of H2O2 signaling confinement via antioxidant systems, Rac1 activity undergoes tight spatiotemporal regulation too; it has been shown that active Rac1 can exhibit a marked differential spatial distribution in several cellular processes, such as axonal elongation and dendritic spine formation [26,48]. Interestingly, and as pointed out below, spatial regulation of Rac1 activity may be directly linked to H2O2-dependent redox control.

2.3. Redox regulation of Rac1 via H2O2-induced fast-cycling activity

Alongside GEFs, GAPs and GDIs, it is widely regarded that Rac1 undergoes post-translational modifications that allow fine-tuned regulation of its activity and localization [5]. In fact, mutations in Rac1’s regulators are prevalent in some type of cancers such as melanoma [49]. In addition, up-regulation of Rac1 activity by increased nucleotide cycling induced via oncogenic mutations are well-known [50,51]; these sort of modifications promote GEF-independent guanine nucleotide exchange activity. Another way of obtaining high GEF-independent guanine nucleotide exchange activity is by cysteine oxidation; hence Rac1 may be considered as a redox target. The latter is supported by evidence showing that ROS and RNS directly affect Rac1 activity. In this sense, Heo and Campbell, 2005 [52] show that peroxide increases Rac1 guanine nucleotide exchange by 10-fold. Later, Hobbs et al. [53] showed that Rac1-Cys18 may be glutathiolated, which is a reversible oxidative modification, upon ROS induction in primary chondrocytes from human joints. Importantly, human articular chondrocytes are known for expressing Nox2 and suffering elevated levels of ROS during osteoarthritis [54]. Moreover, isolated glutathiolated Rac1 showed a 200-fold increase in nucleotide exchange rate in comparison to non-oxidized Rac1 [53]. Also, a mimicking form of this redox fast-cycling Rac1 generated enhanced lamellipodia formation in Swiss 3T3 cells, while expression of a redox-insensitive Rac1 variant did not show changes in the lamellipodia, whilst increased activity of the mimicking form of Rac1 redox fast-cycling was confirmed via pull-down activity assays [53]. More recently, it has been reported that glutathionylation...
of Rac1 on cysteine 81 and 157, residues near the nucleotide binding site, inactivate Rac1 in endothelial cells under conditions of diabetes and hyperlipidemia [55]. Taken together, redox-dependent modulation of nucleotide cycling in Rac1 seems to be a relevant layer of regulation, though it could function either as a promoter or inhibitor of Rac1 activity. Even though redox signaling and NOX-derived ROS have been shown to play key roles in several neuronal processes [56], Rac1 redox regulation via glutathionylation has yet to be explored in neurons.

2.4. Structural aspects involved in Rac1-mediated ROS- and actin-related events

Spatiotemporal regulation of Rac1 activity may cooperate with other systems that control redox events, such as peroxidases, in such a way that multilayered-regulation would be articulated. Structurally, it is not clear how those mechanisms work in concert over Rac1 domains. In this sense, it is well-established that Rac1 interacts with p67phox. Such an interaction requires residues belonging to the N-terminal (S22, T25, N26, F28, G30 and E31 residues) which are near or inside the switch I region, as well as residues from the C-terminal (A159, L160, and Q162 residues) [57]. Mutations that lead to constitutively active forms of Rac1 (Rac1-G12V and Rac1-Q61L) do not affect its function regarding NOX activation and effectively promote NOX activity [58,59]. Other Rac1 domains such as the insert region have been regarded as not crucial for NOX assembly and activation [60]. Several Cdc42- and Rac-interactive binding (CRIB) effectors of Rac1 such as PAK, WAVE and the scaffold IRSP53, as well as non-CRIB scaffolding proteins including IQGAP and Sra/CYFIP have been found to interact with the Rac1 N-terminal domain, as does p67phox [61].

3. Rac1-dependent ROS and actin regulation in neuronal functions

NOX-mediated ROS have an important role as physiological messengers. One remarkable example regarding such a function is during axonal formation. In this line, increased p40phox/NOX2 levels and co-localization at growth cone contact sites with apCAM beads and interacting growth cones have been observed. Thus apCAM-clustering promotes actin rearrangement and NADPH oxidase activation during neurite outgrowth [62]. Based on the latter, Munnamalai et al. [62] proposed that cytosolic NADPH oxidase subunits such as p40 are associated with actin structures in unstimulated growth cones. Here, NOX2 subunits p47phox, p67phox, p40phox, and Rac1 translocate to the plasma membrane (with or without F-actin) and activate NOX2 upon growth cone stimulation by external cues. In addition, it has been shown that in order to sustain axonal development, Rac1 is activated via RyR-mediated Ca\(^{2+}\) release from the ER [63]. In this mechanism, ER Ca\(^{2+}\) release promotes Rac1-activation, which in turn activates NOX2 leading to ROS production. Since RyR activity is promoted by ROS and Ca\(^{2+}\) [64], a feed-forward loop in which activated Rac1 maintains axonal growth and NOX-mediated ROS production was established [63]. Notably, this loop could be aborted by applying NSC23766, which blocks the interaction of Rac1 with its GEFs Tiam1 and TRIO. Thus ROS production would be sustained in neurons via RyR-mediated Ca\(^{2+}\) release/GEFs/Rac1/NOX pathway.

4. Involvement of Rac1 regulators in redox and actin events

4.1. Rac1-GEFs involved in NOX-mediated ROS and actin cytoskeleton events

Coordination between upstream and downstream effectors may enable localized activation of Rac1, thus establishing and maintaining actin-related events [15] and/or focalized NOX-mediated production of ROS [65]. As the latter relies on GEFs [66], the most-characterized Rac1-GEFs that modulate actin-related functions and NOX-dependent ROS production are discussed below.

4.1.1. βPIX

The GEF for Rac1 and Cdc42 known as βPIX (β form of the PAK-interacting exchange factor; also referred as Cool-1 or ARHGEF7) was first identified in a search for components directly upstream or downstream of Cdc42 and Rac1, being found enriched in focal complexes and necessary for PAK recruitment [67]. The subcellular localization of βPIX is associated with focal adhesions sites, while tyrosine phosphorylation of βPIX appears to determine its spatial regulation [68]. In neurons, βPIX can be found in dendritic spines [69], where it may be recruited to synapses by its interaction with the scaffold Shank [70]. βPIX has been linked with activity-dependent synaptogenesis [71], as well as to dendritic and neurite outgrowth [72,73]. βPIX participates in several pathways downstream of receptor tyrosine kinase (RTK), G protein-coupled receptor (GPCR), integrins and T-cell receptor (TCR) [74]. A relevant feature of βPIX is that it contains a binding domain for GIT1 (G-protein-coupled receptor kinase-interacting target; [75]), a GAP for the small GTPase known as Arf (ADP-ribosylation factor) [76]. Together, GIT1 and βPIX function as a module that can be targeted to scaffold and coordinate Rac1-mediated actin regulation [77,78]. Focal adhesion establishment is among the best-studied GIT1/βPIX/Rac1-mediated events. Targeting the GIT1/βPIX complex to focal complexes depends on direct binding of GIT1 to paxillin [79], an integrin-recruited adaptor protein [80]. Since βPIX also binds to PAK, which is one of the main downstream effectors for Rac1, adhesion and protrusion-related processes can be elicited as Rac1 is locally activated via GIT1/βPIX axis [68,81]. In neurons, the GIT1/βPIX/Rac1 axis leads to PAK-dependent phosphorylation of the myosin II light chain (MLC), thereby promoting dendritic spine and synapse formation [69]. Furthermore, it has been shown that maintenance and clustering of surface GABA\(_{\beta}\) receptors are achieved via the GIT1/βPIX/Rac1/PAK axis, thus keeping the integrity and strength of inhibitory synapses [82]. On the other hand, βPIX has been related to NOX-mediated ROS production as the PI3K products PtdIns(3,4,5)P\(_3\) and PtdIns(3,4)P\(_2\) enhance βPIX function as a module that can be targeted to scaffold and coordinate Rac1-mediated actin regulation [77,78]. Focal adhesion establishment is among the best-studied GIT1/βPIX/Rac1-mediated events. Targeting the GIT1/βPIX complex to focal complexes depends on direct binding of GIT1 to paxillin [79], an integrin-recruited adaptor protein [80]. Since βPIX also binds to PAK, which is one of the main downstream effectors for Rac1, adhesion and protrusion-related processes can be elicited as Rac1 is locally activated via GIT1/βPIX axis [68,81]. In neurons, the GIT1/βPIX/Rac1 axis leads to PAK-dependent phosphorylation of the myosin II light chain (MLC), thereby promoting dendritic spine and synapse formation [69]. Furthermore, it has been shown that maintenance and clustering of surface GABA\(_{\beta}\) receptors are achieved via the GIT1/βPIX/Rac1/PAK axis, thus keeping the integrity and strength of inhibitory synapses [82]. On the other hand, βPIX has been related to NOX-mediated ROS production as the PI3K products PtdIns(3,4,5)P\(_3\) and PtdIns(3,4)P\(_2\) enhance βPIX activation to promote Rac1 activity and NOX1 activation [83]. The stimulation of ROS production mediated by βPIX has been confirmed by Kaito et al., (2014) [84], who reported that phosphorylation of βPix positively regulates NOX1 activity. More recently it has been shown that the polarity protein SCRIB may interact with p22phox/βPIX/Rac1 to promote ROS production [85].

4.1.2. Dock180

This is a specific Rac1 GEF (180-kDa protein downstream of CRK) that was initially identified as a binding protein for CRK, a homolog of the oncogene product v-Crk from the CT10 retrovirus able to transform 3T3 fibroblasts [86]. Usually, GEFs interact with Rho-GTPases through their Dbl homology-pleckstrin homology (DH-PH) domain. This is not the case for DOCK180, which does not contain the DH-PH domain; instead it interacts with Rac1 by its Docker domain [87]. In order to achieve an efficient activation and localization of Rac1, DOCK180 forms a complex with the scaffold protein, ELMO1 (engulfment and mobility) [88,89]. Elevated PtdIns(3,4,5)P\(_3\) levels that are locally-generated at the leading edge, promote membrane localization of Dock180/ELMO and polarized activation of Rac1 during elongation and migration in LR73 cells (a variant of the CHO cell line) [90]. Although it has been observed that Dock180 may promote epithelial cell migration [88,91] or even metastasis [92], this GEF is known to localize at both integrin- and cadherin-based adhesions sites [93]. Similarly to βPIX, Dock180 has been localized in dendritic spines along with its interacting partners RhoG and ELMO1, where Dock180 promotes spine morphogenesis [94]. Furthermore, roles of Dock180 in axonal guidance and pruning have been reported [95,96]. Interestingly, there is some evidence indicating that ELMO1/Dock180 can promote NOX-mediated ROS production. It has been reported that the brain-specific angiogenesis inhibitor 1 (BAI1) directly interacts with ELMO1/Dock180, and...
together they activate Rac1 [97,98]. In this sense, Billings et al. [65] reported that BA11 promotes NOX2 activation via ELMO1/Dock180-mediated Rac1 activation in macrophages.

4.1.3. Vav

Named after the sixth letter of the Hebrew alphabet, Vav was first characterized as an oncogene responsible for tumorigenesis in nude mice injected with NIH3T3 cells transduced with DNA from human esophageal carcinomas [99]. Vav is a GEF of Rac1, RhôA and Cdc42, and functions downstream of GPCR and phosphorylation via Src and Syk tyrosine kinases that release Vav from its auto-inhibitory conformation [100]. It has been shown that Vav3 localizes to membrane rafts in immune cells [101], while Vav1 locally promotes cytoskeletal rearrangements that take place in the peripheral area of immunological synapses [102]. Also, Vav1 is localized at lamellipodia of pancreatic tumor cells where it promotes Rac1-mediated invasive migration [103]. Vav2 and Vav3 are known to regulate neutrie-outgrowth and branching, axon guidance and the collapse of the growth cone [104,105]. Moreover, Vav2 and Vav3 are essential for theta-induced LTP and spine enlargement [106]. It has been proposed that targeting of Vav to different downstream pathways may rely on the regulation of multiple phosphorylation steps [107]. Vav2 functions as a mediator of growth factors and mecano-transduction, with well-known roles in cell-cell adhesion, migration and angiogenesis [108,109]. On the other hand, Vav1 is particularly expressed in hematopoietic tissues, and plays key roles in lymphocyte development and function [110]. Vav1 is essential for T-cell receptor (TCR)-mediated cytoskeletal reorganization [110,111]. Importantly, Vav1 and PI3K are linked, as PtdIns(3,4,5)P₃ enhances phosphorylation and activation of Vav1 by Lck [112] and promotes its recruitment to the plasma membrane [113]. Regarding NOX-related events, Price et al. [114] showed in COS phox cells that stimulation of endogenous Rac1 by expressing of a constitutively-active p67 phox promotes its recruitment to the plasma membrane [113]. Regarding NOX-related events, Price et al. [114] showed in COS phox cells that stimulation of endogenous Rac1 by expressing of a constitutively-active p67 phox promotes its recruitment to the plasma membrane [113]. Regarding NOX-related events, Price et al. [114] showed in COS phox cells that stimulation of endogenous Rac1 by expressing of a constitutively-active p67 phox promotes its recruitment to the plasma membrane [113].

4.1.4. Tiam1

The Rac1-GEF known as Tiam1 (T-lymphoma invasion and metastasis 1) was originally identified as an invasion-inducing gene in the context of proviral insertional mutagenesis assays [118]. Tiam1 has been linked to the formation of cell-cell adhesions mediated by cadherin and cell migration suppression, while disassembly of cell-cell adhesions mediated by downregulation of Tiam1 may promote cell migration [119,120]. Membrane translocation of Tiam1 is crucial for its capacity to induce Rac1-mediated effects such as actin-related events and NOX-derived ROS generation [121,122]. Tiam1 has been related to several events located at membrane ruffles and others (Tiam1 subcellular locations have been reviewed by Boissier et al. [123]). In neurons, Tiam1 is involved in the formation of dendritic spines, being highly enriched at the post-synaptic density [124]. Also, as Rac1-mediated actin events are crucial to ensure proper function of dendritic spines [125], the Tiam1/Rac1 axis has key roles in synaptic plasticity [126]. Importantly, Tiam1/Rac1-mediated actin dynamics at dendritic spines may be modulated via calcium-dependent phosphorylation of Tiam1 [127]. Tiam1-mediated activation of Rac1 has been linked with lowered cell scattering upon induction with hepatocyte growth factor (HGF) and EGF [128]. It has been shown that expression of wild-type Tiam1 promotes E-cadherin localization at cell-cell contacts, in a process that is likely to be mediated by the interaction of Tiam1 and Rac1 with the Ras GTPase-activating-like protein 1 (IQGAP1) [128,129]. In contrast, upon integrin-induced signaling, Tiam1, probably in association with PAR3 and PKCζ, may promote Rac activation, cell migration and tumor invasion [130,131]. Besides, Tiam1 has also been linked to NOX-mediated ROS production. In this sense, Price et al. [114] showed that activation of endogenous Rac1 by expression of a constitutively-active form of Tiam1 activates NADPH oxidase in COS phox cells. Additionally, at the initial stages of diabetes the Tiam1-Rac1-NOX2 axis leads to increased intracellular ROS [122]. Remarkably, a significant interaction between Vav2 and Tiam1 has been reported in endothelial cells undergoing shear stress. In this study, Liu et al. [132] showed that Vav2 conducts Rac1 loading of GTP while Tiam1 acts as a scaffold linking Rac1 to the flow-sensitive polarity complex Par3/VE-cadherin and to the NADPH oxidase, thus favoring flow-dependent ROS production.

4.1.5. P-Rex

The PtdIns(3,4,5)P₃-dependent Rac exchanger 1 or P-Rex was first purified from neutrophil cytosol and identified as a factor able to activate Rac via PI3P stimulation; it was thus originally linked to ROS production [133]. This GEF locates at membranes at GPCR and PI3K-mediated signaling microdomains; the sub-cellular localization of P-Rex1 has been recently reviewed by Welch et al. [134]. In neurons, P-Rex1 localizes at neurite shafts, distal tips and at the growth cone [125,126,136]. The P-Rex family are Rac1-GEFs activated by PtdIns(3,4,5)P₃ and by the Gβγ subunit of the heterotrimeric G protein complex linked to G protein-coupled receptors (GPCR). Accordingly, it has been suggested that P-Rex acts as a “coincidence detector” for both signals [137]. The gelsolin protein superfamily member, flightless-1 homolog (FLII) has been recently identified as an interacting partner for Rac1 and P-Rex [128]. FLII is pivotal for actin cytoskeleton events, regulating capping in actin barbed-ends and severing [138,139]. Marei et al. [128] reported that FLII binds to active Rac1, thus serving as a scaffolding protein for P-Rex1 which in turn enhances the interaction of Rac1 with FLII. Together, this promotes P-Rex1/Rac1-driven cell migration [140]. As can be appreciated, Rac1 activation by Tiam1 or P-Rex1 may yield opposing actin-related outcomes; suppression or promotion of migration, respectively. This has been proposed by Marei et al. [128] who reported that activation of Rac1 by Tiam1 or P-Rex1 may result in distinct actin rearrangements, each one leading to different phenotypes. Further, there is considerable evidence showing that P-Rex regulates NOX-mediated ROS. For instance, a differentiating human pro-myelocytic cell line treated with P-Rex1 antisense oligonucleotide has reduced C5a-stimulated ROS production [133]. Also, in a study on neutrophils from P-Rex1 knockout mice, it was found that disruption of P-Rex1 impairs Rac2 activation and ROS production upon FMLP exposure [141]. In a similar study done in mouse neutrophils, it was found that P-Rex1 cooperates with Vav1 during FMLP-stimulated ROS production; in fact, mice lacking both P-Rex1 and Vav1 showed severe reduction in Rac1 and Rac2 activities [142]. Moreover, Nie et al. [143] reported that P-Rex1 expression mediates FMLP-stimulated ROS generation in COS phox. They also observed that superoxide generation is further enhanced by expression of PKCδ and by overexpression of Akt. Consistently, it has been proposed that the assembly of the phagocyte NADPH oxidase complex via GPCR/PtdIns(3,4,5)P₃/P-Rex also involves PtdIns(3,4,5)P₃-mediated phosphorylation of p40phox and p47phox through Akt and PKCζ activity [144].
Table 1: Featured Rac-mediated actin and ROS-related events.

<table>
<thead>
<tr>
<th>GEFs</th>
<th>Racl-mediated actin events</th>
<th>Racl-mediated ROS events</th>
</tr>
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<tbody>
<tr>
<td>βPIX</td>
<td>Focal adhesion establishment [79], dendritic spine, synapse formation [69] and neurite outgrowth [72,73]</td>
<td>NOX-mediated ROS production enhanced by PtdIns(3,4,5)P3 and PtdIns(3,4)P2 [83]. ROS production induced via SCRIB/p22phox/βPIX/Racl or binding to NOXO1 instead of p22phox [85].</td>
</tr>
<tr>
<td>Dock180</td>
<td>Epithelial cell migration [88,91], cell adhesion [93], spine morphogenesis [94] and axon guidance [96,224.]</td>
<td>BAII/Elmo1/Dock180-mediated Rac1 activation and subsequent Racl-mediated ROS production [65].</td>
</tr>
<tr>
<td>Tiam1</td>
<td>Cell-cell adhesion and events located at membrane ruffles [123]. Cell migration and tumor invasion [130,131]. Formation and function of dendritic spines [125] and roles in synaptic plasticity [126].</td>
<td>Interplay with Vav2 and PAR3/VE-cadherin to promote Racl-mediated ROS production [132].</td>
</tr>
<tr>
<td>P-Rex</td>
<td>GPCR and PI(3)K-mediated signaling events [134]. Actin events at neurite shafts, distal tips and at the growth cone [135,136].</td>
<td>NOX-mediated ROS production stimulated by C5a or fMLP in several phagocytic cells [133,142].</td>
</tr>
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</table>

Table 1 highlights the main findings regarding the GEFS-controlled dual function of Racl.

4.2. Redox control of GEF-dependent Racl regulation

In a further line of evidence, Src family kinases are well-known regulators of actin cytoskeleton re-arrangements upon cell adhesion and migration [145], and ROS-dependent activation of Src is crucial for cell adhesion [146]. In fact, Src activation and hence actin-related events such as adhesion may be suppressed via antioxidant-mediated ROS removal [147]. Racl activity may be regulated by Src-dependent tyrosine phosphorylation of Vav2 and Tiam1 [148]. In this sense, Gianni et al. [149] demonstrated in human HT29 colonic adenocarcinoma cells that c-Src promotes Racl-mediated ROS (in NOX1) by increasing the levels of active Racl through the activation of Vav2 by tyrosine phosphorylation; this mechanism did not depend on Tiam1. Interestingly, transfection with a constitutively active form of Src promotes NOX1-dependent ROS production in HT29 cells [149].

4.3. RhoGDIs involved in Racl-mediated ROS production and actin events

Racl dissociation from the RhoGDI-Racl complex has been investigated during FcγR-mediated phagocytosis. Here, Racl is translocated to the phagosomes as a RhoGDI (or RhoGDII)-Racl complex, concomitant with ROS production [22]. Regarding dissociating mechanisms, Griner et al., (2013) [150] reported inactivation of RhoGDII via PKCα phosphorylation of Ser 31 in a region that contacts Racl in response to phorbol 12-myristate 13-acetate stimulation in HEK 293 T cells, thus promoting translocation of active Racl and suggesting that PKCα may be pro-tumorigenic. It has been shown that increases in intracellular Ca²⁺ and subsequent activation of PKCα generates serine phosphorylation in RhoGDII, thus inducing membrane translocation and Tiam1-induced activation of Racl. This leads to cytoskeletal rearrangement in NIH/3T3 Fibroblasts and PC3 (human prostate cancer line) cells [151]. On the other hand, PKGs phosphorylation on RhoGDII at Serine 96 releases Racl to mediate apical amylase secretion upon cholecystokinin-stimulated pancreatic acini cells [152]. The effect of other post-translational modifications over RhoGDII such as lysine acetylation [153] on Racl activity and possible interactions between RhoGDII with unphosphorylated Racl have yet to be determined. Also, it is well-established in macrophages that Racl-dependent ROS production is regulated via RhoGDII. In this line, the dissociation of the cytoplasmic complex RhoGDII-Racl-GDP and subsequent membrane localization of GTP-Racl is crucial to activate NOX2 [19]. In neurons, it has been observed that RhoGDIIa is necessary for the maintenance of mature-mushroom-shaped spines in rat hippocampal neurons; however, the precise RhoGDII-mediated mechanisms were not investigated [48].
5. Crosstalk between actin and ROS-related events mediated by Rac1

In the following section, evidence concerning Rac1-mediated crosstalk phenomena is addressed, focusing on cellular functions and dysfunctions where the dual function of Rac1 might play a key role.

5.1. Crosstalk events regulated by the HACE1/Rac1 axis

Crosstalk between events related to the actin cytoskeleton and Rac1-mediated ROS production can be found in studies focusing on Rac1 degradation by the ubiquitin-proteasome system (UPS) [165,166]. In this sense, by using MDCKII cells and hepatocyte growth factor treatment, Castillo-Llucha et al. [167] found that HACE1 (HECT domain and Ankyrin repeat containing E3 ubiquitin-protein ligase 1) antagonizes migration via poly-ubiquitylation of active Rac1 and subsequent UPS degradation. Indeed, HACE1 selectively targets membrane-associated Rac1, thus decreasing migration. Conversely, marked accumulation of actin and Rac1 at the leading edge, as well as increased duration of migration are observed when the HACE1-mediated degradation of active Rac1 is disrupted [167]. In another study, Goka and Lippman [168] showed that HACE1 deficiency results in the accumulation of activated Rac1 enhancing migration and invasion as well as anchorage-independent growth in the human mammary epithelial cell line MCF12A. Furthermore, the same study reported that strong tumorigenic transformation is observed when knocking-down HACE1 and overexpressing HER2/neu, a well-established activator of several Rac1 GEFs in breast cancer [66,169]. Therefore, Rac1 degradation via HACE1 is linked to actin-related events, such as cell mobility. Besides actin-related events, there is strong evidence showing that the HACE1-Rac1 axis also participates in NOX-mediated ROS production. In this respect, Daugaard et al. [170] showed that in vitro and in vivo that HACE1 activity correlates with NOX-mediated ROS levels, while the underlying mechanism is direct targeting of active Rac1 bound to the subunit NOXA1. Thereby, they demonstrated that the ubiquitylation and degradation of Rac1 mediated by HACE1 is directly-related to NOX-mediated ROS production. Although NOX2 may also be involved, this aspect was not investigated further [170]. Supporting the latter, Centibas et al. [171] discovered that Hor− MEF cells undergo ROS-induced cell death upon glutamine starvation. In fact, the ROS-induced cell death of Hor− MEFs was mediated by Rac1 and NOX activation [171]. In summary, these studies clearly show that Rac1 displays a dual function mediated by the HACE1-Rac1 axis. On the other hand, there is some evidence suggesting that the HACE1/Rac1 axis might be involved in neuronal development and degeneration. In this way, Hollstein et al. [172] identified loss of function mutations in HACE1 leading to autosomal recessive neurodevelopmental disorders associated to intellectual disability, spasticity, and abnormal gait. Also, there is evidence of increased oxidative stress in striatal regions of the brain of Hor KO mice, whereas HACE1 ectopic expression correlates with neuroprotection against oxidative stress in striatal neuronal progenitor cells [173]. The same study also reports decreased HACE1 levels in the striatum of postmortem patients with Huntington’s disease. Though the authors of the latter study highlight the role of the transcription factor Nuclear Factor Erythroid 2-related Factor (NRF2), the role of Rac1 may be relevant. Another line of evidence allows relating actin cytoskeleton and Rac1-mediated ROS through RHO inhibition-induced migration. Rounded-amoeboid melanoma cells, which are characterized by high RHO-ROCK-actomyosin activity, present mechanisms for rapid migration via inhibiting Rac1-mediated cell adhesion [174]. Notably, Herrera et al. [175] observed that increased Rac1 activity along with ROS levels inversely correlate with RHO-ROCK actomyosin activity in melanoma cells. Moreover, they obtained a highly invasive phenotype by applying antioxidant treatments that increased actomyosin contractility.

5.2. Crosstalk events related to cell adhesion and polarity

In endothelial cells (ECs), mechanosensing-induced activation of Rac1 at cell-cell junctions is essential for aligning actin stress fibers in response to fluid shear stress [176]. The mechanosensory complex involved in this process consists of the platelet endothelial cell adhesion molecule-1 (PECAM-1), vascular endothelial cadherin (VE-cadherin), and vascular endothelial growth factor receptor 2 (VEGFR2) [177]. An interesting feature of this complex is that PECAM-1 transduces mechanical signals via Src-mediated phosphorylation of Vav2, Rac1 activation and localized NOX-activation, where the latter is mediated by a complex composed of Tiam1, VE-cadherin, p67phox and Par3 [132]. Hence, Rac1-mediated ROS production downstream of Vav2 is linked with cell-adhesion and polarity. A similar mechanism can be found in neurons where the interaction of L1 adhesion molecule and b1 integrins generates Src-dependent tyrosine phosphorylation of Vav2, and subsequent activation of Rac1, Pak1, MEK, and the MAP kinases ERK [178]. This is essential for neuronal migration, axonal growth and guidance, as well as for process branching during development [105,179]. It remains to be investigated whether or not this process involves Rac1-mediated ROS production. Further evidence showing that Rac1 is key during axonal formation comes from a feedback loop with PI3K. In this mechanism, Rac1 is activated by the specific Rac1-GEFs, Tiam1 and STEF/Tiam2 [180,181]. In turn, these GEFs are induced by their interaction with the polarity protein Par3 associated with Par6 and the atypical Protein Kinase C (aPKC), while this inter- 

section is promoted via PI3K-induced Cdc42 activation [182,183]. As activated Rac1 can bind PI3K [184], and PI3P can recruit Vav2 to activate Rac1 [185], a positive-feedback loop promoting Rac1-mediated actin filament reorganization may be established during neuronal polarization [186,187]. In this context, the Rac1-PI3K feedback loop could signal via the Sra-1/WAVE1 complex [188] and PI3K-mediated phosphorylation of Shoost1 [189]. It has been observed in epithelial cells that the apical polarity complex Crumbs (Crb) breaks a similar Rac1-PI3K positive-feedback loop, thereby repressing the activation of Rac1 as well as PI3K signaling at the apical membrane, whereas the Rac1-PI3K loop restricts Crb function [190]. Notably, Crb can also repress NADPH oxidase-dependent superoxide production in epithelia via Crb-dependent inhibition of Rac1 [191]. Conversely, the same study reported that loss of the inhibitory function of Crb results in NOX-mediated ROS overproduction. Taken together, studies on Rac1-PI3K positive-feedback in neuronal polarity and its Crb-dependent inhibition in epithelia suggest that Rac1 can display both ROS- and actin-related functions in tight association with polarity-related effectors. All crosstalk events described so far in this section are summarized in Fig. 2. Furthermore, scaffolding events conducted by the polarity protein Scrib also suggest crosstalk between ROS- and actin-related Rac1 outcomes. The GIT1/βPIX/Rac1 axis is involved in several polarity events due to the interaction between βPIX and Scrib [192]. In this line, it has been shown that Scrib is crucial to activate and localize Rac1 at the leading edge upon directed epithelial migration in MCF10A mammary cells [193]. Also, Nola et al. [194] reported that Rac1-mediated PIK activation at the leading edge of migrating MEF cells depends on the Scribble/βPIX/GIT1 axis. On the other hand, Zhan et al. [195] observed that in order to maintain mammary epithelial polarity, Scrib locally promotes Rac1 activation at cell-cell junctions, suggesting that the GIT1/βPIX complex and Scribble may be interacting in such an event. Additionally, Boczonadi et al. [196] showed that Scrib assembles with βPIX and Rac1 to regulate junctional complexes in cardiomyocytes. Remarkably, Zheng et al. [85] recently reported a direct interaction between Scrib and the NADPH oxidase subunit p22phox, whereby Scrib acts as a scaffold recruiting βPIX to induce Rac1-mediated ROS production in macrophages. Interestingly, they found that Scrib also binds to NOXO1 (subunit of NOX1), thereby showing an extended role for the Scrib/βPIX/Rac1 axis regarding NOX-mediated ROS production. In neurons, Scrib may promote Rac1/PAK-regulated actin polymerization.
During dendritic spine formation by interacting with the neuronal nitric oxide synthase adaptor protein (NOS1AP) and βPIX/GIT1 [197]. Also, it has been suggested that Scrib directs cytoskeleton elongation during neurite outgrowth mediated by nerve growth factor in PC12 cells; here, membrane-located Scrib forms a complex with βPIX, Rac1, HRas and ERK1/2 [198]. Interestingly, Scrib and Rac1 are crucial in the process known as active forgetting in the mushroom body neurons of Drosophila [199]. In this study, a direct interaction between Scrib, Rac1, PAK3 and cofillin was found to promote active forgetting downstream of dopaminergic signaling. More recently, Liu et al. [200] confirmed in mice that Rac1 activity favors active forgetting while Rac1 inactivation promotes memory retention. These and other studies strongly suggest that active forgetting heavily relies on Rac1-mediated actin regulation [201,202]. A possible scaffolding role for Scrib regarding Rac1-mediated ROS production in neuronal functions has yet to be investigated.

6. Crosstalk between redox and scaffolding events in Rac1-dependent actin regulation

6.1. Key scaffolding events in Rac1-mediated actin cytoskeleton regulation

There is strong evidence showing that Rac1-mediated actin events are tightly-regulated by scaffolding interactions. This fact contrasts with what is known about Rac1-mediated ROS production, which does not appear to rely on scaffolding proteins. As scaffolding interactions would be the way by which Rac1 outcomes might be selected, such events are discussed in the following sections. Of note, and as discussed later, redox protein modification of scaffolds indicates crosstalk between the two Rac1-mediated outcomes. Rac1-mediated actin regulation requires scaffolding events to take place, as shown by the fact that interaction between Tiam1 and the Arp2/3 complex links activation of Rac1 to actin polymerization [203], and Sra-1 and Nap1 link Rac1 to actin assembly thus driving lamellipodia formation [204]. Another well-studied scaffolding protein that spatially restricts Rac1 activity is IQGAP1, that binds and crosslinks actin filaments, while active Rac1 promotes the oligomerization of IQGAP1 [205,206]. Moreover, IQGAP1 can enhance actin polymerization by interacting with (Arp) 2/3 [207]. Another case of fine modulation of Rac1 activity is provided by the Tiam1 interacting proteins spinophilin and insulin receptor substrate protein 53 kDa (IRS53) [208]. In this study, Rajagopal et al., [208] showed in fibroblasts that Tiam1-dependent Rac1 activation may be mediated by IRS53 or spinophilin, depending on the sort of upstream signaling; in fact each scaffold may lead to totally different independent events such as IRSp53/Tiam1/Rac1-dependent adhesion or spinophilin/Tiam1/Rac1-dependent migration. Rajagopal et al. [208] suggested that the interaction between Tiam1 and distinct scaffolding proteins allows the selection of specific Rac1-dependent outcomes.

6.2. Actin cytoskeleton scaffolding involves redox regulation

It is well-documented that actin itself and some of its regulators undergo reversible cysteine oxidation (specific modifications have been recently reviewed by Xu et al. [209]). Several lines of evidence suggest that actin polymerization is increased or decreased depending on the specific cysteine residue that is oxidized, cell type and experimental settings [209]. Also, it has been proposed that thiol oxidation, and hence H2O2-mediated signaling, plays a pivotal role in actin events related to cell migration [210], neuronal development [56,63,211,212] and synaptic plasticity [213,214]. Considering that Rac1 is directly involved in ROS production, it is interesting to note that some of the Rac1-related effectors that modulate the actin cytoskeleton undergo hydrogen peroxide-inducible redox modifications, namely cysteine oxidation. Remarkable evidence of crosstalk between Rac1-mediated ROS- and actin-related events stems from the redox control of IQGAP. It has been reported that cysteine oxidation of IQGAP residues co-localizes with p47phox and F-actin at the leading edge during migration in endothelial cells [215].

7. Deregulated Rac1 activation in neurodegeneration

It has been reported that fibrillar amyloid-beta peptide, which is observed in AD, promotes increased Rac1 activity via Tiam1 activation through a Ca2+-dependent mechanism, this phenomenon also involves enhanced actin polymerization [216]. Here, amyloid beta mediates Rac1 activation through phosphorylation and translocation of Tiam1; in fact, Fibrillar Al-42 induced a significant increase (1.5-fold) in the level of Thr phosphorylation of Tiam1. Calcium-dependent PKC activity was observed in AD, promotes increased Rac1 activity via Tiam1 activation through phosphorylation and translocation of Tiam1; in fact, Fibrillar Al-42 induced a significant increase (1.5-fold) in the level of Thr phosphorylation of Tiam1. Calcium-dependent PKC activity was responsible for Tiam1 phosphorylation. Therefore, upon amyloid beta exposure, Rac1 is over-activated by Ca2+ signaling (conventional PKC) that promotes Tiam1 activation (through phosphorylation and translocation of Tiam1). A link between actin dynamics and ROS production upon amyloid beta exposure has been proposed by Tsyo et al. [218], whom by using immortalized cerebral endothelial cells (bEnd3) observed that Aβ42 promoted ROS production by up to 83% after 60 min of treatment. They also demonstrated that Aβ42 favored actin polymerization, while pretreatment with the antioxidant N-acetylcyesteine (NAC) suppressed Aβ-induced actin polymerization and cytoskeletal rearrangement [218]. Manterola et al. [219] also found a positive correlation between Aβ42 exposure and Rac1 activation in SN4741 cells (a line originated from substantia nigra dopaminergic cells derived from transgenic mouse embryos), primary embryonic cortical neurons from rats and in neuronal organotypic cultures of the hippocampus and the entorhinal cortex. They observed that Aβ1–42 peptide stimulates the Rac1 pathway through Tiam-1 phosphorylation by novel PKCs. These kinases are not calcium dependent. Similarly, in a mice model of the Fragile X syndrome, characterized by thin, long and immature high
density dendritic spines, Bongmba et al. [220] found that Rac1 is over-activated in the mouse brain. It has been observed that NOX2-derived ROS is locally produced at synapses and that NOX2 has a post-synaptic localization [214,221,222]. Remarkably, Abdel-Rahman et al. [223] showed that NOX2 rather than the mitochondria is the major source of synaptic ROS in forebrain synaptosomes from mice. Therefore, it might be possible that neurodegenerative hyper-activation of Rac1 could be inducing deregulated NOX activation.

8. Concluding remarks

Rac1 offers a remarkable example on convergence between signaling pathways leading to actin modification and ROS production. These two seemingly independent cellular events should not be understood as separate entities since their co-occurrence and crosstalk are reversible events can take place. Furthermore, as Rho-GTPase regulators and their crosstalk, thus evaluating the effect of redox-driven modulation of Rac1 against other post-translational modifications is also needed. Novel regulatory loops might be discovered once those phenomena were better characterized. It is worth noting that scaffolding events are observed both in actin- and NADPH oxidase-related downstream outcomes. In addition, some cellular functions and dysfunctions that show crosstalk between actin regulation and ROS production are related to direct regulation of Rac1 levels, for example, HACE1-mediated Rac1 degradation. Taken together, dual function of Rac1 relies substantially on integrating patterns and multi-layered regulation. Such features need to be explored to understand complex cellular functions that require coordinated dynamic interplay between actin cytoskeleton rearrangements and ROS production.

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