The functional cycle of the Rac1 GTPase involves a large number of steps, including post-translational processing, cytosolic sequestration by RhoGDIs, translocation to specific subcellular localizations, activation by GDP/GTP exchange, inactivation by GTP hydrolysis, and re-formation of cytosolic Rac1/RhoGDI inhibitory complexes. Here, we summarize the current knowledge about the regulation of those steps. In addition, we discuss a recently described, cytoskeletal-dependent feedback loop that favors the efficient translocation and activation of Rac subfamily proteins during cell signaling. This route is mediated by a heteromolecular protein complex composed of the cytoskeletal protein coronin1A, the Dbl family member ArhGEF7, the serine/threonine kinase Pak1, and the Rac1/RhoGDI dimer. This route promotes the translocation of Rac1/RhoGDI to F-actin-rich juxtamembrane areas, the Pak1-dependent release of Rac1 from the Rac1/RhoGDI complex, and Rac1 activation. This pathway is important for optimal Rac1 activation during the signaling of the EGF receptor, integrins, and the anti- genic T-cell receptor.

Rac1, one of the best characterized members of the Rho/Rac GTPase subfamily, regulates ubiquitous processes such as the formation of membrane ruffles and lamellipodia, cell adhesion, proliferation, intercellular attraction/repulsion, and transcriptional dynamics. In addition, it modulates cell-type-specific processes such as axon migration/guidance, phagocytosis, or the formation of the immunological synapse. To trigger most of those functions, Rac1 has to fulfill two basic conditions. One of them is to be anchored at the plasma membrane to make it possible the subsequent activation of its primary effectors in the correct subcellular localization. The second condition is that it has to be bound to GTP, since this is the only conformational state compatible with the interaction of most downstream effectors. These requirements only change in few signaling scenarios, such as the cell cycle-regulated transfer of Rac1 to the nucleus or the indistinctive binding of GDP-Rac and GTP-Rac1 to mTOR. Like the mythical Greek Odysseus, the travel of Rac1 from the cytosol to the plasma membrane is a stepwise mechanism subjected to multiple regulatory challenges (Fig. 1). The first step required to reach its particular Ithaca is the attachment of a geranyl-geranyl group to the most C-terminal cysteine residue of the translated Rac1 protein, a modification catalyzed in the cytosol by type I geranyl-geranyl transferase (GGT1)1,4 (Fig. 1, step 1a). Rac1 then moves to the endoplasmic reticulum, where it is subjected to further processing by the protease Rce1 and the methyltransferase Icmt.1,5 Rce1 cleaves off the C-terminal Rae1 LLL tripeptide (Fig. 1, step 1b). Icmt incorporates a methyl group at the a-carboxyl group of the C-terminal cysteine that becomes exposed upon the completion of the Rce1-catalyzed reaction (Fig. 1, step 1c). Based on data gathered using Ras subfamily proteins, it has been

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Like the mythical Greek Odysseus, the travel of Rac1 from the cytosol to the plasma membrane is a stepwise mechanism subjected to multiple regulatory challenges (Fig. 1). The first step required to reach its particular Ithaca is the attachment of a geranyl-geranyl group to the most C-terminal cysteine residue of the translated Rac1 protein, a modification catalyzed in the cytosol by type I geranyl-geranyl transferase (GGT1)1,4 (Fig. 1, step 1a). Rac1 then moves to the endoplasmic reticulum, where it is subjected to further processing by the protease Rce1 and the methyltransferase Icmt.1,5 Rac1 cleaves off the C-terminal Rae1 LLL tripeptide (Fig. 1, step 1b). Icmt incorporates a methyl group at the a-carboxyl group of the C-terminal cysteine that becomes exposed upon the completion of the Rce1-catalyzed reaction (Fig. 1, step 1c). Based on data gathered using Ras subfamily proteins, it has been
assumed that the prenylation, cleavage, and methylation steps were condition sine qua non for the biological activity of Rac1 proteins.1,4,5 Such model is consistent with current experimental evidence,7 although some recent reports have unexpectedly shown that Rac1 can be functional in primary GTT1 deficient macrophages8 and in embryonic fibroblasts obtained from Rac1−/− mice.4

After its transit through the endoplasmic reticulum, Rac1 moves back to the cytosol where it stays in an inactive reservoir until the reception of extracellular signals by the cell. This pool is stabilized by the formation of stoichiometric complexes with RhoGDIs10 (Fig. 1, step 2). RhoGDIs perform both negative and positive actions in this complex. On the negative side, they inhibit the usually high intrinsic GDP/GTP exchange of Rac1, thus favoring the maintenance of the bound GTPase in the inactive conformation in non-stimulated cells. This function is mediated by the direct interaction of the RhoGDI molecule with the Rac1 switch regions.10,11 In addition, they use a deep hydrophobic cavity to trap the Rac1 prenyl group, a mechanism that keeps the GTPase away from the membrane.10

GTPase/RhoGDI complex.

Fig. 1, step 3). Whether this step requires GEFs, re-associates with RhoGDIs molecules (Fig. 1, step 6b) and moves back to the inactive cytosolic reservoir until a new stimulation cycle starts.12 In addition to this standard model, recent data demonstrated that Rac1 can undergo activation/inactivation cycles by shuttling between the plasma membrane and endocytic compartments.13,14 The shuttling of Rac1 in and out of the nucleus requires the Rac1 polybasic region and karyophilin β2,15 an importin that transfers cargo molecules inside the nucleus (Fig. 1, step 3b). Whether this step requires GEFs, carrier proteins, or intracellular docking proteins is unknown as yet. The mechanism by which Rac1 is returned to the cytosol remains ill defined (Fig. 1, step 6c).

Whereas the GTPase/RhoGDIs complex and the catalytic steps involved in Rac1 GDP/GTP exchange and GTP hydrolysis are well understood in structural terms,16,17 the dynamic aspects that modulate the release of Rac1 from RhoGDIs during the activation process are not well understood. For instance, we do not know whether Rac1 dissociates from the RhoGDIs before contacting the upstream GEFs or, alternatively, whether the GEFs promote both the dissociation and activation of the GTPase. In either case, it is unclear the intracellular regions used by either Rac1 or the Rac1/RhoGDIs complex to reach the subcellular regions where the stimulated GEFs are localized. Finally, we do not know whether these Rac1 mobilization-related steps are general and/or cell type-specific. Recent inroads in this subject suggest that the release of Rac1 is mediated by a phosphotyrosine-dependent mechanism that can associate with RhoGDIs by either upstream (Src, protein kinase C) or downstream (Pak1) kinases.18,19 In any case, it is still difficult to explain how the cytosolic RhoGDIs/Rac1 complexes manage to get close to those kinases during cell stimulation.

To shed light on this process, we decided to search for proteins involved in the regulation of the translocation of Rac1 to the plasma membrane using a genome-wide functional screen (Fig. 2). This approach led to the isolation of coronin1A (Coro1A) as a protein capable of inducing the translocation and activation of Rac1 during cell signaling (Fig. 2, bottom).20 Coro1A belongs to a large family of cytoskeletal regulators that show a phyla-specific distribution from unicellular eukaryotes to humans.21 These proteins control the bundling of F-actin filaments, the growth and orientation angle of new F-actin branches, and the disassembly of old actin cables.22 Such activities suggested to us that Coro1A could be possibly involved in a cross-talk between the F-actin cytoskeleton and the process of Rac1 translocation and/or activation. Consistent with this idea, we could demonstrate that the overexpression of Coro1A induced the F-actin-dependent translocation and activation of Rac1 in the plasma membrane. Conversely, its inactivation led to ineffective activation of Rac1 and Rac1-downstream routes. This pathway was active in a number of cell types (COS1, 293T, Jurkat cells) and cellular stimulation conditions (EGF signaling, integrin-mediated adhesion, T-cell receptor stimulation).

Mechanistic studies revealed that Coro1A promotes the translocation and activation of Rac1 via the formation of a Rac1–Coro1A–Act52 heteromolecular complex with ArhGef7, Pak1, RhoGDIs and Rac1. According to our proposed model (Fig. 3), the formation of this Rac1
translocation complex requires upstream signals such as Rac1 activity, aggregated lipid rafts in the plasma membrane and the presence of F-actin in cells (Fig. 3, stage 1). In the absence of those upstream signals, Csoo1A can only associate in a stable manner with AdsGEF7 in the cytoplasm (Fig. 3, stage 2). Upon conditions triggering F-actin polymerization, this
The inert complex is tethered to membrane ruffles and lamellipodia, a process made possible by the intrinsic F-actin binding properties of Coro1A (Fig. 3, stage 3). The F-actin bound complex binds then Pak1 and Rac1/RhoGDI (Fig. 3, stage 4), leading to the phosphorylation of RhoGDI by Pak1, the release of Rac1 from the phosphorylated RhoGDI and, finally, to Rac1 activation (Fig. 3, stage 5). Several properties of this new translocation process are important from a signaling point of view. First, the F-actin binding properties of Coro1A offer a rather simple explanation for understanding how the cytosolic Rac1/RhoGDI pool is shuttled toward the plasma membrane during cell stimulation conditions. Second, its dependency on upstream Rac1 signals suggest that the Coro1A route is involved in the generation of secondary waves of Rac1 activation rather than being directly involved in the initial burst of Rac1 activity that takes place upon the reception of the extracellular signal. Thus, according to our proposed regulatory model, cells will have to trigger Rac1 or RhoG activation in order to engage the downstream Coro1A-dependent relay mechanism (Fig. 3). Finally, the need of pre-formed F-actin cytoskeletal structures to engage the Coro1A-dependent translocation route suggests that F-actin can induce a positive

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**Figure 2.** Scheme of the cellomic screen used in the work reviewed here. The first screening was conducted using an expression library of 135,000 independent cDNA clones obtained from human T cells. To this end, we used a reporter HEK293T stably expressing a cytoplasmic EGFP-Rac1 protein (step 1). To increase the efficiency of the screening, this cell line also expressed anti-apoptotic proteins to avoid the loss of clones due to the presence of pro-apoptotic molecules in the transfected cDNA pools. The screening was conducted by transfecting separate pools of 90 cDNAs in the reporter cell line using the calcium phosphate precipitation method (step 2) and the subsequent score of cells showing plasma membrane localized EGFP-Rac1 using epifluorescence microscopy (step 3). Positive pools were progressively subdivided and transfected in the reporter cell line (steps 4a and 4b) until the isolation of the cDNA clones responsible for the Rac1 translocating activity (step 5). The crystal structure of Coro1A, one of the clones identified in this screening, is shown at the bottom. The structure is composed of WD40 domains arranged in a prototypical β-propeller conformation. See inset at the bottom for the shape and color code used for the indicated proteins used in these experiments.
A feed-back mechanism that will allow the generation of additional waves of Rac1 activation and F-actin polymerization at the plasma membrane (Fig. 3). Despite these advances, the role of Coro1A in the translocation and activation of Rac1 is far from being an open and shut case. Thus, the mechanism that regulates the assembly of Pak1 and the RhoGDI/Rac1 pair onto the Coro1A/ArhGEF7 complex has not been fully elucidated yet. Our data indicate that such assembly requires the presence of ArhGEF7 in the Coro1A complex, a result consistent with the known physical interaction between ArhGEF7 and Pak1. However, the requirement of F-actin for the formation of the entire Coro1A/Pak1/RhoGDI complex also indicates that additional ancillary partners and/or signals must also contribute to this process. Whether these extra elements are proteins, membrane lipids, and/or other upstream signals remains to be determined. It is also unclear the Rac1 GEF in charge of activating Rac1 upon its release from the Coro1A-nucleated protein complex (Fig. 3). One option is ArhGEF7 itself, since it is obvious that its presence in the Coro1A complex will ensure its close proximity with the released Rac1 molecules (Fig. 3). However, the catalytic activity of ArhGEF7 is controversial, suggesting that other GEFs could be involved (Fig. 3, protein labeled as X). It is possible therefore that such activation step is at the hands of other GEFs that,
due to their localization in F-actin cables or the plasma membrane, could be in close proximity to the Coro1A-nucleated complex. The resolution of all those lingering issues will need further experimental work in the near future. It is also worth noting that recent data obtained in our laboratory suggest that Coro1A can use alternative mechanisms to induce the translocation of Rac1 to the plasma membrane. Hence, we have observed that Coro1A can trigger the membrane localization of Rac1R66E, a mutant protein that cannot bind RhoGDI. This alternative route is mechanistically distinct from that characterized in this reviewed publication, because it cannot be inhibited by blocking Pak1 function (Castro-Castro A, Bustelo XR, unpublished observations). Although these results are probably not physiologically relevant given the lack of significant amounts of RhoGDI-free Rac1 proteins in the cytosol, they are interesting because they reveal a pathway that can further enhance the membrane anchoring of Rac1 upon its liberation from the RhoGDI complexes by the Coro1A/ArhGEF7/Pak1 complex. Although this phenomenon is not understood, it is possible that the increase in the membrane-localized pool of Rac1 can be achieved by routes alternative to those reported here. For example, our cellomic screen has also identified transmembrane proteins that favor the membrane localization of Rac1 by blocking the internalization of lipid rafts. Unlike the case of Coro1A-dependent route, this alternative pathway cannot be inhibited by either Rac1 dominant negative mutants or Pak1 interference strategies. Taken together, these results suggest that cells can resort to many pathways to regulate the translocation of Rac1 during cell signaling.

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