Rho GTPases

Deborah J. G. Mackay‡ and Alan Hall§

From the §Medical Research Council Laboratory for Molecular Cell Biology, §Cancer Research Campaign Oncogene and Signal Transduction Group, and ¶Department of Biochemistry, University College London, Gower Street, London, WC1E 6BT, United Kingdom

The mammalian Rho GTPase family currently consists of seven distinct proteins: Rho (A, B, and C isoforms), Rac (1 and 2 isoforms), Cdc42 (Cdc42Hs and G25K isoforms), Rhod, RhoG, RhoE, and TC10. Like other members of the Rho superfamily, Rho proteins act as molecular switches to control cellular processes by cycling between active, GTP-bound and inactive, GDP-bound states (Fig. 1). Activation of the GTPase, through GDP-GTP exchange, is promoted by guanine nucleotide exchange factors (GEFs),1 whereas inactivation (by an intrinsic GTPase activity) is stimulated by GTPase-activating proteins (GAPs). Rho guanine nucleotide dissociation inhibitors (Rho-GDIs) appear to stabilize the inactive, GDP-bound form of the protein. Activated Rho GTPases interact with cellular target proteins or effectors to trigger a wide variety of cellular responses, including the reorganization of the actin cytoskeleton and changes in gene transcription. This review aims to summarize current views of Rho GTPase signaling pathways; for reasons of space, however, this will be limited primarily to mammalian Rho, Rac, and Cdc42. More detailed information can be found elsewhere (1, 2).

Rho Proteins and the Actin Cytoskeleton

Rho proteins control the organization of the actin cytoskeleton in all eukaryotic cells. Activation of Rho in fibroblasts has been shown to cause the bundling of actin filaments into stress fibers and the clustering of integrins and associated proteins into focal adhesion complexes; activation of Rac promotes de novo actin polymerization at the cell periphery to form lamellipodial extensions and membrane ruffles, and activation of Cdc42 triggers actin polymerization to form filopodia or microspikes (Fig. 2) (3-7). Actin filaments found in lamellipodia and filopodia are, like stress fibers, associated with integrin adhesion complexes, although the function of these complexes is not clear (5, 7). Cross-talk between Rho proteins has been observed; in particular, Cdc42 is a strong activator of Rac, such that filopodial extensions are usually seen associated with lamellipodial protrusions (5, 6).

The biological implications of these findings are wide ranging, and Rho GTPases are likely to play a regulatory role wherever filamentous actin is used to drive a cellular process. Already Rho, Rac, and Cdc42 have been implicated in cell movement, axonal guidance, cytokinesis, and morphogenetic processes involving changes in cell shape and polarity (1, 2).

Rho Proteins and Gene Transcription

The JNK/stress-activated protein kinase (SAPK) and p38 MAP kinase cascades are known to control gene transcription in response to cellular stresses such as UV light and osmotic shock or challenge with inflammatory stimuli (8). Numerous groups have now reported that these two MAP kinase pathways can be activated by Rac and Cdc42, suggesting an analogous role to that played by Ras in the ERK MAP kinase cascade (Fig. 3) (9, 10). The exact role of the Rho GTPases in MAP kinase activation is, however, far from clear; overexpression of constitutively activated Rac or Cdc42 leads to only modest activation of JNK reporter plasmids in cotransfection assays, and there are still very few examples where physiological activation of the JNK pathway has been shown to be dependent on endogenous Rac or Cdc42 activity. The genetic analysis of dorsal closure in Drosophila has, however, recently provided experimental support for a role for Rac in JNK regulation (11). Interestingly, in the Saccharomyces cerevisiae pheromone response pathway, where both Cdc42 and a JNK-like MAP kinase cascade are required, the GTPase is not required for activation of the kinase cascade per se but instead appears to be required for the correct cellular localization of the MAP kinase-containing signaling complex (12).

Rho, Rac, and Cdc42 have each been reported to activate serum response factor (SRF)-dependent transcription (via an as yet unidentified mechanism) and to activate the transcription factor, NFkB (13, 14) (Fig. 3). In the latter study, the authors suggested that the generation of reactive oxygen species by Rac might be the trigger for NFkB activation, an interesting suggestion because it is known that Rac regulates an NADPH oxidase enzyme complex in phagocytes to produce superoxide (15). Rho, Rac, and Cdc42 activities are required during G1 cell cycle progression, but whether this is because of their effects on the actin cytoskeleton and integrin adhesion complexes or whether it is because of more direct effects on gene transcription is not known (16-20). It was shown in 1992 that oncogenic Ras is a potent activator of Rac, and mutational analysis of Ras has revealed that Rac activation is an essential downstream signal required for Ras-induced malignant transformation (4, 21). Again it is not clear which Rac-regulated activity contributes to the transformed phenotype, but this has important implications in cancer biology (22).

Regulators of Rho Proteins

When considering biological responses such as directed cell migration or axonal guidance, it is clear that the activation of GTPases must be both temporally and spatially controlled; in this respect it seems likely that GEFs will play a major role. A surprisingly large family (>20) of Rho GEFs has been identified, each of which shares two common motifs, the Dbl homology domain, which in some cases at least has been shown to encode the catalytic nucleotide exchange activity, and a pleckstrin homology domain, whose function is unclear but might determine subcellular localization (1, 23). Experimentally some GEFs appear to be specific for an individual GTPase, e.g. Lbc for Rho, Tiam1 for Rac, and FGD1 for Cdc42, whereas others

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1 The abbreviations used are: GEF, guanine nucleotide exchange factor; GAP, GTPase-activating protein; GDI, guanine nucleotide dissociation inhibitor; JNK, Jun N-terminal kinase; MAP, mitogen-activated protein; ERK, extracellular regulated kinase; SRF, serum response factor; LPA, lysophosphatidic acid; PDGF, platelet-derived growth factor; ERM, ezrin/radixin/moesin; PIP2, phosphatidylinositol 4,5-bisphosphate.
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**Effectors of Rho Proteins**

The yeast two-hybrid selection system and biochemical affinity purification methods have been used extensively to identify Rho, Rac, and Cdc42 interacting proteins, and a large number of candidate effectors have been identified (1). 

_Rho_—Although around eight targets for Rho have been identified, one of these, p160Rho kinase, has received much of the attention to date. The activity of this Ser/Thr kinase, which belongs to the myotonic dystrophy family of kinases, is enhanced (though not by much) after binding to Rho-GTP, and when overexpressed in cells, it has been reported to induce stress fibers independently of Rho (35–37). Furthermore, two substrates for the kinase have been identified, the myosin binding subunit of myosin light chain phosphatase and myosin light chain itself (1, 38, 39). Phosphorylation of these two substrates would be expected to lead to an increase in myosin light chain phosphorylation, myosin filament assembly, and F-actin bundling, thereby leading to stress fibers. It seems unlikely, however, that p160 Rho kinase will turn out to be the only Rho target required for the organized cytoskeletal changes induced by Rho; we have observed, for example, that a Rho mutant (with a single effector site amino acid substitution) no longer interacts directly with p160Rho kinase, yet this version of Rho induces cytoskeletal changes indistinguishable from wild type Rho.2 The biological function of stress fibers is also

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2 D. Drechsel and A. Hall, unpublished data.
As already mentioned, the product of this enzymatic activity, PIP\textsubscript{2}, is known to affect actin filament assembly, and it has been reported that in permeabilized platelets, both Rac and PIP\textsubscript{2} are essential for the release of capping proteins from the barbed ends of actin filaments, a prerequisite for actin polymerization in response to thrombin (54).

Unlike Rho and Rac, there have been no reports so far that Cdc42 affects phosphatidylinositol 4-phosphate 5-kinase activity, and in fact, activated Cdc42 can stimulate actin polymerization in a cell-free system independently of PIP\textsubscript{2} (55). In vitro assays such as this will undoubtedly have a major impact on the field in the near future. One Cdc42 target that has been reported to be essential for filopodia formation is N-WASP, a relative of the human Wiskott-Aldrich syndrome protein, WASP (56–58). It appears that overexpression of N-WASP can potentiate, though not bypass, the ability of Cdc42 to induce filopodia (58). Another Cdc42 target, IQGAP, has been shown to interact directly with F-actin; it is not known if it plays any role in filopodia formation, but there is some data linking it to the assembly of actin filaments during cytokinesis (59).

It is clear then, that we are still some way off a biochemical description of the pathways linking Rac and Cdc42 to the JNK cascade and to actin polymerization. Amino acid substitutions in Rac have been identified which block JNK activation, but which do not block actin changes and vice versa (18–20). This shows that these pathways can be biochemically separated and that Rac must interact with at least two distinct cellular targets to trigger the two responses. Despite these observations implying bifurcating pathways, the analysis of Cdc42 in yeast suggests that the two are likely to be coordinately controlled through the formation of a multimolecular signaling complex. So far there is no evidence for such a complex in mammalian cells, but this is likely to be a focus of attention in the future.

**Conclusion**

Rho GTPases play a central role in all eukaryotic cells, coordinately controlling the organization of the actin cytoskeleton with other cellular activities such as gene transcription, cell cycle progression, and adhesion. Elucidation of the underlying biochemical pathways will have a significant impact on understanding normal cell behavior as well as human diseases such as cancer and inflammation.

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