Rho GTPases and the Actin Cytoskeleton

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The actin cytoskeleton mediates a variety of essential biological functions in all eukaryotic cells. In addition to providing a structural framework around which cell shape and polarity are defined, its dynamic properties provide the driving force for cells to move and to divide. Understanding the biochemical mechanisms that control the organization of actin is thus a major goal of contemporary cell biology, with implications for health and disease. Members of the Rho family of small guanosine triphosphatases have emerged as key regulators of the actin cytoskeleton, and furthermore, through their interaction with multiple target proteins, they ensure coordinated control of other cellular activities such as gene transcription and adhesion.

The story begins back in the early 1990s with the analysis of Rho, then a newly described member of the Ras superfamily of small guanosine triphosphatases (GTPases). In Swiss 3T3 fibroblasts, it was shown that Rho can be activated by the addition of extracellular ligands [for example, lysophosphatidic acid] and that Rho activation leads to the assembly of contractile actin-myosin filaments (stress fibers) and of associated focal adhesion complexes (Fig. 1, C and D) (*1*). It was concluded that Rho acts as a molecular switch to control a signal transduction pathway that links membrane receptors to the cytoskeleton. Rac, the next member of the Rho family to be analyzed, could be activated by a distinct set of agonists (for example, platelet-derived growth factor or insulin), leading to the assembly of a meshwork of actin filaments at the cell periphery to produce lamellipodia and membrane ruffles (Fig. 1E) (*2*). More recently, activation of Cdc42, a third member of the Rho subfamily, was shown to induce actin-rich surface protrusions called filopodia (Fig. 1G) (*3*, *4*). As with Rho, the cytoskeletal changes induced by Rac and Cdc42 are also associated with distinct, integrin-based adhesion complexes (Fig. 1, F and H) (*3*). Moreover, there is significant cross-talk between GTPases of the Ras and Rho subfamilies: Ras can activate Rac (hence Ras induces lamellipodia), Cdc42 can activate Rac [hence filopodia are intimately associated with lamellipodia (Fig. 1G)], and Rac can activate Rho (although in fibroblasts, this is a weak and delayed response) (*2*, *3*). These observations suggest that members of the Rho GTPase family are key regulatory molecules that link surface receptors to the organization of the actin cytoskeleton. The aim of this article is to present some of the recent evidence that supports and extends this view.

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Not Just Fibroblasts

Although the effects of Rho GTPases on the organization of the actin cytoskeleton are perhaps still best characterized in fibroblasts, there is now compelling evidence of a similar role for these proteins in all eukaryotic cells. Some of the most exciting observations have been in neuronal cells, where mechanisms of axonal growth and guidance are being intensively studied. Axonal extension is driven by actin polymerization within the growth cone, a highly dynamic structure at the tip of the axon, consisting of filopodial and lamellipodial protrusions (see Fig. 2 and compare with Fig. 1G) that respond to both positive and negative external guidance cues. Activation of Rac and Cdc42 in a neuroblastoma cell line, N1E-115, has been shown to promote the formation of lamellipodia and filopodia, respectively, along neurite extensions. More informatively, lamellipodia and filopodia formation induced by a concentration gradient of an external agonist can be specifically blocked by introducing dominant negative Rac or Cdc42 into these cells (*5*). Activation of Rho in neuronal cells has been shown by a number of groups to induce neurite retraction and cell rounding, and although this appears strikingly different from what is seen in fibroblasts after Rho activation, the underlying biochemical cause seems to be the same: the Rho-dependent formation of contractile actin-myosin filaments (*5*, *6*). The difference is that fibroblasts, unlike neuronal cells, can maintain a flattened shape through the formation of strong focal adhesion attachment sites. It has been proposed that the opposing effects of Rac or Cdc42 and Rho might be a

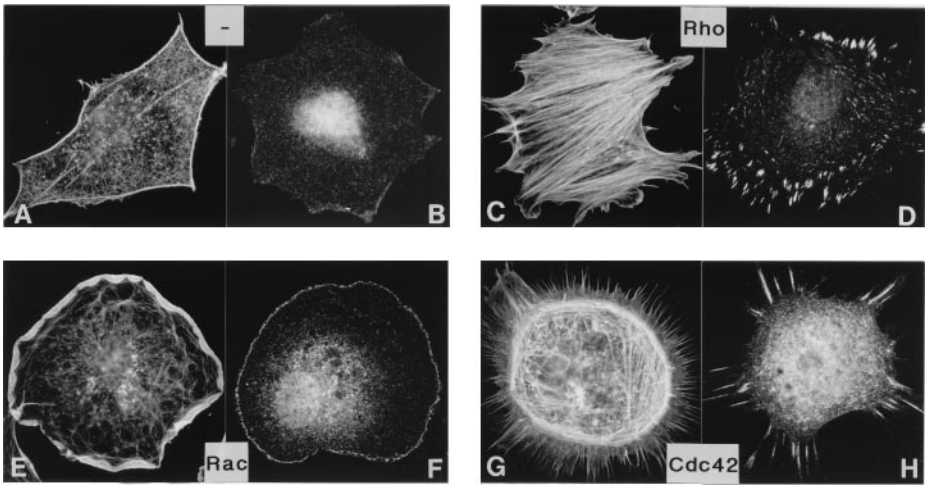

Fig. 1. Rho, Rac, and Cdc42 control the assembly and organization of the actin cytoskeleton. Quiescent, serum-starved Swiss 3T3 fibroblasts (-) contain very few organized actin filaments (**A**) or vinculin-containing integrin adhesion complexes (**B**). The effects of Rho, Rac, or Cdc42 activation in these cells can be observed in several different ways such as with the addition of extracellular growth factors, microinjection of activated GTPases, or microinjection of guanosine diphosphate (GDP)–guanosine triphosphate (GTP) exchange factors. Addition of the growth factor lysophosphatidic acid activates Rho, which leads to stress fiber (**C**) and focal adhesion formation (**D**). Microinjection of constitutively active Rac induces lamellipodia (**E**) and associated adhesion complexes (**F**). Microinjection of FGD1, an exchange factor for Cdc42, leads to formation of filopodia (**G**) and the associated adhesion complexes (**H**). Cdc42 activates Rac; hence, filopodia are intimately associated with lamellipodia, as shown in (G). In (A), (C), (E), and (G), actin filaments were visualized with rhodamine phalloidin; in (B), (D), (F), and (H), the adhesion complexes were visualized with an antibody to vinculin. Scale: 1 cm = 25 μm. [Figure courtesy of Kate Nobes]

general feature of these GTPases (*5–7*); in the case of neurons, Rac and Cdc42 might be under the control of chemoattractants, whereas Rho could be activated by chemorepellants, leading to either localized protrusion or retraction of the growth cone.

Activation of Cdc42 and Rac in macrophages has similar effects on the actin cytoskeleton as it does in fibroblasts and neurons, that is, it induces the formation of filopodial and lamellipodial protrusions (*8*). Moreover, filopodia and lamellipodia induced by the macrophage chemoattractant colony-stimulating factor–1 (CSF-1) are blocked by dominant negative Cdc42 and Rac, respectively, although it has yet to be established whether CSF-1–induced chemotaxis is blocked by either or both. Activation of Rho, on the other hand, induces a contractile actin-myosin filament network but no focal adhesions; as a consequence, macrophage cells round up in a similar way to neuronal cells (*7, 8*).

Distinctive effects of Rho, Rac, and Cdc42 activation on the organization of the actin cytoskeleton have been observed in many other cell types, including epithelial cells, endothelial cells, and astrocytes, as well as in circulating cells such as lymphocytes, mast cells, and platelets (*9–11*). The specific response of different cell types can be modified by other parameters, in particular, the cell's ability to assemble integrin-based cell-matrix or cadherin-based cell-cell adhesion complexes. This then leads to another interesting chapter in the Rho-Rac-Cdc42 story, namely, their ability to regulate other cellular activities coordinately with actin.

Not Just Actin

The observation that both stress fibers and focal adhesion complexes are assembled when Rho is activated in fibroblasts did not come as much of a surprise; the two macromolecular structures appear to go hand in hand (*1*). Further analysis has revealed that Rho activity is required to maintain focal adhesions in attached cells such that within 15 min of inactivating cellular Rho, integrin clusters can no longer be seen at the cell surface (*12*). It is not clear whether the GTPase promotes assembly of the adhesion complex directly, by modification of one or more of its constituents, or indirectly, through cross-linking of actin filaments (to which many of the constituents bind). In any case, the results have important implications. Integrin complexes are the source of adhesion-dependent signals required for cell cycle progression and survival; because their assembly is controlled by Rho, then so must be their signaling activity (*13*). It was somewhat more surprising to find that the actin structures induced by Rac and Cdc42 are associated with integrin adhesion complexes (Fig. 1, F and H) (*3*). These complexes contain many of the same constituents as classical focal adhesions, but in the case of those induced by Rac at least, they are morphologically quite distinct. The role of these integrin complexes is not clear—they do not seem to be required for the formation of lamellipodia, but they may be required for cell movement or perhaps for signaling (*14*).

Cadherin-based adherens junctions, found between polarized epithelial cells for example, are also intimately associated with the actin cytoskeleton, and a recent and exciting revelation has been that Rho (Fig. 3) and Rac are required for their assembly in keratinocytes (*15*). These observations raise some interesting issues. First, components of adherens junctions participate in signal transduction pathways that affect gene transcription; thus, Rho and Rac may influence these pathways (*16*). Second, previous work has shown that activation of Rac contributes to scattering of Madin-Darby canine kidney epithelial cells treated with hepatocyte growth factor, whereas in the keratinocyte experiment referred to above, Rac promotes cell-cell adhesion (*9, 15*). These apparently contradictory responses may well be explained by differences in the activity or availability of cadherins or other junctional components, suggesting that the effects of GTPase activation in different cell types may be difficult to predict.

Rho, Rac, and Cdc42 have been reported to regulate the c-Jun NH_2-terminal kinase (JNK) and the p38 mitogen-activated protein (MAP) kinase cascades and thereby regulate gene transcription in a more direct way than through their effects on adhesion complexes (*17*). Although these results have been largely obtained with overexpressed proteins and transfected tissue culture cells, there is now compelling evidence from *Drosophila* genetics that regulation of kinase pathways does in fact represent a physiological function of these GTPases. The ability of Rho GTPases to coordinately regulate the actin cytoskeleton and MAP kinase pathways is an emerging theme and will be discussed below.

Although the activation of MAP kinase pathways and the stimulation of integrin complex assembly offer ample opportunities for Rho, Rac, and Cdc42 to affect gene transcription, there is evidence that there may be yet other mechanisms. In an apparently JNK- and p38-independent manner, the GTPases have been reported to stimulate transcription from the cyclin D promoter and to activate the serum response transcription factor (SRF) (*18, 19*). Rho GTPases trigger progression of the G_1 phase of the cell cycle when introduced into quiescent fibroblasts, and their activities are essential for serum-induced G_1 progression and for Ras-induced cell transformation (*20, 21*). The signals responsible for these effects are clearly of great interest, but what are they? Activation of G_1 progression by Rac correlates well with its ability to stimulate lamellipodia, but not its ability to regulate JNK, suggesting that actin filaments or integrin adhesion complexes might be the source of a triggering signal (*22, 23*). Another suggestion that has been made is that reactive oxygen species (ROS) may be important. Rac is known to regulate the nicotinamide adenine dinucleotide phosphate (reduced) oxidase enzyme complex in professional phagocytes to generate superoxide and ROS, but until recently it had been assumed that this was a highly specialized function of Rac in these cells. There is now evidence to suggest that Rac-

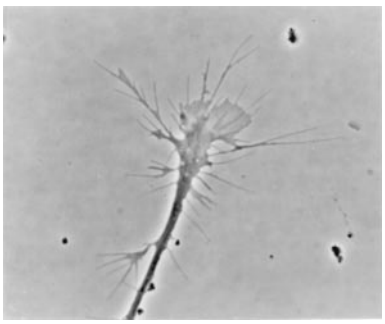

Fig. 2. Filopodial and lamellipodial activity in neuronal growth cones. A migrating growth cone at the end of an axon isolated from a chicken dorsal root ganglion was visualized with phase contrast microscopy. Scale: 1 cm = 20 μm. [Figure courtesy of Dennis Bray]

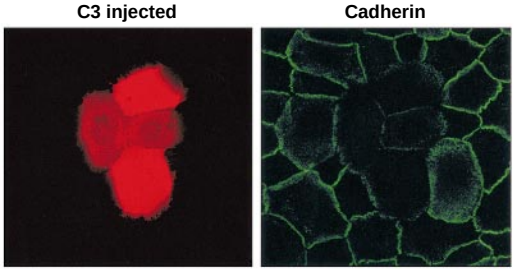

Fig. 3. Rho is required for the establishment of cadherin-based adherens junctions. Primary human keratinocytes were plated and allowed to assemble cell-cell contacts over a period of 3 hours. A group of cells was injected with an inhibitor of Rho (C3) and visualized 30 min later with an injection marker (red) and an antibody to E-cadherin (green). Scale: 1 cm = 60 μm. [Figure courtesy of Vania Braga]

induced generation of ROS occurs in other cell types (*24*). ROS have been implicated in the activation of a variety of cellular responses, including those involving the transcription factor nuclear factor kappa B, which might provide an essential signal for G_1 progression or cellular transformation. Characterization of the underlying biochemical pathways of ROS generation in nonphagocytic cells is needed to sort out this potentially interesting story.

Fishing Upstream and Downstream

To drive processes such as directed cell movement and axonal extension, the activity of Rho GTPases must be restricted to discrete intracellular locations specified ultimately by extracellular cues. The key to understanding this aspect of GTPase function is likely to lie in their upstream regulation.

Upstream activators. About 10 GTPase-activating proteins and three guanine nucleotide dissociation inhibitors, both potential down-regulators of GTPase activity, have been described, but little is known about their mode of action (*25*). The 15 guanine nucleotide exchange factors (GEFs) (the Dbl or DH family) described to date have attracted more attention, in part because many were originally identified as potent oncogenes capable of transforming NIH 3T3 cells to a malignant phenotype (for example, Dbl, Vav, and Lbc) (*25*). There is little doubt that the oncogenic activity of Dbl-related GEFs is mediated through activation of Rho GTPases, but whether subsequent changes to the actin cytoskeleton play a role is not clear. Interestingly, the constitutively activated versions of Rho, Rac, and Cdc42 are at best only weak oncogenes; one explanation for this apparent contradiction is that perhaps the GTPases must cycle between GTP- and GDP-bound states to exert optimal oncogenic effects. Another explanation is that the GEFs play additional roles in signaling, perhaps by promoting the formation of a larger molecular complex. So far there have been no reports of genetic alterations directly affecting DH proteins or Rho GTPases in human cancer.

Two members of the DH family deserve further comment. The gene encoding Tiam-1 was originally identified as being capable of conferring an invasive phenotype when introduced into a noninvasive lymphoma cell line. Tiam-1 is now known to act as a Rac-specific GEF, and indeed Rac itself will also induce an invasive phenotype in these cells (*26*). These observations have raised the possibility that deregulated Rac activity may contribute to the metastatic or invasive phenotype of human cancers. *FGD1* was identified by positional cloning as the locus for the human genetic syndrome faciogenital dysplasia and later shown to be a GEF specific for Cdc42 (*27*). This disease is characterized by severe defects in skeletogenesis, suggesting an important developmental role for Cdc42 in bone morphogenesis.

The mechanisms by which GEFs are activated by membrane receptors are still far from clear. Exchange activity is encoded within their DH domain, but it is notable that in all GEFs this is immediately followed by a pleckstrin homology (PH) domain. It is thought that the PH domain plays a crucial role in membrane localization by interacting with specific lipids, and it is known that the generation of phosphatidylinositol-3,4,5-trisphosphate (PIP_3), by phosphatidlyinositol 3-kinase (PI 3-kinase) activity, is essential for receptor-mediated activation of Rac in mammalian cells, and that a PI 3-kinase homolog, TOR2, controls Rho1p activation in *Saccharomyces cerevisiae* (*28*). A major problem in this field has been the lack of reliable reagents to measure the concentrations and intracellular locations of the active forms of the GTPases. An exciting possibility is that target proteins (see the next section) could be used to recognize the GTP-bound forms of Rho, Rac, or Cdc42 specifically. Another problem is that, unlike Ras, which is constitutively in the membrane, Rho GTPases are thought to be at least partially cytosolic (associated with a guanine nucleotide dissociation inhibitor) and therefore must translocate to the plasma membrane (where they would presumably meet a GEF). How they do so is unknown.

Finally, Rho and Cdc42 are required late in the cell cycle for formation of the actin-myosin contractile ring (*29*). It is not known whether the GTPases act through similar biochemical pathways during G_1 and cytokinesis; however, the upstream regulation of GTPases at the end of mitosis must be tied in to the cell cycle machinery rather than to extracellular signals.

Downstream targets. To understand the biochemical mechanisms through which Rho GTPases regulate the organization of the actin cytoskeleton and other associated activities, there has been an enormous effort to identify cellular targets (effectors). Yeast two-hybrid selection and affinity purification have proved to be powerful techniques, and at least 20 candidate targets have been identified so far that represent a wide variety of enzymatic activities and protein-protein interaction domains. The research has been reviewed elsewhere, and the following discussion will focus on just a few points of current interest (*25*).

The Ser-Thr kinase p160ROCK interacts with Rho in a GTP-dependent manner, and when overexpressed or constitutively activated, it has been reported to mimic Rho. It would seem, then, that this is an excellent candidate for mediating Rho-induced changes to the actin cytoskeleton (*30*). Moreover, two substrates of this kinase, myosin light chain phosphatase and myosin light chain, are known to regulate the assembly of actin-myosin filament bundles, and recent work has shown that Rho-induced stress fiber assembly occurs primarily through bundling of preexisting filaments rather than de novo actin polymerization (*14, 31*). Whether p160ROCK is the only downstream target of Rho required to induce stress fibers remains to be seen. Assembly of stress fibers is blocked by cytochalasin D, suggesting that some actin polymerization might be required, but work from our own laboratory suggests that the actin-myosin filaments induced by this kinase are not correctly organized nor are they contractile as they are when induced by Rho (*1, 32*).

Although not direct targets of Rho, the ERM proteins (ezrin, radixin, and moesin) are emerging as key regulators of the actin cytoskeleton. In vitro binding assays have revealed that their interaction (through their NH_2-termini) with a transmembrane protein, CD44, can be regulated by Rho, and their COOH-terminal ends interact with filamentous actin (F-actin) (*33*). Furthermore, with a permeabilized cell reconstitution assay, it has been shown that ERM proteins are essential for both Rho- and Rac-induced cytoskeletal effects (*34*). A reasonable interpretation of these experiments is that ERM proteins behave as regulatable scaffold proteins that anchor actin filaments to the membrane and that this is an essential prerequisite for Rho and Rac (acting through target proteins) to induce stress fibers and lamellipodia, respectively (Fig. 4).

Mutational analysis suggests that the induction of actin polymerization and of JNK activity are mediated by bifurcating pathways triggered by the interaction of Rac with two distinct target proteins (*22, 23*). A similar conclusion has been reached for Cdc42 (*22*). Although a dozen or so target proteins have been identified for Rac and Cdc42, one of these, the Ser-Thr kinase $p65^{PAK}$, has received much of the attention to date. Its kinase domain is most closely related to yeast Ste20p, a known regulator of MAP kinase pathways, suggesting that it might mediate activation of JNK by Rac and Cdc42. Although some groups have provided evidence in support of this, others have failed to find a link, and it is still not

resolved whether $p65^{PAK}$ is a physiological regulator of JNK (*18, 35*).

A role for $p65^{PAK}$ in Rac-induced actin polymerization has also been proposed, although on the face of it, the data seem contradictory. Three groups reported that Rac mutants that do not interact with $p65^{PAK}$ still induce lamellipodia, suggesting that the kinase is not involved, whereas another group reported that a kinase-dead mutant of $p65^{PAK}$ mimics Rac and induces lamellipodia, suggesting that $p65^{PAK}$ is involved (*18, 22, 23, 36*). Clearly both cannot be true. Closer examination of the data supports a conclusion that the interaction of Rac with $p65^{PAK}$ is neither the trigger nor is it required for actin polymerization, but $p65^{PAK}$ can interact with a molecular complex that does control actin polymerization. Whether $p65^{PAK}$ is essential for actin polymerization remains to be seen. The idea that GTPase-induced effects are mediated by multimolecular complexes and not by linear pathways of biochemical cascades should not be so surprising because it has clearly been established for Cdc42 in yeast (see below).

Another target of Rac that may play a major part in actin polymerization is phosphatidylinositol-4-phosphate 5-kinase (PIP 5-kinase), the enzyme that converts PIP to phosphatidylinositol-4,5-bisphosphate (PIP_2). In platelets, Rac can stimulate PIP_2 formation, leading to barbed-end uncapping and severing of actin filaments (*11*). This provides a bolus of nucleation sites for actin monomer addition, resulting in rapid actin polymerization and lamellipodium formation. There is a growing list of actin-associated proteins (for example, gelsolin, vinculin, and ERMs) that interact with PIP_2; the analysis of the enzymes that control the synthesis of this lipid should provide important insights into the mechanisms of F-actin assembly.

The product of the human Wiskott-Aldrich syndrome gene, *WASP*, has been identified as a Cdc42-specific target (*37*). Although it has no catalytic activity, the presence of numerous protein-protein interaction domains has generated much speculation as to its likely function. Mutational analysis has revealed that the interaction of Cdc42 with WASP is not the trigger for filopodia formation; however, it remains to be seen whether WASP plays an active role in F-actin assembly, or whether it interacts with assembled F-actin and contributes to other Cdc42-induced effects such as gene transcription (*22, 37*). Support for the first suggestion has come from yeast where Bee1p, a WASP-related protein, has been shown to be essential for actin polymerization (*38*).

There is clearly a long way to go to define the biochemical pathways regulated by the Rho GTPases, and in this, as in other problems of signal transduction, the genetic analysis of simpler eukaryotes is playing an increasingly important role.

The Power of Genetics

The genetic analysis of developmental pathways in *Drosophila* and *Caenorhabditis elegans* is rapidly making major contributions to our insight into the physiological role of Rho, Rac, and Cdc42. The area is already too large to be covered here, so just a few examples will be given. During embryonic development, cells undergo a variety of changes in their shape and polarity and some migrate to new sites within the embryo in response to specific cues. An emerging theme underlying these morphogenetic processes is that they often require coordinated changes in gene transcription and in the organization of the actin cytoskeleton. One of the clearest examples of this is dorsal closure, where two symmetrical sheets of epithelial cells elongate and migrate over the embryo, eventually to fuse at the midline. A driving force for this morphogenetic movement is a change in the actin cytoskeleton at the leading edge of the migrating cells, but in addition, activation of the JNK cascade is essential. Inactivation of Rac in the *Drosophila* embryo disrupts both actin changes and JNK activation and blocks dorsal closure (*39*). JNK activity is also required for the morphogenesis of a variety of other epidermal cell types. In the *Drosophila* eye, for example, the development of cell polarity is under the control of the *frizzled* (*fz*) receptor; in this case there is evidence that Rho acts downstream of *fz* to mediate JNK activation (*40*). These results confirm some of the observations made in tissue culture cells and demonstrate the importance of Rho GTPases in coordinating actin changes with the regulation of MAP kinase pathways.

Drosophila RhoL may be the exception that proves this rule. This novel member of the Rho GTPase family lacks a tyrosine residue at codon 40 (conserved in all other family members) and does not activate JNK (*41, 42*). Nevertheless, RhoL is required during oogenesis for the morphogenetic changes in the follicular cells that surround the oocyte (*41*). Perhaps in this case, MAP kinase activity is not required to act in concert with changes in the actin cytoskeleton. No mammalian homolog of RhoL has yet been reported.

Genetic analysis has demonstrated the importance of Rho GTPases in directed cell movement. Inactivation of Rac (but not Cdc42 or RhoL) in the *Drosophila* ovary, for example, prevents the migration of border cells during oogenesis from the anterior tip, through the nurse cells, to the oocyte (*41*). Interestingly, the transcription factor C/EBP and the *Drosophila* fibroblast growth factor

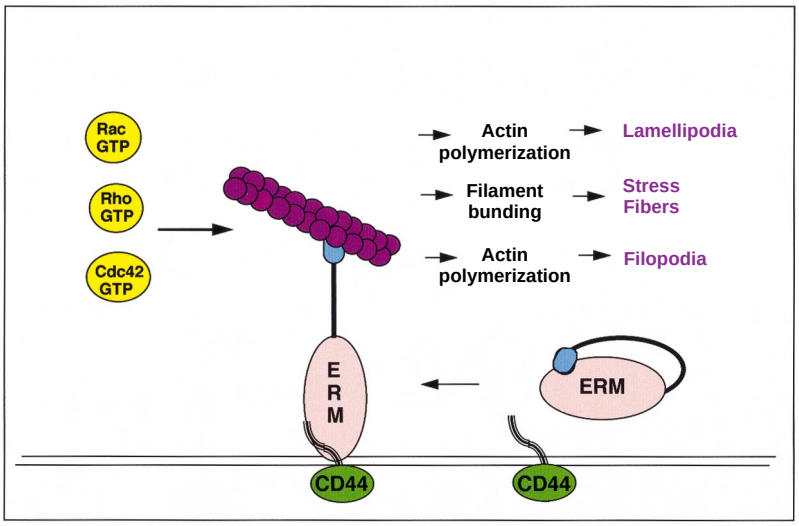

Fig. 4. ERM proteins are required for GTPase-mediated cytoskeletal changes. It has been proposed that ERM proteins exist in a closed (inactive) conformation and an open (active) conformation. There is evidence to suggest that this transition can be regulated by Rho, perhaps through the activation of a Ser-Thr kinase or a lipid kinase (through PIP_2). In the active conformation, the NH_2-terminus of ERM proteins (pink) can interact with transmembrane proteins, such as CD44, whereas the COOH-terminus (blue) interacts with F-actin. This membrane–ERM–F-actin unit is an essential prerequisite for the Rho GTPases to induce cytoskeletal changes. In the case of Rho, this is likely to be mediated by the bundling and reorganization of preexisting actin-myosin filaments to generate stress fibers, whereas in the case of Rac and Cdc42 it is likely that preexisting filaments are uncapped to allow actin polymerization and filament growth leading to the formation of lamellipodia and filopodia.

receptor are also required for border cell migration, but whether Rac is involved in this pathway is not known. Mutations in the *mig-2* locus in *C. elegans* lead to migratory defects in a variety of cell types, including neurons, mesodermal cells, and sex myoblasts (*43*). *Mig-2* encodes another member of the Rho GTPase family, having ~60% amino acid identity to *C. elegans* Rac and Cdc42. Whether there is a mammalian homolog is not known; however, the exciting observation that a constitutively activated *mig-2* causes defects in axonal guidance, but not axon outgrowth, seems likely to encourage a search for additional members of the Rho GTPase family in mammalian cells.

Paradigms from Yeast

Turning to yeast, it becomes clear that the biochemical complexity of GTPase signaling pathways is only just beginning to be appreciated, and any notion that pathways are linear, with occasional points of interaction, is unrealistic. Much progress in understanding the role of Rho1p and Cdc42p (two of the five Rho proteins) in *S. cerevisiae* has been made and details can be found elsewhere; however, some important general lessons about the nature of GTPase signaling have emerged. First, and in agreement with observations made in mammalian cells, both Rho1p and Cdc42p coordinately regulate multiple pathways. For example, three specific targets for Rho1p have been identified to date: Bni1p (which affects actin assembly), Pkc1p (an upstream regulator of a MAP kinase pathway required for cell wall biosynthesis), and glucan synthase (required for cell wall synthesis) (*44*). Two targets of mammalian Rho, PKN and mDia, are related to Pkc1p and Bni1p, respectively, and it seems likely that the coordinated regulation of the actin cytoskeleton and of MAP kinase pathways by Rho GTPases is conserved in all eukaryotic species (*45*). Although there is no mammalian analog of glucan synthase, it is interesting to note that Rho has been shown to control the assembly of extracellular matrix fibers to create a microenvironment surrounding mammalian cells—perhaps this is somehow analogous to the cell wall function of Rho1p in yeast (*46*).

A second take-home message is that components of GTPase-mediated pathways assemble into multimolecular complexes held together by scaffold proteins, a good example of which is Bem1p. This Src-homology 3 (SH3) domain–containing protein interacts with Cdc24p (a GEF for Cdc42p), Rsr1p (an upstream GTPase that also interacts with Cdc24p), Ste20p (a target for Cdc42p), actin, and Ste5p (another scaffold protein that is an essential component of the MAP kinase pathway) (Fig. 5) (*47*). Cdc42p can affect the activity and the localization of this complex. Furthermore, GTPases and their targets can assemble into different multimolecular complexes and thereby participate in different cellular processes. For example, Cdc42p and Ste20p are required in both the pheromone-induced activation of a MAP kinase cascade (leading to cell cycle arrest) and in the starvation-induced activation of a different MAP kinase pathway (leading to filamentous growth) (Fig. 5). Interestingly, the interaction of Cdc42p with Ste20p is not required for the pheromone response, but it is required for the starvation response (*48*). It remains to be seen whether multimolecular complexes are used universally and in higher eukaryotes in GTPase signaling pathways.

A final message to emerge from the analysis of yeast is that GTPase pathways are often linked in a hierarchical fashion. Thus, Cdc42p, which is required for the assembly of components at the bud site during cell division, acts downstream of another GTPase, Rsr1p (closest mammalian homolog Rap1), which is required for localization of the bud site (*49*). In fact Rsr1p interacts directly with Cdc24p, a GEF for Cdc42p, and thereby ensures that components of the bud are assembled only at the bud site. Rho appears to act later in this pathway to promote growth of the bud, but how its activation is coordinated with Cdc42 is not clear (*50*).

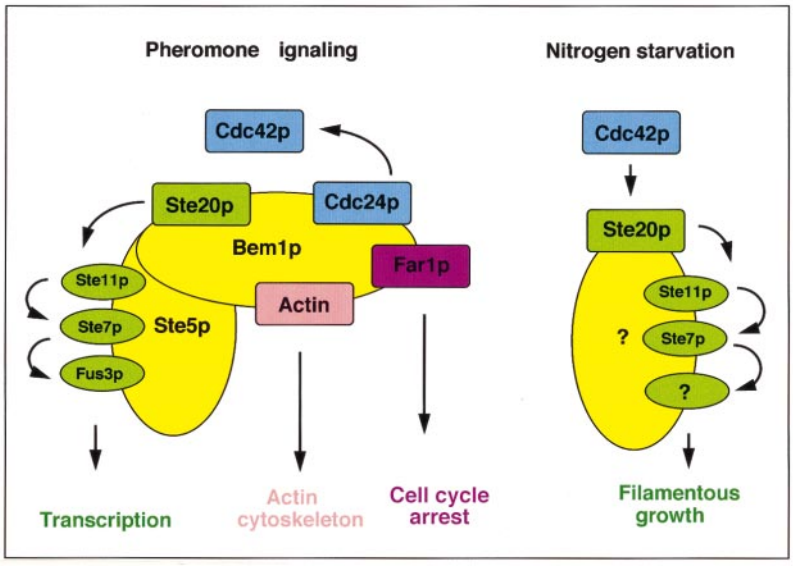

Fig. 5. Cdc42p-associated signaling complexes in *S. cerevisiae*. Cdc42p is essential for both pheromone-induced mating and for nitrogen starvation–induced filamentous growth. In the pheromone response, Bem1p acts a scaffold protein and interacts with Cdc24p (an exchange factor for Cdc42p), Far1p (a cell cycle inhibitor), actin, Ste20p (a Ser-Thr kinase that can interact with Cdc42p), and Ste5p. Ste5p is a scaffold protein that interacts with components of a MAP kinase cascade. Ste20p activates the MAP kinase cascade, but no interaction with Cdc42p is required. A role of Cdc42p is to localize this signaling complex to the mating projection. Cdc42p and Ste20p are also required to activate a distinct MAP kinase pathway (still not completely defined) in response to nitrogen starvation. In this case, the interaction between Cdc42p and Ste20p is essential.

Conclusions

Rho GTPases act as molecular switches. In response to extracellular signals, they induce coordinated changes in the organization of the actin cytoskeleton and in gene transcription to drive a large variety of biological responses including morphogenesis, chemotaxis, axonal guidance, and cell cycle progression. It can be predicted with some confidence that the biochemical and genetic analysis of the signaling pathways controlled by Rho GTPases will lead to a better understanding of these fundamental processes.

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51. I would like to thank the Cancer Research Campaign (United Kingdom), The Wellcome Trust (United Kingdom), and the Medical Research Council (United Kingdom) for their financial support. I thank D. Mackay and C. Nobes for critical reading of this manuscript and C. Nobes, D. Bray, V. Braga, and D. Mackay for help with the figures.

A Structural Scaffolding of Intermediate Filaments in Health and Disease

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The cytoplasm of animal cells is structured by a scaffolding composed of actin microfilaments, microtubules, and intermediate filaments. Intermediate filaments, so named because their 10-nanometer diameter is intermediate between that of microfilaments (6 nanometers) and microtubules (23 nanometers), assemble into an anastomosed network within the cytoplasm. In combination with a recently identified class of cross-linking proteins that mediate interactions between intermediate filaments and the other cytoskeletal networks, evidence is reviewed here that intermediate filaments provide a flexible intracellular scaffolding whose function is to structure cytoplasm and to resist stresses externally applied to the cell. Mutations that weaken this structural framework increase the risk of cell rupture and cause a variety of human disorders.

In contrast to microfilaments and microtubules, whose components are highly evolutionarily conserved and very similar within cells of a particular species, intermediate filaments (IFs) display much diversity in their numbers, sequences, and abundance (*1*). In humans, there are more than 50 different IF genes, which are differentially expressed in nearly all cells of the body. Intermediate filaments generally constitute approximately 1% of total protein, although in some cells, such as epidermal keratinocytes and neurons, IFs are especially abundant, accounting for up to 85% of the total protein of fully differentiated cells. Thus, IF cytoskeletons seem to be tailored to suit specific structural needs of each higher eukaryotic cell.

Despite their diversity, members of the IF superfamily share a common structure: a dimer composed of two α-helical chains oriented in parallel and intertwined in a coiled-coil rod. First discovered in the 1950s in the keratins constituting hair (*2*), this mechanism of dimerization through coiled-coil interaction is now universally found throughout biology. The highly conserved ends of the IF rod associate in a head-to-tail fashion, and mutations in these rod ends have deleterious consequences for the assembly process of most if not all IF proteins (*3*, *4*). The association of dimers results in linear arrays, four of which associate in an antiparallel, half-staggered manner to produce protofibrils; and three to four protofibrils intertwine to produce an apolar intermediate filament 10 nm in diameter (Fig. 1). Generally, the assembly equilibrium is heavily in favor of IF polymer.

Although IFs share similar structures, their properties can be quite unique. Keratin IFs of hair and epidermal cells are highly insoluble, and even their noncovalently linked dimer subunits do not fully dissociate in 9 M urea (*5*). In contrast, nuclear lamin IFs that line the inner surface of the nuclear membrane and vimentin IFs of fibroblasts are dynamic, dissociating and reforming in a cell cycle–dependent manner (*6*). Indeed, despite very small intracellular pools of unassembled subunits, both recovery after photobleaching of fluorescently labeled IFs (*7*) and introduction of small peptide inhibitors of IF assembly (*8*) have demonstrated that individual IFs apparently have in vivo

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