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Role of Rho family GTPases in epithelial morphogenesis

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Epithelial cell sheets line the organ and body surfaces and the specialized barrier functions of these epithelia regulate the exchange of substances with the outside environment and between different body compartments. Epithelia play a role in a wide range of physiological processes such as digestion, excretion, and leukocyte trafficking. In addition, during development, some epithelia form transient primitive structures, including the neural tube and somites, which are essential for the development of more complex organs.

The establishment and maintenance of epithelial cell polarity is critical for the development and functioning of multicellular organisms (Nelson 2000). A multi-step model for the establishment of cell polarity has been proposed by Drubin and Nelson (1996). Cell polarity is initiated by a spatial cue, such as generated by cell–cell contact sites. This cue is interpreted and marked by the formation of signaling complexes that relay the spatial information to the actin cytoskeleton. Localized actin assembly then leads to the formation of a targeting patch, which functions to reinforce the initial cue. Subsequently, this cue can further be propagated via a reorganization of the microtubule cytoskeleton, which in turn causes a redistribution of the membrane trafficking apparatus.

In addition to actin cytoskeletal dynamics and vesicle trafficking, epithelial morphogenesis also depends on cell–substrate and cell–cell adhesion. Members of the Rho family of GTPases play essential roles in each of these processes (for reviews, see Hall 1998; Kaibuchi et al. 1999a,b; Braga 2000; Ellis and Mellor 2000; Schwartz and Shattil 2000; Ridley 2001a,b) and therefore it is not surprising to see that Rho GTPases have emerged as critical players at multiple stages of epithelial morphogenesis. In this review we will discuss the involvement of Rho family members in the development and maintenance of epithelial morphology and highlight recent advances in our understanding of the roles of these GTPases in the establishment of epithelial polarity. We

will also discuss the participation of these GTPases in epithelial remodeling during wound-healing and epithelial-mesenchymal transitions.

As other members of the Ras superfamily, Rho GTPases cycle between a GDP-bound (inactive) state and a GTP-bound (active) state. In the active state, these GTPases relay signals from growth factors, cytokines, and adhesion molecules to regulate a wide range of biological processes, including actin cytoskeleton organization, transcriptional regulation, and vesicle trafficking (Van Aelst and D'Souza-Schorey 1997; Hall 1998).

The nucleotide state of Rho family proteins is controlled by three classes of regulatory proteins: guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs), and guanine nucleotide dissociation inhibitors (GDIs) (Boguski and McCormick 1993). GEFs catalyze the exchange of GDP for GTP by facilitating the release of GDP and transient stabilization of the nucleotide-free protein. GAPs promote the intrinsic GTP hydrolyzing activity of Rho proteins, thereby enhancing their conversion to the GDP-bound form. GDIs preferentially bind to GDP-bound GTPases and prevent spontaneous and GEF-catalyzed release of nucleotide, thereby maintaining the GTPases in the inactive state. Although activation of Rho GTPases in response to extracellular signals in principle could occur either via the activation of GEFs or inhibition of GAPs and GDIs, studies on oncogenic forms of GEFs suggest that nucleotide exchange is the rate-limiting step in GTPase activation.

The localized activation of GEFs is likely to be of critical importance in polarity establishment and morphogenesis. Localized control of GEFs and GTPases has been extensively characterized in the budding yeast *Saccharomyces cerevisiae*. Polarized growth is important at several stages of the budding yeast life cycle, including bud formation during vegetative growth and shmoo formation during mating. Recent genetic and biochemical analyses of the roles of the GTPase Cdc42 and its GEF Cdc24 in these processes has led to a model in which GEF activity is regulated in four distinct steps: GEF recruitment to the plasma membrane and subsequent activation, stabilization by adaptor proteins, and termination of signaling by GEF inactivation (Gulli and Peter 2001). Less is known about the regulation of GEFs in

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other organisms (Symons and Settleman 2000) and the budding yeast model may therefore serve as a more general paradigm for the analysis of localized GEF activation.

Establishment of apical-basolateral polarity

Epithelia can display two types of polarity, apical-basolateral and planar polarity (see below). Apical-basolateral polarity depends on the segregation of different protein and lipid constituents to the apical and basolateral membrane domains and is generated through direct interaction of the cell with its environment, for example, extracellular matrix and intercellular contacts (Eaton and Simons 1995; Yeaman et al. 1999). Apical-basolateral polarity is critical for the function of transporting epithelia, such as those found in the kidney and gastrointestinal tract. These epithelia establish permeability barriers between distinct compartments and vectorial transport along the apical-basal axis enables the maintenance of ionic homeostasis.

Different types of cell–cell junctions can be distinguished in epithelia. Adherens junctions and desmosomes provide mechanical strength to the epithelial cell contacts. The tight junctions (zonula occludens) control the selective permeability of the epithelial layer to ions and small solutes (gate function). Tight junctions also prevent the free mixing of proteins and lipids of apical and basolateral membrane compartments (fence function). Finally, gap junctions allow the passage of chemicals between adjacent cells.

The formation of cell–cell junctions can be described as a sequence of discrete steps, an analysis that is largely based on the use of the calcium-switch model (Braga 2000). On addition of calcium, cadherins become competent for homophilic binding to cadherins of neighboring cells (step 1). This adhesive interaction leads to cadherin clustering at cell–cell contact sites in a process that remains poorly understood (step 2) (for review, see Adams and Nelson 1998). Cadherin clusters are immobilized and stabilized by interaction with the actin cytoskeleton (step 3). This is followed by a reorganization of the circumferential actin belt that typifies isolated cells in culture (step 4). Concomitant with step 4, basolateral transport vesicles are rerouted to targeting patches that are localized at the contacting membranes, where they dock and fuse. In the next stage, these targeting patches and the apical junctional complex (tight junction) become restricted to the apex of the lateral membrane, leading to the establishment of a fully polarized epithelial phenotype with distinct apical and basolateral membrane compartments (step 5).

Adherens junctions

Studies using a wide range of cell lines have demonstrated that inhibition of Rho by C3 transferase, a bacterial exoenzyme, interferes with the establishment of

adherens junctions (Braga et al. 1997, 1999; Takaishi et al. 1997). Interestingly, inhibition of Rho causes removal of cadherins from junctions before significant changes in cell morphology (i.e., cell rounding and retraction) can be observed, suggesting that Rho may play a role in adherens junction formation by stimulating cadherin clustering (Braga et al. 1997, 1999). Rho also is thought to function in the intracellular targeting of proteins, such as c-Src and ERM (Fincham et al. 1996; Kotani et al. 1997; Shaw et al. 1998; Timpson et al. 2001). An alternative scenario for the role of Rho in adherens junction formation therefore could be that Rho recruits accessory proteins to nascent junctions.

The downstream signaling pathways that mediate the effect of Rho on adherens junctions largely remain to be elucidated. Adherens junction formation in keratinocytes has been shown to depend on the activity of the tyrosine kinase Fyn (Calautti et al. 1998). This effect is possibly mediated via phosphorylation of adherens junction proteins such as β - and γ -catenins or p120-catenin. Recent studies have shown that constitutively active Rho stimulates Fyn tyrosine phosphorylation of catenins and cell–cell adhesion, and that Rho-induced phosphorylation of catenins is reduced in Fyn-deficient keratinocytes (Calautti et al. 2002). These results indicate that Fyn and possibly other Src family kinases can function downstream of Rho in the establishment of adherens junctions. A possible Rho effector that could mediate this function is PRK2, as PRK2 kinase activity increases with keratinocyte differentiation and overexpression of PRK2, like activated Rho, stimulates Fyn phosphorylation of catenins and cell–cell adhesion.

Early evidence for a role of Rac in the regulation of adherens junctions came from studies showing that expression of dominant negative Drac1 inhibits actin assembly at adherens junctions in the developing wing disc epithelium in *Drosophila* (Eaton et al. 1995). In mammalian cells, Rac has been shown to be activated by E-cadherin-mediated cell–cell contact (Nakagawa et al. 2001; Noren et al. 2001; Kovacs et al. 2002) and Rac activity is necessary for the establishment of adherens junctions in epithelial and endothelial cells (Braga et al. 1997, 1999; Takaishi et al. 1997). Making use of an assay that presents the ectodomain of E-cadherin on a solid substrate, it was shown that both phosphatidylinositol 3-kinase (PI3K) and Rac are recruited to nascent cadherin contacts and that Rac is activated in a biphasic manner. Interestingly, the early phase (15 min) is independent of PI3K activation, whereas the later phase (30 min) is abrogated by PI3K inhibitors (Kovacs et al. 2002).

Similar to the observations on the wing epithelium in *Drosophila*, inhibition of Rac activity interferes with the accumulation of polymerized actin at adherens junctions (Braga et al. 1997; Takaishi et al. 1997), suggesting that Rac may mediate the reorganization of the actin cytoskeleton that is necessary to stabilize cadherin receptors at cell–cell contact sites. Support for such a mechanism comes from observations showing that recruitment of actin to cadherins that are clustered by antibodies is specifically blocked by inhibition of Rac, but not Rho (Braga

et al. 1997). It is interesting to note that Rac also functions in the clustering of integrin receptors (Hotchin and Hall 1995; D'Souza-Schorey et al. 1998; Rottner et al. 1999), suggesting that it also could play a similar role in clustering of cadherins.

Rac activation leads to a dramatic reorganization of intercellular contacts in MDCK cells. Whereas in wild-type MDCK cells, tight contact is restricted to the apical area of lateral membranes, in cells expressing constitutively active Rac1 tight contact extends over the entire lateral membrane (Takaishi et al. 1997; Jou et al. 1998). In addition, the lateral membranes display extensive interdigitation. Interestingly, the effect of constitutive activation of Rac on adherens junctions dramatically depends on the cell type under investigation. In keratinocytes constitutively active Rac causes disassembly of adherens junctions (Braga et al. 2000), which is essentially the opposite of what is observed in MDCK cells (Hordijk et al. 1997; Sander et al. 1999). The molecular mechanisms that underlie this cell type dependence need to be clarified. It is important to note, however, that these differences in response to Rac activation are critical for the role of Rac in epithelial-mesenchymal transitions and invasion, as discussed below.

A downstream target of Rac that has been implicated in the regulation of adherens junctions is IQGAP (Kuroda et al. 1998). IQGAP is a binding partner of both Rac and Cdc42 and localizes to cell-cell junctions (Kuroda et al. 1996). IQGAP competes with α -catenin for binding to β -catenin, and thereby can displace α -catenin from E-cadherin complexes. These findings suggested a model in which IQGAP keeps E-cadherin complexes in a "weak adhesion" state and Rac/Cdc42 binding to IQGAP acts as a switch to promote a "strong adhesion" state (Kaibuchi et al. 1999b). This model, however, is not corroborated by the phenotype of IQGAP1-deficient mice (Li et al. 2000). Indeed, even in tissues that do not express IQGAP2, a protein with a high degree of homology to IQGAP1 (Brill et al. 1996), no obvious developmental defects could be detected.

The potential role of Cdc42 in the regulation of adherens junctions is less well documented as that for Rac. The effects of constitutively active Cdc42 in MDCK cells resemble those of activated Rac1, although the interdigitation of the lateral membranes is much less pronounced (Kodama et al. 1999). Similar to Rac, Cdc42 is activated by E-cadherin-mediated cell-cell contact in a PI3K-dependent fashion (Kim et al. 2000) and Cdc42 activity is necessary for E-cadherin-dependent cell interactions (Fukata et al. 1999). It is interesting to note, however, that in *Drosophila* expression of a dominant negative version of DCdc42 does not have any significant effect on cell-cell junctions in the developing wing disc epithelium (Eaton et al. 1995).

Tight junctions

Several studies have shown that Rho activity is essential for the proper functioning of tight junctions. Early work showed that inhibition of Rho by C3 transferase dis-

places the tight-junction protein zonula occludens 1 (ZO-1) from junctions and increases the paracellular permeability of polarized human epithelial cells (Nusrat et al. 1995). Expression of dominant negative Rho in MDCK cells also abolishes the fence function of tight junctions in the absence of overt changes in tight junction organization or protein composition (Jou et al. 1998). The more pronounced effect of C3 transferase on tight junctions probably reflects the weaker inhibitory effect of the dominant negative mutant of Rho.

A downstream target of Rho that is likely to mediate tight junction control is the Rho-associated kinase (Rok), as chemical inhibition of this effector enhances the paracellular permeability of polarized intestinal epithelia (Walsh et al. 2001). Inhibition of Rok also interferes with the recruitment of ZO-1 and occludin to newly forming intercellular contacts in these cells, suggesting that Rho/Rok signaling also plays a role in the assembly of tight junctions (Walsh et al. 2001).

It is interesting to note that expression of either dominant negative or constitutively active mutant forms of RhoA at relatively low levels perturbs both the gate and fence functions of tight junctions in MDCK cells (Jou et al. 1998). This observation as well as numerous other instances where both inhibition and constitutive activation of Rho GTPases interfere with the same biological function are often interpreted to imply that GTP hydrolysis is a necessary step in the regulation of this biological function (Symons and Settleman 2000). Another potential mechanism, however, could be that tight junction behavior critically depends on the level of RhoA activation or alternatively that the inhibitory effects of the dominant-negative and GTP-hydrolysis-defective mutants are caused by interference in distinct functions that cooperatively control tight junction properties. Support for the latter scenario comes from electron microscopy observations showing that expression of constitutively active RhoA inhibits the formation of protein strands in the tight junctions, whereas dominant negative RhoA does not significantly affect tight junction morphology (Jou et al. 1998).

As is the case for Rho, both dominant negative and constitutively active mutants of Rac1 interfere with gate and fence functions of tight junctions in MDCK cells (Jou et al. 1998). Interestingly, however, in contrast to the effect of expression of constitutively active RhoA, constitutively active Rac enhances tight junction strand formation. The signaling mechanisms that mediate the regulation of tight junctions by Rac remain to be elucidated. One possible mechanism is that Rac modulates tight junction properties via its effect on the dynamics of perijunctional actin filaments (Madara 1998).

The effects of dominant negative and constitutively active mutants of Cdc42 on the function and morphology of tight junctions in MDCK cells appears to be similar to that of the cognate mutants of Rac1. Both dominant negative and constitutively active mutants of Cdc42 perturb gate and fence functions and constitutive activation of Cdc42 induces the formation of junctions

well below the position where tight junctions normally form (Rojas et al. 2001).

Insights into the signaling mechanisms that control tight junctions downstream of Cdc42 are emerging. Cdc42 binds to Par-6, an adapter protein that in turn binds to PKC λ/ι or PKC ζ , the two members of the atypical protein kinase C (aPKC) subfamily (Joberty et al. 2000; Lin et al. 2000). Both Par-6 and either one of the aPKCs bind to an additional adapter protein, Par-3, forming a ternary complex (Fig. 1). In addition to being implicated in the establishment of epithelial tight junctions in mammals, this evolutionary conserved Par-aPKC complex plays a critical role in a large number of other processes that involve cell polarity. These include asymmetric cell division in the *Caenorhabditis elegans* one-cell embryo and *Drosophila* neuroblasts and the maturation of *Xenopus* oocytes (Ohno 2001; Wodarz 2002).

Studies on the formation of cell-cell junctions in MDCK cells show that the Par-aPKC complex is recruited to early cell-cell contacts at initial stages of cell polarization. The complex remains associated with the

apical-most zone of the developing cell-cell junctions and is still present at the tight junctions of fully polarized cells. What is the role of Cdc42 in the regulation of this complex? Cdc42 has been shown to activate aPKC in the Par-aPKC complex in a Par-6 dependent manner (Qiu et al. 2000; Yamanaka et al. 2001). The precise role of aPKC activity is not yet clear. One scenario is that phosphorylation of Par-3 by aPKC may change the conformation of the complex, allowing the interaction with junctional proteins that tether the Par-aPKC complex to the plasma membrane (Ohno 2001). A good candidate for such a protein is the recently identified transmembrane protein junctional adhesion molecule (JAM) (Ebnet et al. 2001; Itoh et al. 2001). JAM localizes at new cell contacts at an early stage of tight junction formation, before the recruitment of the Par-aPKC complex (Ebnet et al. 2001; Itoh et al. 2001). Subsequently, the Par-aPKC complex could provide a scaffold for the assembly of additional signaling complexes involved in the organization of the actin cytoskeleton and regulation of vesicular trafficking.

Interestingly, whereas overexpression of kinase-dead aPKC or truncated versions of Par-6 or JAM interferes with the establishment of new tight junctions, it does not appear to alter the morphology or composition of mature junctions (Joberty et al. 2000; Ebnet et al. 2001; Itoh et al. 2001; Suzuki et al. 2001; Yamanaka et al. 2001). This suggests that the role of the Par-aPKC complex may be restricted to tight junction development rather than maintenance. In contrast, modulation of Cdc42 activity also perturbs the function of established tight junctions, suggesting that Cdc42 utilizes additional signaling elements that are independent of the Par-aPKC complex to control these junctions.

Membrane trafficking in epithelial polarity

The composition of apical and basolateral surface domains in polarized cells is not only affected by the fence function of tight junctions, but also by membrane trafficking. Polarized membrane trafficking can be divided in direct and indirect routes (Mostov et al. 2000). In the direct mechanism, proteins are sorted in the trans-Golgi network and subsequently directly routed to either the apical or basolateral surface. The indirect mechanism involves endocytosis, usually from the basolateral surface, followed by transcytosis to the opposite surface. Evidence has accumulated in the recent literature for an important role of Rho family GTPases in endocytosis and other aspects of vesicular trafficking (Ellis and Mellor 2000; Ridley 2001b).

Early studies showed that inhibition of Cdc42, but not Rac, induces an accumulation of the basolateral membrane protein gp58 at the apical surface, indicating a depolarization of the basolateral surface (Kroschewski et al. 1999). More recently, Cohen and coworkers (2001) investigated whether this effect of Cdc42 could be attributable to a general disruption of polarized protein traffic or whether it is confined to proteins that are targeted to the basolateral surface. They showed that expression of ei-

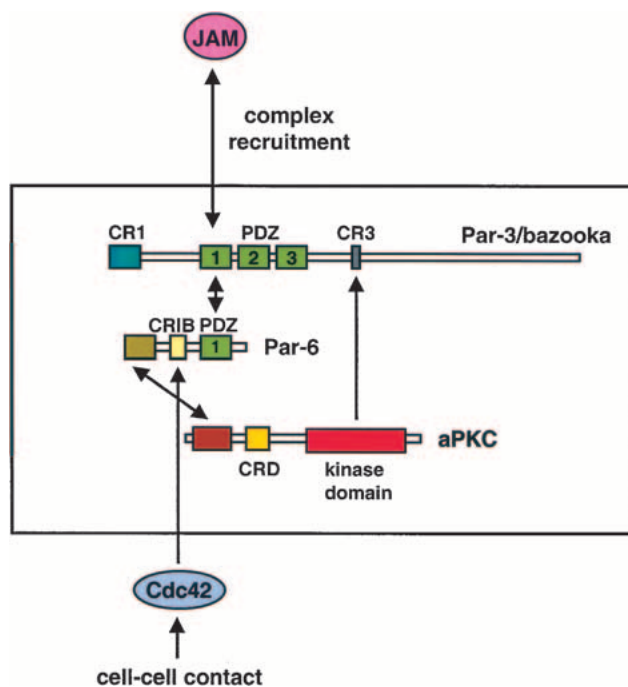


Figure 1. Par-aPKC complex. Control of the Par-aPKC complex by Cdc42. Par-3, Par-6, and a member of the aPKC family form a ternary complex that is evolutionary conserved and plays a role in a large number of functions that involve cell polarity. A model is emerging for the role of this complex in the establishment of epithelial tight junctions in mammals. Cdc42 is activated by initial cell-cell contacts. Cdc42 activates aPKC, thereby loosening the complex and allowing access to the junctional protein JAM, that recruits the complex to nascent tight junctions. The Cdc42-induced conformational changes also could lead to the recruitment of additional signaling components involved in the organization of the actin cytoskeleton and regulation of vesicle trafficking.

ther constitutively active or dominant negative mutants of Cdc42 indeed reverses the polarity from basolateral to apical of two different basolateral membrane proteins, vesicular stomatitis virus G protein and low-density lipoprotein receptor. Modulation of Cdc42 activity, however, does not have any effect on polarized secretion of soluble basolateral proteins or on the targeting of apical membrane or soluble proteins.

Cdc42 is associated with the Golgi apparatus (Erickson et al. 1996; Kroschewski et al. 1999) and expression of either constitutively active or dominant negative mutants of Cdc42 affects Golgi integrity (Kroschewski et al. 1999; Rojas et al. 2001). Therefore, one possible mechanism that could account for the role of Cdc42 in basolateral membrane polarity is that Cdc42 regulates biosynthetic trafficking. Indeed, recent studies demonstrated that modulation of Cdc42 activity slows down the exit of the basolateral protein, neuronal cell adhesion molecule (NCAM), from the TGN, while stimulating the exit of an apical membrane protein (Musch et al. 2001). These results seem to be in contrast with recent data from Apodaca and coworkers who find that dominant negative Cdc42 can actually somewhat enhance biosynthetic delivery of the polyimmunoglobulin receptor to the basolateral surface (Rojas et al. 2001). Whereas this apparent discrepancy could result from methodological differences, it may also reflect the possibility that different proteins can utilize distinct secretory pathways.

Another potential mechanism that has been proposed to contribute to the role of Cdc42 in basolateral membrane polarity is that Cdc42 is involved in the fusion of transport vesicles with the surface (Cohen et al. 2001). Indeed, targeting and fusion of basolateral, but not apical, transport vesicles is mediated by the exocyst, a multiprotein complex that localizes to tight junctions (Mostov et al. 2000), and in budding yeast Cdc42 interacts directly with Sec3, a key component of the exocyst (Zhang et al. 2001).

Coordination of apical-basal polarity with tissue architecture

Tissue morphogenesis and function depend on the coupling of epithelial apical-basolateral polarity to the extracellular environment, that is, the apical domain faces the lumen of the organ and the basal surface contacts the basal lamina. In mammals, interaction with the extracellular matrix is thought to be essential for the proper orientation of the apical-basal axis (Vega-Salas et al. 1987; Wang et al. 1990). A recent study identified Rac as a critical player in the orientation of apical polarity in cysts that are formed by MDCK cells in three-dimensional matrices (O'Brien et al. 2001). Whereas control cells embedded in collagen form cysts in which the apical pole faces the cyst interior, expression of dominant negative Rac inverts this polarity. This reversal of polarity is accompanied by a defect in the assembly of laminin at the cyst surface and proper polarity can be restored by the addition of exogenous laminin. A possible mechanism

for the role of Rac in laminin assembly is suggested by the observation that dominant negative Rac inhibits the expression of the $\alpha 3$ integrin subunit in the cysts, as the $\alpha 3 \beta 1$ integrin has been shown to be required for laminin assembly (O'Brien et al. 2001). Together, these data suggest a model in which Rac regulates the assembly of extracellular laminin and the assembled laminin in turn provides a spatial signal that is necessary for polarity establishment.

Planar polarity

In addition to the apical-basal polarity described above, the specialized function of some tissues requires an additional axis of polarity to be established within the epithelium. This is uniform polarity of single cells or multicellular units within the plane of the epithelium, commonly referred to as planar polarity or tissue polarity (Eaton 1997; Mlodzik 1999). Planar polarity in the oviduct, for example, permits the formation of cilia that beat in the same direction, allowing ovum transport towards the uterus (Chailley et al. 1989). Planar polarity in sensory epithelia of the vertebrate inner ear is reflected in the organization of stereocilia (actin-rich finger-like protrusions) in a staircase-like fashion, with the longest stereocilia positioned on one end of the hair cell, followed by stereocilia of decreasing height positioned towards the opposite end. This precisely polarized organization is essential for auditory and vestibular sensory functions (Tilney et al. 1992; Kollmar 1999; Müller and Littlewood-Evans 2001). Other examples include hair outgrowth in *Drosophila* wings, in which each wing epithelial cell that initially has been polarized along the apicobasal axis orients itself proximally to distally, generating hairs that are all oriented distally, and the uniform orientation of ommatidial clusters of photoreceptors in the compound eye of *Drosophila*. In the latter case, groups of cells make a coordinated decision (Fig. 2). In particular, studies in *Drosophila* have shed light on how planar polarity is established and has led to the identification of signaling components that control planar polarity (for review, see Eaton 1997; Shulman et al. 1998; Mlodzik 1999, 2000; Strutt and Strutt 1999; Bray 2000; Adler and Lee 2001; Strutt 2001). Among these components, the Rho GTPases have emerged as key players and will be discussed in detail below.

In contrast to apical-basal polarity, planar polarity is established in the absence of obvious permanent cues, and is therefore thought to be generated by long-range signals. Genetic analyses in *Drosophila* have led to a model for planar polarity signaling in which cells receive and interpret information from the extracellular environment and then translate this signal into changes in cytoskeletal polarity and transcriptional activation (for reviews, see Eaton 1997; Shulman et al. 1998; Mlodzik 1999; Strutt and Strutt 1999; Bray 2000; Adler and Lee 2001; Strutt 2001). The identity and source of the polarizing signals largely remain to be determined, although it is clear that they are tissue specific. For example, the presumptive signal seems to polarize the wing from the

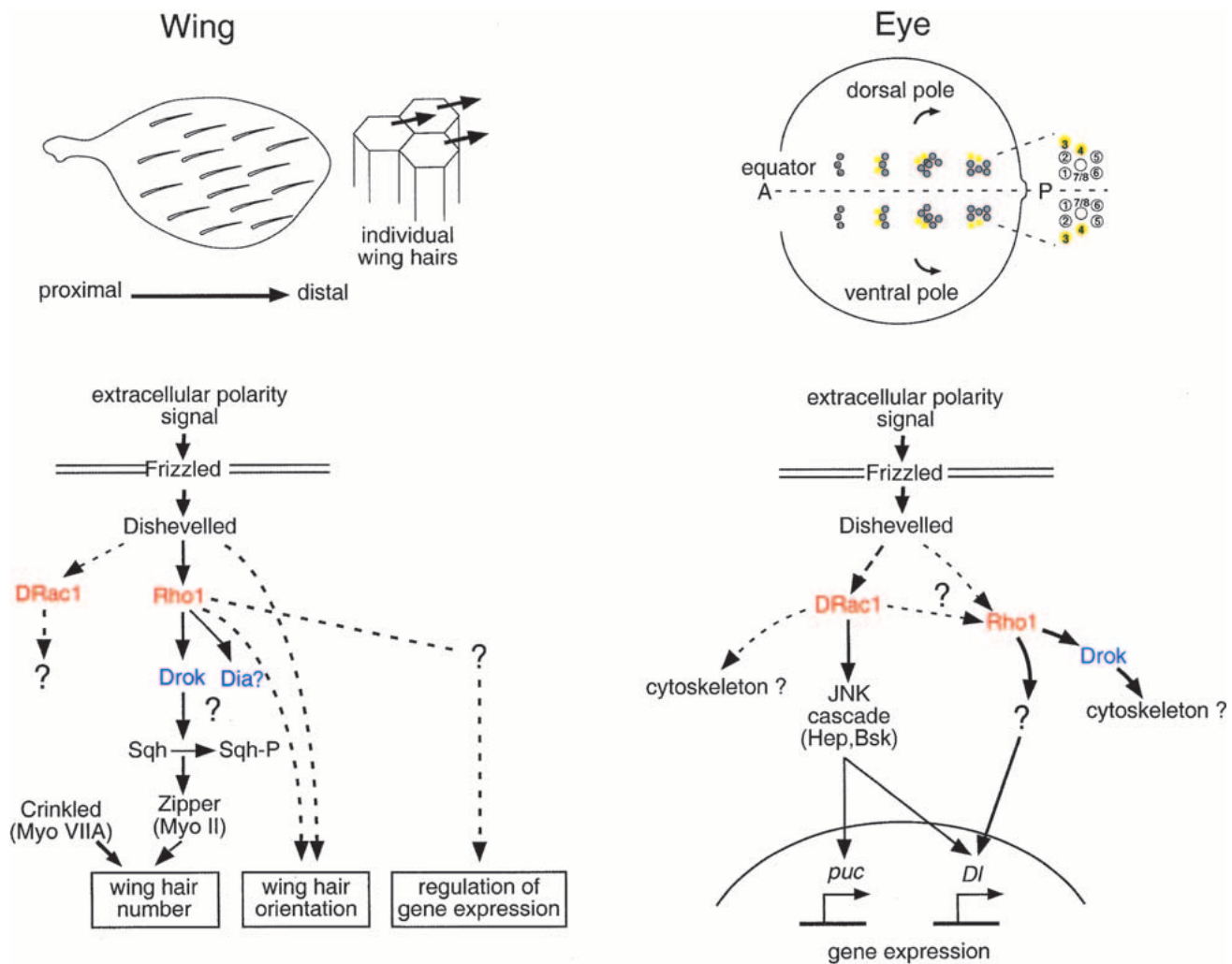


Figure 2. Polarity determination in the *Drosophila* wing (left) and eye (right). The developing *Drosophila* wing develops from a monolayer epithelium in which cells are originally polarized only along their apicobasal axis (left). During pupal development, each wing epithelial cell orients itself proximally to distally, generating a single hair at the distal vertex. A model for the wing polarity signaling pathways is depicted below. The seven-pass transmembrane protein, Frizzled, functions as a receptor and signals to Dishevelled. Rho, and likely also Rac, act downstream of Frizzled and Dishevelled. Drok (*Drosophila* Rho-kinase), Sqh (spaghetti squash or *Drosophila* nonmuscle myosin regulatory light chain), and Zipper (*Drosophila* nonmuscle myosin heavy chain) function downstream of Rho and are important in the regulation of the number but not the orientation of F-actin-based prehairsts. A separate pathway downstream of Rho1 and/or Dishevelled to the cytoskeleton is likely to regulate wing hair orientation. The role of Dia (Diaphanous) in wing planar polarity remains to be defined. Crinkled (*Drosophila* unconventional myosin VIIA) has opposing effects to that of Zipper in regulating prehair assembly. The pathway downstream of Rho leading to transcriptional activation is less well defined. The *Drosophila* eye develops from a monolayer epithelium of identical cells (right). Photoreceptor differentiation occurs in a wave from posterior to anterior such that ommatidia at the anterior site are more mature than those located more posteriorly. Photoreceptor cells are sequentially recruited into the nascent ommatidia as differentiation proceeds (the R3/R4 photoreceptor pair is colored yellow, the other photoreceptors are colored gray). The immature clusters initially possess internal-image symmetry, however, as differentiation proceeds, they rotate 90° and become asymmetric, with the R3/R4 cell closest to the equator taking the R3 fate. Clusters on either side of the equator rotate in opposite directions and thus take an opposite chiral form. A model for the wing polarity signaling pathways is depicted below. Rac1 functions downstream of Frizzled and Dishevelled. Rho1 appears to act downstream or in parallel with DRac. Both DRac and Rho1 signal to the nucleus, likely through distinct effectors to regulate *Dl* (*Delta*) expression. In addition, DRac regulates the expression of Puckered through the activation of the JNK pathway.

proximal edge to the distal edge, whereas the eye is polarized from the equator (the dorsal-ventral midline) to the dorsal and ventral poles (Fig. 2). Components of the signal transduction machinery that interpret the signal have been identified and these appear to be generally

conserved amongst different tissues. The seven-pass transmembrane protein Frizzled (Fz) functions as a receptor required for the reception and transmission of polarity signals (Vinson et al. 1989). The next known downstream component of the polarity-signaling pathway is

the scaffold protein Disheveled (Dsh) that is recruited from the cytoplasm to adherens junctions by Fz (Klingensmith et al. 1994; Theisen et al. 1994; Krasnow et al. 1995). Homologs of both Frizzled and Disheveled have been identified in vertebrates. It is noteworthy that although both Fz and Dsh also participate in the Wingless (Wg) signal transduction system(s), Wg and planar polarity signaling appear to use different pathways downstream of Dsh in *Drosophila* (Axelrod et al. 1998; Boutros et al. 1998; but see Habas et al. 2001).

A number of studies suggest that the Rho GTPases are important signaling elements that function downstream of Dsh in the establishment of both eye and wing planar polarity in *Drosophila* (Eaton et al. 1995, 1996; Strutt et al. 1997; Fanto et al. 2000). Loss- and/or gain-of-function mutants of *DRac1* and *Rho1* display planar polarity defects in the eye and the wing, similar to those described for *dsh* mutants. In the wing, cells expressing a dominant negative mutant form of *DRac1*, *DRac1N17*, fail to restrict outgrowth to a single site and give rise to multiple wing hairs. This defect is associated with disturbances in the organization of junctional actin as well as with disruption of microtubules associated with the junctional region. Similarly, clones of *Rho1* hypomorphic alleles display multiple wing hairs and abnormal wing hair polarity. In contrast to a prominent role for *DRac1* and *Rho1* in establishing wing planar polarity, thereby restricting the site at which hairs grow out, *DCdc42* functions in the control of localized actin polymerization in the extending hair, rather than in polarity generation itself. Expression of a dominant negative mutant form of *DCdc42* abolishes both actin polymerization and hair outgrowth (Eaton et al. 1995, 1996; Strutt et al. 1997). In the eye, interfering with *DRac1* and *Rho1* signaling disrupts ommatidial polarity. In a wild type, ommatidial preclusters, when they initially emerge from the furrow, are arranged symmetrically in the anteroposterior axis. Subsequently, they rotate 90° towards the equator and become asymmetric, with the R3/R4 cell closest to the equator taking on the R3 fate (Fig. 2). Clusters on either site of the equator rotate in opposite directions and take on opposite chiral forms. In eye discs expressing an activated or dominant negative mutant form of *DRac1*, ommatidial rotation is affected early in development and the R3/R4 pairs often are incorrectly oriented with respect to the neighbors and position in the eye disc. Eye clones carrying hypomorphic *Rho1* mutations also are incorrectly rotated relative to the equator and sometimes form inappropriate chiral forms. No role for *DCdc42* in the establishment of planar polarity in the eye has been reported (Fanto et al. 2000). Taken together, these studies suggest that *DRac1* and *Rho1*, but not *Cdc42*, play a role in planar polarity generation. One potential caveat is that the above-described *Rac1* studies relied mainly on the use of dominant negative and constitutively active mutant forms of *Rac*. In more recent studies, an extensive phenotypic analysis of loss-of-function mutations in each of the endogenous *Rac* genes (including *DRac1*, *DRac2*, and *Mtl*) was performed (Hakeda-Suzuki et al. 2002; Ng et al. 2002). Consistent with

the dominant negative and activated *DRac* mutant analyses, these studies showed that the *DRac* genes have overlapping functions in the control of dorsal closure, myoblast fusion, and axon growth and guidance. In contrast, however, no defects in planar polarity establishment were observed in clones of cells in the eye and the wing that were triply mutant for null alleles of *DRac1*, *DRac2*, and *Mtl*. These data indicate that *DRac* proteins are not essential in the generation of planar polarity and that the effects seen with the dominant negative *Rac* mutant proteins may be attributable to cross-inhibition or cross-activation of other pathways (Hakeda-Suzuki et al. 2002). The latter studies do not, however, exclude a possible involvement of the *DRac* proteins in signaling pathways that are activated by Fz.

Genetic interaction and rescue experiments have placed *DRac1* and *Rho1* downstream of Dsh. For example, in the eye, the gain-of-function phenotype resulting from overexpression *fz* or *dsh* are dominantly suppressed by deficiencies that remove *DRac1* and *DRac2*, as well as by a reduction in the gene dose of *Rho1*, but not by the removal of *DCdc42*. Furthermore, ectopic expression of *DRac1* and *Rho1* largely rescues the eye polarity phenotype of a hypomorphic allele of *dsh* (Strutt et al. 1997; Boutros et al. 1998; Fanto et al. 2000). Similar experiments were performed for *Rho1* in the wing (Strutt et al. 1997). Taken together, the above studies suggest a role for *DRac1* and *Rho1* downstream of Dsh in the control of planar polarization of both wing hairs and photoreceptor clusters. Consistent with the findings in *Drosophila*, Habas et al. (2001) recently demonstrated biochemically in mammalian cells that Fz/Dvl (mouse Disheveled) signaling activates Rho and weakly Rac, but not *Cdc42*. In contrast with the above findings in *Drosophila*, which do not implicate Wnt signaling in Fz planar cell polarity or Rho GTPase regulation, they demonstrate that the mammalian Wnt-1 and Wnt-11 activate Rho in mammals and in *Xenopus*. Furthermore, they identified Daam1 as a novel component, which is essential for Wnt/Fz activation of Rho and for *Xenopus* gastrulation, a process involving planar polarization (Habas et al. 2001). Daam1 is a member of the family of formin-homology proteins that are thought to act as platforms that direct localized actin polymerization (Wasserman 1998). It is important to note, however, that the sequencing of the *Drosophila* genome has revealed in addition to Wingless six Wnt homologs in *Drosophila* (Llimargas and Lawrence 2001). At present, their involvement in Fz planar cell polarity or Rho GTPase regulation remains to be determined.

More recent studies in *Drosophila* have provided insights into the downstream signaling components of the Rho GTPases in both eye and wing, providing links as to how Fz/Dsh may relay signals to the cytoskeleton and the nucleus. Several lines of evidence indicated that signaling components of the Jun N-terminal kinase (JNK) signaling module play an important role in mediating the effects of Fz in planar polarity generation (Weber et al. 2000; Ciapponi et al. 2001). Additional studies suggested a role for *DRac1* upstream of the JNK pathway in

the eye polarity generation (Fanto et al. 2000) (Fig. 2). A reduction in gene dosage of components of the JNK cascade (such as basket/JNK, hemipterous/JNKK or D-jun) strongly suppresses the ommatidial rotation defect in eyes expressing DRac1V12. Furthermore, expression of DRac1V12 triggers a strong up-regulation of puckered expression. *Puckered* encodes a MAPK phosphatase whose expression is regulated by the JNK pathway (see below). In addition to *puckered*, DRac1V12 also up-regulates *Delta* expression. *Delta* is the only known transcriptional target of frizzled signaling in the R3 precursors (Fanto et al. 2000). Genetic interaction and rescue experiments have placed Rho1 downstream or in parallel to DRac1 (Fanto et al. 2000). However, whereas an activated mutant form of Rho1 is able to trigger an increase in *Delta* expression, it does not up-regulate *puckered* expression. These data suggest that Rho1-induced signaling to the nucleus is not mediated by JNK and that DRac1 and Rho1 utilize different downstream effectors. The identity of Rho1's downstream effector pathway remains to be defined. A potential link between the Rho GTPases and actin cytoskeleton reorganization in the eye remains more elusive, but as discussed below, the Rho-associated kinase (Drok), a Rho1 effector, may be a potentially important player in mediating Rho1's effects on the cytoskeleton.

Recent studies by Winter et al. (2001) provide evidence that Drok mediates a branch in the planar polarity pathway involved in ommatidial rotation in the eye and the restriction of hair bundle (trichome) formation to a single site in the wing. Eye clones homozygous for Drok loss-of-function mutation show a similar size as their siblings, however ~50% of ommatidia exhibit an increase or decrease in photoreceptor numbers when compared to wild-type clones. Of those ommatidia with the correct number of photoreceptors, ~60% were misrotated. In *Drok* loss-of-function wing clones multiple hairs are generated, whereas in wild-type wings each cell produces a single, distally oriented hair. These *Drok* loss-of-function phenotypes resemble those of Rho1 mutants. Genetic interaction experiments performed in the wing show that Drok functions downstream of the Fz/Dsh in regulating the wing hair number. Together with the findings that Drok interacts directly with an activated mutant form of Rho1 and that Rho1 acts downstream of Fz/Dsh in the establishment of eye and wing polarity, these data place Drok as a downstream effector of Rho1 in the Fz signaling pathway. In mammalian cells, the nonmuscle myosin regulatory light chain (MLC) is a critical substrate of Rok (the *Drosophila* counterpart of Drok) that mediates Rho-stimulated myosin contractility (Kaibuchi et al. 1999a). Consistent with this, the *Drosophila* ortholog of MLC, Spaghetti squash (Sqh), is an important substrate of Drok. Biochemical and immunostaining experiments demonstrate that Drok modulates Sqh phosphorylation in vivo. Furthermore, the multiple wing hair phenotype as well as lethality of *Drok* loss-of-function mutants can be suppressed by the presence of a *sqhE20E21* transgene. The *sqhE20E21* transgene contains mutations in the primary

(Ser 20) and secondary (Thr 20) phosphorylation sites (which are phosphorylated by Drok) changing them to glutamic acid, thereby mimicking phosphorylation on both sites. In addition, the associated myosin heavy chain (myosin II) of Sqh (which is referred to as Zipper in *Drosophila*) is also required for normal trichome formation in the wing (Winter et al. 2001). Reduction in Zipper activity results in the multihair phenotype. Both Sqh and Zipper have been shown to function downstream of Fz/Dsh in regulating actin prehair development. Thus in the wing, a linear pathway from Fz to the regulation of actin structures important for the restriction of the number of F-actin-based prehairst has been established (Fig 2). The precise role of Drok in regulating the process of ommatidial rotation in the eye is less clear. The authors postulate that Drok may function as an effector of Rho1 in mediating ommatidial rotation or that loss of Drok may lead to the disruption of the actin cytoskeleton and cell-cell contacts that support intracellular signaling essential for ommatidial rotation. Interestingly, the studies by Winter et al. (2001) also show that Crinkled (myosin VIIa) acts in conjunction with the Fz/Dsh/Rho1/Drok/myosin II pathway in regulating wing hair number. Crinkled has opposing effects to that of myosin II in regulating prehair assembly and a balance between the activities of myosin II and myosin VIIA appears to be important in regulating wing hair number. *Drosophila* Crinkled is the ortholog of the Usher Syndrome 1B gene that causes deafness in humans and mice deficient in myosin VIIa show defects in the polarity of the stereocilia staircase in the inner ear (Self et al. 1998; Müller and Littlewood-Evans 2001). These findings raise the possibility that a similar Fz/Dsh cytoskeletal pathway regulates a branch of polarity in sensory epithelia of the vertebrate inner ear.

In contrast to Dsh and Rho1, Drok and myosin II are not involved in the determination of the site/orientation of the F-actin based prehairst in the fly wing. These observations suggest that there is a bifurcation of pathways at the level of Rho1 and/or Dsh; one pathway is required for the regulation of the number of wing hairs while the other pathway is required for the orientation of these hairs (Winter et al. 2001). The identities of the signaling components important for wing hair orientation remain to be determined. Noteworthy is the observation that mutations in vertebrate diaphanous, another extensively characterized effector of Rho that is a member of the formin-homology family of proteins (Watanabe et al. 1997), lead to the autosomal dominant hearing-loss syndrome DFNA1 (Lynch et al. 1997). In mammalian cells, Dial has been found to work in concert with Rok to induce stress fibers in transfected fibroblasts (Watanabe et al. 1999). In this system, Rok activates the actin-based molecular motor myosin II to exert tension force on actin filaments during stress fiber formation, whereas Dial seems to contribute to the determination of nucleation sites for actin filaments. More recent studies in fibroblasts and HeLa cells demonstrate a role for Dial as mediator of RhoA's effects on microtubule stabilization in the leading edge (Ishizaki et al. 2001; Palazzo et al.

2001a). Based on these observations, it has been speculated that Dial1 could play a role in regulating the assembly or maintenance of the hair cell cytoskeleton (Müller and Littlewood-Evans 2001). In *Drosophila*, the Diaphanous protein has been shown to play a role in cytokinesis by organizing actin-mediated events involving membrane invagination (Castrillon and Wasserman 1994; Afshar et al. 2000). It would be interesting to see whether the *Drosophila diaphanous* gene product acts in concert with Drok in the regulation of the number and/or orientation of the F-actin based prehair in the wing. In light of this, disruption of the microtubule cytoskeleton in the fly wing has been shown to result in wing cells forming multiple hairs without any obvious effects on polarity. It is also important to mention that expression of a dominant negative mutant form of DRac1 disrupts the microtubule cytoskeleton in the wing (Eaton et al. 1996). In vertebrates, a molecular pathway linking Rac1 to the growth of microtubule plus ends also is emerging and involves the activation of the serine/threonine kinase Pak, which in turn leads to the phosphorylation and inactivation of stathmin/Op18 (Daub et al. 2001). Stathmin was initially identified as a phosphoprotein highly overexpressed in leukemias, breast, and ovarian cancers and was later found as a protein that binds tubulin dimers, thereby destabilizing microtubules. Two different mechanisms have been suggested by which stathmin may stimulate destabilization of microtubules: Stathmin either sequesters tubulin dimers, which would reduce the concentration of tubulin available for assembly, or it stimulates microtubule plus end catastrophe, the transition from microtubule growth to shortening (Belmont et al. 1996; Belmont and Mitchison 1996; Howell et al. 1999; Larsson et al. 1999; Andersen 2000; Cassimeris 2002). Whether or not a similar pathway exists in *Drosophila* remains to be seen. Furthermore, the relationship between DRac1 and Rho1 in regulating planar polarity in the wing remains to be established.

Obviously, further research will be required to determine all the relevant players downstream as well as upstream of the Rho GTPases that are important in the establishment of planar polarity. It is intriguing that several of the downstream components of the Rho GTPases that are important in planar polarity are commonly found in other cell types, such as fibroblasts, where they also regulate nuclear events and cytoskeleton remodeling. Thus, information gained from fibroblast studies may be useful in further unraveling the components involved in planar polarity determination. In addition, the completion of the *Drosophila* genome sequencing is also likely to advance the identification of the signaling components functioning upstream and downstream of the Rho proteins in establishing eye/wing polarity.

Wound healing

Directed cell migration is a critical process during many stages of embryonic development. In adult organisms, cell migration plays a crucial role in numerous physiological and disease related processes, including inflam-

matory responses, wound healing, and metastasis. Whereas inflammatory responses involve the migration of single cells, such as dendritic cells or leukocytes, wound healing and metastasis also involve migration of a complex epithelium. Data accumulated over several years from both in vivo experiments and tissue culture cells have demonstrated that Rho family members are key regulators of both types of motile behavior (for reviews, see Van Aelst and D'Souza-Schorey 1997; Jones et al. 1998; Keely et al. 1998; Montell 1999; Nabi 1999; Evers et al. 2000; Hall and Nobes 2000; Schmitz et al. 2000; Price and Collard 2001; Ridley 2001a). Here we will focus on the role of the Rho GTPases in the wound healing process and in a closely related process, dorsal closure, which occurs during *Drosophila* development. Both of these processes involve extensive and directed movements of two epithelial sheets facing each other. The movement and sealing of epithelial sheets can, in general, be subdivided into the following steps: The leading cells are first specified and brought into position. The cells then make a coordinated forward movement by changing their cell shape and/or migrating over a substrate. Subsequently, epithelial cells of the opposing sites merge and fuse (Jacinto et al. 2001).

Two distinct mechanisms have been suggested to account for the movement of epithelial cell sheets during wound healing (Jacinto et al. 2001). In the first process, which is typical for the repair of adult skin wounds, closure of the wound occurs by active protrusion of filopodia and lamellipodia at the edge of the wound, resembling in fact the crawling behavior of free cells (Lauffenburger and Horwitz 1996; Martin 1997). The cells at the migrating leading edge at the wound bore a passageway enabling them to crawl beyond the cut basal lamina and over the provisional matrix and healthy dermis (Martin 1997). In the second process, which is characteristic of wounded embryonic epidermis, repair occurs by a purse-string mechanism that pulls the wound edges together (Martin and Lewis 1992; Martin 1997; Grose and Martin 1999; Kiehart 1999). This involves contraction of actomyosin cables that run around the circumference of the leading epithelia that are linked by adherens junctions. The lamellipodial crawling and purse-string mechanisms are, however, not strictly limited to adult and embryonic tissues, respectively. For instance, epithelial wounds to the adult cornea and gut appear to close through the purse-string mechanism (Heath 1996; Danjo and Gipson 1998). There are also cases where the two types of epithelial motility take place simultaneously or at different stages of the wound healing process. For example, closure of small wounds in intestinal cultured cells involves formation of both lamellipodia and purse-string structures (Bement et al. 1993).

Analogous tissue movements as observed for the repair of embryonic and adult epithelia take place during *Drosophila* and *C. elegans* embryogenesis. In *C. elegans*, the enclosure of the ventral surface of the embryo involves a two-step process in which two functionally distinct groups of hypodermal cells meet and fuse to seal the ventral midline; one is accompanied by filopodia for-

mation and the other by purse-string contraction (Williams-Masson et al. 1997; Simske and Hardin 2001). During *Drosophila* embryogenesis, the epidermis undergoes a morphogenetic movement, termed dorsal closure (DC), to establish the dorsal ectoderm (Knust 1997; Goberdhan and Wilson 1998; Noselli 1998; Stronach and Perrimon 1999). Similar to the mechanism described for reepithelialization of embryonic skin and some tissue culture wounds, one of the major forces that drives the movement of the epithelial sheets in the *Drosophila* embryo is the contraction of a cable of actin and myosin running around the circumference of the leading epithelial margin (see below).

Significant progress has recently been made in defining the signaling components mediating wound healing and embryonic tissue movements (for review, see Jacinto et al. 2001) and different members of the Rho-like GTPases have emerged as key players in the purse-string and crawling modes of epithelial motility.

Molecular mechanisms underlying cell movements during wound closure

In cases where closure of the wound involves lamellipodial crawling, for example in wounds induced in MDCK epithelial cell monolayers, repair of the wound is dependent on Rac1 activity (Fenteany et al. 2000). Cells at the wound margin extend lamellipodia in the direction of the wound, which are followed by a tandem movement of the submarginal cells. Inhibition of Rac activity in the first three rows of cells prevents lamellipodia formation and subsequent wound healing. Interestingly, if Rac1 activity is only blocked in the first row of cells wound healing still occurs, suggesting that cells behind the margin can generate force independently of the first row (Fenteany et al. 2000). Although these cells do not display obvious lamellipodia, they have sites that promote Rac-dependent actin filament assembly, which may provide actin-based force generation in these cells. In contrast to Rac1, inhibition of RhoA or Cdc42 activity in the first three rows does not prevent wound closure, although it occurs in a significantly less regular fashion (Fenteany et al. 2000).

In similar wound healing assays using primary rat embryonic fibroblasts (REFs), the activities of all three GTPases appear to be required for efficient closure of the wound (Nobes and Hall 1999). In this system, Rac1 is required for the protrusion of lamellipodia and forward movement of the cells. Cdc42 activity is required to establish polarity in the migrating cells, whereas basal RhoA activity is required to maintain cell substrate adhesion of the migrating cells (Nobes and Hall 1999). Thus, Rac, Cdc42, and Rho appear to function cooperatively to drive the forward movement of the REF cells.

More recently, the role of members of the Rho GTPases and the underlying mechanisms have been examined in wound-induced astrocyte migration (Etienne-Manneville and Hall 2001). Scratching a confluent monolayer of astrocytes induces a microtubule-dependent, but actin-independent, polarization of the leading

edge cells. This is characterized by the formation of a pseudopodium-like structure, referred to as a protrusion, and by the reorientation of the microtubule-organizing center (MTOC) and the Golgi apparatus in the direction of migration. Detailed analysis of this wounding-induced cell polarization shows that Cdc42 plays a critical role in this process and suggests the following model (Fig. 3A). On localized activation of integrins at the front of the cells, Cdc42 becomes activated, which leads to the recruitment of the above-described Par6/PKC ζ complex to the plasma membrane at the leading edge, and to the activation of PKC ζ . Localized activation of PKC ζ may then act on the motor protein dynein to establish cell polarity (Etienne-Manneville and Hall 2001). Consistent with this model, Cdc42 also mediates MTOC reorientation in a dynein-dependent manner in wounded NIH 3T3 fibroblast cells (Palazzo et al. 2001b). In the astrocyte model, Cdc42 is in addition required for the formation of protrusions, although only at early stages after wounding. In contrast, Rac is not required for the establishment of cell polarity, but is essential for the development and the maintenance of protrusions during migration. A potential mechanism for this function of Rac is that Rac, via PAK, could phosphorylate and inactivate stathmin, thereby promoting microtubule elongation (Andersen 2000; Daub et al. 2001; Cassimeris 2002). Interestingly, Rac has been shown to be activated by microtubule polymerization (Waterman-Storer et al. 1999). This suggests the possibility of a positive feedback loop that is localized to the leading edge of the moving cell and coordinates microtubule growth with actin polymerization: Rac, via inactivation of stathmin, stimulates microtubule elongation, which in turn enhances Rac activity. Presently, it remains unclear as to whether Rac is activated via Cdc42 or directly by an integrin-based mechanism (Fig. 3A).

In situations where wound healing occurs by means of a contractile purse string, the function of RhoA appears to be essential. Studies in embryonic wound healing models, for example wounds in the embryonic chick wing bud, have shown that the assembly and the contraction of actin cables is dependent on RhoA activity (Brock et al. 1996). Loading of wound edge epidermal cells with C3 transferase prevents assembly of an actin cable and causes a failure of healing. In contrast, expression of a dominant negative mutant of Rac does not interfere with the assembly of an actin cable or with healing. A possible candidate for mediating Rho's effect on the actin filament cable is Rok, as this molecule is a key Rho effector in the regulation of actomyosin contractility. Furthermore, a role for Rok in wound-induced migration has been demonstrated in rat hepatic stellate cells (Tangkijvanich et al. 2001). In studies using mouse corneas with central epithelial debridement wounds, which heal by the contractile purse-string mechanism, it was noted that the actin filament cable is anchored by E-cadherin-mediated adherens junctions at the leading edge, thereby joining the cells to form the purse-string. In light of the role of Rho in the establishment of adherens junctions, as discussed above, these findings sug-

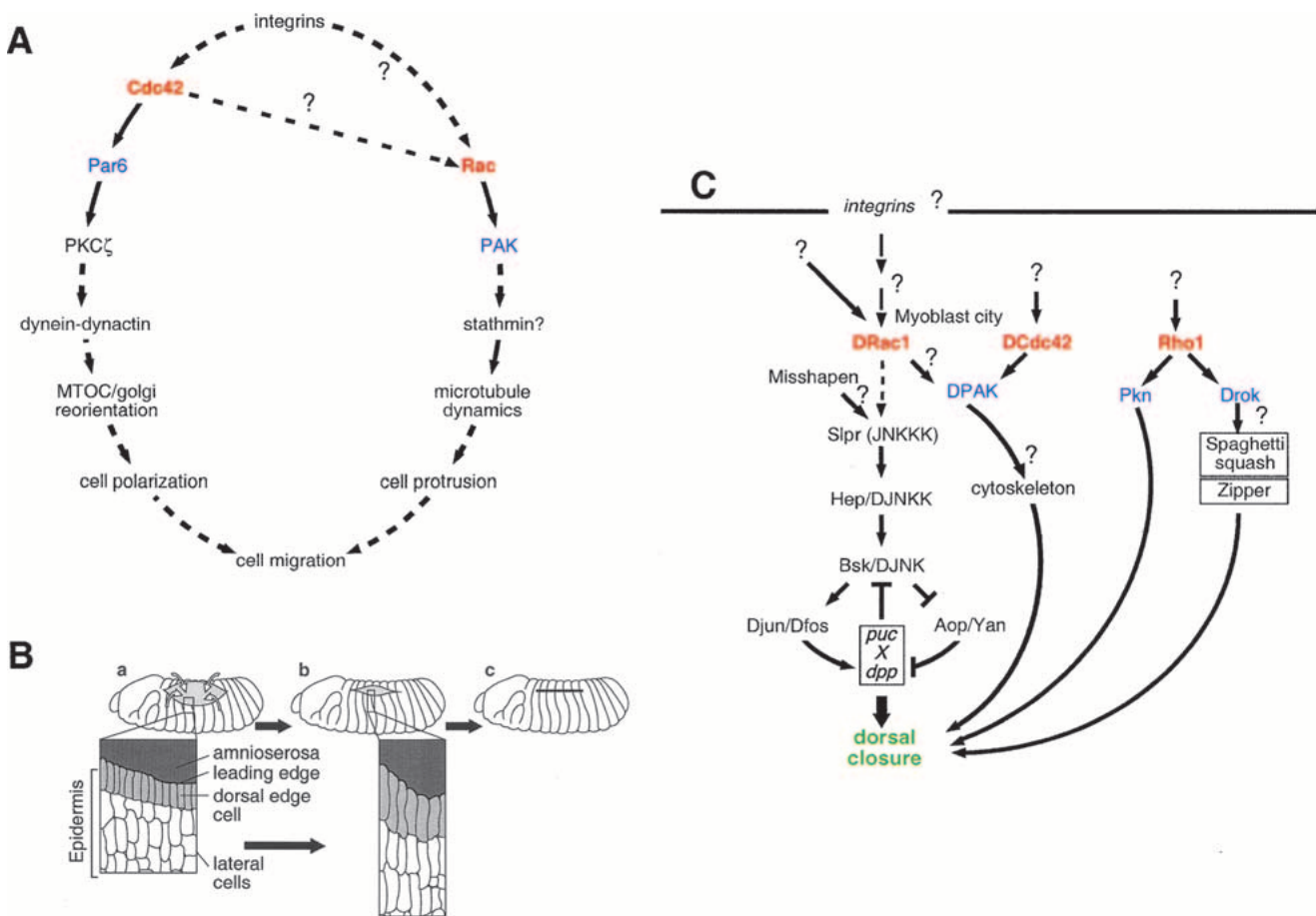


Figure 3. (A) Model for signal transduction pathways underlying wound-induced astrocyte migration. On integrin engagement, Cdc42 becomes activated causing the recruitment of Par6 and PKC ζ to the leading edge plasma membrane, followed by the activation of PKC ζ . Activated PKC ζ then leads to the recruitment and/or activation of dynein, contributing to the reorientation of the MTOC toward the front of the cell. Rac activation (through integrins and/or Cdc42) is essential for the microtubule-dependent formation of protrusions. One potential mechanism by which Rac could exert this effect involves activation of its effector PAK, which in turn phosphorylates and inhibits stathmin. (B) Dorsal closure. Dorsal closure can be divided into three major steps: initiation (a), spreading (b), and suture (c). Initially, cells at the leading edge (LE) of the dorsal epidermis elongate dorsally. This is associated with the accumulation of actin and nonmuscle myosin beneath the dorsal-most cell membranes, which form the 'leading edge' of the epithelial sheet. The initial elongation of the LE cells is followed by a spreading phase. The more lateral ectodermal cells undergo progressive dorsal-ventral elongation and promote spreading of the more lateral sheets over the amnioserosa. Recent studies indicate also that the amnioserosa may provide some contractile forces (Kiehart et al. 2000). These dramatic shape changes eventually cause the entire dorsolateral epithelium to cover the region that was previously occupied by the amnioserosa. During the last phase of DC, ectodermal sheets meet at the dorsal midline and adhere to one another and suture. (C) Schematic representation of the signaling pathways mediated by the Rho GTPases that direct the DC process. Activation of DRac and DCdc42 reorganize the LE actin cytoskeleton, leading to the activation of the JNK–MAPK pathway in the LE. An important outcome of JNK activation is the induction of *dpp* (TGF- β homolog). The latter controls morphogenesis of the more lateral ectoderm cells. Restriction of JNK activation to the LE is in part caused by the repressor function of the phosphatase Puckered (Puc). Rho1 activity is required for the integrity of the LE cytoskeleton, however, its effects on the cytoskeletal assembly are restricted to cells flanking the segment borders. Potential mediators of Rho1 are Drok and PKN. In contrast to DRac1 and DCdc42, Rho1 is not involved in JNK activation and induction of *dpp* expression.

gest that Rho may control this type of wound healing both by regulating the contractile properties of the purse-string and by anchoring it (Danjo and Gipson 1998).

Dorsal closure in Drosophila as a model system for wound repair

Additional insights into the molecular mechanisms operating during wound repair come from studies of analo-

gous morphogenetic movements that occur in genetically tractable embryos, such as DC in *Drosophila*. In late stages of development, the dorsal trunk region of the *Drosophila* embryo remains without ectoderm and is instead covered by a transient epithelial structure, the amnioserosa. During the process of DC, the dorsal-most, or 'leading edge' (LE) cells of the lateral epidermis elongate along the dorsal-ventral axis, which is associated with the accumulation of actin and nonmuscle myosin be-

neath the dorsal-most cell membranes. It has been proposed that the accumulation of F-actin and myosin at the leading edge forms the actomyosin contractile apparatus driving the elongation of the LE cells. Subsequently, cells located more laterally gradually lose their polygonal shape and elongate as a result of the contractions at the LE, thereby shifting the whole epidermis dorsally toward the midline. Finally, the leading edges of both sides meet at the dorsal midline and fuse (Fig. 3B; for reviews, see Knust 1997; Goberdhan and Wilson 1998; Noselli 1998; Stronach and Perrimon 1999).

Genetic analyses have revealed the involvement of all three Rho GTPases (DRac1, DCdc42, Rho1) in the DC process. Expression of dominant negative mutant versions of any of the three GTPases result in DC defects (Harden et al. 1995, 1999). Moreover, loss-of-function mutations have been generated in the *Drosophila* *DCdc42* and *Rho1* genes, and both these mutant embryos show DC defects (Magie et al. 1999; Genova et al. 2000). It is noteworthy that expression of constitutively active forms of the three GTPases also causes DC defects, suggesting that cycling between the GTP- and GDP-bound states of the GTPases and/or correct spatial activation are required (Harden et al. 1999). Alternatively, as already mentioned above, one can not exclude the possibility that the dominant negative mutant form of a Rho GTPase has an inhibitory effect and a constitutively active mutant form produces a gain-of-function phenotype.

Although interference with either DRac1, DCdc42, or Rho1 activity results in cuticles with dorsal holes, the three GTPases appear to play distinct roles in the DC process. DRac1 is required for the formation and/or maintenance of the cytoskeleton all along the LE, as interfering with DRac1 activity causes severe disruption of the LE cytoskeleton (Harden et al. 1995, 1999). Rho1 is required for the integrity of the LE cytoskeleton, especially in cells flanking the segment borders (Barrett et al. 1997; Harden et al. 1999). Expression of a dominant negative mutant of Rho1 leads to a loss of LE components and a loss of anterior–posterior contraction in cells flanking each segment border. DCdc42 has also a role in the initial assembly and/or maintenance of the LE cytoskeleton, although to a much lesser extent than DRac. Furthermore, DCdc42 appears to be involved in regulating the levels of DPAK, a downstream effector of DCdc42 and DRac, at the LE and the mechanics of the DC process (Harden et al. 1999; Ricos et al. 1999). Taken together, the observations made on the *Drosophila* DC process indicate that coordinate actions of the Rho GTPases (which may take place in different subpopulations of LE cells) are essential for DC.

The exact mechanism as to how DRac and DCdc42 affect the actin cytoskeleton in the leading edge is not yet clear. However, a few studies suggest a role for the *Drosophila* JNK pathway, which is activated by DRac1 and DCdc42 (see below) in the regulation of cell shape changes and actin reorganization. JNK pathway mutants seem to fail in accumulating the proper cytoskeletal network in the LE cells required to fully elongate dorsally

(Hou et al. 1997; Harden et al. 1999; Ricos et al. 1999). Furthermore, in a screen for JNK responsive genes in the *Drosophila* embryo, the gene *chickadee*, which encodes for a *Drosophila* profilin, was identified as one of the up-regulated genes. Chick-deficient embryos fail to execute the JNK-mediated cytoskeletal rearrangements during dorsal closure (Jasper et al. 2001). In addition, Otto et al. (2000) recently identified a novel DJNK-interacting protein, p150-Spir, which belongs to the Wiscott-Aldrich syndrome protein (WASP) homology domain 2 family of proteins, which have been shown previously to be involved in actin organization.

As alluded to above, DRac1 and DCdc42 also function in the LE cells to promote the activation of components of the MAPK pathway (Fig. 3C) (for review, see Stronach and Perrimon 1999). These include DJNKK (Hemipterous; Hep) (Glise et al. 1995), which subsequently activates DJNK (Basket; Bsk) (Sluss et al. 1996; Glise and Noselli 1997). In addition, the Ste20-related kinase, Mischapen (Msn) has been shown to function upstream of Hep and Bsk to stimulate dorsal closure (Su et al. 1998), and more recently, Stronach and Perrimon (2002) have identified Slipper (Slpr) as the upstream activator of DJNKK required for dorsal closure. A model is proposed that Msn, Slpr, and DRac1 participate in a ternary signaling complex to stimulate DJNK signal transduction (Stronach and Perrimon 2002). It is well established that DJNK regulates the activity of several transcription factors. In particular, DJNK phosphorylates and activates Djun, which dimerizes with DFos to form a typical AP-1 complex (Hou et al. 1997; Kockel et al. 1997; Riesgo-Escovar and Hafen 1997a,b; Zeitlinger et al. 1997; Stronach and Perrimon 1999). DJNK also phosphorylates and inactivates the ETS-domain repressor Anterior open (Aop) (Riesgo-Escovar and Hafen 1997b). The concomitant assembly of AP-1 complexes and inactivation of Aop then leads to the transcriptional up-regulation of *dpp* and *puc* expression in the LE cells. *Puc* encodes a MAPK phosphatase which down-regulates DJNK/Bsk activity and thus negatively feeds back into the JNK signaling pathway (Martin-Blanco et al. 1998). *Dpp* is a secreted signaling molecule of the transforming growth factor β (TGF- β) family (Padgett et al. 1987). Two *Dpp* receptors, Thick vein (Tkv) and Punt (Put), have been identified in flies, as well as a number of components acting downstream of these receptors, including Mad, Medea, and the Zinc finger protein Schnurri (Brummel et al. 1994; Nellen et al. 1994; Penton et al. 1994; Arora et al. 1995; Grieder et al. 1995; Letsou et al. 1995; Ruberte et al. 1995; Stronach and Perrimon 1999). Loss-of-function mutations in *hep*, *bsk*, *DJun*, and *DFos* all lead to severe DC defects that are characterized by a complete failure of the entire lateral ectoderm, including the LE cells, to elongate dorsally. This indicates that activation of the JNK pathway is required for the elongation of all cells of the lateral ectoderm. On the contrary, in loss-of-function mutants of *tkv*, *put*, and *schnurri*, the cells of the LE still elongate, while those of the lateral ectoderm do not, indicating that the *Dpp* signal emanating from the LE cells is necessary for the elongation of the adja-

cent ectodermal cells, but not for the elongation of the LE cells themselves (Knust 1997). Although the JNK signaling pathway has not been directly implicated in wound repair in mammalian systems, it should be noted that a pivotal role for TGF- β in the tissue-repair process is well documented (Grande 1997; Martin 1997; O'Kane and Ferguson 1997; Massague 1999; Ashcroft and Roberts 2000).

In contrast to DRac1 and DCdc42, Rho1 does not appear to stimulate activation of the DJNK pathway. While *Rho1* loss-of-function mutant embryos showed defects in LE stretching, wild type levels of *dpp* expression were observed in the LE cells (Lu and Settleman 1999a,b). The effects of Rho1 on DC appear, at least partially, to be mediated by PKN kinase (Lu and Settleman 1999a), a Rho1 effector that is conserved in mammals and has been shown to mediate insulin-induced reorganization of the actin cytoskeleton (Dong et al. 2000). As observed for Rho1, PKN is required for DC, but is dispensable for *Dpp* expression (Lu and Settleman 1999a). Indirect evidence also implicates Drok1, the *Drosophila* homolog of vertebrate Rho-kinase, in DC. In particular, Zipper (myosin II), which as described above functions downstream of Drok in the generation of wing planar polarity, is required for DC (Young et al. 1993). Furthermore a *Zipper-Rho1* genetic interaction has recently been reported during *Drosophila* morphogenesis (Halsell et al. 2000). Thus, it is likely that Drok participates in DC as well. Given that Rok is a key Rho effector that mediates actomyosin contractility in mammalian cells, it is possible that a major role of Rho1 in the DC process is to regulate contractility during purse-string action.

The nature of the upstream signaling components of the Rho GTPases in DC is less well-defined (Settleman 2001). One likely candidate is encoded by the gene called *myoblast city* (*mbc*), which was initially identified in a screen for dominant suppressors of a Rac-induced rough-eye phenotype (Nolan et al. 1998). *Mbc* mutant embryos exhibit a DC phenotype (Erickson et al. 1997; Nolan et al. 1998). Homologs of *mbc* have been identified both in *C. elegans* (called *ced5*) and in mammals (called DOCK180) (Hasegawa et al. 1996; Wu and Horvitz 1998). In mammals, DOCK180 has been shown to trigger the activation of Rac1, potentially through interaction with a specific GEF that activates Rac, given that as yet no in vitro GEF activity has been demonstrated for DOCK180 itself (Kiyokawa et al. 1998; Nolan et al. 1998). Furthermore, DOCK180, CrkII and Rac1, along with another adaptor protein p130Cas, have been linked to cell migration downstream of integrin activation (Klemke et al. 1998; Cheresh et al. 1999). More recently, an additional Dock180-interacting protein called ELMO-1 has been found that functions together with CrkII and Dock180 to activate Rac1, which in turn is required for cell migration (Gumienny et al. 2001). A homolog of ELMO-1, designated Ced12, has been identified in *C. elegans* and has been shown to be a component of the Ced-2 (CrkII)/ced5 (Dock180)/ced-10 (Rac) pathway that is involved in the regulation of cell migration in *C. elegans* (Conradt 2001; Gumienny et al. 2001; Wu et al. 2001; Zhou et al. 2001). Future studies will undoubtedly examine the potential

function of these proteins in DC and wound repair. It is noteworthy in this respect that both in the DC and wound healing processes integrins have proven to play a major role. (Harden et al. 1996; Lotz et al. 1997, 2000; Stark et al. 1997; Goldfinger et al. 1999; Mercurio et al. 2001). For example, mutations in the *Drosophila* integrin receptor β -subunit (*mysospheroid*) and in the $\alpha 3$ subunit (*scab*) of the integrin receptor lead to a DC defect (MacKrell et al. 1988; Stark et al. 1997).

Molecular mechanisms underlying sealing of epithelial sheets

During the final steps of enclosing a natural occurring hole or wound, it is critical that cell movement stops and that cells of the two opposing epithelial faces sense each other and adhere tightly to their opposite numbers to form a permanent seam (Abercrombie 1967; Martin-Blanco and Knust 2001). Insights as to how epithelial cells correctly adhere and subsequently fuse initially came from analyses of the late stages of ventral enclosure in *C. elegans* (Williams-Masson et al. 1997; Raich et al. 1999). These analyses revealed that when the two epithelial sheets move towards each other, the epidermal cells extend filopodia and that on contact of two opposing filopodia, adherens junction proteins cluster at the tips. Subsequently, these initial contact sites evolve into mature adherens junctions.

An almost identical actin-based protrusion-mediated mechanism has been demonstrated to occur during the final zipper stages of *Drosophila* DC. Jacinto et al. (2000) demonstrated that the induction of filopodia on opposing epithelial faces during the final stages of DC are critical in the correct matching of the opposing cells and in the adhesion of the epithelial fronts along the fusion seam. The induction of filopodia appears to require Cdc42 activity, as expression of a dominant negative mutant form of DCdc42 interferes with this process, resulting in incorrect fusing of opposing cells. Interestingly, disruption of the DJNK pathway in the LE cells also blocks filopodia formation mediated by DCdc42, suggesting that the DCdc42-triggered actin assembly in these cells requires JNK activation (Jacinto et al. 2000).

Recently, a similar mechanism of filopodia extension has been reported to take place during junction formation in epithelial cells in culture (Adams et al. 1998; Vasioukhin et al. 2000; Vasioukhin and Fuchs 2001). Exposure of these cells to calcium triggers the formation of filopodia, which on contact slide along each other and penetrate the membrane of the opposing cell. As is the case during ventral closure, adherens junction proteins accumulate at the embedded tips of filopodia, generating a two-rowed zipper of embedded puncta. After the two cell surfaces are pulled together, they are clamped by desmosomes. In addition to E-cadherin and catenins, the adherens junctions include also proteins such as VASP, Mena, vinculin, and zyxin (Vasioukhin et al. 2000). It has been postulated that Vasp and Mena are involved in directed actin polymerization at stabilized puncta, as such generating the necessary force to push the two rows of

puncta together into a single row and further seal the membranes into an epithelial sheet (Vasioukhin and Fuchs 2001). Given the recent finding that Cdc42 triggers filopodia formation by promoting the formation of an IRSp53/Mena complex (Krugmann et al. 2001), it is tempting to speculate that Cdc42 also has a role in adhesion junction formation in skin epithelial cells.

Epithelial-mesenchymal transitions

During epithelial-mesenchymal transitions (EMT), epithelial cells lose many of their distinctive epithelial features and take on a more mesenchymal fibroblastic phenotype. In particular, epithelial cells lose their polarized morphology, display less organized cell-cell junctions, and become more migratory. EMTs occur at many instances during embryogenesis, such as emigration of neural crest cells from the neural tube, gastrulation movements in many species, and the formation of cardiac valves (Duband et al. 1995; Hay 1995; Viebahn 1995; Markwald et al. 1996; Selleck and Bronner-Fraser 2000; Pla et al. 2001). It is important to note that EMT and loss of epithelial polarity also are recapitulated during later stages of tumor development (Hay 1995; Thiery and Chopin 1999). Numerous pathways have been described that control EMT transition in a variety of cell models (for reviews, see Hay 1995; Boyer et al. 2000; Savagner 2001). We will focus here on the role of the Rho proteins in EMT in some of these model systems.

EMT transitions can be initiated by numerous extracellular signals, including scatter factor/hepatocyte growth factor (SF/HGF) (Stoker et al. 1987; Stoker 1989; Rosen et al. 1990; Gherardi and Stoker 1991; Vande Woude et al. 1997) and members of the TGF- β family (McLeod et al. 1990; Cui et al. 1996; Oft et al. 1998; Portella et al. 1998). Scattering of MDCK kidney epithelial cells induced by HGF has emerged as a powerful paradigm to study the role of Rho GTPases in EMT. HGF induces multiple effects that promote MDCK cell scattering. These include the induction of actin cytoskeletal rearrangements that contribute to the centrifugal spreading, followed by the disruption of cell-cell junctions, and finally cell scattering. HGF triggers the activation of Cdc42 and Rac, accompanied by the induction of filopodia and lamellipodia (Royal et al. 2000; Zondag et al. 2000), and introduction of dominant negative versions of these GTPases interfered with the formation of filopodia and lamellipodia respectively and inhibits scattering (Ridley et al. 1995; Royal et al. 2000). HGF also stimulates Rho activity (Zondag et al. 2000). The precise role of Rho in HGF-stimulated scattering remains controversial however. In one study using MDCK cells, inhibition of Rho was shown to promote scattering and introduction of constitutively active Rho interfered with this process (Ridley et al. 1995), whereas in another study using keratinocytes, inhibition of Rho interferes with HGF-induced scattering (Takaishi et al. 1994). Cell type specificity and the extent of Rho inhibition may both contribute to this discrepancy. The potential roles of Rho GTPases in the disruption of cell-cell junctions by HGF

also need to be further clarified. In MDCK cells, both inhibition and constitutive activation of Rac interfere with HGF-induced breakdown of adherens junctions (Hordijk et al. 1997; Potempa and Ridley 1998). Similar results were obtained in the case of Ras-induced disruption of adherens junctions (see below).

Members of the Rho GTPases also have been found to play a role in TGF- β - and integrin-induced cell scattering. Although TGF- β is regarded as a major tumor suppressor during early tumor development through inhibition of cell cycle progression and cell growth, TGF- β can also act as a promoter of tumor progression by stimulating the EMT process. In fact, many late stage tumors are resistant to growth inhibition by TGF- β and even secrete TGF- β . Ras-transformed mammary epithelial cells for instance can undergo TGF- β -induced EMT, maintained via a TGF- β autocrine loop (McLeod et al. 1990; Cui et al. 1996; Oft et al. 1998; Portella et al. 1998). Treatment of epithelial cells with TGF- β results in disruption of cell-cell adhesions and a change in cell morphology to a spindle shape, accompanied by the loss of E-cadherin junctional localization and the acquisition of stress fibers (Piek et al. 1999; Bhowmick et al. 2001). Moses and colleagues provided evidence that one of the mechanisms by which TGF- β mediates EMT involves a Rho/Rok-dependent signaling pathway. TGF- β triggers a rapid activation of RhoA in several nontransformed epithelial cell lines. Furthermore, expression of a dominant negative mutant form of RhoA or its effector Rok inhibits TGF- β -mediated EMT (Bhowmick et al. 2001).

A large number of studies have highlighted the importance of cross talk between integrins and cadherins in the regulation of cell scattering/EMT (for review, see Pignatelli 1998). In one model system, expression of β 1 integrins in β 1-deficient epithelial cells has been shown to induce the disruption of intercellular adhesions, followed by cell scattering. This is accompanied by a decrease of cadherin and α -catenin protein levels and the activation of the RhoGTPases, RhoA and Rac, but not Cdc42 (Gimond et al. 1999). Expression of dominant negative mutant forms of Rac and RhoA prevents these β 1-integrin-induced changes, suggesting that these GTPases contribute to the loss of cell-cell adhesion. Although no apparent activation of Cdc42 by β 1-integrins can be detected, basal levels of Cdc42 seem to be required for scattering, as expression of a dominant negative mutant form of Cdc42 also inhibits cell scattering and partially restores cell-cell adhesion (Gimond et al. 1999).

Taken together, the above data support a critical role for Rho GTPases in mediating EMT (cell scattering) triggered by different extracellular factors. A similar transition from epithelial to mesenchymal phenotype is observed in oncogenic Ras-transformed epithelial cells. These cells show loss of E-cadherin-mediated cell-cell adhesion, and an increase in focal adhesions and stress fibers, as well as invasiveness (Behrens et al. 1989; Birchmeier et al. 1991; Vleminckx et al. 1991). Several studies have implicated the Rac and Rho GTPases in mediating this Ras-induced morphological transformation (Hordijk

et al. 1997; Zhong et al. 1997; Quinlan 1999; Braga et al. 2000; Zondag et al. 2000). Interestingly, whereas an increase in Rac activity is required for HGF/SF and $\beta 1$ integrin-triggered EMT, different studies show opposing data for the activation status and function of Rac in Ras transformed epithelial cells. In keratinocytes, inhibition of Rac prevents disassembly of adherens junctions caused by oncogenic Ras (Braga et al. 2000). Moreover, as mentioned above, in these cells, sustained expression of an activated form of Rac is sufficient to disassemble cadherin-mediated contacts (Braga et al. 2000). These data are consistent with the observation that activated Rac promotes cell-cell adhesion breakdown in T47D mammary carcinoma cells (Keely et al. 1997). In addition, Rac activation has been shown to contribute to the Ras-dependent perturbation of cell-cell contacts in breast cancer cell lines (Quinlan 1999). In MDCK cells, however, the opposite is observed. In these cells, expression of Tiam1, a Rac-specific GEF, or activated Rac restores cadherin-mediated cell adhesion and concomitantly inhibits the Ras-induced invasive phenotype (Hordijk et al. 1997). Concordant with these findings, Collard and colleagues found that sustained signaling by oncogenic Ras downregulates Rac activity through the MAPK signaling pathway in MDCK cells (Sander et al. 1999; Zondag et al. 2000). A number of factors may reconcile the paradoxical observations made for Rac's involvement in transformation-induced EMT. The nature of the extracellular matrix on which the cells are plated is likely to contribute to the differences. Indeed, Tiam-expressing RasV12-transformed MDCK cells showed a fibroblast-like migratory phenotype when plated on collagen, but an epithelial phenotype when plated on laminin or fibronectin (Sander et al. 1998). Differences in cell type and/or the levels and duration of expression of Ras and Rac proteins also may be important.

Studies performed in Ras transformed MCF10A cells illustrate that activation of RhoA also contributes to the fibroblastic phenotype of these cells (Zhong et al. 1997). A possible mechanism for this is that increased contractility that is stimulated by RhoA promotes the formation of stress fibers and focal adhesions and contributes to the disruption of adherens junctions. This RhoA-induced increase in contractility, however, is not sufficient for the disruption of adherens junctions, because inhibition of Rho only partially restores the epithelial phenotype of Ras transformed cells and expression of an activated mutant form of RhoA does not mimic the Ras transformed phenotype (Zhong et al. 1997).

Once epithelial cells dissociate from each other and, at least partially, from the extracellular matrix (ECM), they become motile. Cell migration can be divided into different steps, each of which appears to be differentially regulated by a specific subfamily of Rho GTPases. A migrating cell senses the extracellular environment, including extracellular matrix and soluble or immobilized cytokines, and this information can be used to establish the directionality of movement. Increasing evidence suggests that Cdc42, which regulates filopodia formation, is required for the interpretation of extracellular cues

(Zheng et al. 1996; Gomez et al. 2001). In a second step, Rac-dependent lamellipodia formation is induced at the leading edge of the cell and subsequently these extensions are stabilized through the formation of new adhesion sites to the ECM. A final step consists of the forward movement of the cell body proper, followed by the detachment and retraction of the tail. Rho is important in the generation of contractile force and moving the body and the tail of the cell beyond the leading edge. Rho proteins are thought to accomplish these functions largely by their effects on the actin cytoskeleton. Multiple downstream effectors of Rac, Cdc42, and RhoA mediating the reorganization of the actin cytoskeleton have been identified over the past several years. The most prominent effectors involved in the control of actin dynamics are depicted in Figure 4. This work has been extensively covered in a number of recent reviews (Van Aelst and D'Souza-Schorey 1997; Keely et al. 1998; Machesky and Gould 1999; Machesky and Insall 1999; Nabi 1999; Ridley et al. 1999; Sander and Collard 1999; Christopher and Guan 2000; Evers et al. 2000; Hall and Nobes 2000; Schmitz et al. 2000; Condeelis 2001; de Curtis 2001; Price and Collard 2001; Ridley 2001a). In addition to their effects on the actin cytoskeleton, the Rho proteins have subsequently been found to regulate other processes that are relevant to cell migration. These include microtubule dynamics (Gundersen et al. 1998; Hollenbeck 2001; Wittmann and Waterman-Storer 2001), vesicle trafficking (Ellis and Mellor 2000; Ridley 2001b), and control of extracellular matrix degrading enzymes (Bourguignon 2001).

Concluding remarks

The observations discussed in this review highlight essential functions of Rho family members in multiple aspects of the establishment and maintenance of epithelial cell polarity and cell morphology. In particular, studies in tissue culture combined with genetic analysis in model organisms such as *Drosophila* have greatly helped to elucidate Rho-regulated signaling mechanisms that regulate epithelial morphogenesis.

The Rho proteins have emerged as key players in mediating the effects of extracellular signals (including secreted factors, cell surface-associated ligands, and adhesion molecules) to the downstream cellular machinery that controls the organization of the actin cytoskeleton, determining cell shape, adhesion, contractility, and movement. A large number of effector molecules of Rho GTPases have been identified that link them to the cytoskeleton. Much less is known about the signaling mechanisms that translate the extracellular cues into spatio-temporal regulation of the Rho GTPases. It is likely, however, that the concerted action of GEFs and GAPs is critical for the precisely timed and strictly localized activation of the Rho GTPases (Symons and Settleman 2000; Gulli and Peter 2001). This tight control may explain how the individual Rho GTPases can mediate multiple temporally and spatially distinct processes. Good examples of the coordinate action of two or

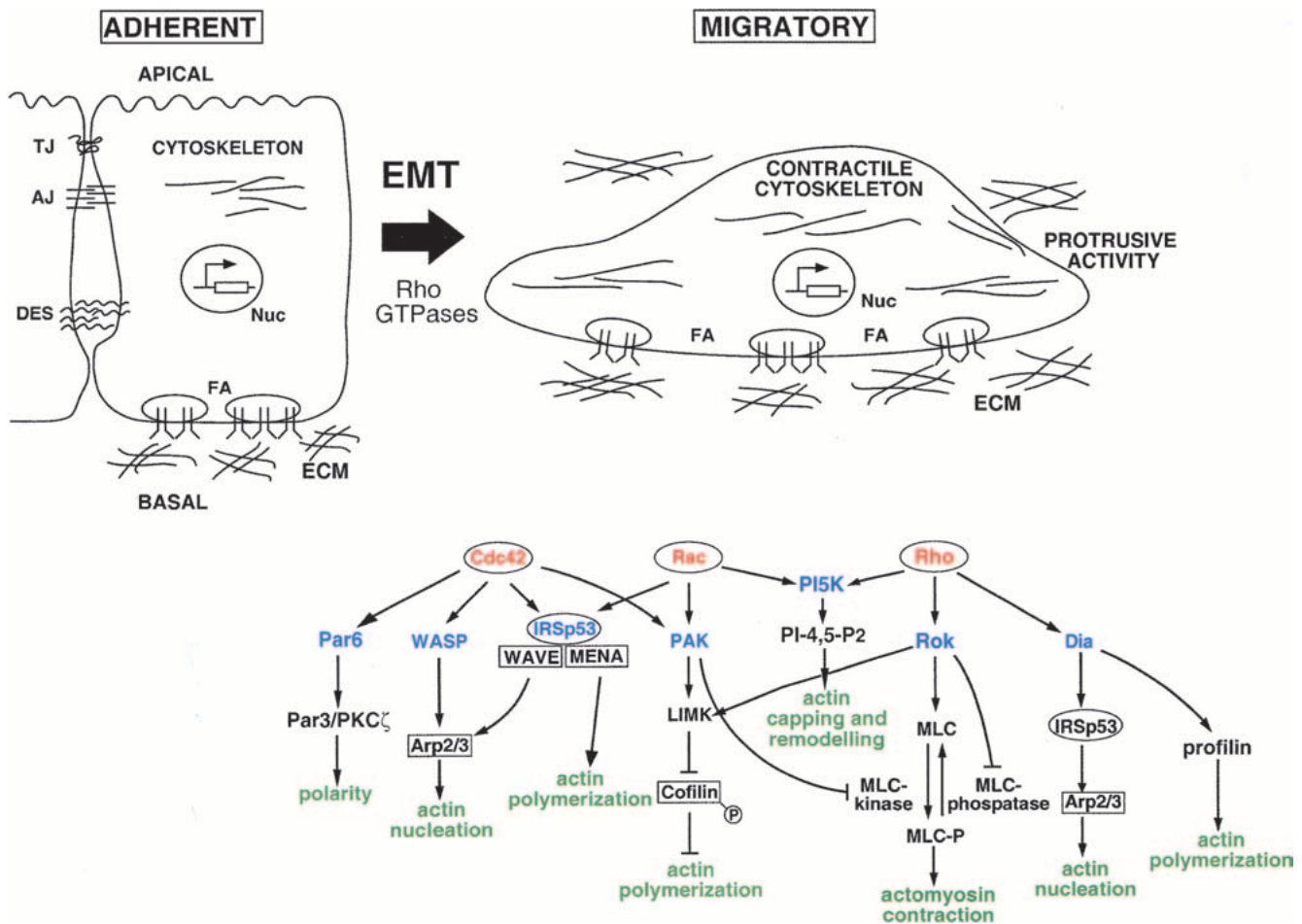


Figure 4. Transition of a typical adherent epithelial cell to a mesenchymal-like motile phenotype. Rho GTPase function is required at different steps during EMT transition (see text). An overview of Rho effectors mediating cytoskeletal rearrangements during cell migration is shown below. Cdc42 acts through several downstream effectors to trigger actin polymerization required for filopodia extension. Among these are the WASP proteins (including WASP and N-WASP), which mediate the activation of the Arp2/3 complex leading to nucleation of new actin filaments on the sides of existing ones. Activated Cdc42 also binds to members of the Pak family, stimulating LIMK, which in turn inhibits cofilin-mediated actin depolymerization. Pak also has been shown to phosphorylate and inactivate MLCK, resulting in the disassembly of stress fibers and focal adhesions, a process that is likely to contribute to motility. Cdc42 also can initiate actin filament assembly via the IRSp53:Mena complex. Finally, Cdc42 interacts with Par-6, a constituent of the Par/aPKC complex that is vital for the establishment of cellular polarity in diverse systems. Rac triggers F-actin accumulation to induce lamellipodia by activating a similar set of effector molecules. Several among these, such as the PAK and IRSp53 proteins are also Cdc42 effectors and analogous mechanisms as described above appears to apply for Rac's effects on actin nucleation and polymerization. In addition, Rac functions via a phosphatidylinositol 4-phosphate 5-kinase (PI 5-kinase) to produce the formation of PI(4,5)P₂. These lipids modulate the activity of capping proteins, removing them from the barbed ends of actin filaments. Activation of Rho regulates actomyosin contractility and causes actin reorganization to induce stress fibers and focal adhesion complexes (FAs). Members of at least two protein families appear to be required for Rho-induced assembly of stress fibers and FAs, the Rho-associated kinases (Rok) and the Dia members. Activation of Rho-kinase triggers an increase in myosin light chain (MLC) phosphorylation through inactivation of MLC phosphatase and likely also through direct phosphorylation of MLC. The increase in phosphorylated MLC stimulates contractility, promoting stress fiber formation. Rok functions in concert with Dia in the formation of stress fibers. Dia may contribute to stress fiber formation through its interaction with profilin, a G-actin binding protein. In addition, Dia has been reported to interact with the Cdc42/Rac effector IRSp53. Rok also stabilizes filamentous actin by phosphorylating LIM-kinase, which subsequently phosphorylates and inactivates cofilin. Similarly as for Rac, Rho may regulate the activity of PI 5-kinase to regulate capping activity.

three Rho GTPases in morphogenic responses are dorsal closure and the establishment of cell-cell junctions. Our deepening understanding of Rho-mediated signaling in epithelial morphogenesis surely will shed new light on the spatio-temporal regulation of Rho proteins. It also will be interesting to see whether the different isoforms

of the above-discussed GTPases (e.g., RhoA vs. RhoB and RhoC) could play a distinct role in epithelial morphogenesis and whether other Rho GTPase family members, such as RhoD, TC21, Wrch-1, are involved and what the underlying mechanisms are. In this regard, recent genomic analysis, combining a protocol selecting

for highly metastatic melanoma cells in mice with microarray analysis, identified the RhoC gene as a promoter of metastatic behavior (Clark et al. 2000). Another intriguing finding is that of the novel Cdc42-like GTPase Wrch-1 as a Wnt-1 transcriptional target (Tao et al. 2001). The Wnt signaling pathway, mostly through mutational analysis of the Wnt-family genes as well as the APC and β -catenin genes, has been implicated in tumorigenesis (Peifer and Polakis 2000; Polakis 2000). The contribution of Wrch-1 to the list of Wnt-responsive genes may help understand the downstream functions effected by Wnt-signaling, including the induction of morphological changes and disruption of cell-cell junctions. An even more challenging issue that remains to be addressed is to determine how processes that are taking place in epithelial monolayers are coordinated with stromal events to direct specific tissue morphogenesis, for example, of kidney tubules, thyroid follicles, or branching structures found in salivary glands and lung.

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