Mini-Review

Untying the Gordian Knot of Cytokinesis: Role of Small G Proteins and Their Regulators

Sergei N. Prokopenko,* Robert Saint,§ and Hugo J. Bellen*‡

*Program in Developmental Biology, ‡Howard Hughes Medical Institute, and Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030; and §Department of Genetics and Centre for the Molecular Genetics of Development, University of Adelaide, Adelaide, SA 5005, Australia

Ability to divide is one of the basic properties of a cell. In metazoans, where cells divide in a context of germ layers, tissues, and organs, cell proliferation must be coordinated with differentiation to prevent developmental abnormalities. Genetic analyses in simple model systems (yeast, slime mold, fruit fly) have demonstrated that defects in either karyokinesis, or nuclear division (e.g., mutations that affect cell cycle checkpoints, mitotic chromosome condensation and segregation, etc.), or cytokinesis (Fig. 1), both may delay or block development.

Early studies of cytokinesis in animal cells took advantage of the ability to easily manipulate and observe large transparent eggs of marine invertebrates, such as echinoderms and ctenophores (reviewed by Rappaport, 1996). These simple but informative micromanipulation experiments have demonstrated that an actomyosin contractile ring is the driving force of cytokinesis and have led to some initial speculations about the nature of the signal inducing cytokinesis. More recently, a combination of genetic screens, genomic sequencing, and biochemical approaches have resulted in the identification of many proteins required for cytokinesis in several model organisms (Goldberg et al., 1998). Not surprisingly, many of these proteins are components of the actin cytoskeleton, actin-interacting or other structural proteins, and microtubule motor proteins. Yet, the identity of proteins required for the spatial and temporal regulation of molecular events during cytokinesis remains largely an open question. Some of the first regulatory proteins shown to be required for cytokinesis were small G proteins of the Ras and Rho (Ras homologous) families.

Small G Proteins: Janus Within

Small GTP-binding proteins (G proteins) of the Ras superfamily act at the crossroads of cell signaling pathways. They relay extracellular or intracellular signals that activate signaling networks regulating cell cycle progression, transcription, vesicle trafficking, nuclear transport, cytoskeletal dynamics, and differentiation. Like other G proteins, small G proteins cycle between inactive (GDP-bound) and active (GTP-bound) states (Fig. 2). Three classes of molecules regulate the GDP/GTP cycling. Small G proteins are activated by guanine nucleotide exchange factors (GEFs)[1], which catalyze the exchange of bound GDP for GTP (Whitehead et al., 1997; Stam and Collard, 1999). GTPase-activating proteins (GAPs) inactivate G proteins by increasing their low intrinsic GTPase activity (Zalcman et al., 1999). In addition, inactive Rho proteins are complexed in the cytosol with guanine nucleotide dissociation inhibitors (GDIs), which keep them in an inactive soluble state by inhibiting the exchange of GDP for GTP and sequestering them from membranes (Zalcman et al., 1999).

Activation of a G protein results in a conformational change, exposing its structural domains and allowing it to interact with and activate downstream effectors. Therefore, the intracellular or subcellular concentration of the GTP-bound form determines a particular cellular response. Given the diversity of cellular and developmental roles of small G proteins one would expect that there is a complex hierarchy of molecules regulating their activity, both spatially and temporally. This may explain how the same G protein often plays multiple cellular or developmental roles. For example, developmental roles of *Drosophila* Rho1 range from cellularization (Crawford et al., 1998), gastrulation (Barrett et al., 1997; Häcker and Perrimon, 1998), segmentation (Magie et al., 1999), dorsal closure (Harden et al., 1999), and cytokinesis (Prokopenko et al., 1999) to the regulation of tissue polarity (Strutt et al., 1997), and dendritic morphogenesis (Lee et al., 2000). This functional diversity may be achieved through tissue, developmental stage, or cell cycle–specific expression of regulatory molecules, such as GEFs and GAPs. An additional level of regulation of G protein signaling can be achieved through subcellular compartmentalization of the molecular machinery (upstream regulators or downstream effectors) that initiates a signaling cascade (see below).

Small G Proteins and Cytokinesis: Caught in the Act

Evidence supporting a requirement for small G proteins in cytokinesis derives from four types of experiments (Table

Address correspondence to S.N. Prokopenko, Baylor College of Medicine, T634, Mail stop BCM235, One Baylor Plaza, Houston, TX 77030. Tel.: (713) 798-5273. Fax: (713) 798-8515. E-mail: sp691305@yahoo.com or to H.J. Bellen at the same address, Tel.: (713) 798-5272. Fax: (713) 798-8515. E-mail: hbellen@bcm.tmc.edu

[1]*Abbreviations used in this paper:* aa, amino acids; GAPs, GTPase-activating proteins; GDIs, guanine nucleotide dissociation inhibitors; GEFs, guanine nucleotide exchange factors; MLC, myosin light chain.

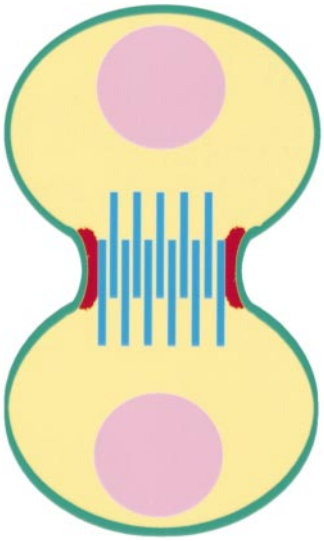

Figure 1. The basics of cytokinesis. Cytokinesis is accomplished through progression of a cleavage furrow (invaginations of a de novo added plasma membrane in green) which divides a cell into two daughter cells. The driving force of this constriction is an actomyosin contractile ring (red) which forms at the cell equator in late anaphase. The positioning of a cleavage plane is thought to depend on a central spindle (blue) and, possibly, spindle poles (i.e., mitotic apparatus). Cytokinesis results in distribution of chromosomes (nuclei in pink) and cytoplasm with organelles (yellow) between daughter cells, thus completing the mitotic cycle.

I). In *Dictyostelium* and *Drosophila*, analysis of the loss-of-function phenotype was the main experimental tool. Analyses in other organisms relied on the ability to mimic mutant phenotypes using one of three approaches: (a) overexpression of constitutively active or dominant-negative forms of small G proteins, (b) injection of antibodies or RNA interference experiments, and (c) administration (through injection, expression or addition to a culture medium) of *Clostridium botulinum* C3 exoenzyme. C3 is an ADP-ribosyltransferase specific for Rho (but not Rac or Cdc42) proteins. It ADP-ribosylates Rho proteins on Asn[41] blocking their translocation to the plasma membrane, which effectively inhibits their biological activity (Fiorentini et al., 1998).

In all documented cases, functional or biochemical inactivation of small G proteins resulted in formation of polyploid cells that contained multiple (two or more) nuclei, suggesting defects in cytokinesis. However, there seem to be different requirements for Rho family members in cytokinesis in different systems, suggesting that there is a significant diversity of evolutionary roles of Rho proteins. Although to date only Rho1 has been implicated in cytokinesis in *Drosophila* (Prokopenko et al., 1999) and *C. elegans* (RHO1; Kodama, Y., A. Sugimoto, and M. Yamamoto, personal communication; Romano, A., and M. Glotzer, personal communication), both Rho and Cdc42 proteins are required for cytokinesis in *Xenopus* and human (Table I). In contrast to an established role of Rho and Cdc42 proteins in cytokinesis (Table I), there is only one Rac protein known to be required for cytokinesis (*Dictyostelium* RacE). Furthermore, the role of Ras protein in cytokinesis has been demonstrated so far only in *Dictyostelium* (RasG; Tuxworth et al., 1997). The involvement of Ras in cytokinesis may be restricted to one or a few phylogenetic groups, since extensive studies of Ras proteins in higher eukaryotes have not provided evidence for their role in cytokinesis.

Formation of multinucleate cells upon inactivation of small G proteins strongly suggests defects of cytokinesis. However, this has been demonstrated directly by the absence of a contractile ring or failure of a cleavage furrow in only few cases (Mabuchi et al., 1993; Drechsel et al., 1996; Gerald et al., 1998; Prokopenko et al., 1999). In addition, G proteins may regulate different steps in cytokinesis (see

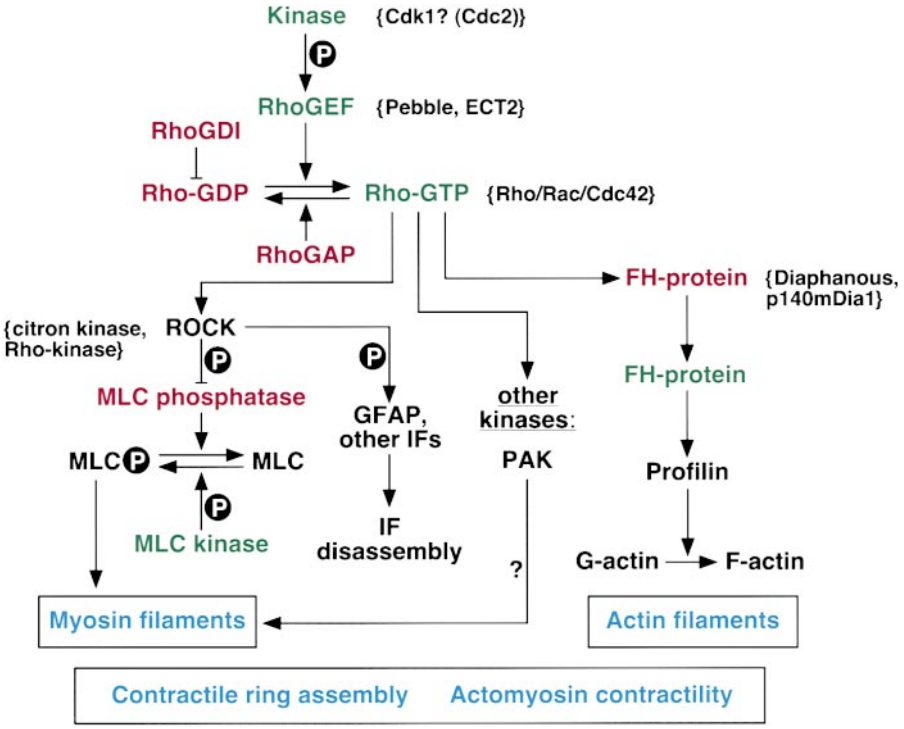

Figure 2. Rho-mediated signal transduction pathways operating during cytokinesis. Positive regulators or active proteins are shown in green. Negative regulators or inactive proteins are shown in red. Specific examples are listed in curly brackets. Although the existence of a Rho•GTP/FH protein–mediated pathway initiating actin polymerization during cytokinesis has been demonstrated, the role of ROCK-like kinases in assembly of myosin filaments during cytokinesis is presumed, based on their known roles in other actin-dependent processes. The assembly of actin and myosin filaments (together with septins and actin-interacting proteins) into a contractile ring and the regulation of actomyosin contractility during cytokinesis are even more poorly understood. Abbreviations: F-actin, fibrous actin; FH-protein, formin homology protein; G-actin, globular actin; GFAP, glial fibrillary acidic protein; IFs, intermediate filaments; P, phosphorylation. For other abbreviations and for details see text.

Table I. Small G Proteins and Their Regulators Implicated in Cytokinesis

Protein	Organ-ism*	Evidence	Experimental Assay	Observations	Reference
			Ras proteins and their regulators		
RasG	*Dd*	In vivo	Null mutation (gene disruption)	Multinucleate cells in suspension, late cytokinetic defect	Tuxworth et al., 1997
DGAP1 (Ras GAP)	*Dd*	In vivo	Null mutation (gene disruption)	Increased growth rate	Faix and Dittrich, 1996
			Overexpression	Multinucleate cells in suspension	
Ras GAP	*Dd*	In vivo	Null mutation (gene disruption)	Multinucleate cells in suspension	Lee et al., 1997
			Yeast two-hybrid assay	Interacts with Ras•GTP, but not Ras•GDP	
		In vitro	GAP assay	GAP for RasD GTPase	
GAPA (IQGAP)	*Dd*	In vivo	Null mutation (REMI)	Incomplete cleavage, reversion of cytokinesis	Adachi et al., 1997
			Rho proteins and their regulators		
RacE	*Dd*	In vivo	Null mutation (REMI)	CR forms, but CF constriction is incomplete	Gerald et al., 1998
RHO1‡	*Ce*	In vivo	RNAi	Formation of multinucleate blastomeres	
T19E10.1‡§ (RhoGEF)	*Ce*	In vivo	RNAi	Formation of multinucleate blastomeres	
Rho1	*Dm*	In vivo	Null mutation or expression of DN Rho1	Failure of CR and CF, formation of multinucleate cells	Prokopenko et al., 1999
Pebble (RhoGEF)	*Dm*	In vivo	Null mutation or expression of DN Pebble	Failure of CR and CF, formation of multinucleate cells	Prokopenko et al., 1999
			Genetic interaction and two-hybrid assay	Pebble interacts with Rho1, but not with Rac1 or Cdc42	
Rho	*Cj, Sm*	In vivo	Injection of C3 transferase in sand dollar eggs	Failure or regression of CR and CF, formation of multinucleate embryos	Mabuchi et al., 1993
RhoA	*Xl*	In vivo	Injection of C3 transferase in embryos	Aberrant CF ingression	Drechsel et al., 1996
			Injection of ACT Rho	Initiation, but no ingression of CF	
Cdc42	*Xl*	In vivo	Injection of ACT or DN Cdc42 in embryos	Aberrant CF ingression	Drechsel et al., 1996
Rho GDI	*Xl*	In vivo	Injection of Rho GDI or C3 transferase in embryos	Failure of CF	Kishi et al., 1993
Rho	*Mm*	In vitro	Transient expression of C3 transferase in EL4 T lymphoma cells	Formation of multinucleate cells	Moorman et al., 1996
RhoD	*Mm*	In vitro	Transient expression of ACT RhoD in C3H 10T1/2 fibroblasts	Formation of multinucleate cells	Tsubakimoto et al., 1999
		In vivo	Injection of ACT RhoD in *Xenopus* embryos or eggs	Formation of multinucleate cells	
Rho	*Rn, Mm, Hs*	In vitro	Injection of C3 transferase in NRK, Swiss 3T3, and HeLa cells	Irregular CF ingressions, ectopic cleavage sites	O'Connell et al., 1999
RhoA	*Hs*	In vitro	Addition of C3 transferase to culture medium of HL60 cells	Reduced cell proliferation, accumulation of binucleate cells	Aepfelbacher et al., 1995
Rho	*Hs*	In vitro	Addition of C3 transferase to culture medium of CMK cells	Increased cell polyploidy	Takada et al., 1996
CDC42Hs	*Hs*	In vitro	Inducible expression of ACT CDC42Hs in HeLa-derived cells	Formation of giant multinucleate cells	Dutartre et al., 1996
ECT2 (RhoGEF)	*Hs*	In vitro	Injection of anti-ECT2 antibodies or expression of DN ECT2	Formation of multinucleate cells	Tatsumoto et al., 1999
			Guanine nucleotide exchange assay	GEF for RhoA, Rac1, Cdc42	

*Species name abbreviations: *Ce*, *Caenorhabditis elegans*; *Cj*, *Clypeaster japonicus*; *Dd*, *Dictyostelium discoideum*; *Dm*, *Drosophila melanogaster*; *Hs*, *Homo sapiens*; *Mm*, *Mus musculus*; *Rn*, *Rattus norvegicus*; *Sm*, *Scaphechinus mirabilis*; *Xl*, *Xenopus laevis*. Other abbreviations: ACT, activated; CF, cleavage furrow; CR, contractile ring; DN, dominant-negative; GAP, GTPase-activating protein; GDI, guanine nucleotide dissociation inhibitor; GEF, guanine nucleotide exchange factor; REMI, restriction enzyme-mediated integration; RNAi, RNA interference.
‡Kodama, Y., A. Sugimoto, and M. Yamamoto, personal communication.
§Romano, A., T. Schedl, and M. Glotzer, personal communication.

Table I). In some cases, inactivation of a Rho protein resulted in late cytokinetic defects with incomplete or aberrant ingression (Drechsel et al., 1996; Tuxworth et al., 1997; Gerald et al., 1998; O'Connell et al., 1999) or even regression (Mabuchi et al., 1993) of the cleavage furrow. Yet, in other instances, Rho proteins were required for the initiation of cytokinesis, since the contractile ring failed to form and there were no signs of cleavage furrow ingression (Mabuchi et al., 1993; Prokopenko et al., 1999). The most compelling evidence that small G proteins are required for cytokinesis was the identification of regulators of Ras (GAPs) and Rho (GEFs and GDIs) proteins (Table I) that upon inactivation or overexpression blocked cytokinesis (Kishi et al., 1993; Faix and Dittrich, 1996; Adachi et al., 1997; Lee et al., 1997; Prokopenko et al., 1999; Tatsumoto et al., 1999; Kodama, Y., A. Sugimoto, and M. Yamamoto, personal communication; Romano, A., T. Schedl, and M. Glotzer, personal communication).

Subcellular localization studies of Rho proteins strongly support a role in cytokinesis. Rho proteins localize to the

cytosol or plasma membrane in resting cells (Adamson et al., 1992; Lang et al., 1993; Robertson et al., 1995), but translocate to the cleavage furrow and midbody during cytokinesis (Takaishi et al., 1995; Nishimura et al., 1998). Remarkably, two RhoGEFs known to be required for cytokinesis, human ECT2 (Tatsumoto et al., 1999) and *Drosophila* Pebble (Prokopenko et al., 1999), have a similar distribution during mitosis, being initially cortical or cytoplasmic and translocating to the cell equator at the onset of cytokinesis. Pebble accumulation at the cell equator parallels the assembly of the contractile ring and progression of the cleavage furrow, suggesting that it is required for the initiation of contractile ring assembly (possibly, by interacting with and activating Rho1 at the cleavage furrow). These data are consistent with the proposed GEF-dependent spatial and temporal regulation of Rho activation, leading to induction of a signal transduction pathway through a direct interaction of Rho•GTP with its downstream effectors. Transient activation of the GEF/small G protein molecular switch dependent on the targeting of proteins to a particular subcellular compartment is likely to be a very common strategy. Although the signaling pathways used by different small G proteins must be different, the basic principle of a G protein working as a switch to turn on downstream effectors remains the same and probably has been used independently multiple times during evolution to regulate a variety of aspects of cellular morphogenesis.

Rho Signaling during Cytokinesis: The Labyrinth of Minotaur

Among a plethora of known effectors of Rho proteins, four recently identified proteins were shown to be required for cytokinesis (Fig. 2). The formin-homology proteins, *Drosophila* Diaphanous (Castrillon and Wasserman, 1994; Wasserman, 1998) and its mouse homologue p140mDia1 (Watanabe et al., 1997), bind to and regulate profilin, an actin-binding protein that promotes F-actin polymerization and is required for cytokinesis (Giansanti et al., 1998; Suetsugu et al., 1999). *Dictyostelium* p21-activated serine/threonine kinase PAKa, a putative Cdc42/Rac effector, is thought to regulate myosin II assembly by inhibiting myosin II heavy chain kinase (Chung and Firtel, 1999). Bovine Rho-associated kinase (cleavage furrow kinase) is required for the regulation of the contractile ring contractility and for phosphorylation of intermediate filaments, leading to their disassembly and segregation into daughter cells, which, in turn, ensure efficient cell separation (Kosako et al., 1997, 1999; Yasui et al., 1998). Finally, mouse citron kinase functions at a later step by regulating actomyosin contraction in a Rho-dependent manner (Madaule et al., 1998). The role of these effectors in Rho signaling during cytokinesis is further suggested by their interaction with GTP-bound forms of Rho proteins, localization to the cleavage furrow, and colocalization with either Rho proteins or components of the contractile ring. However, a role of Rho-kinase and citron kinase in cytokinesis is suggested from experiments with dominant-negative mutants, and this conclusion awaits further proof in loss-of-function studies.

How many Rho effectors does a cell need to undergo cytokinesis? Cytokinesis is a complex event involving assembly of actin, myosin, septins, and actin-interacting proteins into a contractile ring, its dynamic contraction, and disassembly at the end of cytokinesis. Most likely, these cytoskeletal events are regulated via several signaling pathways that converge on the contractile ring, with kinases featuring prominently among Rho effectors (Fig. 2). These pathways are likely to act cooperatively, as demonstrated recently for two Rho effectors, p140mDia1 and serine/threonine kinase ROCK, in the formation of actomyosin stress fibers (Watanabe et al., 1999). Rho-activated ROCK phosphorylates and inhibits myosin light chain (MLC) phosphatase, thus promoting accumulation of phosphorylated MLC generated by MLC kinase. Phosphorylated myosin II assembles into myosin filaments and associates with actin to form stress fibers. Interestingly, the kinase domain of citron shows the highest similarity to that of ROCK (Madaule et al., 1998), though it remains to be demonstrated if citron kinase regulates myosin II polymerization during cytokinesis. Since the contractile ring is a cortical structure more complex and dynamic than stress fibers, one can expect a high degree of complexity of Rho-mediated signaling pathways regulating its function. We propose that there is an elaborate hierarchy of proteins regulating cytoskeletal dynamics at the cleavage furrow, in particular polymerization/depolymerization of molecules making up the contractile ring. Since Rho proteins, their upstream regulators, and downstream effectors all localize at the cell equator during cytokinesis, the cleavage furrow is likely to function as a workshop where protein complexes that initiate and regulate cytokinesis are assembled and disassembled.

The limited knowledge we have about signal transduction pathways that initiate and regulate cytokinesis tells us that there are at least two basic regulatory mechanisms operating during cytokinesis: (a) protein–protein interaction or binding of small molecules and (b) phosphorylation. Small G proteins undergo conformational change and become biologically active in response to GTP binding. A similar mechanism, involving protein–protein interaction, has been proposed recently for p140mDia1 (Watanabe et al., 1999). Binding of Rho•GTP to the Rho-binding domain of mDia1 is thought to disrupt the intramolecular interaction between protein termini releasing the FH1 and FH2 COOH-terminal domains required to induce actin polymerization. How common is such an activation mechanism? We know that Rho•GTP/effector interactions are necessary to initiate a signaling cascade. However, one can imagine that the signal may also be transduced via the formation of ternary protein complexes alone, without interaction-dependent conformational change as suggested for p140mDia1. Intermolecular interactions as well as conformational changes are likely to be featured in this "protein dance". A second mechanism, likely to be universal, is regulation by phosphorylation. Kinases and phosphatases play prominent roles in downstream pathways (Rho effectors), but may also regulate the upstream components of the cytokinetic signaling machinery. ECT2 appears to be activated by phosphorylation which occurs specifically in G2/M phases and this phosphorylation is required for its exchange activity (Tatsumoto et al., 1999). Cdk1 or a Cdk1-regulated kinase may phosphorylate ECT2, since

it contains several consensus phosphorylation sites for Cdk1 (Tatsumoto et al., 1999). Interestingly, other ECT2-related RhoGEFs implicated in cytokinesis also contain several Cdk1 phosphorylation sites, one of which is conserved in three species (amino acids [aa] 771–774 in *Drosophila* Pebble, aa 671–674 in mouse Ect2, and aa 814–817 in human ECT2).

Finally, it is difficult to rationalize the unexpected cell cycle–dependent nuclear localization of three RhoGEFs required for cytokinesis in *Drosophila* and human cells (Prokopenko et al., 1999; Tatsumoto et al., 1999) or cell polarization in yeast (Cdc24p; Toenjes et al., 1999). All three proteins localize to the nucleus in interphase cells, their levels diminish before nuclear division (or upon nuclear envelope breakdown), and proteins reappear in divided nuclei. Is it evidence for a direct link between the cytokinetic machinery and the mitotic apparatus? Or do these proteins play some role in the nucleus that is unrelated to their roles in cytokinesis? Or is it just a common mechanism to inactivate a regulatory molecule by sequestering it into the nucleus (Pines, 1999)? Answers to these and other questions await a better understanding of the molecular pathways initiating and regulating cytokinesis.

The authors would like to thank Michael Glotzer (Research Institute of Molecular Pathology, Vienna, Austria), Toru Miki (National Cancer Institute, Bethesda, MD), and Asako Sugimoto (University of Tokyo, Tokyo, Japan) for communicating results before publication and Richard C. Atkinson and Anthony L. Lau for comments on the manuscript. We apologize to those whose work could not be cited directly because of space limitations.

H.J. Bellen is Investigator of the Howard Hughes Medical Institute.

Submitted: 6 January 2000
Revised: 1 February 2000
Accepted: 2 February 2000

References

Adachi, H., Y. Takahashi, T. Hasebe, M. Shirouzu, S. Yokoyama, and K. Sutoh. 1997. *Dictyostelium* IQGAP-related protein specifically involved in the completion of cytokinesis. *J. Cell Biol.* 137:891–898.

Adamson, P., H.F. Paterson, and A. Hall. 1992. Intracellular localization of the $p21^{rho}$ proteins. *J. Cell Biol.* 119:617–627.

Aepfelbacher, M., M. Essler, K. Luber de Kintana, and P.C. Weber. 1995. ADP-ribosylation of the GTP-binding protein RhoA blocks cytoplasmic division in human myelomonocytic cells. *Biochem. J.* 308:853–858.

Barrett, K., M. Leptin, and J. Settleman. 1997. The Rho GTPase and a putative RhoGEF mediate a signaling pathway for the cell shape changes in *Drosophila* gastrulation. *Cell*. 91:905–915.

Castrillon, D.H., and S.A. Wasserman. 1994. *diaphanous* is required for cytokinesis in *Drosophila* and shares domains of similarity with the products of the *limb deformity* gene. *Development.* 120:3367–3377.

Chung, C.Y., and R.A. Firtel. 1999. PAKa, a putative PAK family member, is required for cytokinesis and the regulation of the cytoskeleton in *Dictyostelium discoideum* cells during chemotaxis. *J. Cell Biol.* 147:559–575.

Crawford, J.M., N. Harden, T. Leung, L. Lim, and D.P. Kiehart. 1998. Cellularization in *Drosophila melanogaster* is disrupted by the inhibition of rho activity and the activation of Cdc42 function. *Dev. Biol.* 204:151–164.

Drechsel, D.N., A.A. Hyman, A. Hall, and M. Glotzer. 1996. A requirement for Rho and Cdc42 during cytokinesis in *Xenopus* embryos. *Curr. Biol.* 7:12–23.

Dutartre, H., J. Davoust, J.-P. Gorvel, and P. Chavrier. 1996. Cytokinesis arrest and redistribution of actin-cytoskeleton regulatory components in cells expressing the Rho GTPase CDC42Hs. *J. Cell Sci.* 109:367–377.

Faix, J., and W. Dittrich. 1996. DGAP1, a homologue of rasGTPase activating proteins that controls growth, cytokinesis, and development in *Dictyostelium discoideum*. *FEBS Lett.* 394:251–257.

Fiorentini, C., M. Gauthier, G. Donelli, and P. Boquet. 1998. Bacterial toxins and the Rho GTP-binding protein: what microbes teach us about cell regulation. *Cell Death Differ.* 5:720–728.

Gerald, N., J. Dai, H.P. Ting-Beall, and A. De Lozanne. 1998. A role for *Dictyostelium* racE in cortical tension and cleavage furrow progression. *J. Cell Biol.* 141:483–492.

Giansanti, M.G., S. Bonaccorsi, B. Williams, E.V. Williams, C. Santolamazza, M.L. Goldberg, and M. Gatti. 1998. Cooperative interactions between the central spindle and the contractile ring during *Drosophila* cytokinesis. *Genes Dev.* 12:396–410.

Goldberg, M.L., K.C. Gunsalus, R.E. Karess, and F. Chang. 1998. Cytokinesis. *In* Dynamics of Cell Division. S.A. Endow and D.M. Glover, editors. Oxford University Press, Oxford. 270–316.

Häcker, U., and N. Perrimon. 1998. *DRhoGEF2* encodes a member of the Dbl family of oncogenes and controls cell shape changes during gastrulation in *Drosophila*. *Genes Dev.* 12:274–284.

Harden, N., M. Ricos, Y.M. Ong, W. Chia, and L. Lim. 1999. Participation of small GTPases in dorsal closure of the *Drosophila* embryo: distinct roles for Rho subfamily proteins in epithelial morphogenesis. *J. Cell Sci.* 112:273–284.

Kishi, K., T. Sasaki, S. Kuroda, T. Itoh, and Y. Takai. 1993. Regulation of cytoplasmic division of *Xenopus* embryo by *rho* p21 and its inhibitory GDP/GTP exchange protein (*rho* GDI). *J. Cell Biol.* 120:1187–1195.

Kosako, H., M. Amano, M. Yanagida, K. Tanabe, Y. Nishi, K. Kaibuchi, and M. Inagaki. 1997. Phosphorylation of glial fibrillary acidic protein at the same sites by cleavage furrow kinase and Rho-associated kinase. *J. Biol. Chem.* 272:10333–10336.

Kosako, H., H. Goto, M. Yanagida, K. Matsuzawa, M. Fujita, Y. Tomono, T. Okigaki, H. Odai, K. Kaibuchi, and M. Inagaki. 1999. Specific accumulation of Rho-associated kinase at the cleavage furrow during cytokinesis: cleavage furrow-specific phosphorylation of intermediate filaments. *Oncogene*. 18: 2783–2788.

Lang, P., F. Gesbert, J.-M. Thiberge, F. Troalen, H. Dutartre, P. Chavrier, and J. Bertoglio. 1993. Characterization of a monoclonal antibody specific for the ras-related GTP-binding protein RhoA. *Biochem. Biophys. Res. Commun.* 196:1522–1528.

Lee, S., R. Escalante, and R.A. Firtel. 1997. A Ras GAP is essential for cytokinesis and spatial patterning in *Dictyostelium*. *Development.* 124:983–996.

Lee, T., C. Winter, S.S. Marticke, A. Lee, and L. Luo. 2000. Essential roles of *Drosophila* RhoA in the regulation of neuroblast proliferation and dendritic but not axonal morphogenesis. *Neuron*. In press.

Mabuchi, I., Y. Hamaguchi, H. Fujimoto, N. Morii, M. Mishima, and S. Narumiya. 1993. A rho-like protein is involved in the organisation of the contractile ring in dividing sand dollar eggs. *Zygote.* 1:325–331.

Madaule, P., M. Eda, N. Watanabe, K. Fujisawa, T. Matsuoka, H. Bito, T. Ishizaki, and S. Narumiya. 1998. Role of citron kinase as a target of the small GTPase Rho in cytokinesis. *Nature.* 394:491–494.

Magie, C.R., M.S. Meyer, M.S. Gorsuch, and S.M. Parkhurst. 1999. Mutations in the Rho1 small GTPase disrupt morphogenesis and segmentation during early *Drosophila* development. *Development.* 126:5353–5364.

Moorman, J.P., D.A. Bobak, and C.S. Hahn. 1996. Inactivation of the small GTP binding protein Rho induces multinucleate cell formation and apoptosis in murine T lymphoma EL4. *J. Immunol.* 156:4146–4153.

Nishimura, Y., K. Nakano, and I. Mabuchi. 1998. Localization of Rho GTPase in sea urchin eggs. *FEBS Lett.* 441:121–126.

O'Connell, C.B., S.P. Wheatley, S. Ahmed, and Y. Wang. 1999. The small GTP-binding protein Rho regulates cortical activities in cultured cells during division. *J. Cell Biol.* 144:305–313.

Pines, J. 1999. Four-dimensional control of the cell cycle. *Nature Cell Biol.* 1:E73–E79.

Prokopenko, S.N., A. Brumby, L. O'Keefe, L. Prior, Y. He, R. Saint, and H.J. Bellen. 1999. A putative exchange factor for Rho1 GTPase is required for initiation of cytokinesis in *Drosophila*. *Genes Dev.* 13:2301–2314.

Rappaport, R. 1996. Cytokinesis in animal cells. Cambridge University Press, Cambridge. 386 pp.

Robertson, D., H.F. Paterson, P. Adamson, A. Hall, and P. Monaghan. 1995. Ultrastructural localization of Ras-related proteins using epitope-tagged plasmids. *J. Histochem. Cytochem.* 43:471–480.

Stam, J.C., and J.G. Collard. 1999. The DH protein family, exchange factors for Rho-like GTPases. *In* Cytoskeleton and Small G Proteins. P. Jeanteur, editor. Springer-Verlag, Berlin. 51–83.

Strutt, D.I., U. Weber, and M. Mlodzik. 1997. The role of RhoA in tissue polarity and Frizzled signalling. *Nature.* 387:292–295.

Suetsugu, S., H. Miki, and T. Takenawa. 1999. Distinct roles of profilin in cell morphological changes: microspikes, membrane ruffles, stress fibers, and cytokinesis. *FEBS Lett.* 457:470–474.

Takada, M., N. Morii, S. Kumagai, and R. Ryo. 1996. The involvement of the *rho* gene product, a small molecular weight GTP-binding protein, in polyploidization of a human megakaryocytic cell line, CMK. *Exp. Hematol.* 24: 524–530.

Takaishi, K., T. Sasaki, T. Kameyama, S. Tsukita, S. Tsukita, and Y. Takai. 1995. Translocation of activated *Rho* from the cytoplasm to membrane ruffling area, cell-cell adhesion sites and cleavage furrows. *Oncogene.* 11:39–48.

Tatsumoto, T., X. Xie, R. Blumenthal, I. Okamoto, and T. Miki. 1999. Human ECT2 is an exchange factor for Rho GTPases, phosphorylated in G2/M phases, and involved in cytokinesis. *J. Cell Biol.* 147:921–927.

Toenjes, K., M.M. Sawyer, and D.I. Johnson. 1999. The guanine-nucleotide-exchange factor Cdc24p is targeted to the nucleus and polarized growth sites. *Curr. Biol.* 9:1183–1186.

Tsubakimoto, K., K. Matsumoto, H. Abe, J. Ishii, M. Amano, K. Kaibuchi, and T. Endo. 1999. Small GTPase RhoD suppresses cell migration and cytokinesis. *Oncogene*. 18:2431–2440.

Tuxworth, R.I., J.L. Cheetham, L.M. Machesky, G.B. Spiegelmann, G. Weeks, and R.H. Insall. 1997. *Dictyostelium* RasG is required for normal motility and cytokinesis, but not growth. *J. Cell Biol.* 138:605–614.

Wasserman, S. 1998. FH proteins as cytoskeletal organizers. *Trends Cell Biol.* 8:111–115.

Watanabe, N., T. Kato, A. Fujita, T. Ishizaki, and S. Narumiya. 1999. Cooperation between mDia1 and ROCK in Rho-induced actin reorganization. *Nature Cell Biol.* 1:136–143.

Watanabe, N., P. Madaule, T. Reid, T. Ishizaki, G. Watanabe, A. Kakizuka, Y. Saito, K. Nakao, B.M. Jockusch, and S. Narumiya. 1997. p140mDia, a mammalian homolog of *Drosophila* diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin. *EMBO (Eur. Mol. Biol. Organ.) J.* 16: 3044–3056.

Whitehead, I.P., S. Campbell, K.L. Rossman, and C.J. Der. 1997. Dbl family proteins. *Biochim. Biophys. Acta.* 1332:F1–F23.

Yasui, Y., M. Amano, K. Nagata, N. Inagaki, H. Nakamura, H. Saya, K. Kaibuchi, and M. Inagaki. 1998. Roles of Rho-associated kinase in cytokinesis: mutations in Rho-associated kinase phosphorylation sites impair cytokinetic segregation of glial filaments. *J. Cell Biol.* 143:1249–1258.

Zalcman, G., O. Dorseuil, J.A. Garcia-Ranea, G. Gacon, and J. Camonis. 1999. RhoGAPs and RhoGDIs, (His)stories of two families. *In* Cytoskeleton and small G proteins. P. Jeanteur, editor. Springer-Verlag, Berlin. 85–113.