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Cell cycle regulation of Rho signaling pathways

Muriel David, Dominique Petit and Jacques Bertoglio*

Inserm U749; Institut Gustave Roussy; Villejuif, France

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The dynamics of the actin cytoskeleton and its regulation by Rho GTPases are essential to maintain cell shape, to allow cell motility and are also critical during cell cycle progression and mitosis. Rho GTPases and their effectors are involved in cell rounding at mitosis onset, in chromosomes alignment and are required for contraction of the actomyosin ring that separates daughter cells at the end of mitosis. Recent studies have revealed how a number of nucleotide exchange factors and GTPase-activating proteins regulate the activity of Rho GTPases during these processes. This review will focus on how the cell cycle machinery, in turn, regulates expression of proteins in the Rho signaling pathways through transcriptional activation, ubiquitylation and proteasomal degradation and modulates their activity through phosphorylation by mitotic kinases.

Biology of the Rho GTPase Pathways

During the past 20 years, GTP-binding proteins of the Rho family have been identified as essential players in many cellular functions, and the basics of their biology have been reviewed extensively.[1-3] This branch of the Ras super family encompasses 22 genes in humans, of which Rho, Rac and Cdc42 are the best characterized. Through regulation of the actin cytoskeleton, Rho GTPases control changes in cell morphology and cell motility triggered by extracellular stimuli (reviewed in refs. 4 and 5). Rho GTPases mediate these functions through a large array of effector proteins and are themselves regulated by GDP/GTP exchange factors (GEFs) and GTPase-activating proteins (GAPs).[6-8] At least 80 genes are present in mammalian genomes encoding Rho GEFs that are subdivided into proteins containing a DH-PH (Dbl homology, Pleckstrin homology) domain and the 11 members of the DOCK protein subfamily. GEF proteins bind the GDP-bound GTPases and function to accelerate the exchange of GDP for GTP, thus generating the active, GTP-bound conformation of the GTPase. On the other hand, a BLAST search using the SMART tool (http://smart.embl-heidelberg.de/) identifies more than 100 proteins (167 including multiple isoforms of some of them) containing a RhoGAP domain in humans, many of them uncharacterized. The GAP proteins act opposite to the GEFs by stimulating the rate of GTP hydrolysis and thus return the GTPase to its inactive, GDP-bound conformation. To add to the complexity, active (GTP-bound) Rho GTPases can bind to over 50 proteins that fit the definition of an effector and have been functionally characterized. Effector and target proteins often contain motifs that recognize the Rho proteins that are bound to GTP and are recruited or activated by the Rho GTPases. These effector/target proteins include many different functional families, such as serine/threonine protein kinases, lipid kinases and adaptor or scaffold proteins.[9] Depending on the cell type or the nature of the stimulus, a single Rho can be activated by several GEFs and, in turn, can trigger an array of various effectors. Now that most of the actors have been identified, if not fully characterized, the challenge is to understand how cells mobilize the appropriate set of Rho GTPases, GAPs, GEFs and effectors and organize them into specific signaling pathways to achieve defined cell functions.

Most of the cellular functions of the Rho GTPases stem from their ability to trigger actin polymerization and bundling of actin cables and therefore to remodel the cytoskeleton. By doing so, they are involved in the control of cell shape and morphology, cell migration and chemotactic responses, axonal guidance and dendrite outgrowth in neurons, endocytosis and intra-cellular vesicle trafficking.

In many instances, Rho GTPases have also been shown to regulate cell cycle entry and cell cycle progression, in particular by regulating expression of a number of genes involved in G_1/S transition, e.g., cyclin D1 or $p21^{waf1}$.[10] Rho GTPases are also critically involved in mitosis. Indeed, at mitosis onset, RhoA activity increases and the resulting activation of its effector, the Rho-associated kinase ROCK, mediates cortical retraction during mitotic cell rounding. During early mitosis, depending on the cell type, either the GEF-H1/RhoA/mDia1 pathway (Rat-2 cells) or the Ect2/Cdc42/mDia3 pathway (HeLa cells) are necessary for spindle assembly and attachment of microtubules to kinetochores.[11,12] Later in mitosis, Rho GTPases are directly involved in cytokinesis by regulating the actin and myosin contractile ring, which eventually forms the cleavage furrow to separate daughter cells.[13-18] One of the pathways that regulate Rho activity in this process involves the GEF Ect2 and the GAP MgcRacGAP. MgcRacGAP is active toward Rac and Cdc42 and, to a lesser extent, toward RhoA. Both Ect2 and MgcRacGAP localize in the nucleus of interphasic cells, associate to the spindle in metaphase and anaphase and accumulate at the midbody during cytokinesis.[19,20] MgcRacGAP associates with the kinesin-like protein MKLP1, resulting in a heterotetrameric complex designated centralspindlin, which is required for microtubule bundling.[21,22] In addition, Ect2 directly binds to MgcRacGAP in the centralspindlin complex.[18,22] However, as shown by mutations of the

*Correspondence to: Jacques Bertoglio; Email: jacques.bertoglio@igr.fr
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catalytic arginine residue in Drosophila or *C. elegans*, the GAP activity of MgcRacGAP may not be necessary for cytokinesis.[23,24] This GAP activity may thus be required to reduce the levels of Rac and Cdc42 in metaphase, whereas at the time of cytokinesis, MgcRacGAP might only act to recruit Ect2 and favor activation of RhoA. Ect2 and MgcRacGAP are not the only Rho regulators in mitosis and successful cytokinesis may also depend on GEF-H1/lfc,[11,25] MyoGEF[26] and p190RhoGAP (the –A isoform).[27] Downstream of the Rho GTPases, some of the effector proteins that have been described to participate in cytokinesis include ROCK, PKN, citron kinase and mDia, which activate myosin II and actin polymerization.[14,28-30] Most of the studies have used si-RNA mediated knockdown or overexpression in HeLa cells to describe these functions. However, different types of cells may vary in their requirements, and at this time it remains to be elucidated which cell type uses which combination of proteins to build the mitotic Rho regulatory pathways.

Thus, Rho GTPases exert critical functions during cell cycling. In this review we will discuss how, in turn, the cell cycle machinery influences Rho signaling pathways to regulate mitosis-specific functions.

Cell Cycle-Regulated Transcription of Rho Pathway Genes

GDP/GTP cycling stimulated by GEFs and GAPs represents the primary mode of regulation of small GTPases. However, in a few instances, it has been shown that cell stimulation or oncogenic processes may involve transcriptional or posttranslational regulation of expression of some GTPases, their effectors or their regulators. Examples of such regulations have been described for some atypical Rho GTPases, meaning these GTP-binding proteins such as Rnd3/RhoE, RhoH or RhoU/Wrch1 that are GTPase-deficient (for review see ref. 31). Indeed, because they are not regulated by the classical GDP/GTP conformation switch, atypical Rho proteins are often regulated at the level of expression: for instance, Wrch1 is induced by Wnt-1 signaling,[32] and RhoE is a p53 target gene that is induced in response to genotoxic stress.[33] More conventional GTPases may also be regulated at the transcriptional level: RhoB was initially characterized as an immediate early gene in growth factor responses and is also induced by TGFβ signaling.[34,35] RhoB mRNA was found to peak in the G_2 phase of cycling HeLa cells.[36] Another example is IQGAP1, an effector of Cdc42 involved in mitosis, which is also an early gene induced in response to TGFβ.[37] In addition, more pathway components than anticipated are regulated at the transcription level in response to growth factors[35,38,39] or during differentiation processes. For instance, ARHGEF8/NET1, DOCK5 and Wrch1 are increased during osteoclastic differentiation of RAW264.7 cells induced by the cytokine RANKL, and silencing either of these genes impedes differentiation.[40] Growth factor stimulation often leads to cell cycle entry and cell cycle progression, and indeed, expression of several GEFs and GAPs seems to correlate with cell cycle phases. In Interleukin 2-stimulated lymphocytes ARHGEF3/XPLN, a GEF specific for RhoA and RhoB, is induced early in the cell cycle (G_1), whereas other GEFs, such as NET1 or Ect2, are induced later, with peak protein expression in G_2/M.[39] Unfortunately, except for Ect2 (see below), the consequences of these phase-specific variations of expression have not yet been investigated.

It is of interest that the levels of expression of the Rho GTPase regulators that are known to be involved during mitosis, such as Ect2, MgcRacGAP, p190RhoGAP and GEF-H1, fluctuate during the cell cycle and reach their highest in G_2/M.[19,27,41] The transcription of Ect2 and MgcRacGAP is induced when synchronized cells enter S-phase. Chromatin immunoprecipitation experiments indicated that the promoters of these two genes contain binding sequences for E2F1 and CUX1 another cell cycle-related transcription factor.[41,42] Thus transcription of Ect2, MgcRacGAP, but also of the centralspindlin component MKLP1 at the G_1/S transition or in early S provides a rationale for the high protein expression in G_2/M. Also identified within the promoter sequences of these genes are CHR elements that mediate repression of these promoters in the G_1 phase of the cell cycle and contribute to reduce the level of expression after mitosis. RhoBTB2, an atypical GTPase of the Rho family, is also a transcriptional target of E2F1; its mRNA is induced in S phase, and its protein level peaks in G_2/M.[43] Although this has not been reported, it is not unlikely that cell cycle variations of the protein levels of p190RhoGAP might also be, at least in part, due to transcriptional regulation.

One experimental procedure that has been used extensively to analyze the role of the Rho pathways in mitosis is gene silencing through siRNA knockdown. Silencing of either RhoA, any of the GEFs Ect2, GEF-H1, MyoGEF, or of the GAPs MgcRacGAP or p190RhoGAP results in multinucleated cells. One physiological condition, however, where failed cytokinesis is the rule, rather than an accident, is the differentiation of megakaryocytes, the hematopoietic cells that give rise to blood platelets. During their differentiation, megakaryocytes become polyploid by a process named endomitosis. Activation of ROCK by RhoA has been known for a long time to be involved in the function of mature platelets, namely, aggregation and mediator release, but it also controls the fragmentation of megakaryocyte cytoplasm, which releases platelets.[44] In addition, downregulation of this Rho/ROCK pathway is required to prevent non-muscle myosin IIA activation at the cleavage furrow to abort ring contraction and allow endomitosis.[45] It was recently reported that these events are under the control of the megakaryocyte transcription factor MKL1, also known as MAL, which represses the transcription of Ect2 and GEF-H1 as cells undergo differentiation.[46] Indeed, exogenous expression of either Ect2 or GEF-H1 forces megakaryocytes to complete cytokinesis, prevents endomitosis and leads to proliferation of 2N megakaryocytes.

Ubiquitylation of Rho Signaling Proteins

Besides transcriptional regulation, many proteins that play key roles during mitosis and cytokinesis are further controlled by ubiquitin-mediated proteasomal degradation.[47] The E3 ubiquitin ligase that is critical at the onset of mitosis is the anaphase-promoting complex/cyclosome (APC/C). Indeed, APC/C mediates

ubiquitylation, thus triggering subsequent proteasome-dependent degradation of key proteins, such as Securin or Cyclins A and B, allowing progression from prometaphase to mitotic exit.[48] The APC is fully active as a ubiquitin ligase once it has bound to its co-activators Cdc20 or Cdh1, resulting in distinct assemblies named APC/Cdc20 or APC/Cdh1. APC/Cdc20 activity is critical for metaphase/anaphase transition, whereas APC/Cdh1 is fully active in late mitosis and G_1 phase. Several lysine residues (K-11, K-48, K-63) of ubiquitin can be used to build polyubiquitin chains, although the APC appears to preferentially assemble K-11 linked polyubiquitin chains to target the complex to the proteasome.[49-51]

The Rho GTPases have been known for some time to be ubiquitylated under certain circumstances, for example, following action of bacterial toxins, and the responsible E3 ubiquitin ligases have been identified.[52-56] The Rac exchange factor DOCK180 is ubiquitylated in response to EGF, depending upon its interaction with the adaptor Crk, and protected from degradation by its association with the scaffold protein Elmo.[57] Thus control of the protein levels by degradation is an integrate part of the regulation of the Rho pathways.

Not surprisingly, ubiquitylation and post-mitotic degradation also target some of the Rho pathway components involved in mitosis (**Table 1**). The protein levels of p190RhoGAP-A decrease in late mitosis through ubiquitin-mediated degradation.[59] Interestingly, expression of non-degradable mutants of p190RhoGAP in HeLa cells results in multinucleation, indicating that reduced levels of p190RhoGAP are required before cytokinesis is completed. As p190RhoGAP acts on RhoA, reducing GAP activity would allow for maintaining the levels of RhoGTP throughout cytokinesis. The other known RhoGAP which is involved in the process, MgcRacGAP, was also observed to be degraded at this time following ubiquitylation by APC/Cdh1 (Bertoglio et al., unpublished). It was also found that Ect2 is a substrate of APC/Cdh1, and its poly-ubiquitylation sends it to degradation at the end of mitosis.[58] Nuclear localization of Ect2, mediated through a bi-partite NLS, is required for this event to occur. Also critical is a region toward the C terminus of Ect2 that contains TEK-like boxes, which have been described by Jin and colleagues as novel motifs that facilitate ubiquitin chain nucleation in APC substrates.[49] Although a precise comparison of the timing of protein degradation is hard to achieve with the current protocols used for synchronizing cells, it would appear that Ect2 is degraded later than MgcRacGAP. To our knowledge, mDia2, a member of the formin family that contributes to contractile ring formation, is the only effector protein that has been described to undergo degradation in mitosis in mammalian cells.[61] In yeast, the paralog of IQGAP1, a Cdc42 effector, is also degraded by APC/Cdh1 at the end of cytokinesis.[62]

Table 1. Ubiquitylation of Rho signaling proteins

	Target	Ligase	References
Rho GTPases	RhoA, Rac1, Cdc42	Smurf-1,Hace-1 XIAP/cIAP1/2	For review, refs. 55, 56
GEFs	Ect2	APC/Cdh1	58
	Dock180	(Cbl ?)	57
GAPs	P190RhoGAP	APC/Cdh1	59, 60
	MgcRacGAP	APC/Cdh1	Bertoglio et al., unpublished
Effectors	mDia2	(APC?)	28
	IQGAP1 (yeast)	APC/Cdh1	62

Phosphorylation of Rho Signaling Proteins by Mitotic Kinases

A shared mode of regulation of many GEFs, GAPs or Rho effectors is through protein phosphorylation. Most often protein phosphorylation on tyrosine and/or serine threonine residues controls the subcellular localization of these proteins as well as protein-protein interactions. In addition, there are many examples where phosphorylation induces a switch from an autoinhibitory to an active conformation. This has been well-documented for GEFs such as Ect2, and it appears to be the rule for effector kinases like PAK or Rho kinases that autophosphorylate upon binding the active GTPase.[9,63] Some of the kinases that target Rho regulatory proteins are still under characterization. Thus, given the scope of this review, we will only expand here on the role of the mitotic kinases.

Mitosis progression is indeed regulated by the activity of a number of kinases that comprise, among others, Cdk1, Aurora A and B kinases and Polo kinases.[61,64] Their roles in mitosis progression are fairly well understood, even though some of their direct targets remain to be identified. Proper mitotic processes require spatio-temporal coordination of activation of the mitotic kinases. Before mitosis entry, Cdk1 is partitioned between cytoplasm and nucleus. Activation of Cdk1 by the Cdc25 phosphatase (by dephosphorylation of T14 and Y15 of Cdk1) at the G_2/M transition allows mitosis entry. Cdk1 phosphorylates various substrates such as histones, nuclear lamins, proteins interacting with microtubules and thus controls the condensation of chromosomes, nuclear envelope breakdown and spindle assembly. Following breakdown of the nuclear membrane, active Cdk1 is localized throughout the cytoplasm, with a strong enrichment on centrosomes and, to a certain extent, on kinetochores and spindle microtubules.[65] At this stage, Aurora A and Plk1 localization is similar to that of Cdk1.[66] At metaphase-anaphase transition, Cdk1 activates the APC/C that induces ubiquitin-dependent degradation of Securin and activation of Separase as well as degradation of Cyclin B1, which, in turn, downregulates Cdk1 activity. Cdk1 inactivation is required for Aurora B relocalization from kinetochores to the spindle midzone.[67] Similarly, Plk-1, which was localized at centrosomes until anaphase onset, relocalizes to the spindle midzone following Cdk1 inactivation.

It has only been recently recognized that these mitotic kinases also participate in regulating the Rho pathways that are active during mitosis (**Table 2**). At mitosis onset, RhoA activity increases and the resulting activation of its effector ROCK is required for cortical retraction during mitotic cell rounding.[70] Inhibition of the p190RhoGAP-A through its phosphorylation by Cdk1 may contribute to this increase in RhoA activity.[70] In addition, three

Table 2. Phosphorylation of Rho signaling proteins by mitotic kinases

Mitotic kinase	Target	Residues	Notes	References
Cdk1	Ect2	T341	Induces conformation change of Ect2 that promotes GEF activity Occurs in metaphase; Inhibits binding to MgcRacGAP Dephosphorylated upon anaphase onset	18, 63
		T814	Slightly increases GEF activity on Rac1 Prevents Ect2 recruitment to plasma membrane prior to anaphase onset	17, 68
		T412	Required for Ect2/Plk-1 interaction Increases GEF activity Induces excessive membrane blebbing	17
	GEF-H1	S959	Inhibits GEF activity; dephosphorylated prior to cytokinesis	25
	MgcRacGAP	T588	NR	69
	PRK2/PKN2	NR	NR	30
	P190RhoGAP	NR	Downregulates GAP activity	70
	IQGAP1	Multiple	Described in yeast	71
Plk-1	Ect2	(BRCT domain)	NR	17, 72
	MgcRacGAP	S170	Phosphorylation increases throughout anaphase Promotes the onset of cytokinesis	72, 73
		S157 S149 S164 S214 S260	Phospho-dependent recruitment of Ect2 to the spindle midzone	72, 73
		S410	NR	74
	Rock2	T967, S1099, S1133, S1374 T489	Plk1-dependent Rock2 phosphorylation activates Rock2	75
	MyoGEF	T574	Required for MyoGEF localization to the spindle. Increases GEF activity toward RhoA.	26
Aurora A	GEF-H1	S885	Inhibits GEF activity Dephosphorylated prior to cytokinesis Allows interaction with 14–3-3	25, 76
Aurora B	MgcRacGAP	S387	From telophase to cytokinesis. Renders the GAP active on RhoA	74
		S144, T145, S185, T186, S187	These residues are phosphorylated during metaphase Occurs at the spindle Phosphomimetic mutants escape inhibition by PRC1 with respect to MgcRacGAP activity on CDC42	74
	mDia3	S196, T882, T66, S820	Phosphorylation of T66 and S196 releases autoinhibition. Phosphorylation of S820 and T882 is involved in FH2 domain function. Globally, phosphorylation of mDia3 reduces binding to microtubules and microtubule stabilization. Non-phosphorylatable mutants induce chromosome misalignment.	77

NR, not reported

proteins with GEF activity on RhoA have been shown to play a role in mitosis: Ect2, GEF-H1 and MyoGEF. In early mitosis, GEF-H1 is maintained inactive through Aurora A- and Cdk1-mediated phosphorylation.[25] In contrast, Ect2 phosphorylation at T341 and T412 by Cdk1 increases the GEF activity of Ect2, through release of the intramolecular interaction between the N-terminal BRCT domains and the catalytic DH-PH region of Ect2.[63,78] This increase in Ect2 GEF activity probably accounts for most of the activation of RhoA at mitosis entry. During early mitosis, however, Cdk1-mediated phosphorylation of T814 and

T341 of Ect2 limits the recruitment of Ect2 to the plasma membrane and prevents precocious binding of Ect2 to MgcRacGAP.[68] These two residues will be dephosphorylated upon anaphase onset.

In prometaphase and metaphase, either the GEF-H1/RhoA/mDia1 pathway (Rat-2 cells) or the Ect-2/Cdc42/mDia3 pathway (HeLa cells) is necessary for spindle assembly and attachment of microtubules to kinetochores.[11,12] In the second model, Aurora B-mediated phosphorylation of Rho signaling proteins plays a major regulatory role. First, Aurora B phosphorylates MgcRacGAP at multiple sites.[74] During metaphase, the mitotic spindle-associated protein PRC1 has been found to bind to MgcRacGAP and to inhibit its GAP activity toward Cdc42.[13,79] The phosphorylation of MgcRacGAP by Aurora B might disrupt the PRC1/MgcRacGAP complex and thus de-repress MgcRacGAP activity on Cdc42. Thus, the balance between PRC1-mediated inhibition and Aurora B-mediated de-repression of MgcRacGAP activity on Cdc42 seems required for adequate formation of the spindle. Second, Aurora B phosphorylates kinetochore-located mDia3 at T66 and S196 which releases mDia3 autoinhibition.[77] In addition, phosphorylation of S820 and T882 promotes function of the FH2 domain of mDia. Proper phosphorylation of mDia on these sites seems critical for chromosome alignment at the metaphase plate as well as formation of stable kinetochore microtubule fibers. These regulatory phosphorylation events must be fine-tuned, since a nonphosphorylatable mDia3 mutant prevents chromosomes alignment at the metaphase plate in contrast to the phosphomimetic mutant that alters formation and stability of the kinetochore fibers.

Upon anaphase onset, MgcRacGAP associates with the kinesin MKLP1 to form the centralspindlin, which is required for microtubule bundling.[21,22] Until the metaphase-anaphase transition, Cdk1-mediated phosphorylation negatively regulates the functions of central spindle proteins, such as PRC1 and MKLP-1.[80,81] These regulatory mechanisms include phosphorylation of PRC1, which prevents formation of the PRC1/Plk1 complex and the relocalization of Plk1 to the central spindle[82] as well as phosphorylation of MKLP1, which prevents it from interacting with microtubules.[80,81] In parallel, Aurora B activity impacts on the targeting of centralspindlin to the midzone by phosphorylating MKLP1.[83,84]

One of the functions of the centralspindlin is to trigger recruitment of Ect2 to the midzone in a Plk1-dependent manner.[13,18,85] Ect2 induces subsequent activation of RhoA at the cell equator, which, in turn, regulates the actin and myosin contractile ring that eventually forms the cleavage furrow to separate daughter cells.[13-18] Recruitment of Ect2 onto the centralspindlin complex is prevented, until the metaphase-anaphase transition, by Cdk1-mediated phosphorylation of Ect2 on T341.[18] Thus, dephosphorylation of this residue after anaphase onset has a permissive effect. In addition, Plk1 phosphorylates MgcRacGAP to promote its interaction with Ect2 and recruitment of Ect2 to the spindle midzone.[72,73]

Ect2 is probably not the only GEF that is involved in RhoA activation during cytokinesis. Indeed, two other GEF active toward RhoA whose RNA interference-mediated knockdown induces failed cytokinesis and cell multinucleation are MyoGEF/PLEKHG6 and GEF-H1. As for Ect2, activity and localization of these GEFs are regulated by phosphorylation events. Indeed, Plk1 has been shown to phosphorylate MyoGEF on threonine 574 to control its localization to the spindle and increase its exchange activity.[26] In addition, GEF-H1 phosphorylations by Aurora and Cdk1 maintain it inactive until just before cytokinesis, at which time it is dephosphorylated and activates RhoA.[25]

Thus, it appears that all of the regulators of Rho GTPases that participate in mitosis are, one way or the other, under the control of mitotic kinases. In addition, several of the downstream effectors of Rho that have been shown to participate in cleavage furrow positioning and constriction can also be substrates for the mitotic kinases. For instance, Plk-1 associates with ROCK2 specifically during mitosis and phosphorylates it on at least four identified residues to enhance its kinase activity in response to RhoA.[75] Plk-1 also binds ROCK1 and citron, although it is still unclear whether it phosphorylates these two proteins. Although the evidence is indirect, it has also been suggested that PRK2 may be phosphorylated by Cdk1 in mitosis.[30]

Miscellaneous

Cdk1 is the orchestrator of mitosis entry and progression and is tightly regulated by endogenous inhibitors (such as $p21^{Cip}$ or $p27^{Kip}$) and activators (such as Cyclins A and B). These proteins that directly affect Cdk1 activity could thus be viewed as indirect regulators of the Rho pathways in mitosis. It is, however, surprising that recent reports indicate that direct effects of $p27^{Kip}$ or cyclin A on the Rho pathways are, in both cases, independent of Cdk1 activity. Cyclin A2, whether wild-type or mutated in its Cdk1 interaction domain, was found to directly associate with RhoA and promote its activation, resulting in actin remodeling.[86] Similarly, $p27^{Kip1}$ binds citron kinase and prevents its activation by RhoA, leading to failed cytokinesis and a multinucleation phenotype. This phenotype was also observed with a form of p27 that is unable to bind or inhibit cyclin-CDK complexes.[87] In both these reports, the experimental setting that lead to these observations does not allow conclusions as to whether these regulatory circuits are indeed involved in or of any importance during normal cell cycling. They may, however, be critical in the context of tumor cells that display deregulated expression of these proteins.

Concluding Remarks

As described above, several proteins of the Rho pathways that contribute to mitosis are regulated both at the transcriptional level and by ubiquitin-dependent degradation. In this respect, such proteins of the Rho pathway are regulated just as cyclins are. Obviously, these mechanisms are efficient and reliable ways for cells to make sure these proteins are present at the time their activity is required and absent later on. Considering that activities of most of these proteins are also regulated by phosphorylation, why do cells need them to be degraded after mitosis? One hypothesis is that expression of these proteins during inappropriate phases of the cell cycle may be detrimental for the cell.

Table 3. Examples of other Rho signaling proteins whose expression vary during cell cycle progression and/or undergo cell-cycle regulated phosphorylation [a]

GEFs	GAPs	Effectors
ECT2	RACGAP1(MgcRacGAP)	CIT
ARHGEF1	ARHGAP1	DIAPH1
ARHGEF5	ARHGAP5	PAK1
ARHGEF6	ARHGAP11A	PAK2
ARHGEF7	ARHGAP12	PAK3
ARHGEF11	ARHGAP17	PAK4
ARHGEF12	ARHGAP21	PKN1
ARHGEF16	ARHGAP29	PKN2
ARHGEF17	CENTD2	ROCK1
ARHGEF18	IQGAP1	ROCK2
DOCK5	IQGAP2	
DOCK7		
DOCK8		
DOCK9		
ITSN1		
ITSN2		
PLEKHG3		
PLEKHG4		

[a]Compiled from references 36, 89 and 90 or from the websites www.phosphosite.org, www.phosida.de and www.cyclebase.org

This could be the case for Ect2, as non-degradable mutants of this RhoGEF have been shown to promote cell transformation.[58] Another possibility is that degradation of these proteins is actually required for cytokinesis to be completed. Expression of non-degradable mutants of p190RhoGAP, constitutively active RhoB, deregulated expression of mDia2 or stable versions of IQGAP1 (in yeast) all lead to cell multinucleation, likely by preventing dismantling of the intercellular bridge linking daughter cells and abscission. In fact, yeast cells with genetic knockdown of the APC activator Cdh1 display abscission defects that result from failed disassembly of the contractile actin ring.[88]

Given the large number of yet-uncharacterized GEFs and GAPs, it would not be unexpected if others than those discussed above turned out to also be regulated in a cell cycle-dependent manner. Large scale gene expression and proteomics analyses in various systems will identify new cell cycle-dependent variations of members of the Rho pathways, which will provide new hints at their functions, for example by analyzing those that are co-regulated. In addition, searching public databases and supplemental information that nowadays come with most publications, indeed yields a huge amount of yet-unexploited expression or phosphorylation data (**Table 3**). One of the widely used models in these studies is the human HeLa cell line. Of course, it should be understood that HeLa cells are not real life, and if indeed it helped in developing new concepts and methodologies, it still will be necessary to carefully analyze the Rho signaling pathways in normal cells of various lineages.

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