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Myosin light chain kinases and phosphatase in mitosis and cytokinesis

Fumio Matsumura*, Yoshihiko Yamakita, and Shigeko Yamashiro

Department of Molecular Biology & Biochemistry, Rutgers, 604 Allison Road, Piscataway, NJ 08854 USA

Summary

At mitosis, cells undergo drastic alterations in morphology and cytoskeletal organization including cell rounding during prophase, mitotic spindle assembly during prometaphase and metaphase, chromatid segregation in anaphase, and cytokinesis during telophase. It is well established that myosin II is a motor responsible for cytokinesis. Recent reports have indicated that myosin II is also involved in spindle assembly and karyokinesis. In this review, we summarize current understanding of the functions of myosin II in mitosis and cytokinesis of higher eukaryotes, and discuss the roles of possible upstream molecules that control myosin II in these mitotic events.

Keywords

myosin II; kinases; phosphatase; mitosis; cytokinesis

Introduction

At mitosis, cultured animal cells show the most drastic alterations in cytoskeletal organization. Adherent cultured cells become rounded during prophase, concomitant with the disassembly of stress fibers. The microtubule cytoskeleton in interphase cells is re-organized to assemble the bipolar mitotic spindle, which captures sister chromatids and aligns them at the metaphase plate. Mitotic cells have a mechanism that places the mitotic spindle at the geographical center of the cell horizontal to the substrate and mostly parallel to the long axis of the mitotic cell. Just after chromatid segregation, cells undergo cytokinesis by assembling the contractile ring. The assembly and activation of the contractile ring are precisely regulated both in time and space: Contractile ring assembly must occur at the equator of the cortex only after sister chromosomes separate. It is well established that signals initiating cytokinesis come from the central spindle (see [1] for review). The central spindle contains a number of the signaling molecules essential for cytokinesis, which include the MKLP1 (CHO1, orthologue of *Pavarotti* or *ZEN-4*)/MgcRacGAP (*CYK-4*) complex, the AuroraB/INCENP/Survivin complex, PRC1, ECT2 (RhoGEF, *Pebble* and the PLK1/MKLP2 (or PLK1/MKLP1) complex. The central spindle is assembled between two separating chromatids, thereby coordinating the sister chromatid segregation and cytoplasmic division.

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*Corresponding author, matsumura@biology.rutgers.edu, Tel: 1-732-445-2838, Fax: 1-732-445-4213.

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Myosin II plays several roles in these processes. First, myosin II is a motor responsible for cytokinesis [2, 3]. Second, myosin II must control cell rounding during prophase because stress fiber disassembly depends on dephosphorylation of the regulatory light chain of myosin II (RMLC) [4, 5]. Third, evidence has recently been accumulated that myosin II is involved in mitotic functions including mitotic spindle positioning and karyokinesis. In this review, we summarize recent progress on the roles of myosin II in mitosis and cytokinesis, and discuss phospho-regulatory systems that control RMLC phosphorylation. We focus on myosin II in higher eukaryotes including vertebrate cells, *C. elegans* and *Drosophila* because nonmuscle myosin II in these cells is mainly regulated by RMLC phosphorylation [6–10].

1. Functions of myosin II in mitosis

A considerable body of evidence has suggested that myosin II plays roles in mitosis including spindle assembly and positioning, cell fate determination, and karyokinesis. All these mitotic processes are interrelated. Spindle orientation, for example, is critical for the regulation of polarity establishment in early embryogenesis, as well as self-renewal and differentiation of stem cells [11–14]

1.1 Spindle assembly and positioning

Many reagents that directly or indirectly inhibit myosin II functions have been reported to affect spindle assembly, orientation and positioning [15–18]. Rosenblatt *et al.* showed that blebbistatin, a myosin II inhibitor, blocked separation of centrosomes, resulting in perturbation of spindle assembly and positioning [15]. Similar perturbation was observed by treatment with Y-27362, a ROCK (also called Rho-kinase) inhibitor, which inhibits myosin II by activating myosin phosphatase. The depletion of myosin II by siRNA also caused similar defects in spindle positioning, supporting the concept that myosin II is a target of these drug inhibitors. While the exact mechanism by which myosin II alters spindle positioning is not well established, it has been proposed that myosin at the cortex somehow controls the interactions between astral microtubules and microtubule motors including dynein. It should be noted that the myosin II-mediated perturbation of spindle orientation is dependent on the timing of nuclear envelope disassembly: If the nuclear envelope stayed intact during assembly of the mitotic spindle, spindle mis-orientation did not occur. The explanation for this difference is that separating centrosomes could use the nuclear membrane as an anchor and move in opposite directions to assemble the bipolar spindle. Myosin II inhibition also appears to affect the positioning of the meiotic spindle, as well as the formation of the second polar body [19].

1.2. Karyokinesis

Defects of spindle assembly resulting from myosin II inhibition cause defective karyokinesis. Indeed, a very recent report by Ma *et al.*, [20] has presented convincing evidence for the role of myosin II in karyokinesis: Knockout of myosin IIB and IIC in cardiac myocytes of mice embryos resulted in severe karyokinesis defects. Mammalian cells have three distinct isoforms of nonmuscle myosin II (myosin IIA, IIB and IIC) [21]. As cardiac myocytes express myosin IIB and IIC but not myosin IIA, knockout of myosin IIB and IIC resulted in complete loss of nonmuscle myosin II. The authors demonstrated that the myosin II null myocytes exhibited severe spindle assembly defects, which was apparently due to more stabilized microtubules (as evidenced by increased tubulin acetylation). The karyokinesis defect was also observed with HL-1 cultured cells that were depleted of myosin IIA by siRNA treatment, indicating that the karyokinesis defect appears to be a direct effect of myosin II ablation and is independent of myosin isoform. As HL-1 cells are from atrial sources, the karyokinesis defect appeared to be specific for cells of atrial origin. It is

unknown, however, how myosin II ablation resulted in the increased acetylation of microtubules.

1.3. Cell fate determination

Actin and myosin II have been reported to be necessary for the asymmetric segregation of cell fate determinants (such as Numb and Pon) in the *Drosophila* nervous system [22] and this actin-dependent localization of Numb required AuroraA kinase [23]. Because myosin II is often suggested to be responsible for the cortical flow, lateral transport of cell fate determinants along the cortex by myosin II was suggested to be responsible for the asymmetric distribution of Numb and Pon. However, FRAP analyses revealed that this seemed not to be the case. Rather, preferential binding of cytoplasmic Numb and Pon to the cortex appeared to explain their asymmetric localization [24]. It is thus unclear how actin and myosin are involved in the preferential localization of these cell fate determinants.

1.4. Upstream signaling molecules

What are the upstream signaling pathways that control myosin II-mediated spindle orientation? Recent reports have suggested several candidate kinases. These include LKB1 and its downstream effector kinase AMPK (AMP-activated protein kinase), and Tousled-like kinase1 (TLK1, also called protein kinase ubiquitous, PKU-beta). These kinases have been reported to control spindle assembly, as well as karyokinesis, by directly or indirectly controlling RMLC phosphorylation. In addition, we have shown that myosin phosphatase is involved in spindle positioning [25].

- a. **LKB1 and AMPK:** LKB1 and its downstream effector kinase, AMPK, are involved in the response to energy deprivation. Recently, they have also been implicated in the control of spindle assembly, cell division and cell polarity in *Drosophila*, as well as in mammalian cells (see [26, 27] for review). Depletion of LKB1, AMPK or AMPK-related kinase resulted in spindle abnormality and defects in chromosome alignment [28]. Consistently, a *Drosophila* null mutant of AMPK was lethal with defects in cell polarity and mitosis (leading to polyploidy) [29], the phenotypes of which are similar to LKB1 mutation [28, 30]. Furthermore, an active form of AMPK has been reported to be associated with centrosomes [31]. Importantly, AMPK directly phosphorylated RMLC at its activating sites, and a phosphomimetic mutant of RMLC rescued null phenotypes of AMPK. Taken together, these studies indicate that the LKB-AMPK pathway controls mitosis via RMLC phosphorylation [29].
- b. **TLK1:** Tousled-like kinase1 (TLK1, also called protein kinase ubiquitous, PKU-beta), while involved in chromatin remodeling and DNA replication, appears to play a role in mitosis of human cells [32]. Depletion of TLK1 resulted in defects in chromatid segregation, which was accompanied by reduction of RMLC phosphorylation. Interestingly, a phosphomimetic mutant of RMLC rescued this defect, suggesting that TLK1-mediated chromatid segregation occurs via myosin II regulation. The exact mechanism remains to be clarified because TLK1 itself did not directly phosphorylate RMLC at Ser18/Thr19.
- c. **Myosin phosphatase:** We have demonstrated that myosin phosphatase targeting subunit1 (MYPT1) controls spindle orientation [25]. Depletion of MYPT1 in HeLa cells resulted in oblique (not horizontal to the substrate) chromatid segregation (Fig. 1). There are two possible explanations for this phenomenon. One is that depletion of MYPT1 raises myosin II activity at the cortex, increasing the contractility of the cortex. Such an increase in cortical tension could change the interactions between microtubules and microtubule motors like dynein at the

cortex, which may result in spindle misorientation or rotation. The other possibility is that myosin phosphatase affects spindle orientation via altering the activity of Polo-like kinase1 (PLK1). We have demonstrated that MYPT1 was associated with PLK1, as a consequence of mitosis-specific phosphorylation of MYPT1 by Cdk1 during mitosis [25]. The mitosis-specific association was also shown by proteomic screening of proteins that bind to the polo-box domain of PLK1 [33]. Importantly, we found that myosin phosphatase antagonized PLK1 [25]. While PLK1 depletion is known to arrest mitosis, simultaneous depletion of MYPT1 and PLK1 rescued the mitotic arrest caused by PLK1 depletion (Fig. 1A). Furthermore, MYPT1 depletion resulted in an increased phosphorylation of PLK1 at its activation site of Thr210 *in vivo*. *In vitro*, purified myosin phosphatase was able to dephosphorylate Thr210-phosphorylated PLK1. Taken together, these results suggest that myosin phosphatase antagonizes PLK1 by directly dephosphorylating PLK1 at its activation site (see the model in Fig. 2) although we cannot exclude an alternative possibility that myosin phosphatase may dephosphorylate PLK1 substrates, thereby counterbalancing PLK1 at its substrate phosphorylation levels. Depletion of MYPT1 would thus result in hyper-activation of PLK1 and/or hyper-phosphorylation of PLK1 substrate. Because one of the targets of PLK1 is dynein [34–36], MYPT1 depletion may aberrantly activate dynein, resulting in spindle mis-orientation and oblique chromatid segregation.

The notion that PLK1 is hyper-activated by MYPT1 depletion is further supported by our recent observation: We found that MYPT1 depletion in metaphase-arrested HeLa cells resulted in precocious chromatid segregation [37]. The precocious segregation required PLK1 activity, and was blocked by the expression of an unphosphorylatable mutant of SA2 (SCC3 homologue 2), a subunit of the cohesin complex and a substrate of PLK1. Because SA2 phosphorylation at centromeres leads to chromosome separation without cohesion cleavage [38–40], these results suggest that myosin phosphatase blocks hyper-activation of PLK1, thereby preventing precocious chromatid segregation before the onset of the metaphase-anaphase transition.

The antagonism between PLK1 and myosin phosphatase may suggest the interesting possibility of a feedback system involving ROCK, PLK1 and myosin phosphatase. If myosin phosphatase were inactivated, PLK1 would be active. Because PLK1 phosphorylates and activates ROCK [33], PLK1, once activated, would increase ROCK activity, which would result in the further inhibition of myosin phosphatase, leading to more activation of PLK1 (Fig. 3A). On the other hand, myosin phosphatase, once activated, would inhibit PLK1, resulting in lower ROCK activity, leading to further activation of myosin phosphatase (Fig. 3B). This feedback system could explain why ROCK inhibition caused spindle mis-orientation: Inhibition of ROCK would activate myosin phosphatase, which would inhibit PLK1, resulting in dynein inhibition and spindle assembly defects. However, it should be noted that this system is not able to explain why myosin inhibition alone caused spindle defects.

2. Regulation of myosin II in cytokinesis

A generally accepted paradigm of the cytokinesis mechanism may be as follows: PLK1 promotes the association between ECT2 (RhoGEF) and MgcRacGAP (also called Cyk4), allowing ECT2 localization to the equatorial plane, which activates RhoA, leading to activation of RhoA-dependent kinases, ROCK and citron kinase, at the cleavage furrow (see [41] for review). This results in myosin II phosphorylation on its RMLC and initiation of cleavage furrowing. The observations that expression of an unphosphorylatable mutant of the RMLC increases multinucleated cells [42, 43] indicate a critical role of myosin regulation in cytokinesis. However, the above model may be over-simplified. For example,

at least two additional RhoGEFs, myoGEF and GEF-H1, appear to control RhoA activity during cytokinesis [44–46] though it is unknown whether these three GEFs have redundant or specific functions. Furthermore, myosin II activity appears to be controlled by multiple pathways in addition to the Rho/ROCK/citron kinase pathway (see [47] for review). For example, ROCK inhibition retarded the rate of cleavage furrowing but did not block cytokinesis of mammalian cells [43, 48]. Multiple layers of myosin regulation may also explain why cytokinesis is a robust process [49]. In this section, we will discuss the roles of possible upstream molecules that control myosin II in cytokinesis.

2.1. ROCK and Citron kinase

Both ROCK and citron kinase are activated by RhoA, placing them as ideal downstream kinases of RhoA [50–53]. Indeed, both kinases are localized at the cleavage furrow [50, 54, 55]. ROCK inhibits myosin phosphatase by phosphorylating MYPT1 [51]. In addition, ROCK is also able to directly phosphorylate RMLC at its activation sites [5, 16, 56]. Citron kinase, on the other hand, does not inhibit myosin phosphatase but it directly phosphorylates RMLC [57]. Inhibition of ROCK by a selective inhibitor, Y-27632, retarded cleavage furrow ingression but did not block cytokinesis in mammalian cells [43, 48]. This result is consistent with a genome-wide screening with mammalian cells [58], which did not identify ROCK as a cell division gene (<http://www.mitocheck.org/>). Similarly, ROCK depletion resulted in retardation of cytokinesis in *C. elegans* [59]. ROCK seems to have a more significant role in *Drosophila* because ROCK depletion in *Drosophila* S2 cells by siRNA resulted in multinucleated cells [60]. Depletion of citron kinase resulted in multinucleated cells in both mammalian cells and *Drosophila* [58, 60, 61]. However, citron kinase did not appear to block the initiation of cleavage furrow ingression, rather it is involved in the completion of cytokinesis [62]. This role of citron kinase in cytokinesis is cell type specific in mammalian cells because cytokinesis failure is limited to certain brain tissues in citron kinase KO mice [61].

There is a discrepancy in the role of ROCK in cell division of sea urchin eggs. Lucero et al. demonstrated that both MLCK and ROCK were necessary for the successful completion of cytokinesis [63]. Uehara et al., on the other hand, suggested that ROCK is not involved in cytokinesis in sea urchin eggs because they observed that ROCK inhibition by Y27632 showed no effect on RMLC phosphorylation and cytokinesis [64]. Interestingly, H1152, another ROCK inhibitor, blocked both RMLC phosphorylation and contraction of the contractile ring, suggesting that another molecule(s) inhibited by H1152 but not by Y-27632 is involved in RMLC phosphorylation in sea urchin eggs [64].

2.2. MLCK

MLCK's role in cytokinesis appears to be species specific. Sea urchin eggs apparently use MLCK as a major RMLC kinase in cell division. Both Lucero et al. and Uehara et al. reported that inhibition of MLCK blocked assembly and contraction of the cleavage furrow in sea urchin eggs [63, 64]. In *C. elegans*, however, MLCK has been reported to play no major role in cytokinesis [65]. In insect cells, there are conflicting studies on the role of MLCK in cytokinesis: Hickson et al., showed that MLCK knockdown had no major role in cytokinesis of *Drosophila* S2 cells [60] while Wang et al. reported that MLCK inhibition by ML-7 resulted in the loss of RMLC phosphorylation and cleavage furrow regression in crane-fly, as well as *Drosophila* [66].

In mammalian cells, MLCK appears to be dispensable for early cell division and embryonic development because MLCK knockout mice are born without a defect in embryogenesis (though they showed neonatal death) [67]. It is possible, however, that this lack of a phenotype may be due to the presence of redundant kinases. Though MLCK may be

dispensable for early embryonic cell division, MLCK appears to be involved in cytokinesis in cultured mammalian cells. Long MLCK, one of the two MLCK isoforms, was localized at the cleavage furrow and activated during cytokinesis, [68]. In addition, long MLCK is phosphorylated at its N-terminus during mitosis probably by aurora B kinase [69]. While the mechanism is unknown, expression of the N-terminal domain of long MLCK increased multinucleate cells and disrupted spindle assembly and spindle pole fragmentation [69]

2.3. Other kinases

DAPK (Death-associated protein kinase) and DAPK-like kinase (also called DAPK3 or ZIPK, zipper interacting protein kinase) are able to phosphorylate RMLC at its activation site and are necessary for stress fiber assembly in the central region of cells upon serum stimulation [70, 71]. This family of kinases may be involved in cytokinesis because ectopic expression of DAP kinases was found in the contractile ring [72], and expression of a C-terminal truncation mutant generated multinucleated cells [73]. In addition, GFP-fusion protein of DAP-like kinase was associated with centrosomes and centromeres [72].

A-kinase anchoring protein12 (AKAP12) has been reported to control the final step of cytokinesis [74]. AKAPs are a family of proteins that bind to the regulatory subunit of PKA, thereby directing the PKA holoenzyme to a specific cellular location. AKAPs also bind to PKC and phosphatases (such as PP1C & PP2B) and thus work as a scaffold protein. AKAP12 was localized to a contractile ring and depletion of AKAP12 resulted in multinucleated cells. Furthermore, inhibition of MLCK altered AKAP12 localization away from the contractile ring, indicating a link between MLCK and AKAP12. Further work is needed to elucidate how this protein is involved in the completion of cytokinesis.

2.4. Motility-dependent cytokinesis

Wang and his colleagues first demonstrated that Botulinum C3 exotoxin, a specific inhibitor of Rho, inhibited cytokinesis of HeLa cells but not adherent NRK cells, indicating that RhoA activation for cytokinesis is cell type dependent [75]. This work was further developed by Kanada et al. [76]. They showed that mammalian adherent NRK cells were able to divide efficiently via contractile ring-independent, but motility-dependent cytokinesis, which is similar to the cytokinesis observed in myosin II-null Dictyostelium [77]. The adherent-dependent furrowing occurred in the presence of blebbistatin, a myosin inhibitor. If neighboring cells blocked polar lamellipodia movements of a dividing cell, adhesion-dependent cytokinesis was inhibited, suggesting that a pulling force by lamellipodia is critical for the adherent-dependent cytokinesis.

This motility-dependent and adhesion-dependent cytokinesis may be related to Rac activation observed in the polar regions in certain cells, which was revealed by FRET analyses that were able to detect active RhoA or Rac during cytokinesis [78]. RhoA activation during cytokinesis at the cleavage furrow was shown to be cell type dependent: RhoA activation was observed in HeLa cells but not in Rat1 cells, which are more adherent. Furthermore, in Rat1 cells, the ECT2-RhoA-ROCK pathway did not seem to act on cytokinesis, which is consistent with the result by Wang's lab [75]. In contrast, Rac1, though suppressed at the cleavage furrow, was activated at the polar regions at the end of cytokinesis in both Rat1 and HeLa cells. This Rac activation may correspond to the lamellipodia-driven cytokinesis described above [76].

The Rac inactivation at the contractile ring may suggest inactivation of PAK (p21-activated kinase) activity, resulting in MLCK activation for cytokinesis because PAK phosphorylates and inactivates MLCK [79]. However, PAK localization in the cleavage furrow needs to be established. In *Drosophila*, Paklike kinase is not concentrated at the cleavage furrow [80].

Furthermore, the genome-wide screening to search for cytokinesis genes did not identify PAK though PAK2 depletion was shown to alter mitotic spindle assembly [58].

2.5. Myosin phosphatase

Myosin phosphatase has been demonstrated to play a role in cytokinesis. Genetic analyses of *Drosophila* MYPT revealed that myosin phosphatase is involved in the assembly of ring canals. Ring canals are derived from incomplete cytokinesis and function as intracellular bridges between nurse cells, thereby allowing cytoplasmic flow from nurse cells to oocytes. MYPT mutation was reported to cause over-constriction of ring canals [81], suggesting a function of myosin phosphatase as a counterbalance of RMLC kinases. In *C. elegans*, MYPT mutation (MEL-11) has been shown to cause cytokinesis failure [9]. In mammalian cells, MYPT1 depletion has also been demonstrated to result in multinucleated cells [25]. We frequently observed that two nuclei in MYPT1-depleted HeLa cells were separated by a temporary structure consisting of dense actin and phosphorylated RMLC (Fig. 4). This observation suggests that myosin phosphatase is required for the disassembly of a contractile ring at the end of cytokinesis. Thus myosin phosphatase must be temporally controlled during cytokinesis: It is first inactivated for the activation of a contractile ring at the initial stage of cytokinesis and then inactivated for the disassembly of the contractile ring. It remains to be elucidated how myosin phosphatase is controlled during this process.

2.6. Asymmetric cell division

Two recent reports have demonstrated the role of myosin II in asymmetric cell division of *Drosophila* and *C. elegans* neuroblasts that is independent of spindle positioning [82, 83]. Unlike the displaced spindle in the first embryonic cell division of *C. elegans*, the metaphase spindle in these neuroblasts formed symmetrically. However, during the anaphase, myosin II shows polarized localization in the cortex of the anterior daughter cell, resulting in contraction of the anterior cell. This contraction, while squeezing the anterior cell, allows the posterior daughter cell to expand, leading to asymmetrical cell division. Interestingly, the cleavage furrow could assemble without the mitotic spindle in *Drosophila* neuroblasts, where the polarity pathway including Pins (Partner of Inscuteable) directs cleavage furrow assembly [83]. It appears that both the mitotic spindle and the polarity pathway are able to redundantly direct cleavage furrow formation in these neuroblasts.

Perspectives

Much progress has been made over the past decade in our understanding of the roles of myosin II in mitosis and cell division. Such progress poses new questions in several key areas. First, we need to know the mechanisms by which myosin II controls spindle assembly and karyokinesis. Two possibilities have been presented. One possibility is that a change in cortical tension by myosin II inhibition would alter the interactions between microtubules and microtubule motors including dynein. However, it is unknown how the cortical tension affects the interactions. The other is that the increased stability of microtubules resulting from myosin II ablation causes defects in spindle assembly and karyokinesis [20]. We need to know the biochemical link between myosin II and tubulin acetylation and why only a certain cell type showed such an alteration in microtubule stability.

Second, it is critical to examine whether motility-dependent cytokinesis could occur in an *in vivo* situation within a whole animal and if so, to what extent. Third, we know very little regarding how myosin II plays a role in mitosis and cell division in a three-dimensional matrix, the environment of which is more analogous to a physiological condition. Fourth, while myosin II is also controlled by its heavy chain phosphorylation, in particular during cell migration [84, 85], we do not know whether heavy chain phosphorylation is involved in

cell division in higher eukaryotes. Finally, we need to investigate the possibility that myosin II and its upstream regulators could be a target of drug development for treatment of diseases including cancer. Such a possibility may be high as judged from the roles of myosin II in karyokinesis and cytokinesis.

Abbreviations

RMLC	regulatory myosin light chain
MYPT1	myosin phosphatase targeting subunit1
PLK1	polo-like kinase1

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Fig. 1.

Myosin phosphatase antagonizes PLK1 (A) and is required for correct spindle orientation (B) A. MYPT1 depletion rescues mitotic arrest caused by PLK1 depletion. Live cell imaging of control, PLK1-depleted, double-depleted and MYPT1-depleted cells. HeLa cells stably expressing histone H2B-GFP were transfected with siRNAs to deplete PLK1, MYPT1 or both and imaged using a DeltaVision Image Restoration Microscope system. Five Z-section images were taken at 1 min intervals, deconvoluted, and projected images were shown. Time in minutes. Bar, 10 μ m. B. Oblique chromatid segregation caused by MYPT1 depletion. Orthogonal images of MYPT1-depleted cells at 90 min and 100 min were generated from the live cell imaging above. The yz plane images show oblique segregation of sister chromatids. Reprinted from ref. [25]. Copyright with permission from Elsevier.

Fig. 2.

A model for the antagonism between myosin phosphatase and polo-like kinase1 (PLK1). Myosin phosphatase targeting subunit1 (MYPT1) is phosphorylated by Cdc2 (Cdk1) at Ser473, generating the Ser-phosphoSer-Pro motif for binding to the polo-box domain (PBD) of PLK1, and resulting in association with PLK1. This association would allow myosin phosphatase to directly dephosphorylate PLK1 at its activation site of Thr210. The 20-kDa subunit of myosin phosphatase is omitted for clarity.

Fig. 3.
Possible feedback regulatory system consisting of MYPT1/PP1C (myosin phosphatase), ROCK and PLK1. **A.** Inactivation (indicated by a blue arrow with number1) of myosin phosphatase (MYPT1/PP1C/20-kDa subunit complex) would activate PLK1 (red arrow with number2), as well as myosin II (red arrow with number2). This PLK1 activation would activate ROCK, which would further suppress myosin phosphatase. PLK1 activation may also activate dynein. **B.** Activation of myosin phosphatase would result in PLK1 inactivation, which would decrease ROCK activity, causing further activation of myosin phosphatase. For the sake of clarity, the 20-kDa subunit of myosin phosphatase has been omitted.

Fig. 4. Myosin phosphatase is required for contractile ring disassembly at the end of cytokinesis. MYPT1-depleted HeLa cells were labeled with DAPI (A) and with an antibody specific to Ser19-phosphorylated RMLC (B). MYPT1 depletion frequently produced cells with double nuclei that were separated by a phosphorylated myosin-rich structure (indicated by an arrow), suggesting that myosin phosphatase is required for the disassembly of the cleavage furrow. The cell boundary is indicated by the dashed white line.