Archives of Biochemistry and Biophysics 510 (2011) 76–82

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Review

Myosin light chain kinases and phosphatase in mitosis and cytokinesis

Fumio Matsumura *, Yoshihiko Yamakita, Shigeko Yamashiro

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

ARTICLE INFO

Article history:
Available online 10 March 2011

Keywords:
Myosin II
Kinases
Phosphatase
Mitosis
Cytokinesis

ABSTRACT

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.

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.

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)[1] [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, *Caenorhabditis elegans* and *Drosophila* because nonmuscle myosin II in these cells is mainly regulated by RMLC phosphorylation [6–10].

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].

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. [15] 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

* Corresponding author. Fax: +1 732 445 4213.
E-mail address: matsumura@biology.rutgers.edu (F. Matsumura).

[1] *Abbreviations used:* RMLC, regulatory myosin light chain; MYPT1, myosin phosphatase targeting subunit1; PLK1, polo-like kinase1.

0003-9861/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2011.03.002

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].

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 (myosins 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.

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.

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