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Nonmuscle Myosin II Regulation Directs Its Multiple Roles in Cell Migration and Division

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myosin II, kinase, migration, division, motility, force

Abstract

Nonmuscle myosin II (NMII) is a multimeric protein complex that generates most mechanical force in eukaryotic cells. NMII function is controlled at three main levels. The first level includes events that trigger conformational changes that extend the complex to enable its assembly into filaments. The second level controls the ATPase activity of the complex and its binding to microfilaments in extended NMII filaments. The third level includes events that modulate the stability and contractility of the filaments. They all work in concert to finely control force generation inside cells. NMII is a common endpoint of mechanochemical signaling pathways that control cellular responses to physical and chemical extracellular cues. Specific phosphorylations modulate NMII activation in a context-dependent manner. A few kinases control these phosphorylations in a spatially, temporally, and lineage-restricted fashion, enabling functional adaptability to the cellular microenvironment. Here, we review mechanisms that control NMII activity in the context of cell migration and division.

Contents

1. INTRODUCTION

Nonmuscle myosin II (NMII) is a major member of the myosin superfamily of molecular motors. Each motor is a hexamer complex formed by two heavy chains (MHCIIs) and four light chains (**Figure 1*a***). The hexamer has a polar structure, divided into the globular N-terminus motor domain and the homodimerization coiled-coil C-terminus domain. Two MHCII molecules form the nucleus of the hexamer as they homodimerize through charge-dependent interactions between the coiled-coil domains (Holmes 2007). Both light chains interact with MHCII at a short junction (often referred to as the neck domain) that separates the motor domain from the coiled-coil domain. There are two types of light chains: (*a*) the ELC (essential light chain), which provides structural integrity to the hexamer; and (*b*) the RLC (regulatory light chain), which controls the function of the whole hexamer by modulating its conformation in response to phosphorylation. The ELC and RLC interact side by side with IQ motifs located in the neck of the MHCII. The ELC IQ-binding site is closer to the N-terminus and the RLC IQ-binding site is closer to the C-terminus of the MHCII (**Figure 1*b*,*c***). At the end of the heavy chain, a nonhelical tail (NHT) domain controls the formation of polymeric structures called mini-filaments (Conti et al. 2007). The length of the NHT domain and other kinetic properties depend on the MHCII isoform. There are three isoforms: MHCII-A (encoded by the gene *Myh9*), MHCII-B (*Myh10*), and MHCII-C (*Myh14*) (Conti et al. 2007, Juanes-Garcia et al. 2017). The different functions of the NMII paralogs, as defined by the MHCII isoform that forms the functional core of the hexamer, and their ability to form mixed mini-filaments have been extensively reviewed elsewhere (Conti & Adelstein 2008, Sellers & Heissler 2019, Vicente-Manzanares et al. 2009).

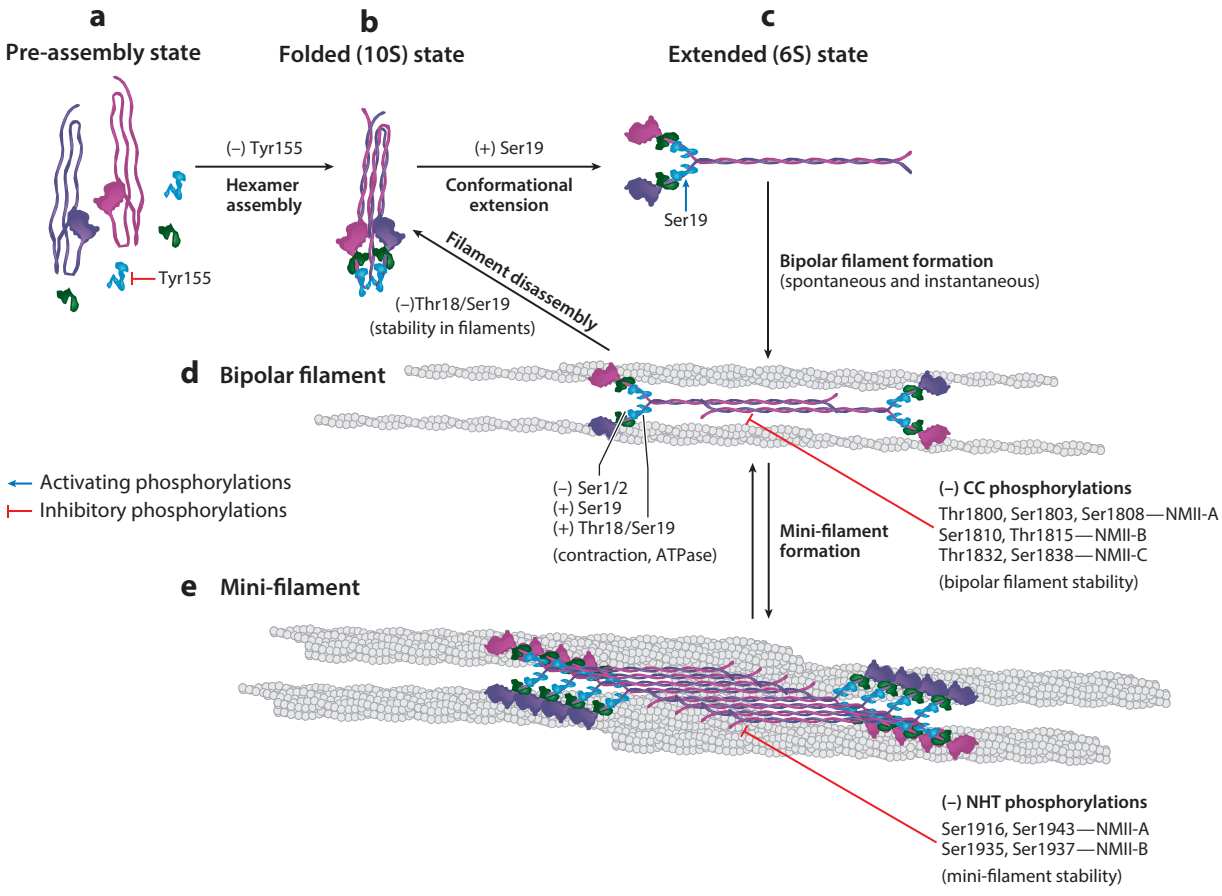


Figure 1

The main phosphorylations of nonmuscle myosin II (NMII) and their effect on its conformation and filamentation states. This figure depicts the different chains and states of NMII regardless of the paralog. (*a*) The pre-assembly stage. Heavy chains are very unstable by themselves and require assembly into the full hexamer, including the essential light chains (ELCs) and the regulatory light chains (RLCs), as shown in panels *b* and *c*. Tyr155 phosphorylation of the RLC prevents the assembly of the pieces shown in panels *a* and *b*. (*b*) The folded (10S) state, assembly-incompetent form of NMII. Head-to-tail interactions prevent the conformational extension of the molecule. (*c*) The extended (6S) state, assembly-competent form, triggered by phosphorylation of Ser19. The extended form is very unstable by itself, either reverting to the form in panel *b* or (*d*) immediately forming a bipolar filament. Diverse phosphorylations of the RLC and the coiled coil (CC) of myosin heavy chain II (MHCII) isoforms affect the contractile ability of NMII (*left*, RLC phosphorylations) or the stability of the bipolar filaments (*right*, CC phosphorylations). As the extended state shown in panel *c* is so unstable, we represent filament disassembly as the transition from the states shown in panels *d* to *b*. (*e*) Bipolar filament growth into mini-filaments, which contain 6–30 subunits. Phosphorylations to the nonhelical tail domain (NHT) of MHCII isoforms govern the stability of the mini-filaments. Some elements of the figure are adapted from Asensio-Juárez et al. (2020); CC BY 4.0.

Previous descriptions of myosin II, including striated and cardiac muscle myosin II, smooth muscle myosin II (SMMII), and NMII, detail the mechanism through which the ATPase-driven conformational change of the head domain enables microfilament motion coupled to actin binding and release. They also cover the dynamics of NMII dimerization and multimerization to form filaments (Conti et al. 2007, Cremo & Hartshorne 2007). Only myosin II and myosin 18 form filaments. While myosin 18 is not contractile (Guzik-Lendrum et al. 2013), it forms filaments that copolymerize with NMII filaments to facilitate their assembly (Billington et al. 2015). Myosin II

filaments generate mechanical force as they move and/or exert tension on actin microfilaments. Striated and cardiac muscle myosin II form very long and stable filaments containing thousands of hexamers, forming the sarcomeric thick band (Reggiani & Bottinelli 2007). SMMII assembles into long monopolar filaments (Dasbiswas et al. 2018). NMII forms smaller structures (mini-filaments) containing 6–30 hexamers (Conti et al. 2007). NMII mini-filaments form stippled patterns along thick actomyosin bundles, e.g., stress fibers (Billington et al. 2015, Shutova et al. 2017, Vicente-Manzanares et al. 2007).

Within mini-filaments, SMMII/NMII hexamers display an extended (6S) conformation (**Figure 1*c,d***). Conversely, nonfilamentous SMMII/NMII appears in a folded, assembly-incompetent (10S) conformation (Scarf et al. 2020, Yang et al. 2020) (**Figure 1*b***). The conversion of 10S into 6S depends on the phosphorylation of the RLC on serine 19 (Ser19), which disrupts the head-to-tail interaction of the 10S conformation (Suzuki et al. 1985). The 6S hexamer binds another 6S hexamer, forming a bipolar filament (Craig et al. 1983). While crucial for NMII function, Ser19 phosphorylation is only one of many mechanisms that control the cellular function of NMII. This review focuses on how NMII regulation controls the diverse roles that NMII plays in motility-related events, particularly cell migration and division.

2. NONMUSCLE MYOSIN II HEXAMER BIOSYNTHESIS AND STABILIZATION

Early experiments showed that the formation of SMMII (and NMII) hexamers requires the RLC (Trybus & Lowey 1988). The deletion of RLC leads to a drastic reduction in the levels of the hexamer and decreased MHCII expression (Aguilar-Cuenca et al. 2020, Park et al. 2011). Interestingly, NMII assembles in the absence of ELC but lacks ATPase activity (Chen et al. 1995). In addition, posttranslational modifications can control the biosynthesis of NMII hexamers. Free RLC can become phosphorylated on Tyr155 by the action of epidermal growth factor receptor (EGFR) and possibly other kinases, impairing its interaction with MHCII (**Figure 1*a***), thus reducing the amount of available NMII (Aguilar-Cuenca et al. 2020).

3. CONFORMATIONAL EXTENSION OF MYOSIN II

Ser19 phosphorylation is the crucial checkpoint that controls the conformational extension of SMMII/NMII from folded (10S) to extended (6S) (**Figure 1*b,c***), enabling the formation of bipolar mini-filaments (Craig et al. 1983). It also increases both the actin-binding ability and the catalytic ATPase of NMII (Adelstein & Conti 1975, Trybus & Lowey 1984) (**Figure 1*d***). The introduction of negative charge by phosphorylation impairs the head-tail contact observed in the 10S conformation (Jung et al. 2008; Scarf et al. 2020; Yang et al. 2019, 2020). The single-hexamer extended (6S) conformation is very unstable in solution (it has been rarely observed and only in in vitro preparations of myosin II). Hexamers in the 6S conformation immediately form bipolar filaments that grow by lateral association with other filaments (Heissler & Manstein 2013) (**Figure 1*d,e***) or return to the 10S conformation (**Figure 1*b***). Several kinases mediate Ser19 phosphorylation, including myosin light-chain kinase (MLCK), Rho-associated coiled-coil-containing kinase (ROCK), myotonic dystrophy kinase–related Cdc42-binding kinase (MRCK), and some others that may act on Ser19 and threonine 18 (Thr18) (see Section 5). Ser19 phosphorylation is also elevated when myosin phosphatase target subunit 1 (MYPT1) is attenuated by ROCK (Kimura et al. 1996). Although Ser19 phosphorylation triggers the 6S conformation in every paralog, reversibility (return to 10S) seems paralog-dependent. This is important to control isoform-specific cellular functions, e.g., cell polarity (Vicente-Manzanares & Horwitz 2010).

4. REGULATION OF NONMUSCLE MYOSIN II ACTIN-BINDING/ATPASE ACTIVITY

In addition to the conformational change that extends NMII, Ser19 phosphorylation also increases its actin-binding/ATPase activity (Adelstein & Conti 1975). Additional phosphorylation of RLC on multiple sites, including Thr18, controls contraction by modulating the ATPase activity and the affinity of the hexamer for actin. The role of Thr18 phosphorylation in NMII function seems twofold: (*a*) It increases the ATPase activity of NMII/SMMII (Ikebe et al. 1986) (**Figure 1*d***), and (*b*) it stabilizes NMII in the 6S conformation, making the filaments less prone to disassemble. Regarding these two phosphorylation events, cells only contain three RLC species: nonphosphorylated, pSer19 and pThr18 + pSer19 (Aguilar-Cuenca et al. 2020). Some evidence indicates that NMII-containing RLC bisphosphorylated in Thr18 and Ser19 has a decreased ability to return to 10S conformation (**Figure 1*b*,*d***). In cells, filament-bound pThr18/pSer19-RLC appears in regions subject to great tensile stress, for example, at the trailing edge of migrating cells (Vicente-Manzanares & Horwitz 2010) or the spindle midzone during metaphase (Matsumura et al. 2011, Tan et al. 2015). This suggests that bisphosphorylated NMII forms filamentous structures that withstand, rather than generate, tension.

Other phosphorylation events inhibit the ATPase activity of SMMII/NMII, e.g., Ser1/2 (Nishikawa et al. 1984) (**Figure 1*d***). These protein kinase C (PKC)-mediated phosphorylation events are involved in actin reorganization in response to growth factors (Komatsu & Ikebe 2007). In live cells, Ser1/2 phosphorylation attenuates mini-filament contraction downstream of conventional PKC (Asokan et al. 2014).

5. KINASE-MEDIATED CONTROL OF THE CONFORMATIONAL EXTENSION AND ACTIN-BINDING/ATPASE ACTIVITY OF NONMUSCLE MYOSIN II THROUGH SER19 AND THR18 PHOSPHORYLATION

As described in Sections 3 and 4, major mechanisms that control NMII function depend on Ser19 and Thr18 phosphorylation. A relatively small subset of Ser/Thr kinases carries out these phosphorylations. The specificity and efficiency of these kinases toward NMII activation via Ser19 and Thr18 is a cornerstone of the field. This section describes these kinases in detail.

5.1. MLCK

MLCK (myosin light chain kinase) was the first identified kinase that could phosphorylate Ser19 of the RLC in SMMII (Driska et al. 1981). MLCK also phosphorylates RLC in NMII, but its role as a regulator of NMII is more controversial, as described in the last paragraph of this section. There are three variants of MLCK, which are encoded by three distinct genes: *MYLK* (on human chromosome 3) is expressed in smooth and nonmuscle tissues, *MYLK2* (also human chromosome 3) is expressed in muscle tissue, whereas *MYLK3* (human chromosome 8) is restricted to cardiac tissue. *MYLK* produces the nonmuscle and smooth muscle variants as well as a short, noncatalytic form of the protein (telokin) that also participates in myosin II regulation. The expression of each variant depends on alternative sites of initiation of transcription (Lazar & Garcia 1999). The nonmuscle variant is 922 amino acids longer than the smooth muscle variant.

The structure of the nonmuscle-specific portion of MLCK comprises a series of immunoglobulin (Ig)-like domains of undetermined function, followed by a calmodulin-binding domain that contains actin-binding motifs and that is common to the nonmuscle and smooth muscle variants. The calmodulin-binding domain is followed by a proline–rich repeat domain, two

Ig-like, actin-binding domains, a fibronectin III–like domain and the kinase domain. Closer to the C-terminus, an autoinhibitory domain is followed by a second calmodulin domain and a myosin-binding domain (IgT) (**Figure 2*a***). The IgT domain is the only portion of MLCK in the shortest version, telokin (Hong et al. 2011).

MLCK is mainly regulated by intracellular calcium. However, its activity toward the diverse variants of myosin II is different (see the last paragraph of this section), and compartmentalization plays a crucial role in its activity. MLCK activation also requires the function of the ESCRT (endosomal sorting complex required for transport) complex and Src during fibroblast polarization (Lobert & Stenmark 2012). Notably, MLCK interacts directly with actin, which is essential for its regulation (Kohama et al. 1996, Smith & Stull 2000). Rare insight into the function of this enzyme emerged from a study from the Cremo group (Hong et al. 2015), which demonstrated that MLCK sliding along actin filaments controls its ability to phosphorylate SMMII or NMII. These observations imply that MLCK mainly phosphorylates filamentous NMII. It follows that NMII would have to already be in an extended conformation as part of preexisting filaments. In this sense, MLCK would contribute to the sustained contractility of NMII filaments, but it is unlikely to trigger the initial formation of seeding bipolar NMII/SMMII filaments.

MLCK phosphorylates RLC on Ser19 but bears little activity toward other residues of RLC or the heavy chains. A recent study showed that MLCK first phosphorylates RLC on Ser19, creating monophosphorylated (Ser19) RLC, leaving it for a second kinase, in this case ROCK, to phosphorylate Thr18 (Kazakova et al. 2020). This could underlie the described segregation of the functions of MLCK and ROCK in polarized fibroblasts, with MLCK phosphorylating NMII at the front (where NMII is mostly monophosphorylated in Ser19) and ROCK at the back of migrating cells, where NMII is prominently bisphosphorylated on Thr18 and Ser19 (Totsukawa et al. 2000, 2004).

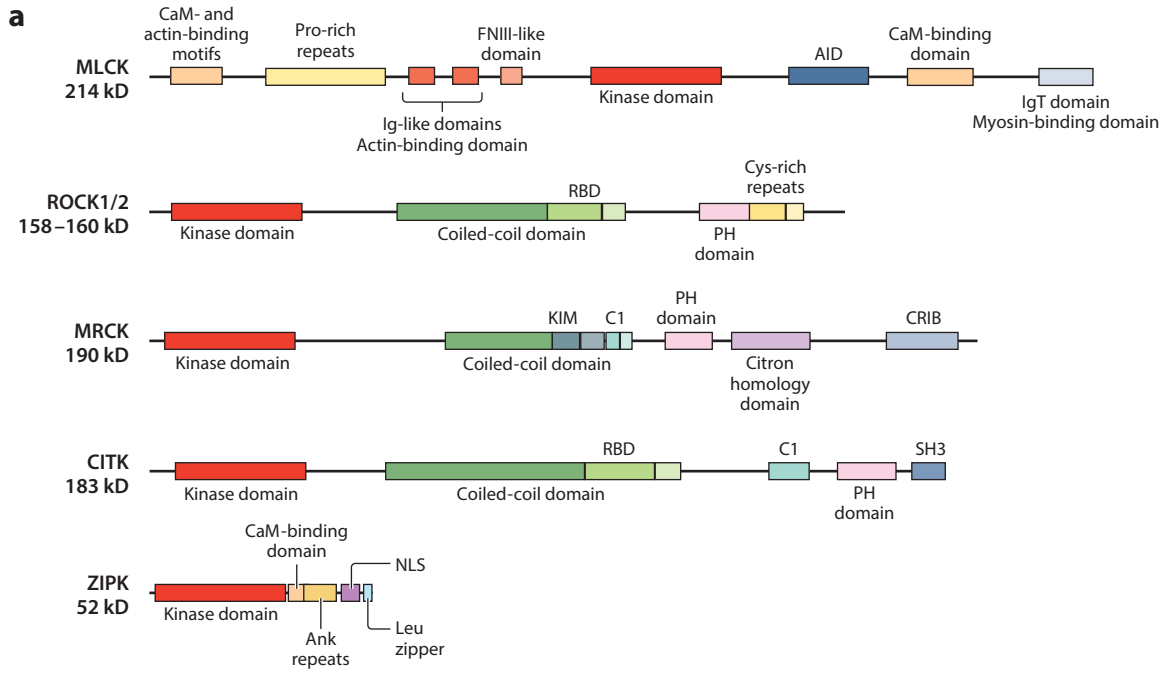
SMMII, not NMII, is the main substrate of MLCK (Alcala et al. 2016). This could explain why MLCK depletion or inhibition in nonmuscle cells has a wide range of outcomes, from no effect at all (Beningo et al. 2006) to strong phenotypes, e.g., focal adhesion and/or stress fiber disappearance (Totsukawa et al. 2000, 2004). Mutations in MLCK cause diseases related to the function of smooth muscle, for example, spontaneous aortic dissection (Wang et al. 2010) or deficient tonic airway contraction (Zhang et al. 2010).

Although SMMII is the main substrate of MLCK, the kinase can also phosphorylate nonmuscle RLC with lower affinity (Alcala et al. 2016). As the difference in specificity is about the same regardless of using free RLC or meromyosin-assembled RLC, the difference seems to reside in a few residues that differ from smooth muscle–specific RLC (encoded by the gene *MYL9*) to nonmuscle RLC (gene *MYL12A/B*).

5.2. ROCK1/2

There are two major isoforms of ROCK, ROCK1/ROCKβ and ROCK2/Rho-kinase/ROKα (Rho-binding kinase alpha). They are AGC Ser/Thr kinases that fold into an autoinhibited conformation that is relieved by binding to GTP-bound RhoA (Ras homolog family member A). ROCK1/2 control NMII function through the direct phosphorylation of RLC on Ser19 (Amano et al. 1996) and by the phosphorylation of MYPT1, which is the phosphatase subunit that dephosphorylates Ser19 (Kimura et al. 1996).

The human genes encoding ROCK1/2 each contain 33 exons and are located on chromosome 18 and 2, respectively. Overall homology between the two isoforms is 62%, and the domain structure is virtually the same (Riento & Ridley 2003). The kinase domain localizes to the N-terminus and is highly conserved in both isoforms. The C-terminus contains a pleckstrin homology (PH)



b

	MLCK	ROCK1	ROCK2	MRCK	CITK	ZIPK	Preferred NMII-related substrates
MLCK	100	53	51	48	50	66	RLC Ser19
ROCK1	100	96	78	69	49		MYPT1, RLC Ser19, Thr18?
ROCK2	100	78	68	50			MYPT1, RLC Ser19, Thr18?
MRCK	100	67	49				MYPT1?, RLC Ser19
CITK	100	53					RLC Ser19, Thr18
ZIPK	100						RLC Ser19, Thr18

Figure 2

Domain structure and homology of the kinase domain of NMII kinases. (*a*) Domain structure of the indicated kinases. For MLCK, the long form is shown. (*b*) Homology of the kinase domains of the human versions, as calculated in PubMed BLAST. Human forms have been used, corresponding to the translations of the coding sequences of the following accession numbers: NM_053025.4 (MLCK), NM_005406.3 (ROCK1), NM_004850.5 (ROCK2), NM_003607.4 (MRCK), NM_001206999.2 (CITK), NM_001348.3 (ZIPK). The table also includes the known preferred NMII-related substrates for each kinase. Abbreviations: AID, autoinhibitory domain; Ank, ankyrin; BLAST, Basic Local Alignment Search Tool; CaM, calmodulin; CITK, citron kinase; CRIB, Cdc42/Rac1 interactive–binding domain; FNIII, fibronectin III; Ig, immunoglobulin; KIM, kinase inhibitory motif; MLCK, myosin light-chain kinase; MRCK, myotonic dystrophy kinase–related Cdc42-binding kinase; MYPT1, myosin phosphatase target subunit 1; NLS, nuclear localization signal; NMII, nonmuscle myosin II; PH, pleckstrin homology; RBD, RhoA-binding domain; RLC, regulatory light chain; ROCK1/2, Rho-associated coiled-coil-containing kinase 1/2; SH3, Src homology 3; ZIPK, zipper-interacting protein kinase.

domain with a cysteine-rich (CR) portion. Between these two domains, a coiled coil contains the RhoA-binding domain (RBD) (Shimizu et al. 2003) (**Figure 2*a***). RBD only binds to the specific switch regions of GTP-bound active RhoA and RhoC (Julian & Olson 2014). The coiled-coil region is structurally similar to that of tropomyosin (Tu et al. 2011). The kinase domains are similar to those present in other kinases with similar function, such as MRCK and citron kinase (CITK) (78% and 69% homology with ROCK1, respectively) (**Figure 2*b***).

The catalytic activation of ROCK1/2 requires their conformational extension. In the absence of stimuli, ROCK1/2 are inactive, with the kinase domains unable to bind their catalytic targets because they are engaged in a head-to-tail interaction (Amano et al. 1999). Importantly, the removal of the RBD and PH domains triggers the constitutive activation of both ROCK isoforms (Amano et al. 1999, Ishizaki et al. 1997). GTP-bound RhoA relieves the autoinhibited conformation (Ishizaki et al. 1997, Leung et al. 1996). Furthermore, some lipids, such as arachidonic acid, can activate ROCK by binding to its PH domain (Araki et al. 2001). ROCKs are dimeric, forming a parallel homodimer joined by the coiled coil that extends upon activation (Shimizu et al. 2003).

Although structurally very similar, ROCK1 and ROCK2 have nonredundant cellular functions. In migrating mesenchymal cells, they both phosphorylate RLC directly. However, their main target seems to be MYPT1 (a Ser/Thr phosphatase that, among other targets, dephosphorylates Ser19 of RLC), increasing contractility. ROCK1 seems more important than ROCK2 during the formation of actomyosin assemblies at the back of the cell that define front-back polarity. Conversely, ROCK2 regulates contractility at the leading edge of migrating cells (Newell-Litwa et al. 2015). This led to the suggestion that their differential effects rely on the ability of ROCK1 to promote RLC bisphosphorylation (phosphorylation of the RLC on Ser19 and Thr18), whereas ROCK2 would only promote RLC monophosphorylation on Ser19 (Newell-Litwa et al. 2015). However, this is unlikely to be the case, as the similarity of the kinase domains (92% identity and 96% homology) (**Figure 2*b***) is not consistent with ROCK1 and ROCK2 having intrinsically different affinities for those sites. Instead, their upstream regulation may diverge, directing ROCK1 and ROCK2 to different subcellular regions and/or controlling their stability and function. A theoretical model is that ROCK1/2-mediated pSer19-RLC accumulation in the posterior regions of the cell increases its local availability as a substrate for a second kinase that mainly triggers phosphorylation in Thr18 once Ser19 is phosphorylated. In this model, the second, yet-to-be-identified kinase (it could be ROCK1 itself) would display a posterior distribution and would not be available at the front. A similar segregation model would explain the differential effect of ROCK1 and ROCK2 in NMII-mediated contractility observed at cell–cell junctions. Another possibility is that the enzyme-substrate interaction is differentially regulated in an allosteric manner by ROCK motifs other than the kinase domain. A third possibility is that the diffusion of the substrates (MYPT1 or RLC) is different at the front and the rear. This is a feasible hypothesis, as RLC diffusion at the front is governed by actin retrograde flow, that is, the centripetal flow of F-actin produced by active actin assembly as it pushes against the plasma membrane (Gardel et al. 2008, Giannone et al. 2007, Ponti et al. 2004), whereas RLC is relatively immobile in the rear (Barbier et al. 2019, Duda et al. 2019). A combination of these models, together with the existence of additional ROCK substrates, likely underlies the divergent functions of ROCK1 and ROCK2 in different contexts. Some specific scenarios in which ROCK1 and ROCK2 have different effects include experimental pulmonary fibrosis (Knipe et al. 2018), cerebral ischemia injury (Lu et al. 2020), cardiac dysfunction and postcapillary pulmonary hypertension (Sunamura et al. 2018), leukocyte recruitment and neo-intima formation following vascular injury (Noma et al. 2008), and oxidized low-density lipoprotein–mediated endothelial dysfunction (Huang et al. 2018).

Consistent with a model of sequential or cooperative phosphorylation of the RLC, dividing cells accumulate ROCK (mainly ROCK2) at the cleavage furrow after anaphase (Kosako et al. 1999, 2000). There, ROCK2 colocalizes with CITK, and its deletion delays but does not prevent the formation of the cleavage furrow (Asano et al. 2009).

Finally, ROCK sharply increases RLC phosphorylation during the early stages of apoptosis. Caspase-3 and granzyme B cleave the autoinhibitory domain of ROCK1, inducing an uncontrolled phosphorylation of the RLC (Coleman et al. 2001, Sebbagh et al. 2001). This triggers the accumulation of active NMII at the cell cortex. Increased contractility in the subcellular cortex alters the hydrostatic balance of the cytoplasm–membrane system, triggering blebbing and nuclear disintegration (Croft et al. 2005). This important mechanism participates in the elimination of pathogen-infected cells by producing zeiosis in the target cell. In this context, target cell zeiosis is caspase-independent but depends on the cleavage of ROCK2 by CTL-secreted granzymes (Sebbagh et al. 2005).

5.3. MRCKs

MRCKs are ubiquitous AGC kinases that act downstream of the small Rho GTPase Cdc42 (Unbekandt & Olson 2014). MRCK proteins are ubiquitously expressed, and their levels are particularly high in the brain (Leung et al. 1998).

There are three main human isoforms, MRCKα, β, and γ (Unbekandt & Olson 2014). Their molecular mass is ≈190 kD. Structurally, they include an N-terminus kinase domain relatively similar to those of ROCK and DMPK, separated from the regulatory portion of the molecule by a coiled-coil stretch of 600–700 amino acids that contains a kinase-inhibitory motif (KIM) (Tan et al. 2008, Zhao & Manser 2015). Close to the C-terminus, MRCKs display a PKC-like C1 domain that weakly binds phorbol esters (S.H. Choi et al. 2008, Tan et al. 2001), followed by a PH domain. These two domains determine the distinct localization of MRCK at the plasma membrane (Tan et al. 2001). A citron homology (CH) domain and a Cdc42/Rac interactive–binding domain (CRIB) follow the C1-PH cluster (**Figure 2*a***). Active Cdc42 (or Rac) disrupts the head-to-tail interaction that keeps the molecule inactive. Similar to ROCK kinases, MRCK is active when it dimerizes. Dimerization is controlled by a stretch of amino acids immediately in front of the kinase domain (Tan et al. 2001).

Native MRCKs form inactive tetrameric complexes of ≈800 kD (Tan et al. 2001, Zhao & Manser 2015). Activation follows binding of Cdc42 to the CRIB domain (Leung et al. 1998). Cdc42 binding also targets MRCK to its specific locations in the cell. The association of MRCK with NMII is mediated by leucine (Leu)-rich adapter protein 35a (LRAP35a), which binds Myo18A and MRCK though a KIM domain, promoting the formation of a tripartite complex that activates MRCK and promotes NMII-A phosphorylation (Tan et al. 2008, Zhao & Manser 2015). Once LRAP35a binds MRCK, the KIM domain no longer inhibits the kinase activity of MRCK. This complex colocalizes and moves with the actomyosin retrograde flow (Tan et al. 2008). LRAP25 mediates both the correct localization of MRCK at the cell front and its interaction with LIMK. Such interaction leads to LIMK phosphorylation by MRCK and cofilin phosphorylation and inactivation, contributing to actin polymerization (Lee et al. 2014). Diacylglycerol interaction with the C1 domain prevents MRCK from refolding into the autoinhibited conformation.

MRCK phosphorylates RLC only on Ser19, not on Thr18 (Tan et al. 2011). It may also inhibit MYPT1 (Murányi et al. 2001). With respect to NMII activation via RLC phosphorylation of Ser19, the roles of MLCK, ROCK2, and MRCK seem redundant, as they coincide in the same region of the cell, the lamellum (Newell-Litwa et al. 2015, Tan et al. 2008, Totsukawa et al. 2000). Upstream signaling and/or differential expression likely determine the main kinase that activates

NMII at the lamellum in a given cell. In this regard, note that their mechanisms of activation are different. MLCK is mainly regulated by calcium and actin binding, whereas ROCK2 is activated by RhoA. Conversely, MRCK activation depends on Cdc42 and PKC. However, this needs to be explored in more detail. Given its role in controlling actin dynamics at the cellular cortex, which is essential during cell division, MRCK targeting is a possible novel approach to the inhibition of cancer invasion and the treatment of metastasis (Unbekandt et al. 2014).

5.4. CITK

CITK (also known as CIT or CRIK) is a RhoA-dependent kinase. CITK phosphorylates NMII and plays crucial roles in cytokinesis and in the development of the central nervous system (CNS).

The *Citk* gene localizes to human chromosome 12, encoding a 183-kD Ser/Thr kinase. In the protein, the kinase domain is located in the N-terminus. The Rho-binding domains localize to a central coiled-coil domain. The C-terminus displays PH, Src homology 3 (SH3), and zinc-finger domains (D'Avino 2017, Madaule et al. 1995). This gene displays two splicing variants, a longer isoform (CITK or CRIK), as previously described, and a shorter isoform (citron-N) that has no catalytic domain (D'Avino 2017, Di Cunto et al. 1998). Citron-N is mainly expressed in the CNS (Di Cunto et al. 1998), whereas CITK is highly expressed in the testis, kidney, and spleen (Di Cunto et al. 1998, Madaule et al. 1995).

The most important activator of CITK is RhoA. Similar to other RLC kinases, its activation is tightly regulated by spatial and temporal segregation. CITK localization in the central spindle during anaphase depends on its phosphorylation by Aurora kinase B (AURKB) (McKenzie et al. 2016). AURKB is part of the chromosomal passenger complex (CPC) (Krenn & Musacchio 2015) that activates the machinery responsible for cytokinesis (Vader et al. 2006). CITK itself is part of the CPC, controlling the localization of the complex in the spindle midzone (McKenzie et al. 2016). CITK is linked with AURKB through a positive feedback loop. CITK phosphorylates the inner centromere protein (INCENP), another component of the CPC, which in turn activates AURKB (McKenzie et al. 2016). AURKB activation in the context of CPC increases CITK phosphorylation in its coiled-coil domain, promoting its localization to the central spindle (McKenzie et al. 2016). However, whether signals that induce the canonical activation of RhoA, e.g., LPA, trigger CITK activation to perform a physiologically relevant function remains unclear.

CITK phosphorylates NMII in both Thr18 and Ser19, and this phosphorylation is a key step during cytokinesis (Yamashiro et al. 2003). Mono- and bisphosphorylated NMIIs are present in the cleavage furrow, although the bisphosphorylated form is more tightly packed within the furrow (Yamashiro et al. 2003). NMII activation generates the forces necessary for the constriction of the cleavage furrow, the midbody abscission, and the generation of both daughter cells. However, the role of these NMII phosphorylations in this context is far from clear (see Section 7).

Interestingly, CITK and RhoA are part of a reciprocal regulatory loop during cell division. Once the mitotic spindle is oriented, the assembly of the contractile ring is initiated by RhoA activation by the Rho guanine nucleotide–exchange factor (Rho-GEF) epithelial cell–transforming sequence 2 (ECT2). Active RhoA is located at the equatorial cell cortex, the future site of the cleavage furrow. Localized RhoA activation promotes the formation of a contractile ring. Initially, RhoA builds actin filaments in this region, which involves formin activation (Narumiya & Yasuda 2006, Piekny et al. 2005). RhoA also increases NMII phosphorylation through ROCK activation (Lai et al. 2005). Further recruitment of active NMII constricts the ring in order to separate both daughter cells, a process called abscission (Wang et al. 2019). However, CITK is also necessary for the initial accumulation of RhoA at the cleavage furrow (Bassi et al. 2011), becoming an essential cog during midbody stabilization and abscission at the end of cytokinesis.

Due to its importance in cytokinesis, CITK has attracted attention as a possible target in cancer therapy. In this regard, CITK is elevated in bladder (Shou et al. 2020) and breast (Meng et al. 2019) cancer cells. In such cells, CITK depletion causes cytokinesis failure, chromosome instability, and apoptosis, which has proven beneficial in medulloblastoma treatment (Pallavicini et al. 2018). A similar approach decreased hepatocellular tumorigenesis in vivo (Fu et al. 2011).

5.5. DAPK3/ZIPK

Death-associated protein kinase-3 (DAPK3) is also known as zipper-interacting protein kinase (ZIPK). It is a protein kinase mainly associated with the onset of apoptosis (Usui et al. 2014).

DAPK3/ZIPK is the smallest NMII kinase, with a molecular mass of approximately 52 kD (**Figure 2*a***). It comprises a kinase domain relatively homologous to those of ROCK1/2, MRCK, and CITK. Although there is a CaM-binding domain adjacent to the kinase domain, the kinase function of the protein is calcium-independent. The middle part of the protein displays several ankyrin repeats (Haystead 2005), a nuclear localization signal, and the Leu-zipper domain ZIPK that underlies its alternative nomenclature.

Another apoptotic kinase, DAPK1, activates DAPK3/ZIPK, promoting its activation in an apoptosis context (Shani et al. 2004). However, ZIPK also interacts with RhoD to regulate adhesion dynamics (Nehru et al. 2013). Dominant-negative forms of RhoA prevent ZIPK from binding to MYPT1, thereby interfering with its ability to control NMII phosphorylation (Endo et al. 2004). How, when, and why ZIPK translocates to the nucleus is unclear. However, it interacts with several transcription factors in the nucleus, including activating transcription factor 4 (ATF4) and signal transducer and activator of transcription 3 (STAT3), which is phosphorylated by ZIPK (Sato et al. 2005). The pro-apoptotic function of ZIPK relies on its phosphorylation-dependent ability to recruit death domain–associated protein (Daxx) to PML oncogenic domains (PODs). A kinase-inactive mutant of ZIPK impairs Daxx translocation to PODs and decreases apoptosis (Kawai et al. 2003). During mitosis, ZIPK is activated by ROCK, which turns on its ability to drive NMII phosphorylation to enable cytokinesis (see Section 7.3.3) (Hamao et al. 2020).

Several studies have indicated that ZIPK phosphorylates RLC in Thr18 and Ser19 (Komatsu & Ikebe 2004, Zhang et al. 2019). The most recent of these studies (Zhang et al. 2019) indicated that ZIPK, and not MLCK, mediates endothelial cell contraction in response to thrombin. This could be due to a comparatively low expression of MLCK in these cells. However, the experimental design of this study did not resolve whether the function of ZIPK was independent of the other NMII kinases, or if its activity was surrogated to them. RLC bisphosphorylation by ZIPK seems particularly important in the context of cytokinesis (Hamao et al. 2020, Hosoba et al. 2015). Bisphosphorylation extends to SMMII independent of the expression of MLCK in smooth muscle cells (Deng et al. 2019). More research is needed to distinguish the context-specific weighting of the regulation of NMII by ZIPK-mediated phosphorylation. Due to its role as a death-associated kinase, ZIPK-mediated phosphorylation of RLC should cause blebbing in apoptotic cells, but the extent of this has not been addressed in a detailed manner.

6. NONMUSCLE MYOSIN II FILAMENT FORMATION AND STABILITY

Bipolar filaments and mini-filament stability depend on the phosphorylation status of the RLC but also on other phosphorylations along the coiled coil and the NHT. While coiled-coil phosphorylations modulate dimerization and mini-filament stability, NHT phosphorylations mainly trigger mini-filament disassembly.

Phosphorylations in the coiled-coil domain introduce negative charge that locally alters its interaction with the complementary region of the other MHCII subunit within the hexamer

(Ricketson et al. 2010). Several phosphorylation sites have been described, including Thr1800, Ser1803, and Ser1808 of MHCII-A; Ser1810 and Thr1815 of MHCII-B; and Thr1832 and Ser1838 of MHCII-C (**Figure 1*e***). These are targets of mechanically activated α-kinases, e.g., TRPM6/7 (Clark et al. 2008).

In contrast, the phosphorylation of several sites within or close to the NHT domain decreases mini-filament stability and increases their turnover. Modeling suggests that the introduction of negative charge in this region does not affect dimerization, instead perturbing lateral interactions between hexamers, which mediate mini-filament stability (**Figure 1*e***). The best-characterized phosphorylation is that of Ser1943 (MHCII-A) downstream of CK-II. Ser1943 phosphorylation destabilizes NMII-A mini-filaments (Dulyaninova et al. 2005), controlling durotaxis (Raab et al. 2012) and hematopoietic stem cell lineage commitment (Shin et al. 2014). Other phosphorylation events with a similar molecular effect include those of Ser1935 and Ser1937 (MHCII-B) that are phosphorylated by atypical PKCζ (Even-Faitelson & Ravid 2006, Juanes-Garcia et al. 2015). They decrease the stability of NMII-B filaments, impairing cell polarity (Juanes-Garcia et al. 2015). At the end of the coiled-coil domain, Ser1916 (MHCII-A) phosphorylation inhibits oligomerization downstream of conventional PKCβ (Beach et al. 2011, Ludowyke et al. 2006). This phosphorylation controls the interaction of NMII-A with metastasis-associated protein 1 (Mts1)/S100A4, which inhibits filament formation (Li et al. 2003).

7. NONMUSCLE MYOSIN II REGULATION IN CELL MIGRATION AND DIVISION: CURRENT MODELS AND PERSPECTIVES

Understanding the diverse levels of NMII regulation has in turn improved our understanding of the role of force generation in cell migration and division. Cells migrate using different mechanisms depending on dimensionality, integrin usage, and degree of confinement. These modes are typically classified as either mesenchymal or amoeboid [reviewed in Friedl & Wolf (2010)]. Mesenchymal cells on bidimensional substrates are slow, polarized, highly contractile, and display numerous structural features rarely seen in other contexts (Naumanen et al. 2008) (**Figure 3*a***). However, important insights have been made in three-dimensional (3D) microenvironments and/or cells under confinement (Kubow et al. 2013, Thomas et al. 2015) (**Figure 3*b***); hence, both scenarios are discussed here.

7.1. Nonmuscle Myosin II Regulation in Migrating Mesenchymal Cells

NMII displays a clear segregation that defines the structure of actin in each subcellular region in mesenchymal cells on bidimensional substrates. This segregation ranges from a complete absence of assembled NMII at the lamellipodium to large and thick actomyosin bundles containing mono- and bisphosphorylated (Ser19 and Thr18 + Ser19) NMII-A and NMII-B at the rear. Sections 7.1.1 and 7.1.2 describe in detail the regulation and functional consequences of this segregation.

7.1.1. Negative regulation of nonmuscle myosin II in protrusive areas. Assembled NMII is absent from the lamellipodium, which constitutes the outer edge of protrusions (**Figure 3*a***). Its local absence enables lamellipodial extension. NMII assembly in this region would interrupt actin retrograde flow, stalling lamellipodial extension (Gardel et al. 2008) (**Figure 3*a***). Periodic contractions of the lamellipodium depend on the local activation of NMII by MLCK (Giannone et al. 2004) and MRCK (Tan et al. 2008), driving focal adhesion elongation at the lamellipodium–lamellum interface (C.K. Choi et al. 2008, Giannone et al. 2007). NMII remains absent in this region due to the local inhibition of NMII hexamer formation (**Figure 1*a***). The phosphorylation

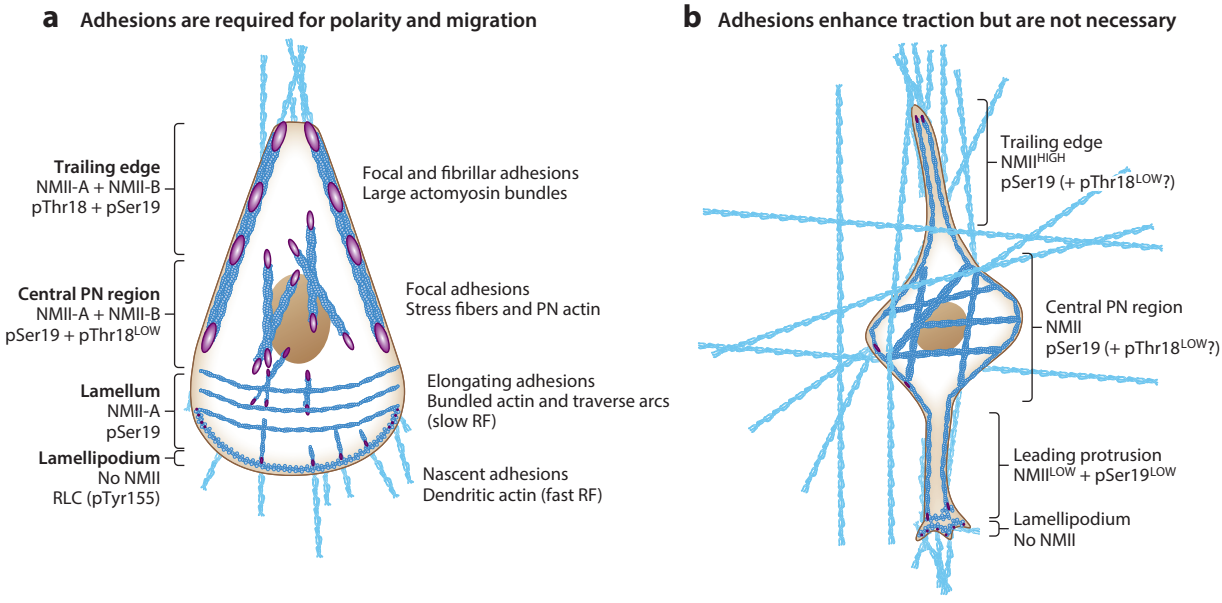


Figure 3

Modeling NMII function in mammalian adherent migrating cells. Both models depict individual adherent cells. (*a*) A model of migration in two dimensions, in which purple spots denote adhesions. Fibers inside the cell (*dark blue*) represent dendritic actin (in the lamellipodium) and/or actomyosin (in the rest of the cell). Outside fibers (*light blue*) represent the extracellular matrix. In this model, the cell is divided into four basic regions: the lamellipodium, the lamellum, the central PN region, and the trailing edge. The lamellipodium is devoid of NMII and shows fast actin RF and nascent adhesions. The lamellum has some NMII (mainly the NMII-A isoform) phosphorylated in Ser19 and some nascent adhesions that begin elongation. Also here, traverse actin arcs move backward with the slow RF typical of this region. The central PN region is characterized by thicker actomyosin bundles heavily phosphorylated in Ser19 (and sometimes also in Thr18) that form contractile (stress) fibers containing both NMII-A and NMII-B. Adhesions are more stable in this region. Note that PN actin, which is often adhesion-associated, is not shown. Finally, the trailing edge contains large fibrillary adhesions connected by thick actomyosin bundles that contain NMII-A/B phosphorylated heavily in Ser19 and Thr18. Adhesions are mandatory for polarization and migration to occur in surfaces. (*b*) The same cell migrating in a three-dimensional environment. The lamellipodium is smaller and is followed by a leading protrusion similar to the lamellum containing scant NMII. Most NMII is concentrated around the nucleus in the PN region and the back, which it contracts to enable nuclear movement. Adhesion is not required, and cells can move due to traction. Abbreviations: NMII, nonmuscle myosin II; PN, perinuclear; RF, retrograde flow (of actin); RLC, regulatory light chain.

of free RLC in Y155 by protrusive factors, e.g., EGFR, impairs its ability to form NMII hexamers, preventing NMII assembly in the lamellipodium. Notably, free pTyr155 RLC is detected in lamellipodia (Aguilar-Cuenca et al. 2020). Also, the phosphorylation of NMII filaments in Ser1 of the RLC inactivates NMII near protrusive regions. At the front, interphase cells display NMII filaments decorated with pSer1 RLC (Komatsu & Ikebe 2007). Phosphomimetic mutations have demonstrated that this mechanism is important for steering mesenchymal cells in chemotactic gradients (Asokan et al. 2014). Whether this mechanism is isoform-dependent has yet to be reported, but it is likely to be more important to curb NMII-A contraction due to its role in adhesion maturation at protruding edges (C.K. Choi et al. 2008).

7.1.2. Contraction and filament stabilization in nonprotrusive areas. Phosphorylated Ser19 RLC is primarily detected in the lamellum, increasing toward the center of the cell (the perinuclear region) and the trailing edge (**Figure 3*a***). Its distribution mimics that of NMII-A, although this phosphorylation controls the actin-binding and ATPase activity of all NMII isoforms

(Kim et al. 2005). Ser19 phosphorylation triggers actomyosin contraction and drives adhesion maturation. This phosphorylation is widely used to measure NMII activation, correlating well with polarization (Vicente-Manzanares & Horwitz 2010), contraction, and tissue stiffness (Miroshnikova et al. 2016).

In contrast, bisphosphorylated NMII (Thr18 and Ser19) is mainly detected in the perinuclear area and the trailing edge. Bisphosphorylated NMII seldom appears in the lamellum. Notably, bisphosphorylation has a dramatic effect on the stability of NMII-B mini-filaments, while it has a modest effect on the stability of NMII-A mini-filaments (Vicente-Manzanares & Horwitz 2010). This may reflect its ability to lock NMII-B, but not NMII-A, in the 6S conformation. The combination of a phosphomimetic mutant of RLC and NMII-B generates cells that are prominently polarized, with both markers concentrated at the rear of the polarized cells (Vicente-Manzanares et al. 2008).

However, the phosphorylation of S1943 (NHT) of MHCII-A also participates in migratory polarization. Ser1943 phosphorylation reduces the size of transient NMII-A mini-filaments, inhibiting cell polarization in soft matrices (Raab et al. 2012). Likewise, the phosphorylation of S1935 of MHCII-B has a similar effect on NMII-B mini-filaments, inhibiting polarization in soft and stiff matrices (Juanes-Garcia et al. 2015). These two sets of data demonstrate that, although the conformational change and activation of NMII via RLC phosphorylation is critical for the role of NMII in cell migration, fine-tuning filament stability by NHT phosphorylation provides additional regulatory flexibility.

The picture is relatively similar in mesenchymal cells migrating in 3D environments. In these, the lamellipodium is smaller, followed by an elongated protrusion (Friedl & Wolf 2010). The amount of NMII in these compartments is very low. Instead, NMII concentrates around and behind the nucleus (Barbier et al. 2019, Friedl & Wolf 2010, Liu et al. 2015, Ruprecht et al. 2015). Together with the nucleus, NMII forms a pistonlike system that pushes the cell ahead (Petrie et al. 2014, 2016). Importantly, NMII-B is necessary for nuclear squeezing through narrow pores (Thomas et al. 2015) and contributes to collagen remodeling (Meshel et al. 2005) (**Figure 3*b***).

7.2. Nonmuscle Myosin II Regulation in Migrating Amoeboid Cells

While mesenchymal cells display a stable and well-delineated spatial and temporal segregation of NMII phosphorylation, this is not the case in amoeboid cells. Cells adopting this migratory mode display a prominent lamellipodium and a posterior appendage of variable length, the uropod (Vicente-Manzanares & Sanchez-Madrid 2004). This is the typical mode of migration of leukocytes in both two and three dimensions. In two dimensions, leukocyte amoeboid locomotion is integrin-dependent; however, it is not in three dimensions (Lammermann et al. 2008). Instead, cells use friction to create transient traction points (Bergert et al. 2015). Friction does not require receptor-based adhesion, but it is enhanced by glycocalyx–extracellular matrix interactions (Schmidt et al. 2020).

The phosphorylation of Tyr155 in RLC is common to mesenchymal and amoeboid cells (C. Llorente-González and M. Vicente-Manzanares, unpublished observations), indicating that the inhibition of NMII hexamer assembly at the leading edge is a common regulatory step. However, amoeboid cells concentrate pSer19 RLC at the uropod. Interestingly, cells displaying mesenchymal traits in two-dimensional (2D) or relaxed 3D environments become amoeboid under high confinement, i.e., when they are so confined their nucleus is squeezed (Liu et al. 2015). Dynamics experiments using cells under high confinement reveal that actin retrograde flow drives NMII filaments to the trailing edge, contributing to nuclear squeezing through narrow pores (Liu et al. 2015, Ruprecht et al. 2015). Under high confinement, damage to the

nuclear membrane is rapidly repaired by the ESCRT complex to prevent DNA damage due to the translocation of cytoplasmic factors (Denais et al. 2016, Raab et al. 2016). Two recent studies (Lomakin et al. 2020, Venturini et al. 2020) showed that cortical NMII translocation to enable 3D migration depends on a mechanosensitive pathway that connects PLA2-dependent RhoA activation with nuclear compression.

Blebbing motion can be classified as a less polarized variant of amoeboid migration, in which cells move due to imbalances in hydrostatic pressure via the selective contraction of cortical NMII at specific regions of the cortex (Charras & Paluch 2008). Confinement triggers this type of motion (Liu et al. 2015); thus, it is likely governed by mechanisms similar to those described previously in this section.

7.3. Nonmuscle Myosin II Regulation During Cell Division

The classical view of mitosis is that it is a microtubule-based process focused on chromosomal segregation (Walczak et al. 2010). Recent evidence, however, demonstrates a key role for actomyosin in this process. NMII mediates initial cell rounding as cells enter prophase and provides constriction during cytokinesis. It is also involved in the formation of the bipolar mitotic spindle in the equatorial plate and in asymmetric division (Shin et al. 2014). The contribution and roles of NMII regulation in mitosis seem to be cell lineage– and context-dependent, except for cytokinesis, which seems to be universally dependent on NMII. Here, we describe a model that integrates the function of NMII in mammalian adherent cell mitosis and cytokinesis (**Figure 4**).

7.3.1. Prophase. The initial morphological manifestation of prophase is cell rounding (Dix et al. 2018), which requires integrin inactivation (**Figure 4**). Global integrin adhesion remodeling is observed at the onset of prophase, which requires turning off the Rap1/RIAM (Rap1-interacting adapter molecule)–signaling circuit (Dao et al. 2009). Integrin inactivation is coupled to stress fiber disassembly due to RLC dephosphorylation (Totsukawa et al. 1999). The disassembly of stress fibers predates the accumulation of NMII at the cortex, which regionally regulates cortical tension and drives the positioning of the mitotic spindle during prometaphase (Sommi et al. 2011).

7.3.2. Metaphase. Metaphase comprises the formation of the mitotic spindle, which is mainly made up of dynamic microtubules (**Figure 4**). Although NMII weakly localizes to the spindle in the equatorial plane (Sandquist et al. 2011), its function seems critical to establishing the symmetric and equal segregation of both genetic and cytoplasmic material between the daughter cells. Experiments using ROCK and NMII inhibitors showed that ROCK-NMII blockade perturbed the assembly and localization of the mitotic spindle and its localization in mammalian adherent cells, also blocking the separation of duplicated centrosomes (Rosenblatt et al. 2004). This process relies on the NMII-dependent contraction of randomly oriented actin filaments around the center of the dividing cell, which become oriented along the cortex, amplifying contraction (Spira et al. 2017). How NMII becomes activated in this spatially restricted manner is still unclear. However, it requires ROCK, as its inhibition impairs spindle positioning (Rosenblatt et al. 2004). Other kinases, e.g., AMP-activated protein kinase (AMPK), participate in this process. AMPK deletion impairs mitosis, which is restored by the expression of phosphomimetic (Thr18/Ser19 to Asp18/Asp19) RLC mutants (Lee et al. 2007). Conversely, experiments in *Drosophila* embryos revealed that a nonphosphorylatable form of RLC (encoded by the *Sqh* gene in *Drosophila*) impaired spindle reorientation (Lam et al. 2020). This suggests that NMII activation is dependent on Ser19 phosphorylation. In agreement with this idea, MYPT1 depletion causes the formation of misaligned spindles in HeLa cells, likely due to increased cortical tension (Matsumura et al. 2011). Also involved in this process, the CDK1-dependent phosphorylation of MYPT1 enables the translocation of

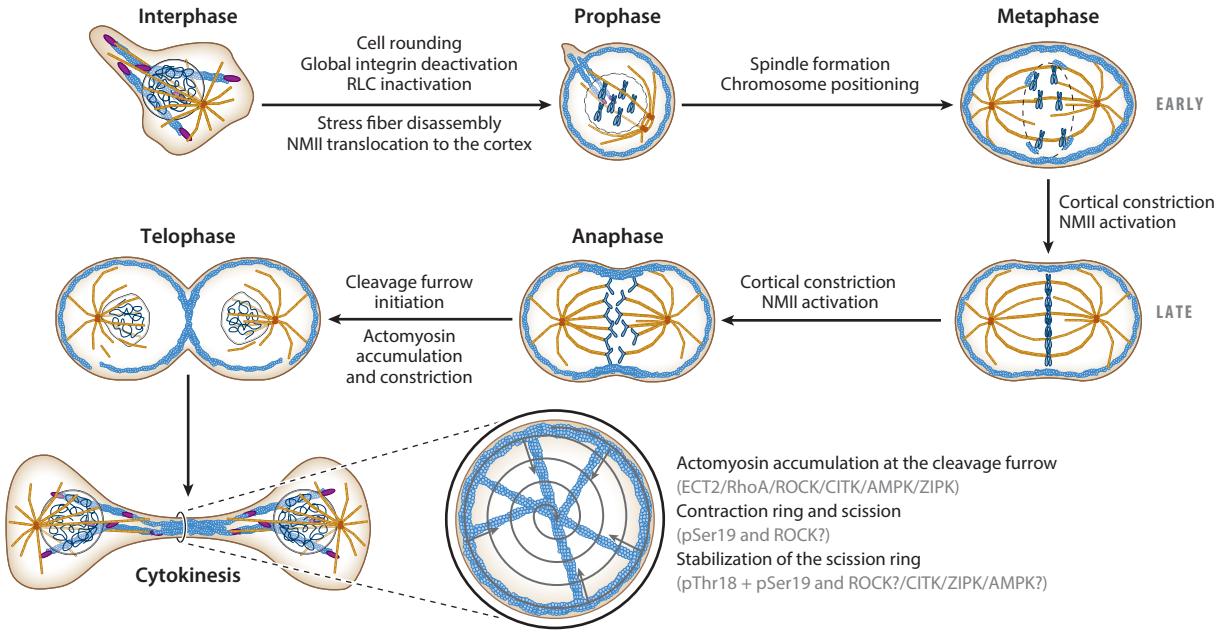


Figure 4

Modeling NMII function in dividing mammalian cells. In cells in interphase, the chromatin appears loose and is surrounded by the nucleus. This model depicts microtubules as gold tubes converging on the centrosome (*dark orange*). In prophase, DNA (*dark blue*) condenses into chromosomes. Chromosome condensation is associated with nuclear envelope breakdown, centrosomal duplication, and cell rounding. In early metaphase, chromosomes are organized around the mitotic spindle that forms within the equatorial plate and are retained in this region by NMII contraction on the actomyosin network associated with the remnants of the nuclear envelope (Booth et al. 2019). In late metaphase, chromosomes are properly congregated and aligned. For the purposes of this review, note the progressive accumulation of actomyosin around the future site of the cleavage furrow, which begins during telophase and culminates in cytokinesis. Cytokinesis is shown to illustrate the narrowing of the connection between daughter cells, which requires both NMII-mediated constriction and ESCRT-mediated scission (*gray spiral*). Abbreviations: AMPK, AMP-activated protein kinase; CITK, citron kinase; ECT2, epithelial cell–transforming sequence 2; NMII, nonmuscle myosin II; RhoA, Ras homolog family member A; RLC, regulatory light chain; ROCK, Rho-associated coiled-coil-containing kinase; ZIPK, zipper-interacting protein kinase.

NMII to the cortex, which locally increases tension (Ramanathan et al. 2015). When phosphorylated, MYPT1 also interacts with PLK1 (Polo-like kinase 1). MYPT1 dephosphorylates PLK1, impairing the activation of dynein and causing aberrant mitotic spindle orientation (Yamashiro et al. 2008). PLK1 may also amplify NMII recruitment to the cortex by phosphorylating ROCK, leading to increased MYPT1 inactivation and additional PLK1 activation (Lowery et al. 2007).

During this process, NMII must remain in the cortex to contract the cleavage furrow in cytokinesis (**Figure 4**). Also, cortical NMII likely prevents inappropriate or premature spreading. Thus, additional factors must impair NMII-dependent stress fiber formation. Because integrins become activated in response to mechanical force (Friedland et al. 2009, Schurpf & Springer 2011), NMII-generated cortical forces cannot activate integrins indiscriminately. This could be accomplished if integrins are physically disconnected from cortical, NMII-dependent forces. The mechanism is currently unknown. We hypothesize that it could involve the competitive interaction of myosin X with the talin-binding domain of integrin. In agreement with this hypothesis, myosin X contains a FERM domain that can outcompete talin without triggering integrin activation (Miihkinen et al. 2020).

NMII-dependent spindle positioning is also dependent on the disassembly of the nuclear envelope. Prior to this disassembly, a perinuclear actomyosin network forms on the cytoplasmic surface of the nuclear envelope in a linker of nucleoskeleton and cytoskeleton (LINC) complex–dependent manner, indicating that actin connections to the nuclear membrane are crucial for its assembly. The perinuclear actomyosin network lingers after nuclear envelope breakdown, providing a physical scaffold that contains chromosomal scattering in a manner dependent on NMII-mediated contraction, facilitating the adequate congregation and segregation of the genetic material between the daughter cells (Booth et al. 2019).

7.3.3. Cytokinesis. While no specific function has been reported so far for NMII in anaphase and telophase, many studies have indicated that NMII is essential for cytokinesis. The localization and activation of NMII at the cleavage furrow provide the mechanical force needed to separate the two daughter cells (**Figure 4**). The deletion or inhibition of NMII in cultured cells impairs cytokinesis, causing multinucleation (Bao et al. 2005, Ma et al. 2010). NMII activation at the cleavage furrow is mediated by the local accumulation of diverse signaling intermediates, e.g., the RhoA GEF ECT2 in a PLK1-dependent manner (Su et al. 2011). During cytokinesis, RhoA becomes part of a complex that includes its effector CITK; this complex localizes to the cleavage furrow (Bassi et al. 2011, 2013), where it is activated by ECT2. Active RhoA at the cleavage furrow triggers ROCK and CITK, locally increasing RLC phosphorylation (Eda et al. 2001, Wagner & Glotzer 2016). Also, RLC phosphorylation is enhanced through localized MYPT1 inactivation (Kawano et al. 1999, Totsukawa et al. 1999). The concurrence of these signals is consistent with a local activation of NMII. However, based on the relative stability and cellular function of mono- and bisphosphorylated NMII and the functional differences between heavy chain isoforms, we postulate a dual role for NMII in this process. Indeed, NMII provides initial constriction, which likely extends and localizes the activation of cortical NMII initiated during metaphase. By analogy with lamellar dynamics, this process is more efficient when NMII (preferentially NMII-A) is monophosphorylated in Ser19. As pSer19 NMII accumulates at the cleavage furrow, it localizes close to other kinases that can mediate its bisphosphorylation, e.g., CITK. A fraction of monophosphorylated NMII becomes bisphosphorylated, locally stabilizing NMII filaments. This would increase the relative resistance to tension of the cleavage furrow to prevent mechanical instability at the constricted region. Based on the role of RLC bisphosphorylation and the superior stability of NMII-B/C filaments when compared to those made of NMII-A alone, the bisphosphorylation of NMII-B/C would be essential for the cleavage furrow to withstand increased tension. Several independent lines of evidence point to this. Cytokinesis fails in cells depleted of CITK, which promotes RLC bisphosphorylation during mitosis (Madaule et al. 1998). Also, NMII-B-depleted cells display a cytokinesis defect that is better restored by the re-expression of NMII-B or NMII-C than of NMII-A (Bao et al. 2005). NMII-B/C have higher duty ratios than that of NMII-A (Heissler & Manstein 2013) and form much more stable filaments than those made exclusively of NMII-A (Vicente-Manzanares et al. 2011). However, bisphosphorylated NMII-B is the main constituent of highly polarized migrating cells due to the stabilization of the rear (Vicente-Manzanares & Horwitz 2010, Vicente-Manzanares et al. 2008), which is consistent with a role for this isoform in the generation of cellular structures designed to resist strain, such as the trailing edge (in cell migration) and the formation of Flemming's body during cytokinesis. Although a relationship between the localization of the ESCRT complex to the cytokinesis ring and NMII-mediated constriction has not been formally proven, it is tempting to speculate that the ESCRT complex is translocated to this region in response to NMII-dependent strain to repair possible damage to the plasma membrane, similar to its role in repairing the nuclear membrane in migrating cells under high confinement (see Section 7.2).

Together, these data support a model in which cytokinesis proceeds in two overlapping phases. The initial constriction of the cytokinesis ring is driven by the dynamic activation of NMII, i.e., its phosphorylation in Ser19 alone. As constriction proceeds and forces become locally higher at the shrinking cleavage furrow, bisphosphorylation- and/or isoform-dependent stable NMII filaments provide a reinforced scaffold that prevents the potential destabilization of the cleavage furrow. Otherwise, the daughter cells would either undergo apoptosis or remain fused, leading to large, multinucleated cells that would trigger post-slippage DNA damage and apoptosis in a manner similar to that observed when cells were treated with the mitotic inhibitor paclitaxel (Zhu et al. 2014). This is a distinct possibility based on the increased cytoplasmic size and degree of multinucleation of NMII-B-depleted cells.

For the purposes of validation, this model needs to include an explanation of how cells expressing only one isoform of NMII undergo division. Most cells that contain only NMII-A, e.g., neutrophils and platelets (Maupin et al. 1994), do not divide. Mouse B16 melanoma cells do not contain NMII-B but are highly transformed and contain NMII-C, which could compensate for such a deficiency (Jacobelli et al. 2004). Regarding genetic models that contain only one isoform, e.g., *Drosophila*, the duty ratio of *zipper* (the only isoform of NMII expressed in *Drosophila*) is more similar to that of NMII-B/C than that of NMII-A (Heissler et al. 2015). This fact supports the superior stability of *zipper*-containing cleavage furrows over mammalian structures made only of NMII-A. Recent evidence also indicates that external tension can add to *zipper*-mediated tension in densely packed regions (Lam et al. 2020), suggesting that external tension, e.g., in regions where cells are densely packed, can compensate for decreased stability in cells only expressing NMII-A.

8. CONCLUSIONS AND PERSPECTIVES

NMII is a pivotal molecule in all cellular phenomena involving movement, most notably cell migration and division. The plasticity of actomyosin structures enables the dynamic changes cells undergo when migrating and/or dividing. Recent research has revealed that the spatial confinement of NMII activity is essential for shape optimization in accordance with the ongoing cellular process. Due to its structure and biochemical composition, which includes different isoforms that can copolymerize in living cells, NMII function can be regulated in myriad ways. These include mechanisms that curb or stimulate force generation, actin binding, and load; filament formation; and even protein biosynthesis. Many of these changes ensue in response to phosphorylation. The compartmentalization of specific kinases that accomplishes these functions provides a high degree of molecular control, adapting NMII organization and function to the spatial and temporal requirements of the cell. Outstanding questions remain, including the ability of the various NMII kinases to promote different degrees of RLC and MHCII phosphorylation, which change the biochemical and biophysical properties of actomyosin filaments in an isoform-dependent manner in migrating cells. In dividing cells, questions remain regarding the retention of NMII in the cortex; isoform dependency; and the lack of force-dependent integrin activation in cells containing a high degree of active NMII, which causes strong adhesion in cells in interphase. Although NMII is not oncogenic, its deregulation controls many aspects of the fate of cancer cells. Future research will address these and many other questions, extending our knowledge of one of the oldest (Kühne 1864) but most fascinating biomolecules at the interface of biochemistry and biomechanics.

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