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Mammalian Nonmuscle Myosin II Comes in Three Flavors

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Abstract

Nonmuscle myosin II is an actin-based motor that executes numerous mechanical tasks in cells including spatiotemporal organization of the actin cytoskeleton, adhesion, migration, cytokinesis, tissue remodeling, and membrane trafficking. Nonmuscle myosin II is ubiquitously expressed in mammalian cells as a tissue-specific combination of three paralogs. Recent studies reveal novel specific aspects of their kinetics, intracellular regulation and functions. On the other hand, the three paralogs also can copolymerize and cooperate in cells. Here we review the recent advances from the prospective of how distinct features of the three myosin II paralogs adapt them to perform specialized and joint tasks in the cell.

Introduction

Myosins constitute a family of molecular motors that use the energy of ATP hydrolysis to move along actin filaments. At present, over 30 distinct myosin classes are known in eukaryotes. The human genome contains 38 myosin genes from 12 of these classes. Class II myosins are unique in their ability to polymerize into bipolar filaments, which can contract an array of oppositely oriented actin filaments and exert large mechanical forces in cells via their ability to act as multimotor ensembles. Mammalian class II myosins include multiple sarcomeric paralogs, as well as one smooth muscle and three nonmuscle myosins, which are closely related to each other and more distinct from sarcomeric myosins. Nonmuscle myosin II (NMII) is present in virtually all animal cell types and involved in numerous cell functions, including migration, adhesion, cytokinesis, intracellular transport, organelle morphogenesis, as well as organization and remodeling of the actin cytoskeleton. Three mammalian NMII heavy chain genes (MYH9, MYH10, and MYH14) encode NMIIA, IIB, and IIC, respectively. Despite overall similarity, NMII paralogs exhibit significant differences in motor kinetics, structure and dynamics of bipolar filaments and cellular functions. NMII paralogs are expressed in cells as various cell type- and tissue-specific combinations [1, 2]. NMIIA and NMIIB are relatively broadly expressed, whereas NMIIC expression is limited to some differentiated tissues, but generally low in fetal tissues and

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stem cells. Comparative analyses of NMIIA and NMIIB in vitro and in cells represent an active focus of current research, whereas studies of NMIIC are still in early stages.

In this review, we focus on our current knowledge of how differences in properties and regulation of mammalian NMII paralogs translate into their specific intracellular functions, and how these paralogs cooperate in cell. Several recent reviews have discussed other aspects of NMII activity, such as kinetics [3], regulation [4], and roles in development and disease [2, 5].

Features of the NMII molecule

Structure

The NMII molecule is a hexamer (Fig. 1A). NMII heavy chains form parallel homodimers that associate with two pairs of light chains – essential and regulatory (myosin regulatory light chain, MRLC) – to form the NMII holoenzyme referred to as NMIIA, NMIIB, and NMIIC depending on the type of the heavy chain. As in all other myosins, the NMII heavy chain contains a conserved *N-terminal motor domain,* which consists of a globular *head* followed by an α-helical *neck,* which is stabilized by light chains and serves as a lever arm to amplify the ATP hydrolysis-dependent conformational change in the head to make a step along the actin filament. The NMII heavy chain C-terminus consists of a long α-helical *rod domain*, which is responsible for heavy chain dimerization through coiled-coil formation, and a short *nonhelical tailpiece,* which is most divergent among NMII paralogs. Two hinge regions in the coiled-coil rod allow the NMII molecule to acquire a folded autoinhibitory conformation.

Motor

Mechanoenzymatic properties of NMII motors vary among NMII paralogs and are tailored to specific intracellular functions [3]. In general, class II myosins have a relatively *high ATPase rate* and a *low duty ratio* (the proportion of the ATPase cycle that a motor spends strongly attached to its track), which makes them *non-processive* motors. However, since NMII in cells works in ensembles (bipolar filaments), NMII can stay associated with actin tracks over multiple ATP cycles as a processive motor [6, 7]. At the same time, the low duty ratio ensures that myosin heads in the filament do not interfere with each other during movement.

NMII paralogs are kinetically distinct. NMIIA is the fastest NMII paralog with highest ATPase rate. NMIIB moves slower with a relatively high duty ratio due to high binding affinity for ADP [8]. Additionally, force resisting the motor powerstroke can increase the duration of actin-myosin interaction, leading to catch-bond behavior [9]. The extent of mechanosensitivity varies among myosins. For example, NMIIB exhibits much stronger catch-bond behavior than NMIIA [10]. Kinetic diversity of NMII motors can be further increased due to alternative splicing of NMIIB and NMIIC heavy chains at two sites in the motor domains [2, 11]. Furthermore, intrinsic mechanokinetic properties of NMII paralogs can be modulated by external factors, such the actin isoform that forms the track [12], the presence of tropomyosins [13], and viscosity of the environment [7, 14].

Polymerization

Polymerization of NMII molecules into bipolar filaments occurs through staggered parallel and anti-parallel interactions between their rod domains. In assembled bipolar filaments, motor domains face opposite directions from the midzone. The filament nucleation depends on two conserved *assembly competence domains (ACD1 and 2)* at the end of the heavy chain rod. ACDs are thought to induce antiparallel dimerization of NMII molecules via electrostatic interactions between their complementary charges [15, 16]. Folded NMII monomers also can form antiparallel dimers in vitro, suggesting that NMII unfolding may occur after filament nucleation [17, 18]. Subsequent addition of NMII subunits to dimeric nuclei depends on interactions between periodically alternating positively and negatively charged segments of the rod [19]. These interactions may also promote unfolding of subunits added in the folded state [18]. The resulting NMII bipolar filament consists of up to 30 molecules for NMIIA and NMIIB and ~14 molecules for NMIIC [17].

Regulation of NMII turnover cycle

NMII undergoes constant polymerization-depolymerization cycles in cells. In the autoinhibitory (folded, 10S) conformation, the coiled coil rod folds at two hinge regions so that the second hinge binds MRLCs at the neck [20–22]. This interaction inhibits both NMII motor activity and polymerization [4]. The NMII dynamic cycle includes activation of autoinhibited molecules and their assembly into bipolar filaments followed by filament disassembly and subunit recycling (Fig. 1B). This cycle allows NMII to build and dismantle the contractile system as needed. Individual steps of the NMII cycle are controlled by phosphorylation and protein-protein interactions.

Activation of the motor

The ATPase activity of autoinhibited NMII molecules is restored by MRLC phosphorylation on Ser19, whereas additional phosphorylation of Thr18 further increases the actin-activated ATPase activity [23]. MRLC can be phosphorylated by multiple kinases, including ROCK, MLCK, MRCK, PAK, and citron kinase [4]. Individual kinases are thought to activate NMII at different subcellular locations and/or in response to different signals. MRLC can also be phosphorylated at Ser1/Ser2/Thr9 by protein kinase C (PKC). Phosphorylation of these residues decreases the rate of MRLC phosphorylation by MLCK in vitro, thereby indirectly inhibiting NMII activity [24]. This regulation was shown to promote PDGF-induced stress fiber disassembly [25] and cell chemorepulsion [26], but not affect NMII assembly in another study [27]. Since MRLC is shared by NMII paralogs, NMII regulation through MRLC phosphorylation is not expected to be paralog-specific, unless the enzymes can recognize paralog-specific sequences in the second hinge region of the heavy chain, which interacts with MRLC in the folded molecule [28, 29].

Regulation of NMII polymerization and depolymerization

Besides restoring NMII motor activity, MRLC phosphorylation releases the MRLC-rod interaction, thus permitting, although not imposing the rod unfolding [18]. Experimental abrogation of the MRLC-rod interaction by deleting the MRLC binding site [30] or

eliminating the MRLC-interacting hinge region [31] caused over-assembly of these mutants in cells, thus confirming an inhibitory role of this interaction for NMII filament assembly.

Disassembly of bipolar filaments is largely regulated through the NMII heavy chain, primarily, through the nonhelical tailpiece and adjacent regions of the coiled coil rod. These regions contain paralog-specific phosphorylation sites and can also bind regulatory proteins [4, 16]. Differences in the C-terminal regions of the NMII heavy chain are largely responsible for different filament dynamics in cells, as well as distinct intracellular localization of the paralogs.

In vitro studies showed that phosphorylation or phosphomimetic mutations of the nonhelical tailpiece inhibit polymerization of rod domains of mammalian NMIIA, NMIIB and NMIIC [32, 33]. Despite similar effects of phosphorylation, deletion of the tailpiece promotes assembly of the NMIIA and NMIIB rods, but weakens assembly of the NMIIC rod [33, 34]. In NMIIC, the dephosphorylated tailpiece flips onto and binds the coiled coil, which promotes rod polymerization, but this activity is lost upon tailpiece phosphorylation [34]. The scenario is likely opposite for NMIIA: the tailpiece may gain affinity for the coiled coil upon phosphorylation, which could inhibit filament assembly [16].

Phosphorylation sites in the NMIIA heavy chain include a putative PKC target site at Ser1916 (human numbering) just before the tailpiece, and a putative casein kinase II (CKII) site at Ser1943 in the tailpiece. Notably, CKII depletion from cells did not affect the level of S1943 phosphorylation suggesting involvement of other kinase(s) [35]. The main regulatory site for the NMIIB heavy chain is a stretch of five serine residues (1935–1941) that can be phosphorylated by PKCγ [36] and aPKCζ [37]. Among several C-terminal phosphorylation sites in NMIIC, only phosphomimetic mutations of PKC sites (T1957D/T1960D) in the tailpiece inhibited polymerization of NMIIC rods in vitro [33].

Cell-based assays, in general, support the insights from in vitro studies about regulation of NMII assembly. Expression of NMIIA heavy chains either lacking the tailpiece or containing the S1943A substitution resulted in over-assembly of NMII in cells [30]. In NMIIB, deletion or phosphomimetic mutations of the tailpiece serine cluster increased NMIIB dynamics in cells [38] and decreased the insoluble (polymerized) NMIIB fraction in cells [36]. Notably, heavy chain phosphorylation unlikely functions as an on/off switch, because NMII heavy chains with phosphomimetic mutations could be found in association with the cytoskeleton in cells [39]. Most likely, heavy chain phosphorylation shifts the balance toward NMII filament disassembly by weakening subunit interactions.

In addition to heavy chain phosphorylation, assembly of NMII filaments is regulated by interacting proteins. The best characterized regulator is S100A4/Mts1, which specifically regulates disassembly of NMIIA [16, 32]. The binding mechanism includes initial recognition of the nonhelical tailpiece and subsequent binding to and partial unwinding of the ACD1-proximal coiled coil, which could eventually lead to dissociation of the NMIIA subunit [40, 41]. Another protein from the same family, S100P, can dissociate NMIIA and NMIIC filaments by a similar mechanism [42]. The cancer suppressor Lgl1 can bind directly to the coiled coil of NMIIA between ACD1 and ACD2, potentially through electrostatic

interactions, and block filament assembly in vitro [43]. This study also showed that ectopically expressed Lgl1 and NMIIA could be coimmunoprecipitated from cells. However, another study revealed an interaction of Lgl1 only with NMIIB, but not NMIIA, using coimmunoprecipitation of endogenous proteins [44]. This discrepancy remains to be resolved, especially because all charged residues within the proposed Lgl1-binding site of NMIIA are conserved in NMIIB. Recently, it was shown that motor-inactive myosin 18A can copolymerize with NMII and might regulate the degree of NMII assembly and/or the mechanical output by reducing the number of force generating NMII heads per bipolar filament [45]. Direct interaction with the NMIIA rod have been also reported for gelsolin [46], gelsolin-like protein flightless-1 [47], Arf GAP ASAP1 [48], and Rho GAP Dlc1 [49], with the latter two interactions affecting stress fiber assembly in cells. The direct interaction between rod domains of NMIIB and kinesin 12 regulated migration of astrocytes [50]. Other interaction partners of NMII, especially of NMIIA, have been reported, but it is not clear whether any of these interactions affect NMII polymerization.

Although the NMII heavy chain-dependent regulatory mechanisms are generally thought to promote disassembly of NMII filaments, in principle, they also can prevent filament assembly. Simultaneous phosphorylation of MRLC and the heavy chain has a potential to produce unfolded motor-active NMII molecules. Indeed, unfolded MRLC-phosphorylated NMIIA and NMIIB monomers were detected in cells and appeared to be functionally important [51–53].

General principles of assembly and remodeling of NMII-containing structures in cells

The major function of NMII filaments in cells is contraction, which is performed in cooperation with actin filaments. The main principles of assembly and dynamics of contractile structures are similar for different NMII paralogs, at least for the best studied ones, NMIIA and NMIIB. The actin-NMII contractile systems consist of non-aligned networks, aligned bundles and intermediate arrays of actin and NMII filaments. These systems gradually evolve in the processes of actin and NMII polymerization and actin-NMII interaction (Fig. 2A).

Assembly of new NMII filaments in cells often begins behind the protrusive cell edges. The NMII filaments then drift away from the cell edge with the actin retrograde flow simultaneously forming clusters, in which NMII filaments often interact at their ends [54, 55], which seems to be an intrinsic property of NMII filaments also observed in vitro [7, 17]. Clusters of NMII filaments embedded into disordered actin filament arrays constitute *actin-NMII networks* capable of large-scale contractile activities including cell body translocation in migrating keratocytes [55], apical constriction of epithelial cells [56], cytokinesis [57], and many others. In the course of contraction, actin-NMII networks can disassemble with subsequent recycling of NMII monomers [56, 58]. The local assembly-disassembly cycles producing pulsatile contraction in the cell lamella are characteristic for NMIIA, but not exhibited by NMIIB, and results from the differences in their motor domains [58]. Alternatively, actin-NMII networks in the course of contraction can reorganize into aligned

actin-NMII bundles [55, 57, 59, 60]. NMIIA and NMIIB both participate in this type of actin-NMII remodeling, but NMIIB typically remains associated with actin bundles for longer time [61].

Stress fibers and *circular bundles* are two main types of aligned bundles of actin and NMII filaments, which can develop greater contractile forces than networks due to their superior organization. Stress fibers participate in cell migration and are often organized into a complex system attached to the substratum by focal adhesions. Circular actin-NMII bundles do not interact with focal adhesions, but apply force to plasma membrane through other protein complexes. They function in cytokinesis, cell-cell adhesion, wound closure and cell extrusion [62]. In actin-NMII bundles, NMII filaments often form registered stacks [54] that can be arranged in a discontinuous manner and alternate with α-actinin-rich zones, thus resembling sarcomeric organization [63]. In the course of contraction, the spacing between the NMII stacks in stress fibers decreases [64] up to a complete loss of gaps (Fig. 2A). Eventually, even long-lived actin-NMII bundles can be disassembled and recycled.

Dynamics and sorting of NMII paralogs

Intracellular segregation of NMIIA and NMIIB

In cells cultured on 2D substrate, NMIIA and NMIIB have overlapping but distinct distributions. At steady state, NMIIB typically acquires more central (in unpolarized cells) or posterior (in front-back polarized cells) localization relative to NMIIA [65, 66]. This phenomenon was initially interpreted as reflecting different sites and/or timing of NMIIA and NMIIB polymerization. However, when the contractile system was allowed to assemble de novo [53, 67], NMIIA and NMIIB initially exhibited indistinguishable distribution and segregated much later, suggesting essentially similar assembly pathways for both paralogs that are followed by their sorting during system maturation.

Maturation of individual stress fibers in cells expressing both NMIIA and NMIIB proceeds through stereotypic temporal changes in their NMII contents [61]. Stress fibers newly formed near the leading edge are enriched with NMIIA, but also contain NMIIB. Over time, while the stress fiber undergoes retrograde flow, it progressively loses NMIIA and becomes enriched with NMIIB. Eventually, NMIIB-rich stress fibers either disassemble or form long-lived ventral stress fibers – the most mature type of stress fibers [68] – at the cell center or rear. In contrast, NMIIA is enriched in the younger stress fiber types – transverse arcs and radial stress fibers – formed at earlier stages of network-to-bundle reorganization of actin-NMII assemblies [55, 64, 69] (Fig. 2B).

Dynamics of NMIIA and NMIIB

Gradual replacement of NMIIA by NMIIB in stress fibers can be explained by similar polymerization and distinct depolymerization mechanisms for two paralogs [61]. The NMII assembly mechanisms are very similar, because they involve phosphorylation of the shared MRLC and interaction between conserved ACDs, which exhibit ~80% identity and ~88% homology between respective NMIIA and NMIIB sequences. Accordingly, NMII paralogs *copolymerize* in cells [53, 67]. The similar assembly properties explain equivalent

incorporation of NMIIA and NMIIB into nascent homo- and heterotypic NMII filaments, which likely occurs according to availability of respective polymerization-competent monomers.

Current ideas about the NMII disassembly mechanisms, on the other hand, implicate the most divergent sequences of the NMII heavy chain – the nonhelical tailpiece [16]. Consistent with this, NMII paralogs exhibit different turnover rates in cells. Specifically, NMIIB was found to exhibit slower rates of fluorescence recovery after photobleaching and larger immobile fractions than NMIIA [70–72]. Analysis of NMIIA/NMIIB chimeras revealed that these differences in NMII turnover depended on the C-terminus of the NMII heavy chain [70]. These findings support the idea that NMII turnover is controlled by the divergent NMII tails, which can be affected by distinct depolymerization mechanisms, such as phosphorylation or binding of interaction partners, to stimulate dissociation of specific NMII subunits from either homotypic or heterotypic bipolar NMII filaments.

Shifting the NMII dynamics toward disassembly often correlates with increased cell migration and invasion, likely due to an increased NMII turnover, which promotes cytoskeleton reorganization. Such behavior was observed upon phosphorylation of S1916 [73] or S1943 [39] on NMIIA. Similarly, several accelerators of NMIIA disassembly positively regulate cell migration and are often upregulated in cancer cells [16, 42, 43, 74]. Phosphomimetic mutations in the NMIIB tailpiece compromised stability of actomyosin bundles and induced protrusive activity at the cell rear [38].

Mechanistic model of NMII self-sorting

The higher rates of NMIIA turnover between monomeric and polymeric states, as compared with that of NMIIB, suggest that NMIIA subunits would dissociate from NMIIA/NMIIB heteropolymers more readily than NMIIB, because of the differences in their tail sequences targeted by the regulators of bipolar filament disassembly. However, new subunits should be added proportionally to the abundance of each paralog in the monomer pool due to their similar assembly properties. Repeating cycles of preferential dissociation of NMIIA subunits and unselective recruitment of new subunits will gradually increase the fraction of NMIIB in the older NMII filaments. Because NMII filaments, as a component of stress fibers, undergo retrograde flow over time, the older NMIIB-enriched filaments become concentrated farther away from the leading edge, as compared with the younger NMIIA-enriched filaments, thus generating the polarized anterior-posterior NMIIA-NMIIB distribution (Fig. 3). This mechanism also explains why NMII chimeras are sorted according to the identity of their C-terminal tails [70]. Since NMIIB is less dynamic, a substantial fraction of NMIIB becomes sequestered in the long-lived stress fibers at steady state, thus decreasing the pool of NMIIB monomers and exacerbating enrichment of NMIIA in nascent stress fibers.

Different motor properties of NMII paralogs can also contribute to paralog segregation. Fast motility and a short duty ratio of NMIIA can accelerate NMIIA dynamics by allowing the dissociated subunits to quickly diffuse away from the parent filament. Conversely, because of its high duty ratio, which further increases under resisting load [6, 8], NMIIB spends much time bound to actin [75], which can further reduce its turnover. Consistent with this

idea, NMIIB polarizes toward the cell rear only on stiff, but not soft substrates [72], suggesting that tension generated by the cell augments paralog segregation.

Distinct properties of actin-NMIIA and actin-NMIIB arrays

Different dynamics and kinetics of NMII paralogs translate into different properties of actin-NMII structures formed by these paralogs [61]. For example, NMIIB favors formation of stable and long-lived ventral stress fibers, whereas NMIIA promotes formation of highly dynamic transverse arcs and radial stress fibers. When different paralogs are present simultaneously in cells, their copolymerization allows for the formation of bipolar filaments with a continuous range of dynamic properties between the extremes characteristic for homotypic NMII filaments. In cells expressing NMIIA, NMIIB exhibits faster dynamics and acquires more disperse distribution compared with cells lacking NMIIA [61]. On the other hand, by forming mixed filaments with NMIIA, NMIIB makes them more processive runners in vitro than the NMIIA-only filaments [7].

Functions in cells

The main function of NMII in cells is generation of contractile forces, which are used in most cell types for many purposes. NMII is best known to function in cell migration, where it contributes to regulation of leading edge protrusion, cell-substrate adhesion, cell body translocation and cell polarity. NMII is also important for cytokinesis, remodeling of the extracellular matrix (ECM), formation of cell-cell adhesions and cell shape determination [76]. Novel NMII roles have been recently revealed in endocytosis, exocytosis and vesicular transport, some of which appear to include functions of motor-active NMII monomers.

Cell-ECM adhesion

Cell adhesion to ECM provides traction to migrating cells. It is typically mediated by adhesion receptors of the integrin family. Integrin-mediated adhesion exhibits catch-bond behavior due to mechanosensitive properties of the adhesion complex [77]. The adhesion-strengthening force is largely generated by NMII, which pulls on actin filaments anchored to integrins through adaptor proteins. In some cell types, this mechanism can lead to the formation of large focal adhesions at the tips of stress fibers. The size and fate of a focal adhesion depends both on how strongly NMII pulls on the attached actin bundle and to what extent actin polymerization at the focal adhesion alleviates the tension. For example, fast actin polymerization at the ends of radial stress fibers delays maturation of the associated focal adhesions until actin elongation is inhibited through phosphorylation of VASP [68].

As a fast motor able to better cope with rapid actin polymerization, NMIIA is expected to have greater contribution to focal adhesion assembly near the leading edge, whereas NMIIB is better posed to stabilize focal adhesions in more central cellular regions, where NMIIB-dependent isometric tension could be sufficient [78]. Nonetheless, NMIIB or NMIIC are able to initiate adhesion formation in cells lacking NMIIA. Compared with NMIIB, NMIIA is more capable of traction force generation, so that NMIIA depletion dramatically decreases the forces that the cell exerts on the substratum [61, 79, 80].

The roles of NMIIC are less understood, but could involve either positive [81] or negative [33, 82] regulation of cell adhesions. The C2 splice variant of NMIIC was found to interact and colocalize with β1 integrin and positively regulate adhesion in neuroblastoma cells [81]. NMIIA also interacts with some leukocyte-specific integrins, among which NMIIA interaction with α4-integrin was detected only at high salt conditions, when NMIIA filaments dissociate, suggesting a possible role of NMIIA monomers in adhesion regulation [83, 84].

Leading edge protrusion

Leading edge protrusion is driven by polymerization of actin filaments that push against the plasma membrane. The membrane resistance results in actin retrograde flow, which is also facilitated by NMII, especially in the cell lamella behind protrusions. How far the leading edge advances depends on a difference between rates of actin polymerization and retrograde flow. Accordingly, NMII activity negatively regulates leading edge protrusion [85–87]. On the other hand, efficient protrusion requires traction that is enabled by adhesion, which is a mechanosensitive process. Nascent adhesions are formed underneath lamellipodia and their formation is mechanically stimulated by retrograde flow [88, 89]. Although NMII appears dispensable for adhesion initiation in some cases [90], NMII motor activity contributes to adhesion initiation and productive leading edge advance in other situations [52]. In the latter case, the underlying mechanism was proposed to extend beyond the classic idea of bipolar filament-mediated contraction and involve motor-active individual NMII molecules.

The negative and positive roles of NMII in protrusion appear to be preferentially played by specific NMII paralogs. For example, NMIIA enables neurite retraction in cultured neuronal cells [91]. In non-neuronal cells, increased expression of NMIIA correlated with reduced cell spreading, probably, due to increased contraction [78, 79], while NMIIA-dependent periodic contractions in the lamella correlated with pauses in lamellipodium advance [92]. Conversely, NMIIB supported axon elongation in neurons [93], as well as cell spreading and lamellipodial protrusion in other cell types [78]. NMIIC also stimulated neurite outgrowth in neuroblastoma cells [82] and lamellipodial protrusion in epithelial cells [75].

Contractile forces

During cell migration, NMII-mediated contraction helps to detach obsolete adhesions, retract the cell rear, and translocate forward the cell body [55, 94, 95]. In neurons, NMII-mediated contraction enables consolidation of the axonal shaft behind the advancing growth cone [96], axon retraction in response to repulsive signals [97], and growth cone turning through its asymmetric retraction [98]. Similar contractile forces applied to compliant ECM contribute to ECM remodeling [99]. This function is particularly characteristic for fibroblasts – mesenchymal cells that organize the ECM in tissues. NMIIA is mainly responsible for generating large contractile forces for cell rear retraction [100] and ECM remodeling [101]. Contribution of NMIIB to these processes is minimal in 2D cultures, but becomes significant in 3D environment, where it promotes translocation of the nucleus through tight spaces [80, 102]. In neurons, however, growth cone retraction in response to a chemorepellent relied primarily on NMIIB [97].

Cell polarity

In migrating cells, actin-NMII bundles and networks undergo retrograde flow and accumulate at the cell rear, where they inhibit protrusive activity of lateral and posterior cell edges, thus supporting front-back cell polarity. This function is thought to largely depend on NMIIB, as it can maintain stable stress fibers [71, 85]. Preferential accumulation of NMIIB at the cell rear in the course of self-segregation of NMII paralogs facilitates the establishment of this polarity. However, excessive expression of NMIIB results in over-stabilized stress fibers and focal adhesions, which retard cell migration [61]. In cases of amoeboid type of cell migration, which is characterized by weak cell-substrate adhesions and an absence of stress fibers (for example, in neutrophils, which do not express NMIIB), NMIIA is responsible for the formation of a stable rear end (called uropod in neutrophils) [103].

Cytokinesis

The constriction of cleavage furrow during cytokinesis is another important NMII-dependent function [104]. Similar to interphase cells, NMII in mature cytokinetic contractile rings is organized into bipolar filaments arranged into stacks and aligned with the contraction axis. However, at earlier stages of cytokinesis, the assembly of the contractile ring follows the network contraction mechanism [57, 60]. Interestingly, motor-impaired mutants of NMIIA and NMIIB that are still able to bind actin filaments in an ATP-dependent manner were able to rescue cytokinesis defects in NMIIB-depleted COS-7 cells, which do not endogenously express NMIIA, although it is not clear how NMII-mediated crosslinking drives constriction of the cleavage furrow [105].

In general, each of NMII paralogs can execute cytokinesis [2]. The cellular preference in employing specific NMII paralogs for cytokinesis depends on their relative abundance in individual cell types and/or efficiency of their recruitment to the cleavage furrow. For example, in immature dividing megakaryocytes, which express both NMIIA and NMIIB, only NMIIB was recruited to the cleavage furrow, because NMIIB could respond to lower levels of RhoA activation than NMIIA [106]. The recruitment to the furrow of NMIIB in this system [107] or NMIIA in COS-7 cells [108] depended on the C-terminal heavy chain regions and did not require the motor domain suggesting an actin-independent targeting mechanism for NMII at the cleavage furrow.

Cell shape

Actin–NMII arrays define cell shape and mechanical properties of the cell surface. Here, NMII can function both as a cross-linker to generate isometric tension and as a motor to maintain dynamic actin-NMII networks. For example, in epithelial monolayers, circumferential actin-NMII bundles associated with apical adherens junctions generate tension to preserve junction integrity [109, 110] and stabilize the constricted shape of the apical domain during epithelium invagination. The apical constriction itself is driven by pulsed contractions of actin-NMII networks in the plane of the apical domain [56, 111], although other contractile mechanisms also contribute [112, 113]. The contractile forces at cell-cell junctions are counterbalanced by pushing forces generated by Arp2/3 complex-

dependent polymerization of branched actin networks, which are required to maintain or expand the junction [110, 113].

The spherical shape and high cortical tension of mitotic cells are maintained by submembrane actin networks jointly assembled by NMII [114] and actin nucleators [115], suggesting their dynamic nature. An actin-NMII cytoskeleton at the dorsal surface of cultured cells contains both bundles and networks and undergoes constant remodeling between these states accompanied by corresponding changes in the mechanical properties of the surface [116].

Participation of stable and dynamic actin-NMII arrays in cell shape determination suggests contribution of NMIIB and NMIIA, respectively. In some epithelial cells, the relatively stable circumferential actin-NMII bundles at apical cell-cell junctions indeed required NMIIB functions [117–119], although NMIIA is also important [120], especially for the initial assembly of adherens junctions [117]. In individual cells, NMIIB and NMIIC promote stability of the cell cortex, which helps to reduce formation of surface blebs. In contrast, NMIIA has greater contribution to the cortex stiffness, contractility and bleb formation [75].

Membrane trafficking

Recent data increasingly point to roles of NMII in membrane organelle morphogenesis, such as exocytosis [121], endocytosis [122], post-Golgi and Golgi-to-ER trafficking [123, 124], and mitochondrion fission [125].

Roles of NMII in exocytosis are especially conspicuous during secretion of viscous cargos, such as salivary mucus [126, 127], lung surfactant [128], and endothelial von Willebrand factor [129, 130]. In these cases, NMII is thought to squeeze the cargo from the secretory vesicle. In salivary glands, both NMIIA and NMIIB are important for different aspects of this function. NMIIB prevents counterproductive expansion of the secretory granule immediately after its fusion, whereas NMIIA stimulates subsequent cargo expulsion [127].

In some membrane trafficking events, NMII might function in a monomeric form. For example, monomers of NMIIA have been found in association with lytic granules in the natural killer cells, where they promoted granule secretion [51], and with Golgi membranes isolated from the intestinal epithelium [123]. The association of NMIIA with the Golgi complex was mediated by its coiled coil rod [131]. It remains unclear whether this interaction is compatible with the rod-mediated NMII filament assembly.

Conclusions

Proper accomplishment of virtually every NMII mission requires fine tuning of the balance between the active contraction and tension maintenance. This task can be achieved through combinatorial engagement of NMII paralogs with distinct dynamic properties. The available data suggest that dynamic features of NMII paralogs often can be correlated with their functions in cells. In general, NMIIA is responsible for fast and powerful force generation in response to changing conditions, whereas NMIIB is more suitable to maintain long-lasting stresses and ensure cytoskeleton stability. Too little is known so far about NMIIC functions

to propose what might be special about this paralog. Future research will bring new insights into paralog-specific regulation of NMII expression, intracellular dynamics, interactions with other proteins, and functions.

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Highlights

1. Nonmuscle myosin II executes numerous mechanical tasks in cells including organization of the actin cytoskeleton, cell adhesion and migration
2. Three mammalian nonmuscle myosin II paralogs have distinct kinetic and dynamic properties in vitro and in vivo
3. Mammalian nonmuscle myosin II paralogs mix and match their abilities to perform both specialized and joint tasks in the cell

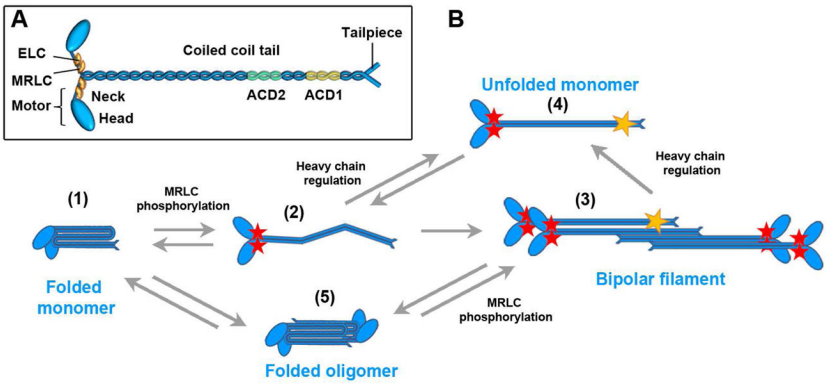

Figure 1.

Structure and dynamics of NMII molecules

A. Structure of a hexameric NMII molecule. ELC, essential light chain; MRLC, myosin regulatory light chain; ACD, assembly competence domains.

B. The basic lifecycle of NMII.

(1) In the autoinhibited conformation, the NMII rod folds onto the heads and blocks motor activity.

(2) Phosphorylation on MRLC (red stars) disrupts the autoinhibition, releases the motors and allows for straightening of the rod.

(3) MRLC-phosphorylated NMII monomers are able to polymerize into bipolar filaments.

(4) Filament disassembly is promoted by heavy chain phosphorylation or protein-protein interaction (yellow star). Combinatorial MRLC phosphorylation and heavy chain regulation may lead to formation of pool of motor-active monomers in an extended conformation.

(5) Folded NMII molecules can associate into antiparallel dimers (or oligomers) that would unfold and join a bipolar filament upon RLC phosphorylation. Alternatively, MRLC dephosphorylation within bipolar filament may lead to formation of folded monomers or oligomers that could serve as storage/transported form of NMII.

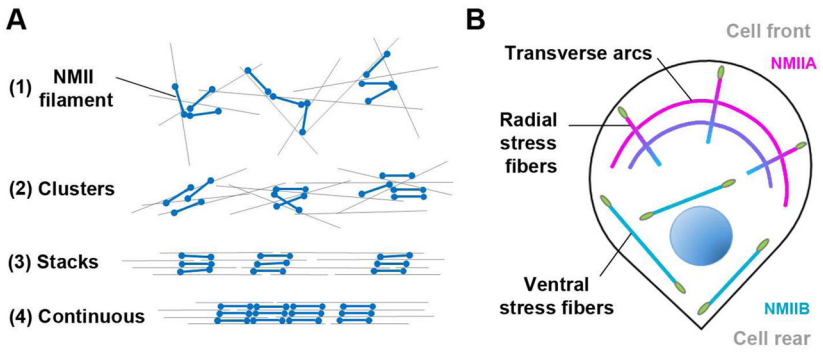

Figure 2.

Development of the actin-NMII contractile system

A. Stages of contractile system evolution.

(1) Newly assembled bipolar filaments form clusters within randomly oriented actin filaments producing an actin-NMII contractile network.

(2) NMII sliding along actin filaments results in coalignment of actin and NMI filaments producing incipient bundles.

(3) Progressive bundling together with gradual registration of NMII filaments into stacks leads to development of quazi-sarcomers in bundles.

(4) Longitudinal contraction of the aging bundle brings stacks of NMII filaments close together resulting in their continuous distribution.

B. Types of stress fibers formed by mesenchymal cells on flat substrate.

Transverse arcs form behind leading edge in the course of actin retrograde flow and NMII contraction. Radial stress fibers have a focal adhesion (green) at the distal end near the leading edge; their proximal ends are often incorporated into transvers arcs. Ventral stress fibers are localized at the basal cell surface and anchored to the substrate by focal adhesions at both ends. They typically develop from merging and straightening of two radial stress fibers and interconnecting arcs.

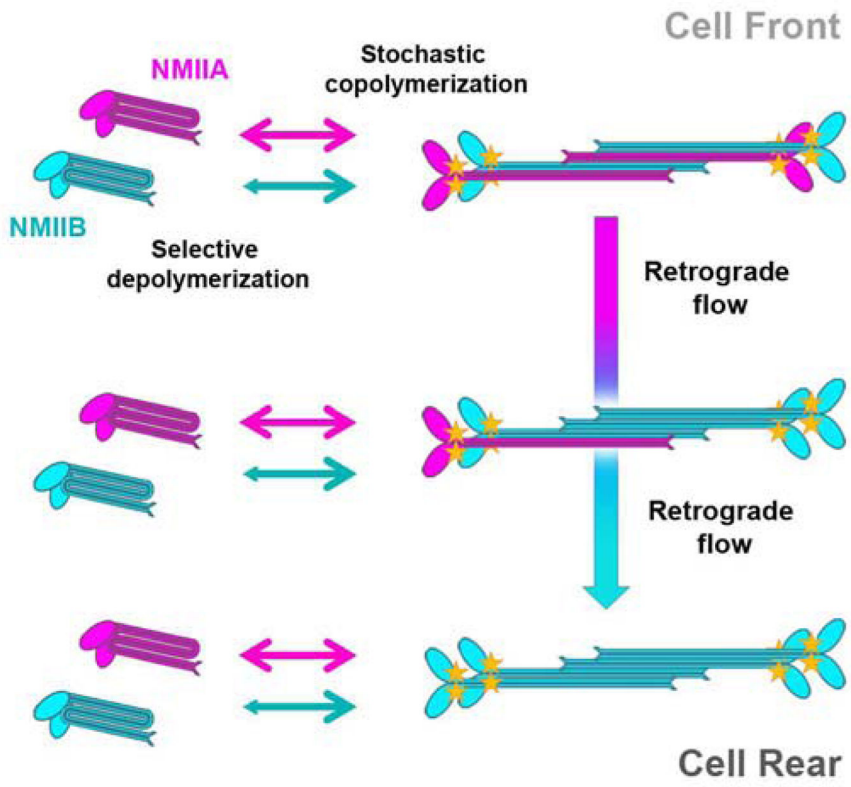

Figure 3.

Self-sorting of NMIIA and NMIIB paralogs during front-back cell polarization, modified from [61]. Monomers of NMIIA (magenta) and NMIIB (blue) incorporate into bipolar filaments with equal efficiency (forward arrows), while the dissociation rates (reverse arrows) are greater for NMIIA than for NMIIB. Faster dissociation of NMIIA subunits together with equivalent addition of new NMIIA and NMIIB subunits leads to gradual enrichment of NMIIB in old filaments that accumulate at the cell rear due to retrograde flow.