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Common and Specific Functions of Nonmuscle Myosin II Paralogs in Cells

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Abstract

Various forms of cell motility critically depend on pushing, pulling, and resistance forces generated by the actin cytoskeleton. Whereas pushing forces largely depend on actin polymerization, pulling forces responsible for cell contractility and resistance forces maintaining the cell shape require interaction of actin filaments with the multivalent molecular motor myosin II. In contrast to muscle-specific myosin II paralogs, nonmuscle myosin II (NMII) functions in virtually all mammalian cells, where it executes numerous mechanical tasks. NMII is expressed in mammalian cells as a tissue-specific combination of three paralogs, NMIIA, NMIIB, and NMIIC. Despite overall similarity, these paralogs differ in their molecular properties, which allow them to play both unique and common roles. Importantly, the three paralogs can also cooperate with each other by mixing and matching their unique capabilities. Through specialization and cooperation, NMII paralogs together execute a great variety of tasks in many different cell types. Here, we focus on mammalian NMII paralogs and review novel aspects of their kinetics, regulation, and functions in cells from the perspective of how distinct features of the three myosin II paralogs adapt them to perform specialized and joint tasks in the cells.

Keywords

actin; nonmuscle myosin II; stress fibers; cytoskeleton; cell motility

Motility is an intrinsic property of living cells and multicellular organisms. The ability of cells to migrate or move their subcellular components is essential for many normal and pathological aspects of physiology of multicellular organisms. In particular, during carcinogenesis, cell motility promotes invasion and metastasis of tumor cells. Diverse motility processes in cell are largely powered by actin cytoskeleton, the major force-generating machinery of the cell. Actin filaments, the main components of the actin cytoskeleton, usually function in cells as networks and bundles formed with the help of various accessory proteins, the most known of which are the myosin superfamily of actin-dependent motors. Among numerous members of myosin superfamily, myosin II is responsible for the generation of large contractile forces involved in many cellular activities.

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Conflicts of Interest

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Myosin II paralogs functioning in striated muscle are organized in a highly ordered and stable sarcomeric arrangement of actin and myosin II filaments. In contrast, the contractile actin–myosin II system in nonmuscle cells exhibits much greater flexibility and is able to adapt to rapidly changing cellular needs. This flexibility largely results from the dynamic properties of nonmuscle myosin II paralogs. In this review, we discuss how differences in the properties and regulation of different mammalian nonmuscle myosin II (NMII) paralogs translate into their specific and joint intracellular functions.

FEATURES OF NMII MOLECULES

Among over 30 classes of eukaryotic myosin, class II myosins are unique in their ability to polymerize into bipolar filaments, which allows them to exert large contractile forces in cells. Mammalian class II myosins include multiple muscle-specific paralogs and three NMII paralogs. NMII functions in virtually all animal cell types in various cell type- and tissue-specific combinations [1]. Similar to other myosins II, the NMII molecule is a hexamer composed of two heavy chains and two pairs of light chains (Fig. 1a). Three mammalian genes – *MYH9, MYH10,* and *MYH14* – encode heavy chains of the NMIIA, NMIIB, and NMIIC proteins, respectively. NMIIA and NMIIB are expressed relatively broadly, whereas NMIIC expression is limited to some differentiated tissues.

Structure.

NMII heavy chain contains a conserved *N*-terminal motor domain consisting of the globular head followed by the α-helical neck region, which binds the essential and the regulatory light chains (myosin regulatory light chains, MRLCs). The neck is followed by a long α-helical rod domain responsible for heavy chain dimerization through the coiled-coil formation. At the extreme *C*-terminus, the NMII heavy chain contains a short nonhelical tailpiece that represents the most divergent part of NMII paralogs. Two hinge regions divide the coiled-coil rod into three nearly equal segments that allow the NMII molecule to acquire its folded autoinhibitory conformation. In this conformation, the rod is sharply folded at the first hinge, whereas the second hinge binds the MRLCs at the neck [2]. This interaction inhibits both the NMII motor activity and polymerization [3].

Motor.

The NMII motor uses ATP energy to move towards the actin filament barbed (plus) end. In general, class II myosins are non-processive motors. However, since NMII works in ensembles (bipolar filaments), it can stay associated with the actin tracks over multiple ATPase cycles, thus acting as a processive motor [4]. The mecha-noenzymatic motor properties vary among NMII paralogs and are tailored to their specific functions [5, 6]. NMIIA has the fastest motor with the highest ATPase rate. NMIIB moves slower with a relatively high duty ratio. Additionally, force resisting motor powerstroke can increase the duration of the actin–myosin interaction leading to the catch-bond behavior, which is much more pronounced in NMIIB than in NMIIA.

Polymerization.

Polymerization of NMII molecules into bipolar filaments occurs through parallel and antiparallel interactions between their unfolded rod domains. The resulting NMII bipolar filament consists of up to 30 molecules for NMIIA and NMIIB and ~14 molecules for NMIIC [7]. In the assembled bipolar filaments, the subunits are staggered and approximately equally distributed between the two opposite orientations with motor domains positioned near the filament ends. The bipolar filament nucleation depends on two assembly competence domains (ACD1 and −2) at the end of the heavy chain rod. ACDs can interact with each other in the antiparallel orientation via electrostatic interactions between their complementary charges [8]. ACDs are highly conserved among NMII paralogs, explaining the ability of NMII paralogs to copolymerize in cells [9, 10] and *in vitro* [4, 11].

A traditional model for NMII polymerization suggests that NMII monomers need to unfold in order to participate in filament assembly. However, folded NMII monomers also can form antiparallel dimers *in vitro* [7, 12], apparently, because their ACDs are still available for the interaction. Moreover, it was recently suggested that NMII polymerization normally occurs by incorporation of folded oligomers (mostly, tetramers) and that unfolding of NMII molecules occurs only after their incorporation into larger structures [13]. In fully formed bipolar filaments, association between both parallel and antiparallel NMII subunits depends on electrostatic interactions between periodically alternating positively and negatively charged rod segments [14]. These interactions may also promote unfolding of subunits that are added in the folded state [12].

REGULATION OF NMII TURNOVER

NMII undergoes constant turnover cycles in cells that include activation of autoinhibited NMII molecules and their assembly into bipolar filaments followed by filament disassembly and subunit recycling (Fig. 1b). These cycles allow NMII to build and dismantle the cell contractile system as needed. Individual steps of the NMII cycle are controlled by phosphorylation and protein–protein interactions.

Regulation of NMII motor activity.

NMII is mainly regulated by MRLC phosphorylation on Ser19, and optionally on Thr18. This phosphorylation restores the ATPase activity of the NMII motor. MRLC can be phosphorylated by multiple kinases, including ROCK, MLCK, MRCK, PAK, and citron kinase [3], which are thought to activate NMII at different subcellular locations and/or in response to different signals. Since MRLC is shared by the 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 that interacts with MRLC in the folded molecule [15].

Regulation of NMII polymerization and depolymerization.

Besides restoring NMII motor activity, MRLC phosphorylation also releases the MRLC–rod interaction, thus permitting, although not imposing rod unfolding [12]. Disassembly of bipolar filaments is largely regulated through the *C*-terminal regions of the NMII heavy

chain, the nonhelical tailpiece, and adjacent regions of the coiled-coil rod that contain paralog-specific phosphorylation sites and can bind regulatory proteins [3, 16]. Because these heavy chain regions are most divergent among the NMII paralogs, the turnover of their filaments is regulated differently.

Phosphorylation or phosphomimetic mutations of the nonhelical tailpiece inhibit *in vitro* polymerization of rod domains of mammalian NMIIB and NMIIC [17, 18]. Although the same effect was originally reported for NMIIA [19], recent study showed that NMIIA polymerization *in vitro* is minimally affected by tailpiece phosphorylation [20]. However, in cells, expression of NMIIA heavy chains either lacking the tailpiece or containing the S1943A substitution in the tailpiece induced over-assembly of NMII filaments [21]. In NMIIB, deletion or phosphomimetic mutations of the tailpiece serine cluster increased NMIIB dynamics in cells [22] and decreased insoluble (polymerized) NMIIB fraction [23].

Disassembly of NMII filaments, at least of NMIIA filament, is promoted by proteins interacting with the *C*-terminal regions of the NMII heavy chain. The best characterized regulator is S100A4/Mts1, which specifically promotes NMIIA disassembly in a calcium-dependent manner [16, 17, 20]. However, other members of the S100 protein family also dissociate NMIIA and NMIIC filaments [20, 24]. Cancer suppressor Lgl1 can block filament assembly of NMIIA [25] and/or NMIIB [26] *in vitro*. Members of the 14-3-3 protein family can solubilize *in vitro* the unphosphorylated tail fragments of various NMII paralogs in a 14-3-3 isoform-specific manner [27]. Motor-inactive myosin 18A can copolymerize with NMII and regulate the degree of NMII assembly [28].

Although 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 heavy chain has a potential to produce unfolded motor-active NMII molecules unable to polymerize. Indeed, unfolded MRLC-phosphorylated NMIIA and NMIIB monomers were detected in cells and appeared to be functionally important [10, 29, 30].

DYNAMICS OF NMII-CONTAINING STRUCTURES IN CELLS

Actin–NMII structures in cells include non-aligned networks, aligned bundles, and composite arrays of actin and NMII filaments. These systems are formed and gradually evolve in the process of actin and NMII polymerization and actin–NMII interaction (Fig. 2a).

Actin–NMII clusters.

New NMII filaments in cells are often formed behind the protrusive cell edges and then drift away from the cell edge with the actin retrograde flow, while simultaneously forming clusters. In clusters, NMII filaments often interact with each other at their ends [31, 32]. Such interaction seems to be an intrinsic property of NMII filaments, as it is also observed with purified filaments *in vitro* [4, 7]. NMII clusters can be formed by congregation of preexisting NMII filaments and/or by nucleation of new NMII filaments next to the pre-existing ones [33, 34] and then stabilized by the head-to-head interaction. Both clustering

mechanisms can rely on higher affinity of NMII for tense actin filaments [35], so that actin filaments stretched by initial NMII filaments would attract additional NMII filaments or monomers [30].

Actin–NMII networks.

Clusters of NMII filaments embedded into disordered actin filament arrays can gradually coalesce and form larger assemblies, actin–NMII networks. Such networks are capable of large-scale contractions that drive the cell body translocation in migrating keratocytes [32], apical constriction of epithelial cells [36], cytokinesis [37], and many others processes. Active actin–NMII networks can disassemble and reassemble in an oscillatory manner [36, 38]. Such pulsatile contractions in the cell lamella are characteristic for NMIIA, but not exhibited by NMIIB because of the difference between the motor domains of these two NMII paralogs [38].

Actin–NMII bundles (stress fibers).

Following contraction, actin-NMII networks can reorganize into aligned actin–NMII bundles [32, 39] often referred to as stress fibers (SFs). SFs can develop greater contractile forces than networks due to their superior organization. SFs represent different types of bundles of aligned actin and NMII filaments and are typically attached to the substratum by focal adhesions. SFs are organized in cells into a dynamic contractile system and can gradually remodel from one SF subtype to another [39, 40]. The earliest SF type – transverse arcs – is formed by coalescence of NMII clusters with concurrent rearrangement of associated actin filaments [32, 39]. Transverse arcs do not directly connect to focal adhesions but are anchored to the substrate through interaction with other SF types. After their formation, transverse arcs move centripetally with the retrograde flow, which is driven in part by their own contraction. Retrograde flow of transverse arcs is accompanied by the formation of radial (or dorsal) SFs that grow due to actin polymerization at their distal end anchored to a focal adhesion and also due to pulling from the rear by a contracting transverse arc anchored to the proximal end of the radial SF [41]. Radial SFs have little if any associated NMII and are poorly contractile [42]. Over time, a system of transverse arcs and radial SFs can resolve into ventral SFs by the straightening of the radial-transverse-radial SF set [39, 43]. Ventral SFs are located at the basal cell surface and anchored to the substrate by focal adhesions at both ends. Eventually, SFs (including even the most stable ventral SFs) are disassembled and their components are recycled.

DIFFERENTIAL DYNAMICS OF NMII PARALOGS

Dynamics of NMIIA and NMIIB.

NMII filaments are not stationary within SFs but undergo constant turnover. When analyzed individually, NMIIB was found to exhibit slower dynamics than NMIIA [44, 45] and spend more time in the actin-bound state [46]. It is unlikely that different dynamics of NMIIA and NMIIB is driven by the differences in the bipolar filament assembly regulated by the shared MRLCs and by interaction between the conserved ACDs. Conversely, the disassembly of NMII filaments depends on the paralog-specific regions of the NMII heavy chain [16]. Consistent with this notion, analysis of NMIIA/NMIIB chimeras revealed that the

differential NMII dynamics is controlled by the *C*-terminus of the NMII heavy chain [44]. Shifting either NMIIA [25, 47, 48] or NMIIB [22] dynamics toward disassembly correlates with increased cell migration and invasion, likely due to increased cytoskeleton reorganization.

Intracellular segregation of NMIIA and NMIIB.

In cells cultured on a stiff 2D substrate, NMIIB typically acquires more central (in unpolarized cells) or posterior (in front-rear polarized cells) localization relative to NMIIA. However, NMIIA and NMIIB exhibit the same distribution at the early stages of contractile system assembly and get segregated much later [9, 10], suggesting an existence of a time-dependent sorting mechanism. Life histories of individual SFs show that their NMII content undergoes stereotypic temporal progression [49]. SFs that are newly formed near the leading edge are enriched with NMIIA, but also contain NMIIB. Over time, while the SF drifts retrogradely, it progressively loses NMIIA and becomes enriched with NMIIB. Eventually, NMIIB-rich SFs either disassemble or form long-lived ventral SFs at the cell center or rear. This process explains why at the steady state, NMIIA is more abundant at the periphery in younger SF types – transverse arcs and radial SFs – and NMIIB is enriched in older and more centrally located ventral SFs. Moreover, a shift in the relative abundance of NMIIA and NMIIB paralogs leads to different properties of actin-NMII structures in the cell [49]. For example, high levels of NMIIB favor formation of stable ventral SFs, whereas abundant NMIIA promotes formation of dynamic transverse arcs and radial SFs [49].

Mechanistic model of NMII self-sorting.

Segregation of NMIIA and NMIIB can be explained by similar polymerization and distinct depolymerization mechanisms for these paralogs [49]. When different paralogs are present simultaneously in the cells, their copolymerization allows for the formation of heterotypic bipolar filaments, which exhibit a new type of dynamics, as compared to homotypic NMII filaments. The higher rates of NMIIA turnover, as compared to those for NMIIB, suggest that NMIIA subunits would dissociate from NMIIA/NMIIB heteropolymers more readily than NMIIB subunits. On the other hand, 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 nonselective recruitment of new subunits will gradually increase the fraction of NMIIB in older NMII filaments. Because NMII filaments, as a component of SFs, undergo retrograde flow, older NMIIB-enriched filaments become concentrated farther away from the leading edge than 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 [44].

Besides enabling spatial segregation of NMII paralogs to enhance cell polarity, copolymerization of NMII paralogs also modulates the dynamic properties of each paralog. In cells expressing NMIIA, NMIIB exhibits faster dynamics and acquires more disperse distribution compared to the cells lacking NMIIA [49]. On the other hand, by forming mixed filaments with NMIIA, NMIIB makes these filaments more processive runners *in vitro*, as compared to the NMIIA-only filaments [4].

DIFFERENTIAL FUNCTIONS OF NMII PARALOGS IN CELLS

The main function of NMII in cells is generation of contractile forces. Migrating cells use them for cell–substrate adhesion, cell body translocation, polarization, and regulation of the leading edge protrusion. NMII is also important for cytokinesis, remodeling of the extracellular matrix (ECM), formation of cell–cell adhesions, and cell shape determination [5]. A new recently discovered role of NMII is its participation in membrane morphogenesis, including the functions of motor-active NMII monomers.

Cell–ECM adhesion.

Cell adhesion to ECM is typically mediated by adhesion receptors of the integrin family and provides traction of migrating cells. Integrin-mediated cell-ECM adhesion can be strengthened by force [50] largely delivered by NMII that pulls actin filaments anchored to the adhesions. As a fast motor able to better cope with rapid actin polymerization, NMIIA has greater contribution to focal adhesion assembly near the leading edge and is more capable of traction force generation [49, 51, 52], whereas NMIIB is better posed to stabilize focal adhesions in more central cell regions [53]. Nonetheless, NMIIB or NMIIC are able to initiate adhesion formation in cells lacking NMIIA. The role of NMIIC in cells is poorly understood, but could involve either positive [54] or negative [18, 55] regulation of cell adhesions.

Leading edge protrusion.

Leading edge protrusion is driven by polymerization of actin filaments and counteracted by retrograde flow, which is driven in part by NMII. In this context, NMII negatively regulates the leading edge protrusion [56, 57]. On the other hand, efficient protrusion requires traction that is enabled by mechanosensitive adhesions. Nascent adhesions under lamellipodia are mechanically stimulated by the NMII-dependent retrograde flow [58, 59], but also more directly by NMII [30]. 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 [60] and reduces cell spreading in non-neuronal cells [51, 53]. Conversely, NMIIB supports axon elongation in neurons [61] and lamellipodial protrusion in other cell types [53]. NMIIC also stimulates neurite outgrowth in neuroblastoma cells [55] and lamellipodial protrusion in epithelial cells [46].

Contractile forces.

During cell migration, NMII-mediated contraction helps to detach obsolete adhesions, retract the cell rear, and translocate the cell body [32, 62]. Similar contractile forces applied to compliant ECM contribute to ECM remodeling [63]. NMIIA is mainly responsible for generating large contractile forces for cell rear retraction [64] and ECM remodeling [65]. Contribution of NMIIB to these processes is minimal in 2D cultures, but becomes significant in the 3D environment, where NMIIB promotes translocation of the nucleus through tight spaces [52, 66].

Cell polarity.

In migrating cells, actin–NMII bundles at the cell rear inhibit protrusive activity, thus supporting the front-rear cell polarity. This function largely depends on NMIIB [45, 56] and is facilitated by preferential accumulation of NMIIB at the cell rear in the course of self-segregation of NMII paralogs [49]. However, excessive expression of NMIIB results in over-stabilized SFs and focal adhesions that retard cell migration [49].

Cytokinesis.

The NMII-dependent constriction of the cleavage furrow during cytokinesis can be executed by each NMII paralog [1]. The cellular preference for employing specific NMII paralogs for cytokinesis depends on their relative abundance in individual cell types and/or efficiency of paralog recruitment to the cleavage furrow [67]. The recruitment to the furrow of NMIIB in dividing megakaryocytes [68] or NMIIA in COS-7 cells [69] depends on the *C*-terminal regions of their heavy chain.

Cell–cell adhesion.

In epithelial monolayers, circumferential actin–NMII bundles associated with the apical cell–cell junctions generate tension to preserve junction integrity [70] and to constrict the apical domain during tissue invagination, although other contraction mechanisms also contribute [36, 71]. The contractile forces at the cell–cell junctions are counterbalanced by pushing forces generated by the Arp2/3 complex-dependent actin polymerization [72]. Circumferential actin–NMII bundles at the apical cell–cell junctions require both NMIIB [73, 74] and NMIIA [75, 76]. NMIIA is especially important for the initial junction assembly [77].

Cell shape.

The spherical shape and high cortical tension of mitotic cells are maintained by the submembrane (cortical) actin networks jointly assembled by activities of NMII [78] and actin nucleators [79]. Similar cortical networks also exist locally in the interphase cells and help to define the cell shape and mechanical properties of the cell surface. In the cell cortex, NMIIB and NMIIC promote cortex stability, which helps to reduce formation of surface blebs, whereas NMIIA supports cortex stiffness, contractility, and blebbing [46]. NMIIA also functions in erythrocytes, where it maintains the biconcave cell shape and regulates membrane tension by interacting with short actin filaments in the submembrane actin–spectrin network [80].

Membrane trafficking.

Accumulated data point to the involvement of NMII in membrane morphogenesis, such as exocytosis [81], endocytosis [82], post-Golgi and Golgi-to-ER trafficking [83, 84], and mitochondrial fission [85]. During secretion of viscous cargos, such as salivary mucus [86, 87], lung surfactant [88], and endothelial von Willebrand factor [89], NMII is thought to forcefully expel cargo from the secretory vesicle. In salivary glands, NMIIB prevents counterproductive expansion of the secretory granule immediately after its fusion with the plasma membrane, whereas NMIIA stimulates subsequent cargo expulsion [87].

In some membrane trafficking events, NMII appears to function in a monomeric form. For example, in natural killer cells, NMIIA monomers associate with lytic granules and promote granule secretion [29]. NMII monomers were also found in association with the Golgi membranes isolated from the intestinal epithelium [83]. The association of NMIIA with the Golgi complex is mediated by its coiled coil rod [90]. It remains unclear whether this interaction is compatible with the rod-mediated NMII filament assembly.

Proper accomplishment of virtually every NMII mission requires fine-tuning of the balance between the active contraction and tension maintenance. This can be achieved through combinatorial engagement of NMII paralogs with distinct dynamic properties. The available data suggest that dynamic features of NMII paralogs often correlate 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 to ensure cytoskeleton stability. At present, too little is known about NMIIC functions to propose what might be special about this paralog. Importantly, copolymerization of NMII paralogs leads to the formation of bipolar filaments with a continuous range of dynamic properties, which is beneficial for the fine-tuning of NMII functions in cells.

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Abbreviations:

ACD1(2)	assembly competence domain 1(2)
ECM	extracellular matrix
MRLC	myosin regulatory light chains
NMII	nonmuscle myosin II
SF	stress fibers

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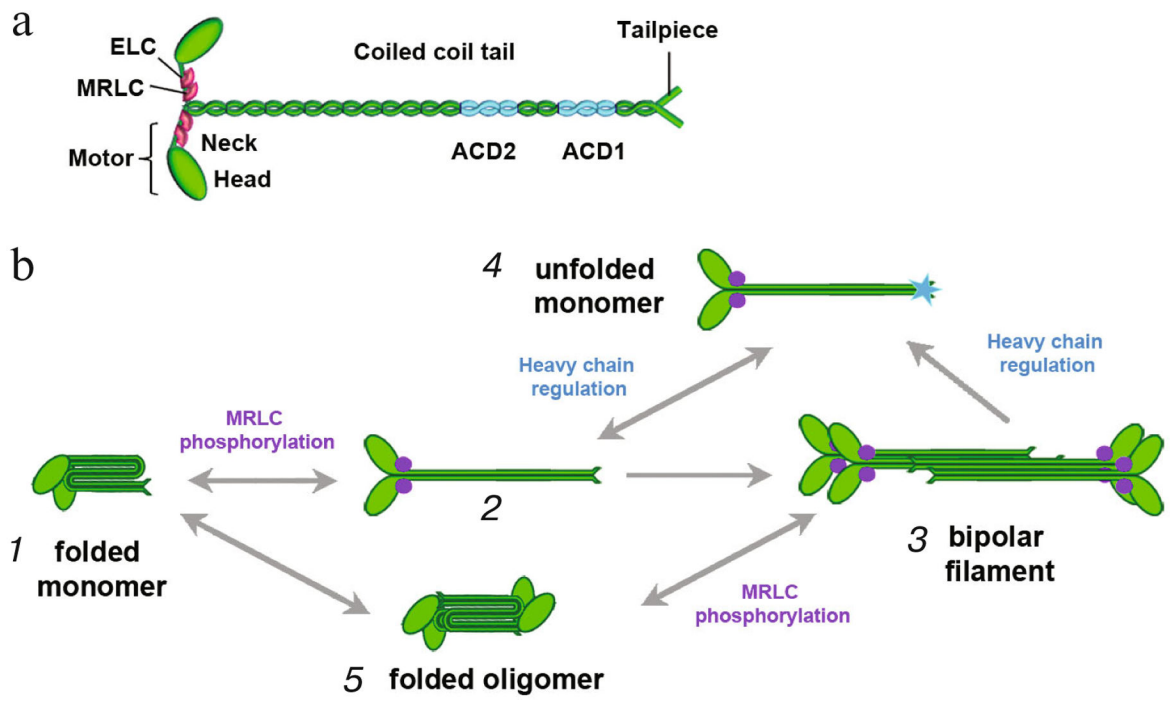

Fig. 1.

Structure and dynamics of NMII molecules. a) Structure of hexameric NMII molecule. ACD1(2), assembly competence domain 1(2); ELC, essential light chain; MRLC, myosin regulatory light chain. 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 (purple) disrupts 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 (blue star). Combinatorial MRLC phosphorylation and heavy chain regulation may lead to the formation of a pool of motor-active monomers in the extended conformation; *5*) folded NMII molecules can associate into antiparallel dimers (or oligomers) that would unfold and join the bipolar filament upon MRLC phosphorylation. Alternatively, MRLC dephosphorylation within bipolar filament may lead to the formation of folded monomers or oligomers that could serve as storage/transported form of NMII.

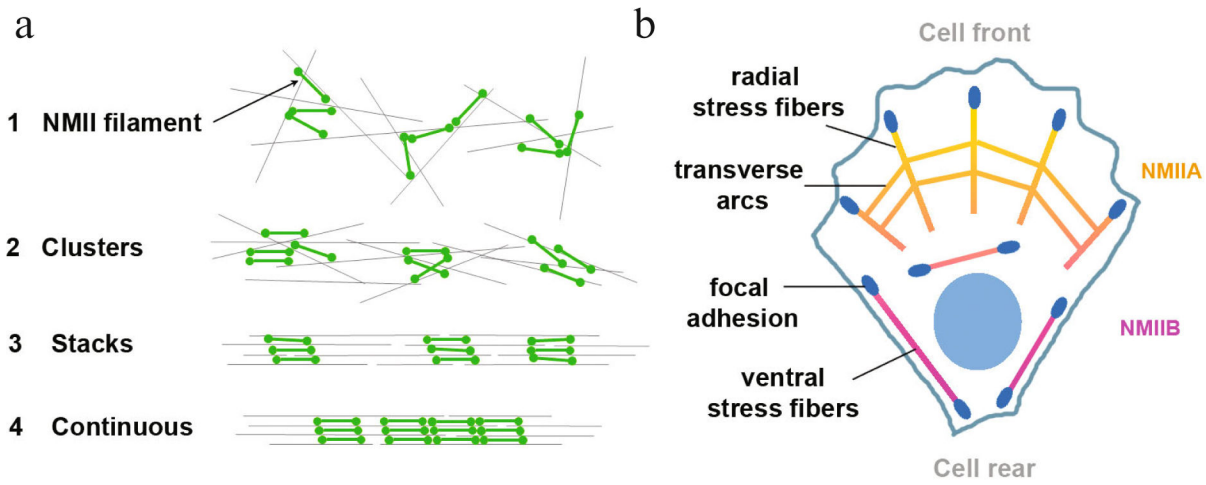

Fig. 2.

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 actin–NMII contractile network; *2*) NMII sliding along actin filaments results in coalignment of actin and NMII filaments producing incipient bundles; *3*) progressive bundling together with gradual registration of NMII filaments into stacks leads to the 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 the leading edge in the course of an actin retrograde flow and NMII contraction. Radial stress fibers have a focal adhesion (blue) 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.

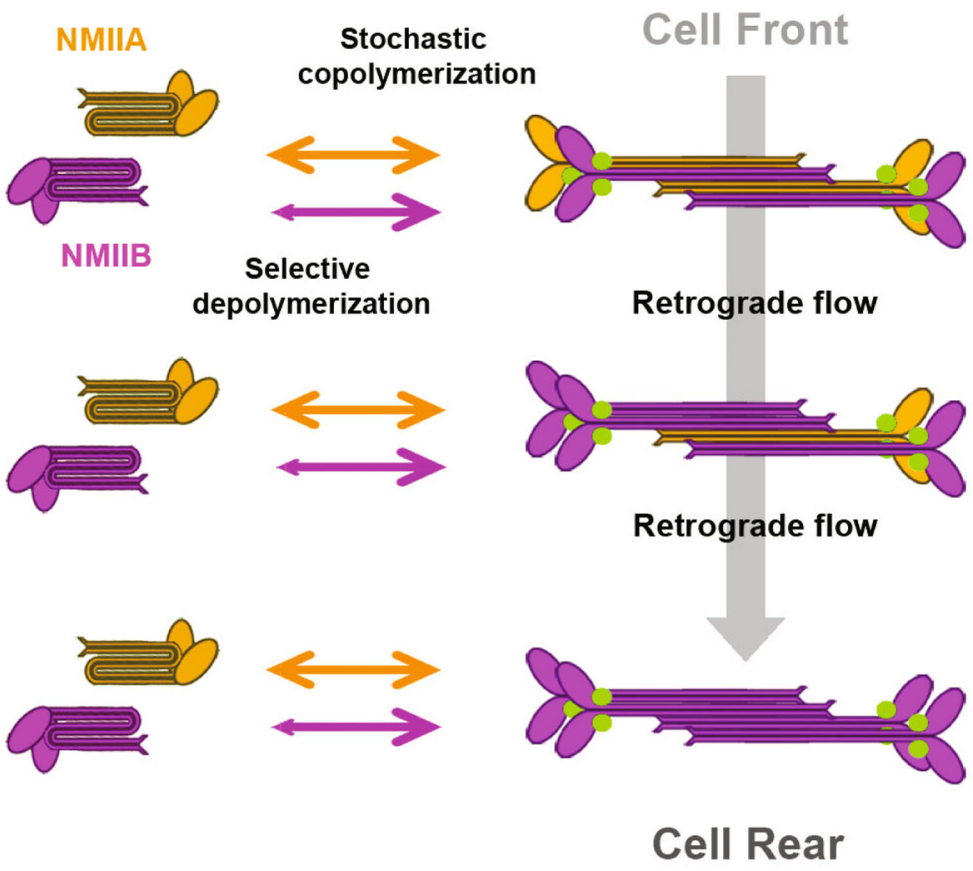

Fig. 3.

Self-sorting of NMIIA and NMIIB paralogs during front-rear cell polarization, modified from [49]. Monomers of NMIIA (yellow) and NMIIB (magenta) 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.