Published in final edited form as:
Nat Rev Mol Cell Biol. 2009 November ; 10(11): 778–790. doi:10.1038/nrm2786.

Non-muscle myosin II takes centre stage in cell adhesion and migration

Miguel Vicente-Manzanares[*], **Xuefei Ma**[‡], **Robert S. Adelstein**[‡,§], and **Alan Rick Horwitz**[*,§]

[*]Department of Cell Biology, University of Virginia School of Medicine, Charlottesville, Virginia 22908, USA

[‡]Laboratory of Molecular Cardiology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892-1583, USA

Abstract

Non-muscle myosin II (NM II) is an actin-binding protein that has actin cross-linking and contractile properties and is regulated by the phosphorylation of its light and heavy chains. The three mammalian NM II isoforms have both overlapping and unique properties. Owing to its position downstream of convergent signalling pathways, NM II is central in the control of cell adhesion, cell migration and tissue architecture. Recent insight into the role of NM II in these processes has been gained from loss-of-function and mutant approaches, methods that quantitatively measure actin and adhesion dynamics and the discovery of NM II mutations that cause monogenic diseases.

Myosins constitute a superfamily of motor proteins that play important parts in several cellular processes that require force and translocation[1–3]. Recent analyses of genomic databases have yielded an increasing number of myosin classes in eukaryotic cells[4,5]. Myosin molecules can walk along, propel the sliding of or produce tension on actin filaments. This requires energy, which is provided by the hydrolysis of ATP, and requires myosins to have catalytic sites with ATPase activity. Myosin catalytic sites are usually found in the amino-terminal (head) region of the molecule, and they are often activated when myosin binds to actin. The carboxy-terminal region of some myosins binds to and moves cargo in a cell, whereas the C-terminal domains of other myosins self-associate into filaments, which allows their heads to tether actin filaments and exert tension. Myosins can also act indirectly through actin to bring adhesion-related proteins, such as integrins, or signal transduction molecules into close proximity[2,3].

Most myosins belong to class II and, together with actin, make up the major contractile proteins of cardiac, skeletal and smooth muscle, in which the sliding crossbridges that connect thick myosin filaments with thin actin filaments provide the force to, for example, pump blood, lift objects and expel babies[6,7]. Importantly, myosin II molecules that resemble their muscle counterparts, with respect to both structure and function, are also present in all non-muscle eukaryotic cells[8–11]. Like muscle myosin II, non-muscle myosin II (NM II) molecules are comprised of three pairs of peptides: two heavy chains of 230 kDa, two 20 kDa regulatory light chains (RLCs) that regulate NM II activity and two 17 kDa essential light chains (ELCs) that stabilize the heavy chain structure (FIG. 1a). Although these myosins are referred to as ‘non-muscle’ myosin IIs to distinguish them from their muscle counterparts, they are also present in muscle cells, where they have distinct functions during skeletal muscle development and differentiation[12], as well as in the maintenance of tension in smooth muscle[13,14].

Correspondence to M.V.-M. miguelvix@virginia.edu.
[§]These authors contributed equally to this work.

NM II has a fundamental role in processes that require cellular reshaping and movement, such as cell adhesion, cell migration and cell division. NM II can use its actin cross-linking and contractile functions, which are regulated by phosphorylation and the ability of NM II to form filaments, to regulate the actin cytoskeleton.

In this Review, we provide an overview of the structure and regulation of NM II, with emphasis on its central role in cell adhesion and cell migration, and also outline the relationship between NM II mutations and disease.

Domain structure of NM II

The two globular head domains of NM II contain a binding site for both ATP and actin and they are followed by neck regions, each of which binds the two functionally different light chains. The neck domain acts as a lever arm to amplify head rotation while the chemical energy of ATP is converted into the mechanical movement of the myosin head[1]. This neck domain is followed by a long α-helical coiled coil, which forms an extended rod-shaped domain that effects dimerization between the heavy chains and terminates in a relatively short non-helical tail (FIG. 1a). The rod domains of NM II self-associate to form bipolar filaments (anti-parallel arrays of myosin molecules), which are considerably smaller than those found in cardiac and skeletal muscle[15,16] (FIG. 1b).

Three different genes in mammalian cells (myosin heavy chain 9 (MYH9), MYH10 and MYH14) encode the NM II heavy chain (NMHC II) proteins (NMHC IIA, NMHC IIb and NMHC IIC, respectively), although there is only one NMHC II gene, *zipper*, in *Drosophila melanogaster*[17]. We refer to the whole myosin II molecule (heavy chains and light chains) as NM II and the heavy chains alone as NMHCs. The NMHC isoform determines the NM II isoform, which are named NM IIA, NM IIB or NM IIC, accordingly. For NM II motors to retain their normal, native and active conformations, the light chains must be bound to the heavy chains. Deletion of a specific NMHC II results in the loss of that NM II isoform.

The mammalian heavy chain pre-mRNAs that are transcribed in humans and mice from *MYH10* and *MYH14* undergo alternative splicing, predominantly in neuronal tissue, which increases the total number of expressed NMHC II proteins to nine[18]. Although there is evidence for alternative splicing of the *MYH9* transcripts at locations homologous to those in *MYH10* and *MYH14*, it is not known whether these transcripts are translated[19]. The light chains are encoded by a different set of genes, which can also undergo alternative splicing, and there is currently no known specificity of light chains for particular NMHC IIs. The two light chain pairs are very tightly, but non-covalently, bound to α-helical stretches of each heavy chain. Despite having a high degree of homology, particularly in their actin-binding globular heads, the myosin isoforms are spatially segregated in some areas of cells, but clearly overlap in others[20,21]. As detailed below, some cellular functions are isoform-specific, whereas others are redundant[22,23].

The short, non-helical tails of NMHC II proteins differ sufficiently among the three mammalian isoforms that peptides can be synthesized to mimic these sequences for the generation of isoform-specific antibodies[24]. There is no evidence for heterodimer formation between the three NMHC II isoforms, and when immunoprecipitation experiments are performed on a mixture of isoforms with specific antibodies only the antibody-specific isoform is detected[24]. Two important kinetic properties that differ among the NM II isoforms are the actin-activated Mg^{2+}-ATPase activity (the increase in ATP hydrolysis by myosin when bound to actin) and the duty ratio (the time that myosin is bound to actin in a force-generating state). NM IIA has the highest rate of ATP hydrolysis of the three NM II isoforms and it propels actin filaments more rapidly than NM IIB and NM IIC25. NM IIb has a significantly higher duty ratio than NM IIA26,27. NM IIB also has an extremely high affinity for ADP when compared to other

myosins, and the release of ADP from NM IIB is markedly slowed by backward strain on the myosin head (the force exerted on myosin heads by the actin filaments, opposite to the direction of myosin head movement)28. Thus, NM IIB is particularly well suited to exert tension on actin filaments for longer periods of time and with less expenditure of energy than NM IIA. Indeed, NM IIB has recently been shown to play an ancillary role in smooth muscle contraction, in which it may be responsible for a portion of the tension maintenance during tonic contractions13. These differences in kinetic properties between NM IIA and NM IIB might help to explain why expressing NMHC IIA under the control of the endogenous *MYH10* promoter can rescue some, but not all, of the abnormalities found in NM IIB-ablated mice[29].

Whereas the different enzymatic and motor activities of NM IIs reside in their N-terminal domain, the C-terminal rod and non-helical tail determine the assembly of myosin filaments and the intracellular localization of the NM II isoforms. Two assembly-competent domains in the NM IIb rod that have a crucial role in filament formation[30], and two regions in the NM IIb rod that are involved in isoform-specific filament assembly, have been identified[31].

Experiments using chimaeras of NM IIA and NM IIB have shown that the intracellular localization of these isoforms is determined by the C-terminal 179–190 amino acids, which contain one of the assembly-competent domains and the non-helical tail[32,33].

Regulation of NM II activity

In contrast to skeletal and cardiac myosins, which are regulated by a separate set of proteins that are bound to the actin filaments, the regulation of Mg^{2+}-ATP hydrolysis and filament formation of NM II involves the reversible phosphorylation of specific amino acids present in the pair of 20 kDa RLCs and the heavy chains. The function of the ELC pair is to stabilize the NMHC and there is no evidence that they undergo reversible phosphorylation.

Regulation of NM II by RLC phosphorylation

The regulation of NM IIA, NM IIB, NM IIC and smooth muscle myosin, but not cardiac or mammalian skeletal muscle myosin, depends on the reversible phosphorylation of the RLC on ser19. This phosphorylation event greatly increases the Mg^{2+}-ATPase activity of myosin in the presence of actin[34] by controlling the conformation of the myosin heads35. However, phosphorylation has little or no effect on the affinity of myosin for actin36. Thr18 can also be phosphorylated, and diphosphorylation of the RLC on ser19 and Thr18 often occurs in cultured cells37. The additional phosphorylation of Thr18 in the presence of phosphorylated ser19 (REF. 38) further increases the total actin-activated Mg^{2+}-ATPase activity at sub-saturating actin concentrations, but does not affect the myosin v_{max} (the maximal actin-activ ated Mg^{2+}-ATPase activity of myosin at saturating actin concentrations) or the rate of movement of actin filaments *in vitro*[39].

There is compelling evidence from *in vitro* experiments that phosphorylation of the RLC also regulates the assembly of NM II filaments[40]. In the presence of ATP, unphosphorylated NM II folds into a compact conformation, in which one head blocks the second head of the same molecule through an asymmetrical interaction (FIG. 1a; assembly-incompetent form)35,41–44. The tail also folds at two points and interacts with the heads to further compact the molecule and prevent filament assembly. Phosphorylation of RLCs disrupts the head–head and head–tail interactions and causes the compact, faster sedimenting (10s) molecule to form an elongated slower sedimenting (6s) species (FIG. 1a), which forms bipolar filaments at physiological ionic strengths (FIG. 1b). Although these dramatic changes can be demonstrated in solution, there is no firm evidence that they occur *in vivo*. The formation of assemblies comprising 14–20 myosin molecules in bipolar filaments promotes the interaction of myosin filaments with actin filaments (FIG. 1b).

More than a dozen kinases have been reported to phosphorylate the RLCs of NM II (FIG. 2), including myosin light chain kinase (MLCK; also known as MYLK), Rho-associated, coiled coil-containing kinase (ROCK), citron kinase, leucine zipper interacting kinase (ZIPK; also known as DAPK3) and myotonic dystrophy kinase-related CDC42-binding kinase (MRCK; also known as CDC42bP)[6,34,45,46]. These kinases phosphorylate RLCs on ser19, Thr18 or both, to relieve the inhibition imposed on the myosin molecule by unphosphorylated RLCs and the head–head interaction outlined above. However, the activating signals for these kinases differ. Ca^{2+}–calmodulin activates MLCK, which seems to be specific for the NM II RLCs, whereas the small GTP-binding protein RHOA activates ROCK and citron kinase. unlike MLCK, ROCK and citron kinase phosphorylate several substrates in addition to the RLCs of NM II. For example, although ROCK can phosphorylate RLCs directly, it primarily acts to inhibit the major myosin phosphatase, protein phosphatase 1 (PP1), which is responsible for the dephosphorylation of NM II[47]. Phosphorylation of the regulatory subunit myosin phosphatase-targeting sub unit 1 (MYPT1; also known as PPP1R12A) of this trimeric phosphatase inactivates the enzymatic activity, resulting in increased NM II phosphorylation and activation. MLCK, at least in some cells, is more peripherally localized, wherase ROCK is more central[48,49]. This differential localization means that the actomyosin structures in the cell centre (such as stress fibres and mature focal adhesions) are much more stable than those at the periphery, which are more responsive to upstream stimuli. Protein kinase C (PKC) differs from the above kinases in that it phosphorylates RLCs on ser1, ser2 and Thr9, which renders the RLC a poorer substrate for MLCK and therefore decreases the activity of NM II[50] (FIG. 2). For example, PKC phosphorylation of the RLC on ser1 or ser2 initiated by stimulation of platelet-derived growth factor receptor inhibits NM II activity to promote the reorganization of actomyosin filaments[51]. As noted below, in addition to phosphorylating the RLCs, PKC also phosphorylates the heavy chains.

Regulation of NM II by NMHC phosphorylation

Although phosphorylation of NMHC II plays an important part in regulating the activity of *Dictyostelium discoideum* myosin II (reviewed in REF. 52), the roles in regulating the structure and activity of mammalian NM II are still being defined. There are several phosphorylation sites near the C-termini of NMHCs, in both the coiled-coil domain and the non-helical tail, including sites that are phosphorylated by PKC53, casein kinase II (CK II)54 and transient receptor potential melastatin 7 (TRPM7)[55] (FIG. 2; see below). In each case phosphorylation of the NMHC either dissociates myosin filaments or prevents their formation *in vitro*. A number of laboratories have reported that ser1917 in rat NMHC IIA (equivalent to ser1916 in human NMHC IIA) is phosphorylated by PKC, and phosphorylation of this site by PKCβ correlates with exocytosis in mast cells[56,57]. ser1943 in the non-helical NMHC tail is a substrate for phosphorylation by CK II, and phosphorylation of this residue, or of ser1917 by PKC, inhibits the assembly of NM IIA rods into filaments54.

Phosphorylation of NM IIA also affects the binding of s100A4 (also known as MTs1) to the NMHC. This protein, which is a member of the s100 family of Ca^{2+}-binding proteins, promotes the invasion of metastatic tumour cells. binding of s100A4 to NMHC IIA prevents filament formation, but s100A4–NMHC IIA binding is blocked by the phosphorylation of ser1943 by CK II[58]. An alpha kinase from the same family as *D. discoideum* NMHC kinase A, TRPM7, and its close homologue TRPM6, phosphorylate NM IIB and NM IIC at several sites in the non-helical tails[59]. Although the effects of these phosphorylation events on filament assembly have not yet been studied, the more specific phosphorylations catalysed by TRPM7 in the helical portion of the rod in NM IIA at Thr1800, ser1803 and ser1808 seem to decrease filament formation and alter the subcellular localization of NM IIA[55]. As yet, there is no evidence for the endogenous phosphorylation of these sites.

Phosphorylation of NM IIB by aPKCζ, which is regulated by p21-activated kinase 1 (PAK1), was studied in a prostate cancer cell line. This phosphorylation, which occurs on ser1937 in the non-helical tail region of NMHC IIB, is stimulated by epidermal growth factor (EGF) and leads to slower filament assembly in cultured cells[53]. Recently, phosphorylation of both the non-helical tail and the rod of the NMHC has been reported for NM IIC. Phosphorylation increases the solubility of NM IIC and also has an effect on cellular localization[60]. Thus, it is clear that considerable regulation of NM II occurs through phosphorylation of the heavy chains, particularly in the tail region (FIG. 2). The continued characterization of the roles of these different phosphorylation events on NM II function and isoform-specific regulation should produce important new insights.

NM II in cell migration, adhesion and polarity

NM II is an important regulator of adhesion and polarity in cell migration. These processes involve the dynamic remodelling of the actin cytoskeleton and the interaction of the cell with its environment. Each of the NM II isoforms affects these processes differently, as discussed below.

NM II regulates protrusion and cell migration

In migrating cells, actin organizes into several distinct structures and its polymerization in cellular protrusions drives cell migration. Protrusions generally contain two actin-based structures: the lamellipodium and the lamellum[61]. Different classes of regulatory molecules organize actin in these two structures. The actin nucleator Arp2/3 generates the lamellipodium. *In vitro*, Arp2/3 binds to the sides of actin filaments and generates branches at a fixed angle (70°) to form a dendritic network[62]. However, the *in vivo* data are still under debate. A recent study has reported actin filaments with variable angles (15°–90°) in the lamellipodium, which challenges the dendritic network notion[63]. The relative positioning of the lamellipodium and the lamellum is also unclear. It was recently proposed that these two actin networks overlap at the leading edge, with the lamellipodium superimposed onto the lamellum64, but a more traditional view is that the lamellipodium is spatially anterior to the lamellum61. In the lamellum, actin filaments coalesce into thicker bundles (FIG. 3a). The lamellipodium and the lamellum are kinetically different: the lamelli podium is distinguished by a fast retrograde flow of actin, whereas the lamellum exhibits slower retrograde flow. The convergent zone between the two is characterized by active depolymerization of the dendritic network and the reorganization of actin[64,65] (FIG. 3b).

NM II does not reside in or seem to play a part in the physical organization of the lamellipodium, but it can affect the net rate of cellular protrusion[22,64,66] (FIG. 3b). When NM II is knocked out, knocked down by small interfering RNA or inhibited with blebbistatin, large actin bundles are not observed in the lamellum, whereas the lamellipodium remains intact[64]. In many cells, advancement of the protrusion is interrupted by NM II-generated pauses[67] that are absent when myosin is inhibited or deleted[22].

one hypothesis for the role of NM II in protrusion formation is that NM II generates the retrograde flow of actin in the lamellum, which is connected to the lamellipodium[64,68] (FIG. 3b). Inhibition of NM II activity with blebbistatin, or genetic deletion of NM II, greatly decreases the rate of actin retrograde flow in the lamellum[64,66] and inhibits coalescence of actin into proto-bundles at the lamellipodium–lamellum interface[69,70], which increases protrusiveness[22,66,71] (FIG. 3b). NM II-generated retrograde flow counters the actin polymerization-mediated advancement of the leading edge and thereby reduces the observed protrusion rate. Thus, periodic contractions of the lamellipodium could cyclically inhibit the protrusion rate. In this model, the protrusion rate is the difference between the actin polymerization and the retrograde flow rates. Adhesion modulates this balance by coupling

actin to the substratum, thereby creating traction points that counter actin retrograde flow[72]. such a mechanism presumably increases the rate of protrusion by diverting the forces created by the retrograde flow of the actin to the substratum. Thus, the linkage between actin and the substratum constitutes a 'molecular clutch'. These observations, first made in neuronal growth cones but later extended to many other cell types, constitute the clutch hypothesis[72–74], which integrates adhesion, retrograde flow and actin polymerization.

NM II in integrin-mediated adhesion

NM II is dispensable for the assembly and disassembly of nascent adhesions inside the lamellipodium[75,76]. However, it is required for their subsequent maturation, which involves elongation and growth. Adhesions mature along thin actin bundles that originate near the transition zone at the lamellipodium and lamellum interface[75] (FIG. 3b). The probability that a nascent adhesion in the lamellipodium matures, rather than disassembles, seems to depend on the level of active NM II[75]. However, the mechanism by which adhesions elongate and grow, and how NM II mediates this maturation, is not completely understood. The actin bundling function of NM II seems to be sufficient, at least for the initial stages of adhesion maturation, because motor-deficient NM II mutants that still bind to actin support growth and elongation of adhesions[75].

An integrin–actin linkage translates the effect of NM II to adhesions and mediates adhesion formation and maturation. Different adhesion proteins seem to participate in this linkage, including α-actinin, vinculin and talin (FIG. 4). There are two non-exclusive hypotheses for how NM II mediates adhesion maturation. one is that NM II bundles actin filaments, and as a consequence adhesion proteins at the ends of these actin filaments are brought together and clustered. This increases molecular inter actions between adhesion proteins and results in increased integrin avidity (the combined strength of multiple integrin–ligand interactions) and signalling. For example, the actomyosin-driven binding of vinculin to talin induces integrin clustering[77]. The second hypothesis is that NM II-generated force induces conformational changes that expose cryptic binding or activation sites in key adhesion components[78–80]. Talin, an actin–integrin linkage protein, binds vinculin when mechanically stretched[79] and is required to translate NM II-generated forces to the substratum[81] (FIG. 4). The adhesion scaffold protein CRK-associated substrate (p130CAS; also known as BCAR1) is also activated when stretched[78]. Furthermore, both ends of the adhesive linkage respond to force. Fibronectin undergoes conformational changes induced by mechanical force that result in the exposure of its cryptic sites[82], and integrins are activated by tension[80]. Finally, NM II itself can also undergo force-dependent conformational changes[83]. Interestingly, the force-dependent modulation of mechanoreactive NM II molecules also impinges on other signalling molecules that are not mechanosensitive themselves. It has recently been proposed that NM II also controls EGF-induced paxillin phosphorylation and its subsequent dephosphorylation, which regulates cellular protrusion and retraction[84]. In addition, zyxin associates and dissociates from adhesions in response to extracellular forces[85], and focal adhesion kinase (FAK), a prominent adhesion-associated tyrosine kinase, is required for durotaxis (that is, the tendency of cells to migrate towards stiffer substrates)[86].

NM II is also an integral part of the cellular response to mechanical stimulation. It reacts to mechanical stimuli through cellular signalling pathways that regulate its activation. In this way, NM II provides a convergence point for external and cell-generated forces. For example, application of external forces produces post-translational modifications such as phosphorylation, or conformational changes in different signalling molecules, which inhibit protrusion formation and lead to adhesion maturation and actin filament bundling[87]. Conversely, cells in which NM II is inhibited do not respond to external forces[88]. In addition, cells sense substrate pliability, in part through the activation of NM II by RLC

phosphorylation[89,90]. When cells are plated on low-pliability substrates, NM II activation is low, actin is not highly bundled and adhesions are small. As rigidity increases, NM II activation increases, actin is visibly bundled and adhesions become large and elongated.

Adhesive signalling in NM II activation

NM II influences adhesive signalling through clustering and/or conformational changes, but adhesive signalling also controls NM II activation (FIG. 4). For example, integrin activation induces Tyr phosphorylation of the adhesion adaptor paxillin on Tyr31 and Tyr118 and of FAK on Tyr397. These phosphorylations trigger the activation and recruitment to adhesions of signalling intermediates such as the p130CAS–CRK and G protein-coupled receptor kinase interacting ArfGAP (GIT)–β-Pix (also known as ARHGEF7) complexes, relaying activation signals to Rho GTPases, particularly Rac (reviewed in REFS 91¯93). Among other functions, Rac triggers actin polymerization through the Arp2/3 complex and also inhibits NM II activation (FIG. 4). other signalling pathways activated by adhesion have the opposite effect and promote NM II activation through RHOA[94,95]. Rac is activated by signals generated in small adhesions close to the leading edge that actively undergo turnover and reassemble. RHOA activation mediates actin filament formation and adhesion maturation as the protrusion moves away and the adhesions become more central. This induces further activation of NM II and the coalescence of thick actomyosin bundles. During adhesion maturation, Rac signalling decreases and RHOA-mediated activation of NM II increases, which results in enhanced actomyosin bundling[87,89,96].

NM II is a dual regulator of protrusion, through its effects on actin retrograde flow and adhesion-generated signalling[22,66]. Increased activation of NM II results in large actin bundles and stable adhesions, decreased signalling to Rac and decreased protrusion. Lower levels of active NM II result in less actin bundling and increased protrusion. This can explain, in part, the underlying differences in migration among different cell types. Specifically, highly migratory cells such as leukocytes do not exhibit large adhesions[97], probably reflecting low levels of NM II activation or an intrinsic inability to re arrange their actin cytoskeleton into large bundles, whereas slow moving cells such as fibroblasts have adhesions that tend to mature into large, elongated structures as a result of NM II activation and robust actin bundling.

NM II activation promotes adhesion maturation through its actin bundling and contractile activities. Cycles of Rac and Rho activation inactivate and activate NM II, resulting in protrusion and actin bundling, respectively. The role of NM II in adhesion depends on its ability to exert force on adhesions, even though it is not physically present in the adhesions but attaches to the actin bundles with which adhesions are associated[22]. Thus, NM II influences adhesion from a distance.

NM II determines the polarity of migrating cell

Directional migration requires an initial symmetry break and the polarization of the migratory machinery. There is strong evidence that NM II contractility initiates symmetry breaking by forming the prospective rear of the cell[98–100], which allows for protrusion to occur at the opposite end. The signals leading to specification of the cell rear and protrusion are spatially segregated[101].

The leading protrusion resides at the cell front, and the cell rear often adopts a defined morphology, such as flat and parallel to the leading edge in keratocytes, or lagging and triangular in some fibroblasts. The molecular composition and organization of these front and rear compartments is distinct[102], particularly with regards to the actin cytoskeleton and the adhesions in these regions. The tail region is usually comprised of larger actin filament bundles that support adhesion disassembly and retraction of the rear, and large, stable adhesions that

do not signal to Rac. By contrast, adhesions at the front are smaller and more dynamic and mediate robust Rac signalling, which drives protrusion.

NM II activation is essential for the formation of a defined rear compartment. NM II localizes to the posterior part of migrating leukocytes and *D. discoideum*[103,104]. Here, it creates stable adhesions and highly bundled actin, which prevent protrusion and thereby generate non-protrusive areas that constitute the sides and the trailing edge of the migrating cell[33,98].

In migrating fibroblasts, the NM II isoforms have different roles in cell polarization. NM IIA is dynamic and assembles actomyosin bundles in protrusions22،66. By contrast, NM IIB incorporates into preformed actin bundles and remains stationary, defining the centre and rear of the migrating cell[33]. In this manner, the cooperative functions of NM IIA and NM IIB induce big, non-dynamic actomyosin structures that define the non-protrusive parts of the cell (the centre and the trailing edge), whereas dynamic filaments in protruding regions of the cell are comprised of NM IIA alone[33]. NM IIA specifically mediates retraction of the trailing edge during migration105 (FIG. 3c). When NM IIA is inhibited, cells become elongated owing to their failure to retract[105,106].

In addition to differences in actin and adhesion organization and function, the position of the nucleus, the microtubule-organizing centre (MTOC) and the Golgi apparatus are also hallmarks of migratory cell polarization. The MTOC and Golgi apparatus reposition in front of the nucleus towards the direction of protrusion[107]. Data from experiments in which NM II activity was inhibited by blebbistatin reveal that NM II also regulates nucleus repositioning108 (FIG. 3d). NM IIB seems to play a prominent part in nuclear dynamics during migration as cells depleted of NM IIB exhibit multidirectional protrusions[109], lack a clear front and back, have a mislocalized Golgi and MTOC, exhibit inappropriate rotation of the nucleus and fail to reposition the nucleus properly during polarized migration[22]. One hypothesis is that NM IIB directly anchors the perinuclear actin at the nucleus by interacting with nuclear transmembrane receptors such as nesprins, which are involved in nuclear redistribution during cell migration[110,111]. Another possibility is that NM IIB provides tension or structural rigidity to the perinuclear actin and this force prevents inappropriate rotation of the nucleus and moves it forwards (FIG. 3d).

NM II in cell–cell adhesion and morphogenesis

NM II is not only pivotal in controlling integrin-mediated adhesion and migration, it also regulates epithelial cell adhesion, polarization and morphogenesis. Well-defined cell–cell junctions are a key feature of epithelial sheets and represent a different type of adhesive structure that is controlled by NM II. Although these cell–cell junctions use cadherins as the main adhesion receptors, they contain scaffolds and signalling intermediates analogous to those found in integrin-mediated complexes. In aggregates, epithelial cells have apical and basolateral regions as well as integrin-based adhesions to the extracellular matrix (ECM) at the basal surface (FIG. 5). Epithelial cell sheets can move as multicellular cohorts, with the leading cells showing protrusions and the trailing cells retracting, or they can detach from the sheet and move away as single cells. In free edges of the sheet, the migrating cells undergo a polarity switch from apicobasal (FIG. 5) to front–back (FIG. 3).

NM II controls the formation and stability of the cell–cell junctions. NM IIA is required for cadherin clustering[112,113] (FIG. 5) and the stability of the cell–cell junction. The initiation of the contact is driven by actin polymerization[114], and NM II-driven contractility mediates the later stages of contact formation (compaction). Active RHOA and phosphorylated RLCs localize to the periphery of the junction and drive the contraction and compaction of the cell–cell contact[114] (FIG. 5). RHOA activation, which activates NM II, also mediates contact inhibition of movement (that is, the inhibition of protrusion by cell–cell contact)[115]. NM IIA

probably plays a similar part during the formation of T cell–antigen-presenting cell conjugates in antigenic presentation116.

NM II-driven mechanisms also govern the three-dimensional organization of epithelial tissues. In three dimensions, the force applied to a single cell is potentially multi-directional, rather than planar or one-dimensional. The rigidity of the cellular microenvironment is crucial. There is much evidence that NM II is part of the mechanism by which the cell both generates and responds to force. Three dimensional responses have been studied in *Xenopus laevis* and *D. melanogaster* during early embryonic development and organogenesis. In both systems, the same general principles of regulation of NM II apply. In *D. melanogaster*, NM II (NMHC encoded by *zipper*) is activated by phosphorylation of the RLCs (encoded by *spaghetti squash*), which is under the control of Rho kinase (ROK)[117–119]. NM II is also implicated in epithelial processes involving contraction, such as gastrulation120, trachea formation[121,122], ventral furrow formation[123,124], dorsal closure[117] and morphogenesis of the vertebrate neural tube[124–126], as well as in the re organization of cell contacts in the plane of the epithelium during convergent extension[127,128]. In *D. melanogaster* eye imaginal disc formation, morphogenetic signalling is under the control of Hedgehog, which activates NM II through the RHOA–ROK pathway[129].

Finally, NM II provides the tension and traction that cells use to form and remodel a functional ECM during tissue morphogenesis and wound repair. This includes NM II-powered collagen fibre remodelling[130] and fibronectin fibrillogenesis[82,131]. Recently, a link between cell–cell contact organization and fibrillogenesis was proposed. Actomyosin reorganization driven by cadherins translates into ECM remodelling through integrins, suggesting that cadherin-generated signals regulate integrin activation by modulating cytoskeletal tension[132] (FIG. 5).

NM II mutations and disease

An early indication that NM IIA, NM IIB and NM IIC have different roles during development came from studies in mice showing that disruption of the genes encoding the heavy chains of these proteins (*MYH9*, *MYH10* and *MYH14*, respectively) leads to different phenotypes. NMHC IIA germline-ablated mice fail to form a polarized visceral endoderm and show defects in cell–cell adhesion that result in death by embryonic day (E) 6.5, before organ formation[133]. Mice in which NMHC IIB has been deleted or mutated survive to E14.5 and show cardiac and brain defects, which reflects the enrichment of the NM IIB isoform in these organs in wild-type mice134,135. As noted below, the brain defects were due to failures in both cell adhesion and cell migration.

To decipher the role of NM II in adhesion and migration, several investigators used mutants that mimic the mutations found in humans in *MYH9* and *MYH14* and expressed the mutant NMHC IIA and NMHC IIC proteins in mice, *D. melanogaster* and cultured cells. To date, almost 40 mutations in NMHC IIA, distributed among the head and rod domains, have been reported to cause *MYH9*-related diseases[136] (TABLE 1). All of the patients with these diseases were heterozygous for *MYH9* mutations. one of the most common abnormalities in patients with *MYH9*-related diseases is associated with dysfunction of the blood platelets, which play an important part in blood clotting and clot retraction, and affected patients suffer from macrothrombocytopenia. Platelets from patients with mutated NM IIA have an abnormal cytoskeletal composition and fail to form and/or reorganize cytoskeletal structures on stimulation with the thrombin receptor activating peptide137. The initial shape change and internal contraction of the platelets following stimulation requires NM IIA activity, which in turn is activated by RLC phosphorylation[138,139]. Baculoviral-expressed catalytic fragments (heavy meromyosins) of NM IIA mutants that mimic the human mutations in the motor domain (N93K and R702C in NMHC IIA), have a reduced actin-activated Mg^{2+}-ATPase activity and

an inability to translocate actin filaments *in vitro*[140]. It is therefore likely that impairment of NM IIA motor function contributes to the platelet defects in these patients.

Both haploinsufficiency and the ability of mutant myosin to interfere with wild-type myosin (dominant-negative effects) have been proposed as mechanisms by which mutated NM IIA leads to *MYH9*-related diseases. A reduction in wild-type NM IIA, accompanied by no or very low levels of the mutant form of NM IIA, has been observed in platelets and their precursors (megakaryocytes) from patients with *MYH9*-related diseases. However, both haploinsufficiency and dominant-negative effects have been reported in different tissues from the same patient. Haploinsufficiency was shown to be the mechanism by which mutated NM IIA leads to *MYH9*-related diseases in platelets, and dominant-negative effects were shown to be the mechanism in granulocytes[141,142]. Mice with NM IIA specifically ablated from megakaryocytes have thrombocytopenia with large platelets similar to those in patients with *MYH9*-related diseases, as well as defects in cytoskeletal reorganization[143,144].

So far there have been no reports of mutations in *MYH10*, which encodes NMHC IIB, associated with human disease. To establish a putative mouse model for such diseases, mice carrying the R709C mutation in NMHC IIB (a homologue of the R702C mutation in human NMHC IIA) have been generated[145]. Homozygous mutant mice die by E14.5 and show major morphogenetic defects, including a failure to close the ventral body wall, resulting in a cleft palate, ectopia cordis and a large omphalocele, as well as defects in heart and diaphragm development (X.M. and R.S.A., unpublished observations). These abnormalities are reminiscent of a rare human syndrome known as Pentalogy of Cantrell146.

Mice ablated for NM IIB, in contrast to mice with point mutations in NMHC IIB, develop hydrocephalus (in which circulation of the cerebral spinal fluid is blocked), one of the most common congenital diseases that affects humans (1–3 per 1,000 live births). This is because loss of NM IIB causes disruption of apical cell–cell adhesion of the neuroepithelial cells that border the spinal canal147. Interestingly, this phenotype can be rescued by expression of the NMHC IIB point mutant R709C, which has a motor that is deficient in enzyme activity, or by using gene substitution to replace NM IIB with NM IIA29،147. NM IIA is normally not detected in the neuroepithelial apical adhesion complex and demonstrates markedly increased enzymatic motor properties compared with NM IIB, as well as a decreased duty ratio (see above). Therefore it is likely that the structural properties of NM IIB, rather than its enzymatic motor activity, are essential for maintaining the integrity of cell–cell adhesion in the neuroepithelial cells that line the spinal canal10.

Finally, five mutations in *MYH14*, which encodes NMHC IIC, have been reported to occur in both the myosin head and tail domains. All of these mutations result in deafness[148,149]. The *in vivo* function of NM IIC is largely unknown and mice ablated for NM IIC show no obvious abnormalities (X.M. and R.S.A., unpublished observations).

The pathophysiological roles of NM II are still not fully understood. Although there is no evidence for heterodimer formation among the NMHC isoforms, there is evidence for colocalization of NM II isoforms in filaments. This raises the interesting possibility that a mutant isoform might interfere with a second (or third) wild-type isoform.

Studies from genetically manipulated mouse models demonstrate that NM II is involved in cell–cell adhesion and cell migration *in vivo*. In maintaining the integrity of cell–cell adhesions, NM IIA and NM IIB are functionally interchangeable and this function seems to be independent of their actin-activated Mg^{2+}-ATPase motor activity. In driving cell migration, however, the ATPase activity of the myosin motor is essential and NM IIA and NM IIB cannot substitute for each other.

Concluding remarks

Through its effects on actin bundling and contractility, NM II acts as a master integrator of the processes that drive cell migration and adhesion. It is also an important end point on which many signalling pathways converge, largely through Rho GTPases. NM II itself is tightly regulated at different levels, including at the level of folding, myosin filament assembly and disassembly, actin binding and ATPase and motor activity. The regulation of the actin cytoskeleton by NM II controls multiple interrelated processes, such as migration, cell–cell and cell–matrix adhesion, cell differentiation, tissue morphogenesis and development. Furthermore, NM II is at one end of the regulatory feedback loops that control the activation of NM II's own upstream signalling pathways, including the conformational activation of adhesion molecules and actin organization. The importance of NM II is highlighted by the profound and varied physiological effects of genetic deletion of NM II isoforms in animal models and the high number of naturally occurring mutations in the genes encoding the NMHC II proteins that cause human disease.

The recent interest in the cellular and tissue functions of NM II has produced many revealing and insightful observations, although many basic and translational questions remain. For example, the spatiotemporal regulation of NM II by subcellular localization and activation of its regulating kinases in different cells and tissues has important ramifications in controlling the NM II function but needs further investigation. Emerging evidence strongly indicates that mechanical forces probably remodel the tumour cell microenvironment through NM II to affect tumour progression and metastasis[150], but the precise mechanism by which NM II responds to and generates the microenvironment remains to be elucidated. In addition, the notion that NM II is a central integrator of external force during cell and tissue differentiation, as well as migration, opens up the possibility of manipulating both the rigidity of the microenvironment and the expression and activation of NM II to guide cell proliferation and differentiation during tissue regeneration and stem cell transplantation. In this way, the manipulation of NM II and its regulators constitutes a potentially valuable approach in regenerative medicine.

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene

MYH9 | MYH10 | MYH14 | MYL9

UniProtKB: http://www.uniprot.org

EGF | MLCK | MYPT1 | p130CAS | PAK1 | RHOA | S100A4 | TRPM7 | ZIPK | zyxin

FURTHER INFORMATION

Alan Rick Horwitz's homepage: http://people.virginia.edu/~afh2n/

Robert S. Adelstein's homepage: http://dir.nhlbi.nih.gov/labs/lmc

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

Acknowledgments

The authors apologize for the numerous studies left out owing to journal limits on the number of references. We thank M. A. Conti and J. R. Sellers (National Heart, Lung and Blood Institute (NHLBI)) for helpful suggestions and critical reading of the manuscript. This work was supported by the US National Institutes of Health (NIH) grant GM23244, the Cell Migration Consortium grant U54-GM064346 (A.R.H.), and the Division of Intramural Research, NHLBI, NIH (R.S.A.).

Glossary

Actin filament	A strand of polymerized globular actin subunits that winds around another strand to form a helix. Actin filaments are one of the three major cytoskeletal elements of a cell, along with microtubules and intermediate filaments.
Integrin	One of a large family of heterodimeric transmembrane proteins that functions as a receptor for ECM or cell adhesion molecules.
Coiled coil	A structural domain that can mediate oligomerization. The myosin coiled-coil rod domain contains two α-helices that twist around each other to form a supercoil.
Tonic contractions	Sustained muscular contractions that develop slowly and show a prolonged phase of relaxation.
Actomyosin filaments	Produced when bipolar myosin filaments interact with polymerized actin filaments to exert tension or produce movement.
Lamellipodium	A 1–2 μm-wide band that is made up of a network of dendritic actin filaments and forms the outer edge of a cell protrusion.
Lamellum	The cell region immediately behind the lamellipodium, characterized by the absence of dendritic actin and the presence of longer, bundled actin filaments and slow retrograde flow.
Blebbistatin	A small-molecule inhibitor with high affinity for myosin II that blocks myosin in an actin-detached state.
Pliability	The mechanical properties of the cellular environment. The environment can show low pliability (that is, be elastic or compliant) or high pliability (that is, be rigid or stiff).
Microtubule-organizing centre	A eukaryotic cell structure from which microtubules emanate. During mitosis, the MTOC organizes the mitotic spindle.
Cadherin	A member of a family of type I transmembrane receptors that mediate cell–cell adhesion through homophilic interactions.
Convergent extension	A phase of gastrulation in which layers of cells intercalate (converge) and become longer (extend). Extension is driven by a rearrangement of the cells of the ventral part of the epithelium, which converge towards the ventral midline.
Fibrillogenesis	A cell-induced reorganization of the surrounding ECM molecules into bundled fibres.
Macrothrombocytopenia	A condition that is characterized by enlarged blood platelets that are approximately the size of red blood cells, are reduced in number and result in prolonged bleeding times.
Haploinsufficiency	A state in which the loss of only one allele of a gene detectably disables the encoded protein’s function.
Ectopia cordis	A congenital displacement of the heart outside the thoracic cavity and chest wall.

Omphalocele	A protrusion that occurs at birth, whereby part of the intestine protrudes through a large defect in the abdominal wall at the umbilicus.

References

1. Holmes, KC. Myosins. Coluccio, LM., editor. The Netherlands: Springer; 2007. p. 35-54.Provides an in depth survey of selective myosin class members, including NM IIs. See also references [2, 3, 6, 7] and [9].
2. Mooseker, MS.; Foth, BJ. Myosins. Coluccio, LM., editor. The Netherlands: Springer; 2007. p. 1-34.
3. El-Mezgueldi, M.; Bagshaw, CR. Myosins. Coluccio, LM., editor. The Netherlands: Springer; 2007. p. 55-93.
4. Richards TA, Cavalier-Smith T. Myosin domain evolution and the primary divergence of eukaryotes. Nature 2005;436:1113–1118. [PubMed: 16121172]
5. Odronitz F, Kollmar M. Drawing the tree of eukaryotic life based on the analysis of 2,269 manually annotated myosins from 328 species. Genome Biol 2007;8:R196. [PubMed: 17877792]
6. Cremo, CR.; Hartshorne, DJ. Myosins. Coluccio, LM., editor. The Netherlands: Springer; 2007. p. 171-222.
7. Reggiani, C.; Bottinelli, R. Myosins. Coluccio, LM., editor. The Netherlands: Springer; 2007. p. 125-169.
8. Clark K, Langeslag M, Figdor CG, van Leeuwen FN. Myosin II and mechanotransduction: a balancing act. Trends Cell Biol 2007;17:178–186. [PubMed: 17320396]
9. Conti, MA.; Kawamoto, S.; Adelstein, RS. Myosins. Coluccio, LM., editor. The Netherlands: Springer; 2007. p. 223-264.
10. Conti MA, Adelstein RS. Nonmuscle myosin II moves in new directions. J. Cell Sci 2008;121:11–18. [PubMed: 18096687]
11. Krendel M, Mooseker MS. Myosins: tails (and heads) of functional diversity. Physiology 2005;20:239–251. [PubMed: 16024512]
12. Swailes NT, Colegrave M, Knight PJ, Peckham M. Non-muscle myosins 2A and 2B drive changes in cell morphology that occur as myoblasts align and fuse. J. Cell Sci 2006;119:3561–3570. [PubMed: 16895968]
13. Yuen SL, Ogut O, Brozovich FV. Nonmuscle myosin is regulated during smooth muscle contraction. Am. J. Physiol. Heart Circ. Physiol 2009;297:H191–H199. [PubMed: 19429828]
14. Morano I, et al. Smooth-muscle contraction without smooth-muscle myosin. Nature Cell Biol 2000;2:371–375. [PubMed: 10854329] The first demonstration that NM II can play a part in smooth muscle contraction. The authors make use of smooth muscle myosin knockout mice to study smooth muscle contraction and show that the sustained phase of contraction is due to NM II. See reference [13] for recent work in this area which suggests that NM IIB might contribute to the smooth muscle 'latch' state.
15. Niederman R, Pollard TD. Human platelet myosin. II. *In vitro* assembly and structure of myosin filaments. J. Cell Biol 1975;67:72–92. [PubMed: 240861]
16. Svitkina TM, Verkhovsky AB, Borisy GG. Improved procedures for electron microscopic visualization of the cytoskeleton of cultured cells. J. Struct. Biol 1995;115:290–303. [PubMed: 8573471]
17. Mansfield SG, al-Shirawi DY, Ketchum AS, Newbern EC, Kiehart DP. Molecular organization and alternative splicing in *Zipper*, the gene that encodes the *Drosophila* non-muscle myosin II heavy chain. J. Mol. Biol 1996;255:98–109. [PubMed: 8568878]
18. Jana SS, et al. An alternatively spliced isoform of nonmuscle myosin II-C is not regulated by myosin light chain phosphorylation. J. Biol. Chem 2009;284:11563–11571. [PubMed: 19240025]
19. Li Y, Lalwani AK, Mhatre AN. Alternative splice variants of MYH9. DNA Cell Biol 2008;27:117–125. [PubMed: 17997715]

20. Maupin P, Phillips CL, Adelstein RS, Pollard TD. Differential localization of myosin-II isozymes in human cultured cells and blood cells. J. Cell Sci 1994;107:3077–3090. [PubMed: 7699007]
21. Kolega J. Cytoplasmic dynamics of myosin IIA and IIB: spatial 'sorting' of isoforms in locomoting cells. J. Cell Sci 1998;111:2085–2095. [PubMed: 9664030]
22. Vicente-Manzanares M, Zareno J, Whitmore L, Choi CK, Horwitz AF. Regulation of protrusion, adhesion dynamics, and polarity by myosins IIA and IIB in migrating cells. J. Cell Biol 2007;176:573–580. [PubMed: 17312025]
23. Bao J, Jana SS, Adelstein RS. Vertebrate nonmuscle myosin II isoforms rescue small interfering RNA-induced defects in COS-7 cell cytokinesis. J. Biol. Chem 2005;280:19594–19599. [PubMed: 15774463]
24. Golomb E, et al. Identification and characterization of nonmuscle myosin II-C, a new member of the myosin II family. J. Biol. Chem 2004;279:2800–2808. [PubMed: 14594953]
25. Kim KY, Kovacs M, Kawamoto S, Sellers JR, Adelstein RS. Disease-associated mutations and alternative splicing alter the enzymatic and motile activity of nonmuscle myosins II-B and II-C. J. Biol. Chem 2005;280:22769–22775. [PubMed: 15845534]
26. Kovacs M, Wang F, Hu A, Zhang Y, Sellers JR. Functional divergence of human cytoplasmic myosin II: kinetic characterization of the non-muscle IIA isoform. J. Biol. Chem 2003;278:38132–38140. [PubMed: 12847096]
27. Wang F, et al. Kinetic mechanism of non-muscle myosin IIB: functional adaptations for tension generation and maintenance. J. Biol. Chem 2003;278:27439–27448. [PubMed: 12704189] Together with reference [26], this paper details the differences in the kinetic properties of NM IIA and NM IIB. These studies provide a mechanistic framework for understanding the putative *in vivo* function of two of the three NM II isoforms.
28. Kovacs M, Thirumurugan K, Knight PJ, Sellers JR. Load-dependent mechanism of nonmuscle myosin 2. Proc. Natl Acad. Sci. USA 2007;104:9994–9999. [PubMed: 17548820]
29. Bao J, Ma X, Liu C, Adelstein RS. Replacement of nonmuscle myosin II-B with II-A rescues brain but not cardiac defects in mice. J. Biol. Chem 2007;282:22102–22111. [PubMed: 17519229]
30. Nakasawa T, et al. Critical regions for assembly of vertebrate nonmuscle myosin II. Biochemistry 2005;44:174–183. [PubMed: 15628858]
31. Sato MK, Takahashi M, Yazawa M. Two regions of the tail are necessary for the isoform-specific functions of nonmuscle myosin IIB. Mol. Biol. Cell 2007;18:1009–1017. [PubMed: 17202408]
32. Sandquist JC, Means AR. The C-terminal tail region of nonmuscle myosin II directs isoform-specific distribution in migrating cells. Mol. Biol. Cell 2008;19:5156–5167. [PubMed: 18843042]
33. Vicente-Manzanares M, Koach MA, Whitmore L, Lamers ML, Horwitz AF. Segregation and activation of myosin IIB creates a rear in migrating cells. J. Cell Biol 2008;183:543–554. [PubMed: 18955554]
34. Somlyo AP, Somlyo AV. Ca^{2+} sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. Physiol. Rev 2003;83:1325–1358. [PubMed: 14506307]
35. Wendt T, Taylor D, Trybus KM, Taylor K. Three-dimensional image reconstruction of dephosphorylated smooth muscle heavy meromyosin reveals asymmetry in the interaction between myosin heads and placement of subfragment 2. Proc. Natl Acad. Sci. USA 2001;98:4361–4366. [PubMed: 11287639]
36. Sellers JR, Eisenberg E, Adelstein RS. The binding of smooth muscle heavy meromyosin to actin in the presence of ATP. Effect of phosphorylation. J. Biol. Chem 1982;257:13880–13883. [PubMed: 6128340]
37. Hirata N, Takahashi M, Yazawa M. Diphosphorylation of regulatory light chain of myosin IIA is responsible for proper cell spreading. Biochem. Biophys. Res. Commun 2009;381:682–687. [PubMed: 19254691]
38. Ikebe M, Hartshorne DJ, Elzinga M. Identification, phosphorylation, and dephosphorylation of a second site for myosin light chain kinase on the 20,000-dalton light chain of smooth muscle myosin. J. Biol. Chem 1986;261:36–39. [PubMed: 3079756]

39. Umemoto S, Bengur AR, Sellers JR. Effect of multiple phosphorylations of smooth muscle and cytoplasmic myosins on movement in an *in vitro* motility assay. J. Biol. Chem 1989;264:1431–1436. [PubMed: 2521481]
40. Scholey JM, Taylor KA, Kendrick-Jones J. Regulation of non-muscle myosin assembly by calmodulin-dependent light chain kinase. Nature 1980;287:233–235. [PubMed: 6893621]
41. Woodhead JL, et al. Atomic model of a myosin filament in the relaxed state. Nature 2005;436:1195–1199. [PubMed: 16121187]
42. Jung HS, Komatsu S, Ikebe M, Craig R. Head–head and head–tail interaction: a general mechanism for switching off myosin II activity in cells. Mol. Biol. Cell 2008;19:3234–3242. [PubMed: 18495867]
43. Craig R, Woodhead JL. Structure and function of myosin filaments. Curr. Opin. Struct. Biol 2006;16:204–212. [PubMed: 16563742]
44. Burgess SA, et al. Structures of smooth muscle myosin and heavy meromyosin in the folded, shutdown state. J. Mol. Biol 2007;372:1165–1178. [PubMed: 17707861] Together with references 35 and 41[–43], this is an outstanding structural study that shows the transformation of the folded, blocked (10S) state to the unfolded, activated (6S) state of single NM II molecules following RLC phosphorylation. These studies also show the interaction of the two myosin heads in the inactive state.
45. Matsumura F. Regulation of myosin II during cytokinesis in higher eukaryotes. Trends Cell Biol 2005;15:371–377. [PubMed: 15935670]
46. Tan I, Yong J, Dong JM, Lim L, Leung T. A tripartite complex containing MRCK modulates lamellar actomyosin retrograde flow. Cell 2008;135:123–136. [PubMed: 18854160]
47. Matsumura F, Hartshorne DJ. Myosin phosphatase target subunit: Many roles in cell function. Biochem. Biophys. Res. Commun 2008;369:149–156. [PubMed: 18155661]
48. Totsukawa G, et al. Distinct roles of ROCK (Rho-kinase) and MLCK in spatial regulation of MLC phosphorylation for assembly of stress fibers and focal adhesions in 3T3 fibroblasts. J. Cell Biol 2000;150:797–806. [PubMed: 10953004]
49. Totsukawa G, et al. Distinct roles of MLCK and ROCK in the regulation of membrane protrusions and focal adhesion dynamics during cell migration of fibroblasts. J. Cell Biol 2004;164:427–439. [PubMed: 14757754]
50. Nishikawa M, Sellers JR, Adelstein RS, Hidaka H. Protein kinase C modulates *in vitro* phosphorylation of the smooth muscle heavy meromyosin by myosin light chain kinase. J. Biol. Chem 1984;259:8808–8814. [PubMed: 6235218]
51. Komatsu S, Ikebe M. The phosphorylation of myosin II at the Ser1 and Ser2 is critical for normal platelet-derived growth factor induced reorganization of myosin filaments. Mol. Biol. Cell 2007;18:5081–5090. [PubMed: 17928407]
52. Bosgraaf L, van Haastert PJ. The regulation of myosin II in *Dictyostelium*. Eur. J. Cell Biol 2006;85:969–979. [PubMed: 16814425]
53. Even-Faitelson L, Ravid S. PAK1 and aPKCζ regulate myosin II-B phosphorylation: a novel signaling pathway regulating filament assembly. Mol. Biol. Cell 2006;17:2869–2881. [PubMed: 16611744]
54. Dulyaninova NG, Malashkevich VN, Almo SC, Bresnick AR. Regulation of myosin-IIA assembly and Mts1 binding by heavy chain phosphorylation. Biochemistry 2005;44:6867–6876. [PubMed: 15865432]
55. Clark K, et al. TRPM7 regulates myosin IIA filament stability and protein localization by heavy chain phosphorylation. J. Mol. Biol 2008;378:790–803. [PubMed: 18394644]
56. Conti MA, Sellers JR, Adelstein RS, Elzinga M. Identification of the serine residue phosphorylated by protein kinase C in vertebrate nonmuscle myosin heavy chains. Biochemistry 1991;30:966–970. [PubMed: 1899200]
57. Ludowyke RI, et al. Phosphorylation of nonmuscle myosin heavy chain IIA on Ser1917 is mediated by protein kinase C βII and coincides with the onset of stimulated degranulation of RBL-2H3 mast cells. J. Immunol 2006;177:1492–1499. [PubMed: 16849455]
58. Li ZH, Bresnick AR. The S100A4 metastasis factor regulates cellular motility via a direct interaction with myosin-IIA. Cancer Res 2006;66:5173–5180. [PubMed: 16707441]

59. Clark K, et al. The α-kinases TRPM6 and TRPM7, but not eEF-2 kinase, phosphorylate the assembly domain of myosin IIA, IIB and IIC. FEBS Lett 2008;582:2993–2997. [PubMed: 18675813]
60. Ronen D, Ravid S. Myosin II tailpiece determines its paracrystal structure, filament assembly properties, and cellular localization. J. Biol. Chem 2009;284:24948–24957. [PubMed: 19553683]
61. Heath JP, Holifield BF. Cell locomotion: new research tests old ideas on membrane and cytoskeletal flow. Cell. Motil. Cytoskeleton 1991;18:245–257. [PubMed: 2049790]
62. Pollard TD, Borisy GG. Cellular motility driven by assembly and disassembly of actin filaments. Cell 2003;112:453–465. [PubMed: 12600310]
63. Koestler SA, Auinger S, Vinzenz M, Rottner K, Small JV. Differentially oriented populations of actin filaments generated in lamellipodia collaborate in pushing and pausing at the cell front. Nature Cell Biol 2008;10:306–313. [PubMed: 18278037]
64. Ponti A, Machacek M, Gupton SL, Waterman-Storer CM, Danuser G. Two distinct actin networks drive the protrusion of migrating cells. Science 2004;305:1782–1786. [PubMed: 15375270] Describes the kinetic and compositional distinction between the lamellipodium and the lamellum — the two actin networks in protrusions.
65. Delorme V, et al. Cofilin activity downstream of Pak1 regulates cell protrusion efficiency by organizing lamellipodium and lamella actin networks. Dev. Cell 2007;13:646–662. [PubMed: 17981134]
66. Cai Y, et al. Nonmuscle myosin IIA-dependent force inhibits cell spreading and drives F-actin flow. Biophys. J 2006;91:3907–3920. [PubMed: 16920834]
67. Giannone G, et al. Periodic lamellipodial contractions correlate with rearward actin waves. Cell 2004;116:431–443. [PubMed: 15016377]
68. Giannone G, et al. Lamellipodial actin mechanically links myosin activity with adhesion-site formation. Cell 2007;128:561–575. [PubMed: 17289574]
69. Anderson TW, Vaughan AN, Cramer LP. Retrograde flow and myosin II activity within the leading cell edge deliver F-actin to the lamella to seed the formation of graded polarity actomyosin II filament bundles in migrating fibroblasts. Mol. Biol. Cell 2008;19:5006–5018. [PubMed: 18799629]
70. Nemethova M, Auinger S, Small JV. Building the actin cytoskeleton: filopodia contribute to the construction of contractile bundles in the lamella. J. Cell Biol 2008;180:1233–1244. [PubMed: 18362182]
71. Even-Ram S, et al. Myosin IIA regulates cell motility and actomyosin-microtubule crosstalk. Nature Cell Biol 2007;9:299–309. [PubMed: 17310241] Establishes the role of NM IIA in the co-regulation of actin and microtubule functions in motile cells.
72. Mitchison T, Kirschner M. Cytoskeletal dynamics and nerve growth. Neuron 1988;1:761–772. [PubMed: 3078414]
73. Lin CH, Forscher P. Growth cone advance is inversely proportional to retrograde F-actin flow. Neuron 1995;14:763–771. [PubMed: 7536426]
74. Jay PY, Pham PA, Wong SA, Elson EL. A mechanical function of myosin II in cell motility. J. Cell Sci 1995;108:387–393. [PubMed: 7738114]
75. Choi CK, et al. Actin and α-actinin orchestrate the assembly and maturation of nascent adhesions in a myosin II motor-independent manner. Nature Cell Biol 2008;10:1039–1050. [PubMed: 19160484]
76. Alexandrova AY, et al. Comparative dynamics of retrograde actin flow and focal adhesions: formation of nascent adhesions triggers transition from fast to slow flow. PLoS ONE 2008;3:e3234. [PubMed: 18800171]
77. Humphries JD, et al. Vinculin controls focal adhesion formation by direct interactions with talin and actin. J. Cell Biol 2007;179:1043–1057. [PubMed: 18056416]
78. Sawada Y, et al. Force sensing by mechanical extension of the Src family kinase substrate p130Cas. Cell 2006;127:1015–1026. [PubMed: 17129785]
79. del Rio A, et al. Stretching single talin rod molecules activates vinculin binding. Science 2009;323:638–641. [PubMed: 19179532]
80. Friedland JC, Lee MH, Boettiger D. Mechanically activated integrin switch controls α5β1 function. Science 2009;323:642–644. [PubMed: 19179533]

81. Jiang G, Giannone G, Critchley DR, Fukumoto E, Sheetz MP. Two-piconewton slip bond between fibronectin and the cytoskeleton depends on talin. Nature 2003;424:334–337. [PubMed: 12867986]
82. Zhong C, et al. Rho-mediated contractility exposes a cryptic site in fibronectin and induces fibronectin matrix assembly. J. Cell Biol 1998;141:539–551. [PubMed: 9548730]
83. Schwaiger I, Sattler C, Hostetter DR, Rief M. The myosin coiled-coil is a truly elastic protein structure. Nature Mater 2002;1:232–235. [PubMed: 12618784]
84. Schneider IC, Hays CK, Waterman CM. Epidermal growth factor-induced contraction regulates paxillin phosphorylation to temporally separate traction generation from de-adhesion. Mol. Biol. Cell 2009;20:3155–3167. [PubMed: 19403690]
85. Yoshigi M, Hoffman LM, Jensen CC, Yost HJ, Beckerle MC. Mechanical force mobilizes zyxin from focal adhesions to actin filaments and regulates cytoskeletal reinforcement. J. Cell Biol 2005;171:209–215. [PubMed: 16247023]
86. Wang HB, Dembo M, Hanks SK, Wang Y. Focal adhesion kinase is involved in mechanosensing during fibroblast migration. Proc. Natl Acad. Sci. USA 2001;98:11295–11300. [PubMed: 11572981]
87. Galbraith CG, Yamada KM, Sheetz MP. The relationship between force and focal complex development. J. Cell Biol 2002;159:695–705. [PubMed: 12446745]
88. Chen CS. Mechanotransduction — a field pulling together? J. Cell Sci 2008;121:3285–3292. [PubMed: 18843115]
89. Beningo KA, Dembo M, Kaverina I, Small JV, Wang YL. Nascent focal adhesions are responsible for the generation of strong propulsive forces in migrating fibroblasts. J. Cell Biol 2001;153:881–888. [PubMed: 11352946]
90. Beningo KA, Hamao K, Dembo M, Wang YL, Hosoya H. Traction forces of fibroblasts are regulated by the Rho-dependent kinase but not by the myosin light chain kinase. Arch. Biochem. Biophys 2006;456:224–231. [PubMed: 17094935]
91. Parsons JT. Focal adhesion kinase: the first ten years. J. Cell Sci 2003;116:1409–1416. [PubMed: 12640026]
92. Deakin NO, Turner CE. Paxillin comes of age. J. Cell Sci 2008;121:2435–2444. [PubMed: 18650496]
93. Mitra SK, Schlaepfer DD. Integrin-regulated FAK–Src signaling in normal and cancer cells. Curr. Opin. Cell Biol 2006;18:516–523. [PubMed: 16919435]
94. Geiger B, Spatz JP, Bershadsky AD. Environmental sensing through focal adhesions. Nature Rev. Mol. Cell Biol 2009;10:21–33. [PubMed: 19197329]
95. Vicente-Manzanares M, Choi CK, Horwitz AR. Integrins in cell migration — the actin connection. J. Cell Sci 2009;122:199–206. [PubMed: 19118212]
96. Ballestrem C, et al. Molecular mapping of tyrosine-phosphorylated proteins in focal adhesions using fluorescence resonance energy transfer. J. Cell Sci 2006;119:866–875. [PubMed: 16478788]
97. Smith A, et al. A talin-dependent LFA-1 focal zone is formed by rapidly migrating T lymphocytes. J. Cell Biol 2005;170:141–151. [PubMed: 15983060]
98. Verkhovsky AB, Svitkina TM, Borisy GG. Self-polarization and directional motility of cytoplasm. Curr. Biol 1999;9:11–20. [PubMed: 9889119]
99. Yam PT, et al. Actin-myosin network reorganization breaks symmetry at the cell rear to spontaneously initiate polarized cell motility. J. Cell Biol 2007;178:1207–1221. [PubMed: 17893245]
100. Mseka T, Bamburg JR, Cramer LP. ADF/cofilin family proteins control formation of oriented actin-filament bundles in the cell body to trigger fibroblast polarization. J. Cell Sci 2007;120:4332–4344. [PubMed: 18042624]
101. Xu J, et al. Divergent signals and cytoskeletal assemblies regulate self-organizing polarity in neutrophils. Cell 2003;114:201–214. [PubMed: 12887922]
102. Ridley AJ, et al. Cell migration: integrating signals from front to back. Science 2003;302:1704–1709. [PubMed: 14657486]
103. Eddy RJ, Pierini LM, Matsumura F, Maxfield FR. Ca^{2+}-dependent myosin II activation is required for uropod retraction during neutrophil migration. J. Cell Sci 2000;113:1287–1298. [PubMed: 10704379]
104. Kolega J. Asymmetry in the distribution of free versus cytoskeletal myosin II in locomoting microcapillary endothelial cells. Exp. Cell Res 1997;231:66–82. [PubMed: 9056413]

105. Chrzanowska-Wodnicka M, Burridge K. Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. J. Cell Biol 1996;133:1403–1415. [PubMed: 8682874] Implicates NM II in trailing edge retraction.

106. Worthylake RA, Lemoine S, Watson JM, Burridge K. RhoA is required for monocyte tail retraction during transendothelial migration. J. Cell Biol 2001;154:147–160. [PubMed: 11448997]

107. Etienne-Manneville S, Hall A. Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCζ. Cell 2001;106:489–498. [PubMed: 11525734]

108. Gomes ER, Jani S, Gundersen GG. Nuclear movement regulated by Cdc42, MRCK, myosin, and actin flow establishes MTOC polarization in migrating cells. Cell 2005;121:451–463. [PubMed: 15882626] Demonstrates the role of NM II in nuclear repositioning during cell migration.

109. Lo CM, et al. Nonmuscle myosin IIB is involved in the guidance of fibroblast migration. Mol. Biol. Cell 2004;15:982–989. [PubMed: 14699073]

110. Warren DT, Zhang Q, Weissberg PL, Shanahan CM. Nesprins: intracellular scaffolds that maintain cell architecture and coordinate cell function? Expert Rev. Mol. Med 2005;7:1–15. [PubMed: 15953398]

111. Nery FC, et al. TorsinA binds the KASH domain of nesprins and participates in linkage between nuclear envelope and cytoskeleton. J. Cell Sci 2008;121:3476–3486. [PubMed: 18827015]

112. Shewan AM, et al. Myosin 2 is a key Rho kinase target necessary for the local concentration of E-cadherin at cell-cell contacts. Mol. Biol. Cell 2005;16:4531–4542. [PubMed: 16030252]

113. Ivanov AI, et al. A unique role for nonmuscle myosin heavy chain IIA in regulation of epithelial apical junctions. PLoS ONE 2007;2:e658. [PubMed: 17668046]

114. Yamada S, Nelson WJ. Localized zones of Rho and Rac activities drive initiation and expansion of epithelial cell-cell adhesion. J. Cell Biol 2007;178:517–527. [PubMed: 17646397]

115. Carmona-Fontaine C, et al. Contact inhibition of locomotion *in vivo* controls neural crest directional migration. Nature 2008;456:957–961. [PubMed: 19078960]

116. Ilani T, Vasiliver-Shamis G, Vardhana S, Bretscher A, Dustin ML. T cell antigen receptor signaling and immunological synapse stability require myosin IIA. Nature Immunol 2009;10:531–539. [PubMed: 19349987]

117. Young PE, Richman AM, Ketchum AS, Kiehart DP. Morphogenesis in *Drosophila* requires nonmuscle myosin heavy chain function. Genes Dev 1993;7:29–41. [PubMed: 8422986]

118. Karess RE, et al. The regulatory light chain of nonmuscle myosin is encoded by *spaghetti-squash*, a gene required for cytokinesis in *Drosophila*. Cell 1991;65:1177–1189. [PubMed: 1905980]

119. Winter CG, et al. *Drosophila* Rho-associated kinase (Drok) links Frizzled-mediated planar cell polarity signaling to the actin cytoskeleton. Cell 2001;105:81–91. [PubMed: 11301004]

120. Skoglund P, Rolo A, Chen X, Gumbiner BM, Keller R. Convergence and extension at gastrulation require a myosin IIB-dependent cortical actin network. Development 2008;135:2435–2444. [PubMed: 18550716]

121. Brodu V, Casanova J. The RhoGAP crossveinless-c links trachealess and EGFR signaling to cell shape remodeling in *Drosophila* tracheal invagination. Genes Dev 2006;20:1817–1828. [PubMed: 16818611]

122. Myat MM. Making tubes in the *Drosophila* embryo. Dev. Dyn 2005;232:617–632. [PubMed: 15712279]

123. Barrett K, Leptin M, Settleman J. The Rho GTPase and a putative RhoGEF mediate a signaling pathway for the cell shape changes in *Drosophila* gastrulation. Cell 1997;91:905–915. [PubMed: 9428514]

124. Pilot F, Lecuit T. Compartmentalized morphogenesis in epithelia: from cell to tissue shape. Dev. Dyn 2005;232:685–694. [PubMed: 15712202]

125. Rolo A, Skoglund P, Keller R. Morphogenetic movements driving neural tube closure in *Xenopus* require myosin IIB. Dev. Biol 2009;327:327–338. [PubMed: 19121300]

126. Hildebrand JD, Soriano P. Shroom, a PDZ domain-containing actin-binding protein, is required for neural tube morphogenesis in mice. Cell 1999;99:485–497. [PubMed: 10589677]

127. Bertet C, Sulak L, Lecuit T. Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. Nature 2004;429:667–671. [PubMed: 15190355]

128. Yamamoto N, Okano T, Ma X, Adelstein RS, Kelley MW. Myosin II regulates extension, growth and patterning in the mammalian cochlear duct. Development 2009;136:1977–1986. [PubMed: 19439495]
129. Corrigall D, Walther RF, Rodriguez L, Fichelson P, Pichaud F. Hedgehog signaling is a principal inducer of Myosin-II-driven cell ingression in *Drosophila* epithelia. Dev. Cell 2007;13:730–742. [PubMed: 17981140]
130. Meshel AS, Wei Q, Adelstein RS, Sheetz MP. Basic mechanism of three-dimensional collagen fibre transport by fibroblasts. Nature Cell Biol 2005;7:157–164. [PubMed: 15654332]
131. Zhang Q, Magnusson MK, Mosher DF. Lysophosphatidic acid and microtubule-destabilizing agents stimulate fibronectin matrix assembly through Rho-dependent actin stress fiber formation and cell contraction. Mol. Biol. Cell 1997;8:1415–1425. [PubMed: 9285815]
132. Dzamba BJ, Jakab KR, Marsden M, Schwartz MA, DeSimone DW. Cadherin adhesion, tissue tension, and noncanonical Wnt signaling regulate fibronectin matrix organization. Dev. Cell 2009;16:421–432. [PubMed: 19289087]
133. Conti MA, Even-Ram S, Liu C, Yamada KM, Adelstein RS. Defects in cell adhesion and the visceral endoderm following ablation of nonmuscle myosin heavy chain II-A in mice. J. Biol. Chem 2004;279:41263–41266. [PubMed: 15292239]
134. Tullio AN, et al. Nonmuscle myosin II-B is required for normal development of the mouse heart. Proc. Natl Acad. Sci. USA 1997;94:12407–12412. [PubMed: 9356462]
135. Tullio AN, et al. Structural abnormalities develop in the brain after ablation of the gene encoding nonmuscle myosin II-B heavy chain. J. Comp. Neurol 2001;433:62–74. [PubMed: 11283949]
136. Burt RA, Joseph JE, Milliken S, Collinge JE, Kile BT. Description of a novel mutation leading to *MYH9*-related disease. Thromb. Res 2008;122:861–863. [PubMed: 18676005]
137. Canobbio I, et al. Altered cytoskeleton organization platelets from patients with *MYH9*-related disease. J. Thromb. Haemost 2005;3:1026–1035. [PubMed: 15869600]
138. Johnson GJ, Leis LA, Krumwiede MD, White JG. The critical role of myosin IIA in platelet internal contraction. J. Thromb. Haemost 2007;5:1516–1529. [PubMed: 17488351]
139. Calaminus SD, et al. MyosinIIa contractility is required for maintenance of platelet structure during spreading on collagen and contributes to thrombus stability. J. Thromb. Haemost 2007;5:2136–2145. [PubMed: 17645784]
140. Hu A, Wang F, Sellers JR. Mutations in human nonmuscle myosin IIA found in patients with May-Hegglin anomaly and Fechtner syndrome result in impaired enzymatic function. J. Biol. Chem 2002;277:46512–46517. [PubMed: 12237319]
141. Kunishima S, Hamaguchi M, Saito H. Differential expression of wild-type and mutant NMMHC-IIA polypeptides in blood cells suggests cell-specific regulation mechanisms in *MYH9* disorders. Blood 2008;111:3015–3023. [PubMed: 18192507]
142. Pecci A, et al. Pathogenetic mechanisms of hematological abnormalities of patients with *MYH9* mutations. Hum. Mol. Genet 2005;14:3169–3178. [PubMed: 16162639] This study analyses 11 patients from 6 families, with 6 different NM IIA mutations, and provides evidence that defects in the megakaryocytic lineage arise from haploinsufficiency of NM IIA, whereas the inclusion bodies in granulocytes are due to the mutant form of NM IIA interfering with the wild-type form.
143. Leon C, et al. Megakaryocyte-restricted *MYH9* inactivation dramatically affects hemostasis while preserving platelet aggregation and secretion. Blood 2007;110:3183–3191. [PubMed: 17664350]
144. Eckly A, et al. Abnormal megakaryocyte morphology and proplatelet formation in mice with megakaryocyte-restricted *MYH9* inactivation. Blood 2008;113:3182–3189. [PubMed: 18984861]
145. Ma X, Kawamoto S, Hara Y, Adelstein RS. A point mutation in the motor domain of nonmuscle myosin II-B impairs migration of distinct groups of neurons. Mol. Biol. Cell 2004;15:2568–2579. [PubMed: 15034141]
146. Cantrell JR, Haller JA, Ravitch MM. A syndrome of congenital defects involving the abdominal wall, sternum, diaphragm, pericardium, and heart. Surg. Gynecol. Obstet 1958;107:602–614. [PubMed: 13592660]
147. Ma X, Bao J, Adelstein RS. Loss of cell adhesion causes hydrocephalus in nonmuscle myosin II-B-ablated and mutated mice. Mol. Biol. Cell 2007;18:2305–2312. [PubMed: 17429076]

148. Donaudy F, et al. Nonmuscle myosin heavy-chain gene *MYH14* is expressed in cochlea and mutated in patients affected by autosomal dominant hearing impairment (DFNA4). Am. J. Hum. Genet 2004;74:770–776. [PubMed: 15015131]

149. Yang T, et al. Genetic heterogeneity of deafness phenotypes linked to DFNA4. Am. J. Med. Genet. A 2005;139:9–12. [PubMed: 16222661]

150. Butcher DT, Alliston T, Weaver VM. A tense situation: forcing tumour progression. Nature Rev. Cancer 2009;9:108–122. [PubMed: 19165226]

151. Medeiros NA, Burnette DT, Forscher P. Myosin II functions in actin-bundle turnover in neuronal growth cones. Nature Cell Biol 2006;8:215–226. [PubMed: 16501565]

152. Miyajima Y, Kunishima S. Identification of the first in *cis* mutations in *MYH9* disorder. Eur. J. Haematol 2009;82:288–291. [PubMed: 19191864]

153. Miyazaki K, Kunishima S, Fujii W, Higashihara M. Identification of three in-frame deletion mutations in *MYH9* disorders suggesting an important hot spot for small rearrangements in *MYH9* exon 24. Eur. J. Haematol 2009;83:230–234. [PubMed: 19459928]

154. De Rocco D, et al. Identification of the first duplication in *MYH9*-related disease: A hot spot for hot unequal crossing-over within exon 24 of the *MYH9* gene. Eur. J. Med. Genet 2009;52:191–194. [PubMed: 19450438]

155. Capria M, et al. Double nucleotidic mutation of the *MYH9* gene in a young patient with end-stage renal disease. Nephrol Dial Transplant 2004;19:249–251. [PubMed: 14671068]

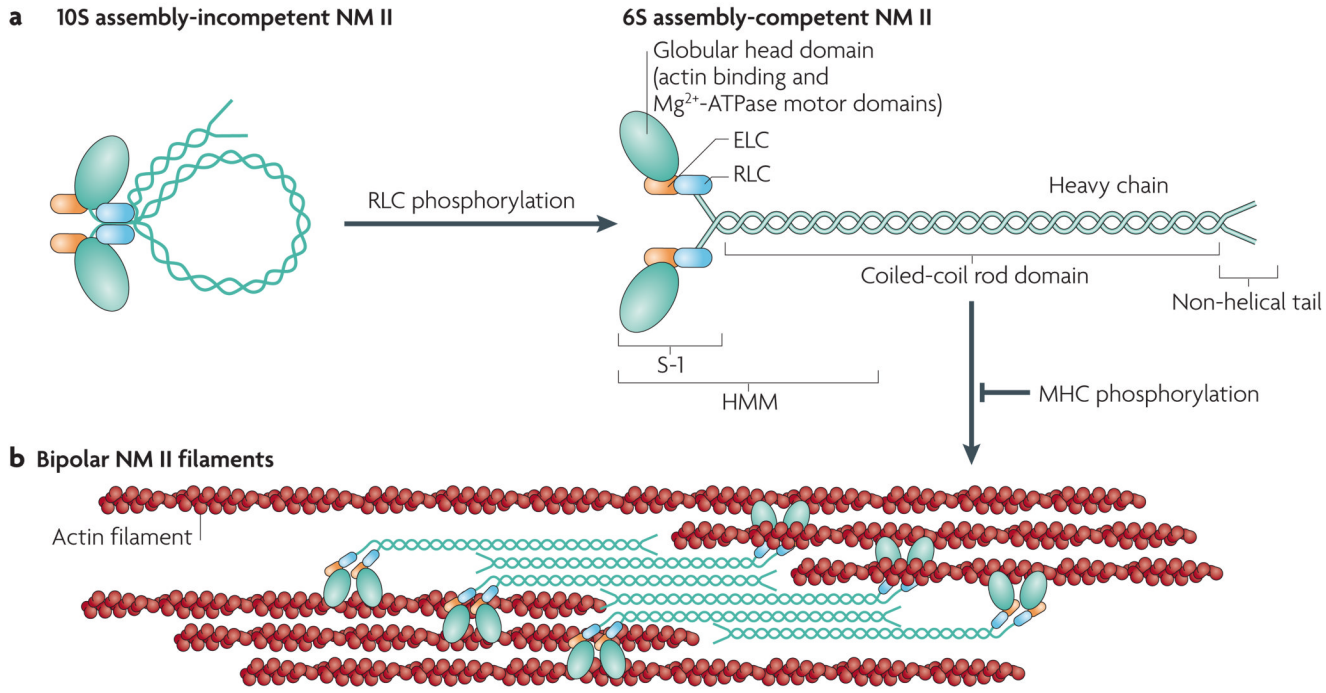

Figure 1. Domain structure of NM II

a | The subunit and domain structure of non-muscle myosin II (NM II), which forms a dimer through interactions between the α-helical coiled-coil rod domains. The globular head domain contains the actin-binding regions and the enzymatic Mg^{2+}-ATPase motor domains. The essential light chains (ELCs) and the regulatory light chains (RLCs) bind to the heavy chains at the lever arms that link the head and rod domains. In the absence of RLC phosphorylation, NM II forms a compact molecule through a head to tail interaction. This results in an assembly-incompetent form (10S; left) that is unable to associate with other NM II dimers. On RLC phosphorylation, the 10S structure unfolds and becomes an assembly-competent form (6S). S-1 is a fragment of NM II that contains the motor domain and neck but lacks the rod domain and is unable to dimerize. Heavy meromyosin (HMM) is a fragment that contains the motor domain, neck and enough of the rod to effect dimerization. **b** | NM II molecules assemble into bipolar filaments through interactions between their rod domains. These filaments bind to actin through their head domains and the ATPase activity of the head enables a conformational change that moves actin filaments in an anti-parallel manner. Bipolar myosin filaments link actin filaments together in thick bundles that form cellular structures such as stress fibres.

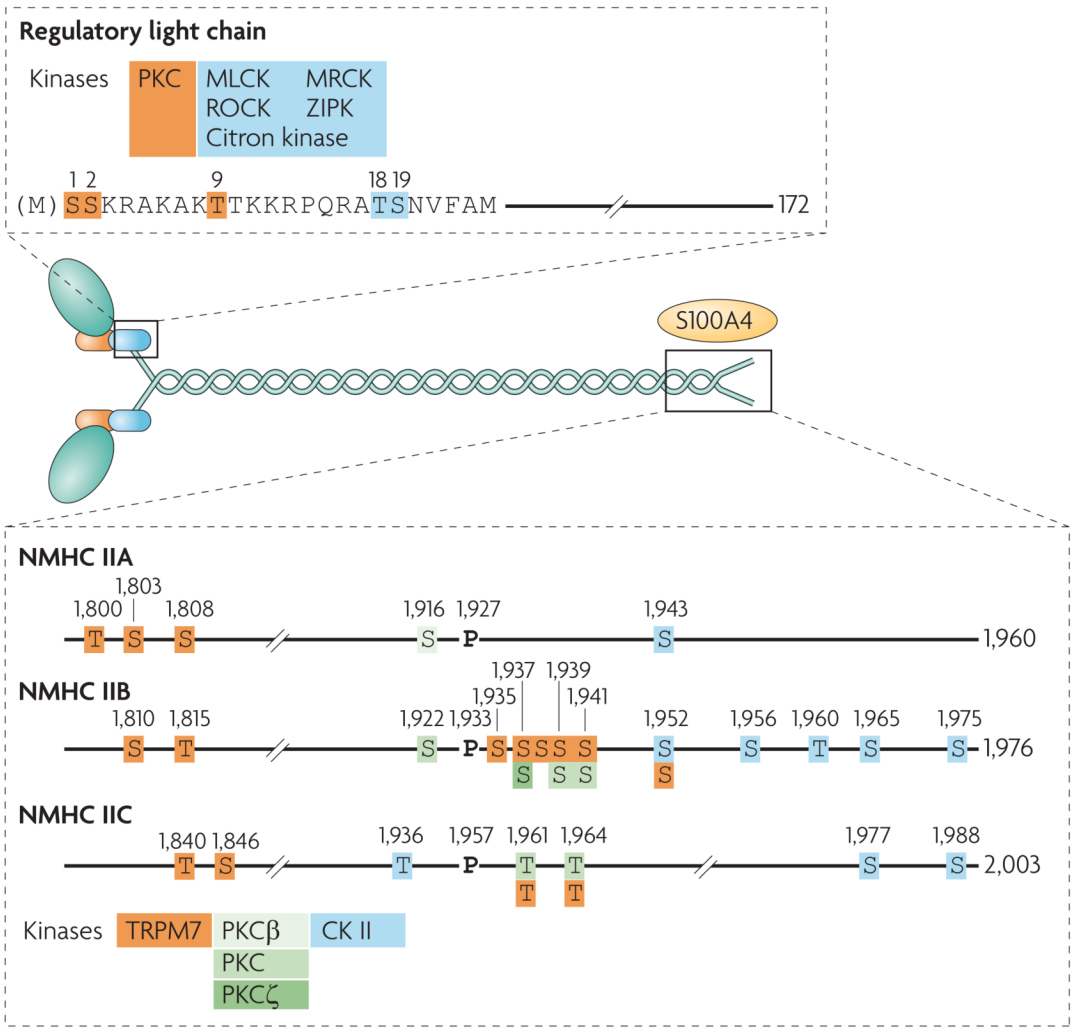

Figure 2. Regulation of NM II activation and filament formation

Multiple kinases, including myosin light chain kinase (MLCK; also known as MYLK), Rho-associated, coiled coil-containing kinase (ROCK), citron kinase, myotonic dystrophy kinase-related CDC42-binding kinase (MRCK; also known as CDC42BP) and leucine zipper interacting kinase (ZIPK; also known as DAPK3) can phosphorylate the regulatory light chain (RLC) of non-muscle myosin II (NM II) on Ser19 or on Thr18 and Ser19 to activate it. Protein kinase C (PKC) can phosphorylate the RLC on Ser1, Ser2 and Thr9 to inhibit NM II. Human RLC is encoded by myosin light chain 9 (MYL9). In addition to RLC phosphorylation, NM II filament assembly is regulated by phosphorylation of the NM II heavy chain (NMHC II) coiled-coil and tail domains. The phosphorylation sites and the corresponding kinases, including transient receptor potential melastatin 7 (TRPM7), PKC proteins and casein kinase II (CK II), are shown for human NMHC IIA, NMHC IIB and NMHC IIC. Phosphorylation of NMHC II by CK II blocks the binding of S100A4 (also known as MTS1) to the tail of NM II to prevent it from inhibiting myosin filament assembly. Human NMHC IIA is encoded by myosin heavy chain 9 (*MYH9*), NMHC IIB is encoded by *MYH10* and NMHC IIC is encoded by *MYH14*. Note that each phosphorylation site and the kinase that phosphorylates it are depicted in the same colour.

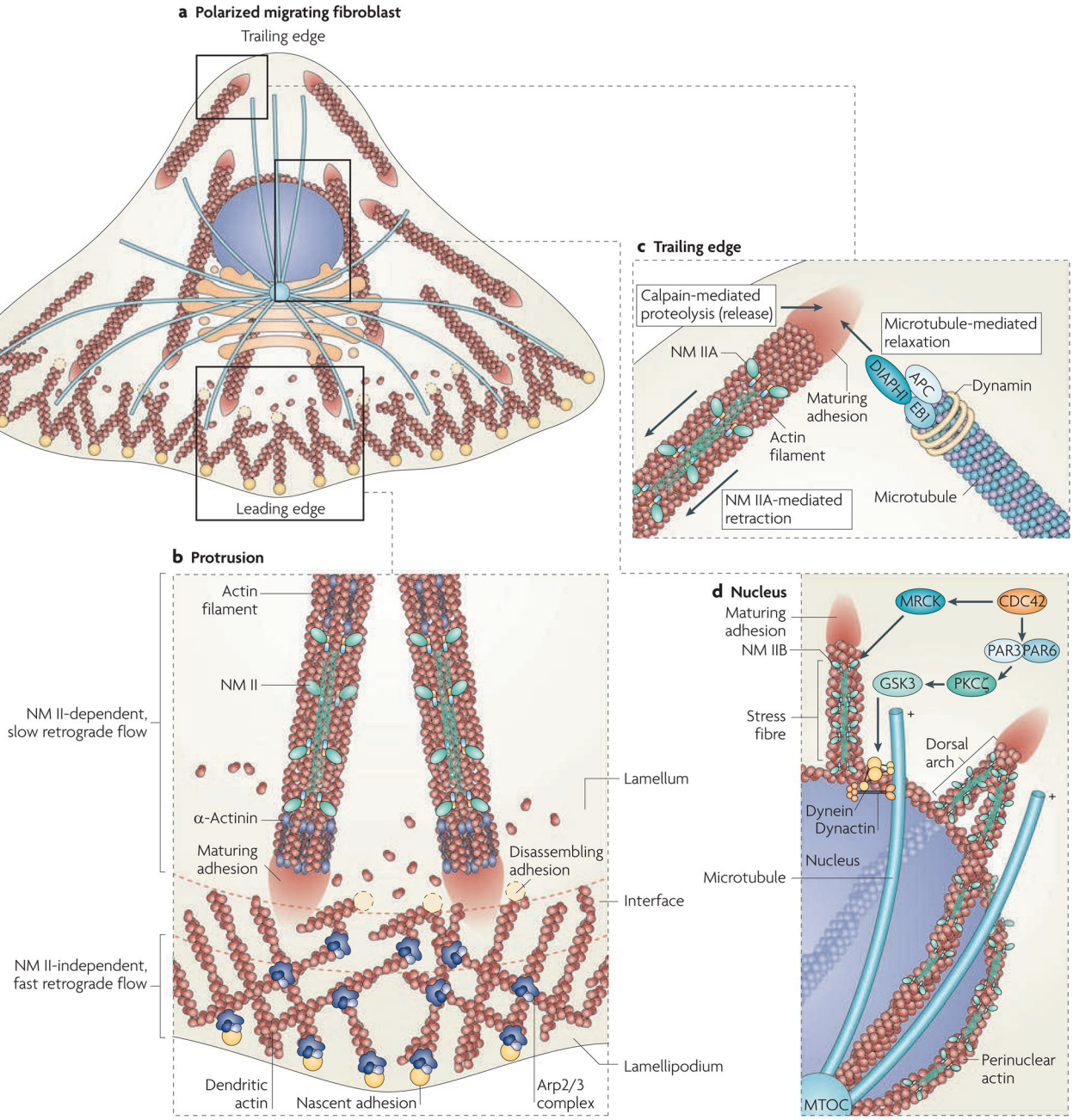

Figure 3. Multiple roles of NM II in cell migration

a | A polarized, migrating fibroblast. Areas of the cell in which non-muscle myosin II (NM II) has an active role are boxed and expanded in parts **b– d**. **b** | NM II regulates retrograde flow in the lamellum and promotes adhesion maturation, thereby limiting protrusion. Nascent adhesions form in the lamellipodium, in which dendritic actin branching mediated by the Arp2/3 complex also occurs. At the lamellipodium–lamellum interface, actin is depolymerized or bundled and adhesions disassemble or mature. A schematic of adhesions maturing in the lamellum is also shown. NM II localizes to actin bundles contacting growing adhesions, forming a striated pattern with α-actinin. In other cells, such as in neuronal growth cones, NM II may have a more direct role controlling retrograde flow in the peripheral zone[151]. **c** | NM II

participates in adhesion disassembly at the rear of the cell. NM IIA-mediated contraction, calpain-dependent cleavage of adhesion components and microtubule targeting coordinately induce adhesion disassembly. **d** | NM II has a role in nuclear positioning and orienting the microtubule-organizing centre (MTOC) and Golgi, which are important for cell polarization. NM II is thought to act in concert with the CDC42–partitioning defective 3 (PAR3) or PAR6–protein kinase Cζ (PKCζ) –glycogen synthase kinase 3 (GSK3) pathway to polarize the cell. Myotonic dystrophy kinase-related CDC42-binding kinase (MRCK; also known as CDC42BP) activates NM II and regulates its effect on nucleus repositioning. APC, adenomatous polyposis coli; DIAPH1, diaphanous 1; EB1, end binding protein 1 (also known as MAPRE1).

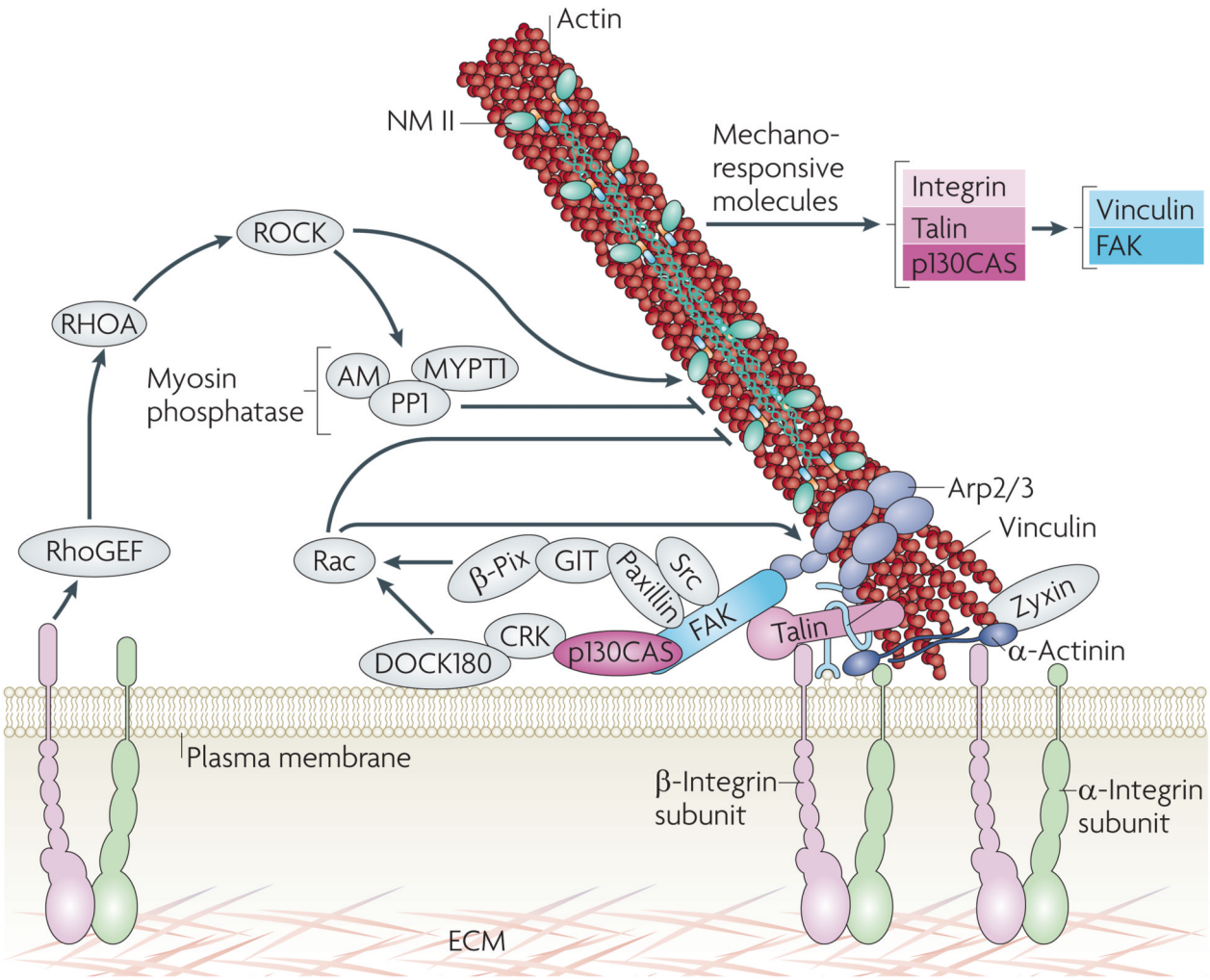

Figure 4. NM II in integrin-mediated adhesion

Integrins that are bound to the extracellular matrix (ECM) are linked to the actin cytoskeleton through an actin linkage that is formed by multiple molecules, including talin, vinculin and α-actinin. Kinases such as focal adhesion kinase (FAK) and Src, and adaptors such as paxillin, are also recruited and trigger the downstream activation of Rho GTPases such as Rac through adaptor and activating proteins. Representative pathways and associations are shown, including the activation of Rac through paxillin by the CRK-associated substrate (p130CAS; also known as BCAR1)–CRK–dedicator of cytokinesis 1 (DOCK1; also known as DOCK180) and G protein-coupled receptor kinase interacting ArfGAP (GIT)–β−Pix (also known as ARHGEF7) pathways. Activated Rac induces actin polymerization through the Arp2/3 complex, which can also interact with some of the molecules of the actin linkage, such as vinculin and FAK. Rac is also thought to locally inhibit NM II activation. The activation of RhoGEFs by integrins, and the subsequent activation of RHOA and Rho-associated, coiled coil-containing kinase (ROCK), activates NM II. ROCK activates NM II directly by phosphorylating the regulatory light chains (RLCs) or by inactivating myosin phosphatase, which in turn promotes RLC dephosphorylation. The pathways are spatially and temporally regulated. Additionally, the activation and inactivation of NM II itself affects adhesive signalling by triggering conformational changes in the mechanoresponsive molecules shown (pink boxes), which induces the clustering of the indicated adhesion proteins (blue boxes) by reinforcing or weakening the linkage of the adhesion and the actin cytoskeleton. AM, adaptor

module; MYPT1, myosin phosphatase-targeting subunit 1 (also known as PPP1R12A); PP1, protein phosphatase 1.

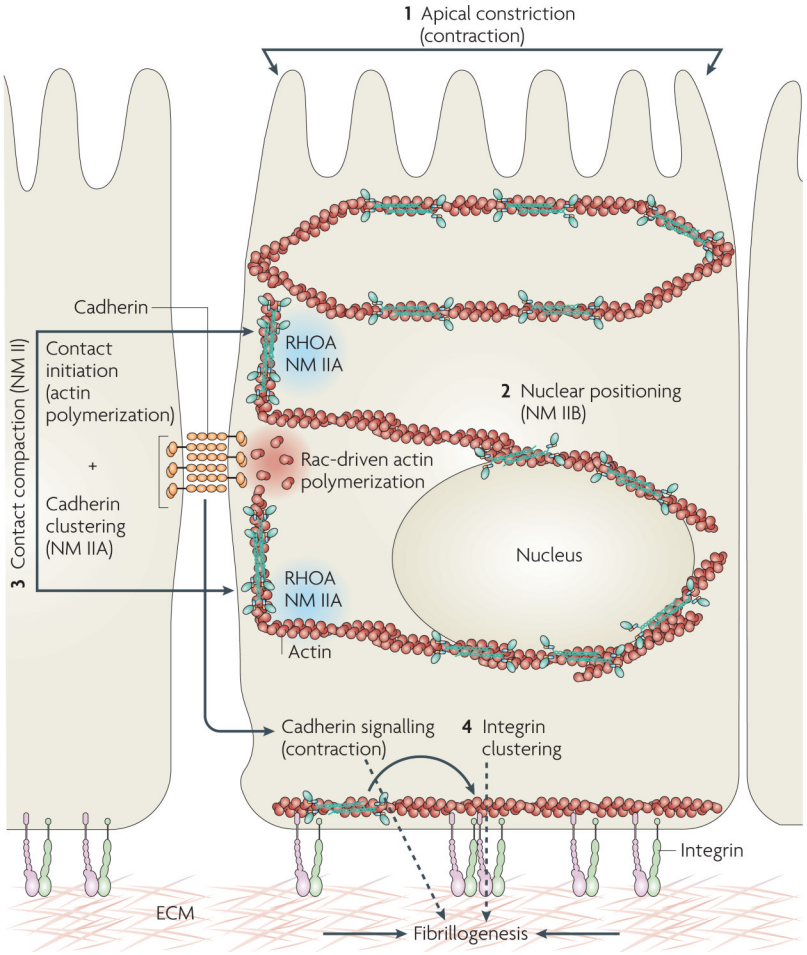

Figure 5. Roles of NM II in epithelial cell polarization

The different roles of non-muscle myosin II (NM II) in epithelial cell polarization. NM II is involved in apical constriction (step **1**), which leads to important morphogenic movements such as dorsal closure (closure of the epidermis over the amnioserosa during embryogenesis) in *Drosophila melanogaster*. In addition, NM II regulates nuclear positioning (step **2**), in a similar manner to how it does this in fibroblasts (see FIG 3). NM II and RHOA signalling also stabilize cell–cell contacts by reinforcing them through actin cross-linking (known as contact compaction; step **3**). The initial contacts are formed as a result of Rac-driven actin polymerization, but NM IIA is required for contact formation and reinforcement and cadherin clustering. NM II also mediates crosstalk between homophilic cadherin contact-initiated signalling and extracellular matrix (ECM) remodelling triggered by integrin activation and clustering (step **4**).

Table 1

The 39 *MYH9* mutations associated with *MYH9*-related diseases*

Exon	Mutation	clinical features (in addition to macrothrombocytopenia)
Head		
Exon 1	W33C and P35A[152] or A95T	Döhle-like inclusions‡
	N93K	Döhle-like inclusions and deafness
	S96L	Deafness and nephritis
Exon 10	K373N§	Döhle-like inclusions
Exon 16	R702C	Döhle-like inclusions, deafness, nephritis and cataracts
	R702H	Deafness and nephritis
	R705H	Deafness
	Q706E or R718W‖	Döhle-like inclusions
Rod		
Exon 24	G1055_Q1068del[153]	Döhle-like inclusions, deafness and nephritis
	E1066_A1072del	Döhle-like inclusions, deafness and cataracts
	E1066_A1072dup[154]	Döhle-like inclusions and cataracts
	E1084del[153]	Döhle-like inclusions
Exon 25	V1092_R1162del or T1155A‖	Döhle-like inclusions
	S1114P	Nephritis
	T1155I	Döhle-like inclusions, deafness and nephritis
Exon 26	R1165C	Döhle-like inclusions, deafness and cataracts
	R1165L	Döhle-like inclusions, deafness and nephritis
	L1205_Q1207del	Döhle-like inclusions
Exon 30	R1400W	Nephritis
	D1424Y, D1424N, D1447V‖ or D1447H	Döhle-like inclusions
	D1424H	Döhle-like inclusions, deafness, nephritis and cataracts
	K910Q and D1424H[155]	Döhle-like inclusions, deafness, nephritis and hypertension
Exon 31	V1516L	Döhle-like inclusions
Exon 37	I1816V	Döhle-like inclusions and nephritis
Exon 38	E1841K	Döhle-like inclusions, deafness, nephritis and cataracts
Exon 40	G1924fs or D1925fs¶	Döhle-like inclusions
Non-helical tail		
Exon 40	P1927fs, R1933fs‖ or D1941fs	Döhle-like inclusions
	R1933X	Döhle-like inclusions and nephritis

Exon	Mutation	clinical features (in addition to macrothrombocytopenia)
	E1945X	Döhle-like inclusions and deafness

*See also REF. 136.

‡Basophilic spindle-shaped inclusion bodies in the granulocytes.

§Originally reported as K371N.

||Clinical features listed are based on common findings for myosin heavy chain 9 (*MYH9*)-related diseases, but were not described in the reference.

¶Deletion of two different nucleotides both cause D1925fs. del, deletion; dup, duplication; fs, frame shift.