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REVIEW

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Actomyosin forces in cell migration: Moving beyond cell body retraction

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Abstract

In textbook illustrations of migrating cells, actomyosin contractility is typically depicted as the contraction force necessary for cell body retraction. This dogma has been transformed by the molecular clutch model, which acknowledges that actomyosin traction forces also generate and transmit biomechanical signals at the leading edge, enabling cells to sense and shape their migratory path in mechanically complex environments. To fulfill these complementary functions, the actomyosin system assembles a gradient of contractile energy along the front-rear axis of migratory cells. Here, we highlight the hierarchic assembly and self-regulatory network structure of the actomyosin system and explain how the kinetics of different nonmuscle myosin II (NM II) paralogs synergize during contractile force generation. Our aim is to emphasize how protrusion formation, cell adhesion, contraction, and retraction are spatiotemporally integrated during different modes of migration, including chemotaxis and durotaxis. Finally, we hypothesize how different NM II paralogs might tune aspects of migration in vivo, highlighting future research directions.

KEYWORDS
actomyosin, cell migration, chemotaxis, durotaxis, intracellular force generation, mechanotransduction, nonmuscle myosin II

1 | INTRODUCTION

Contractile force generation is a prerequisite for cell migration and thus of fundamental importance for embryonic development, wound healing, and homeostasis of adult tissues.[1] In nonmuscle tissues, intracellular forces are generated by nonmuscle myosin motorproteins of the class II (NM II). Three different NM II paralogs have evolved in vertebrates, each exhibiting specific kinetic features that differentially affect actomyosin force generation.[2,3] Using actin fibers as a substrate, these NM II motors generate forces in the pN range.[4] Although tiny compared to sarcomeric counterparts, such forces play an integral role in the coherent locomotion of single cells and collectives.[5–9]

In textbooks, single-cell migration is typically depicted in four consecutive steps (Figure 1A): (i) polarization via protrusive actin activity at the leading edge, (ii) leading edge adhesion to the extracellular matrix (ECM) via focal adhesions (FA), (iii) contraction of the cell body, and (iv) cell body retraction upon focal detachment at the cell rear. This depiction of locomotion is usually described as mesenchymal cell migration and captures the main events during the migratory cycle of most adherent cell types. However, beyond this simple depiction, coherent locomotion with persistence in speed and direction requires the spatiotemporal integration of protrusion formation, cell adhesion, and actomyosin contraction.[10–16] Seminal ideas about how this integration could work on the mechanistic level were formulated already

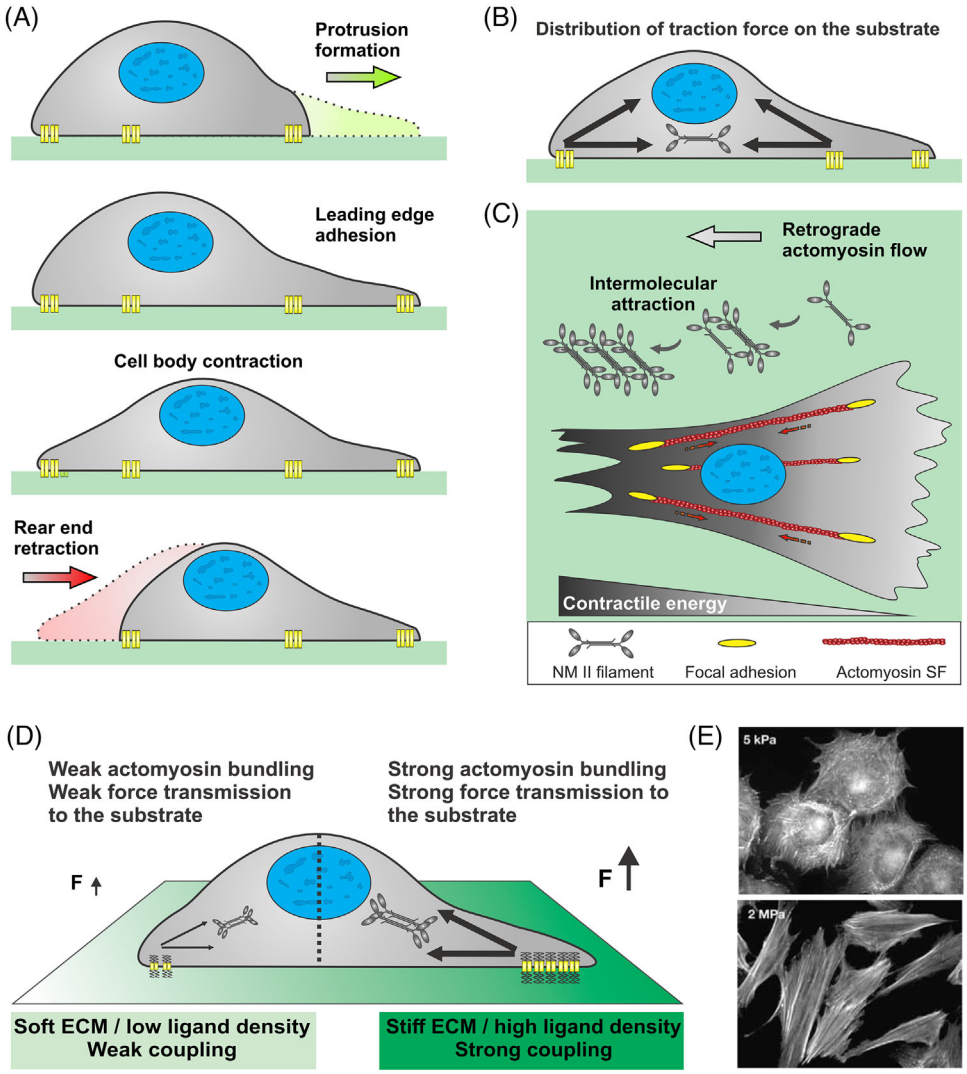

FIGURE 1 Actomyosin contractility during mesenchymal cell migration. (A) Classic scheme of a migrating mesenchymal cell, consisting of four consecutive steps: (i) protrusion formation at the leading edge, (ii) leading edge adhesion, (iii) contraction of the cell body, and (iv) cell body retraction upon focal adhesion (FA) detachment at the cell rear. (B) Contractile energy is transmitted to substrate traction via FA, in accordance with the molecular clutch model. During cellular locomotion, traction forces on the substrate emerge at the leading edge and trailing edge of the cell, with the contractile energy being directed to the cell center each, allowing to pull the cell forward while retracting the rear upon FA detachment. (C) To direct traction forces to the cell center, actomyosin filaments move centripetally with the retrograde flow with their growth being spatially primed in retrograde direction. Due to spatially restricted biochemical signaling and intermolecular attraction between nonmuscle myosin II (NM II) molecules, actomyosin eventually accumulate in the cell center. (D) Reciprocal feedback between extracellular matrix (ECM) elasticity and actomyosin contractility during cell migration. Actomyosin stress fiber (SF) are physically connected to the ECM meshwork via FA, and a positive correlation between actomyosin bundling and ECM fiber elasticity exists. Stiffer ECM substrates increase force transmission, leading to stronger actomyosin assembly, FA growth, and faster cell spreading. (E) Fluorescent micrographs, showing that fibroblasts form longer and more bundled SFs on stiff (2 MPa) versus soft (5 kPa) substrates. Modified with permission from Ref. [21]

more than 35 years ago.[17] With the introduction of compliant cell culture substrates and the development of traction force microscopy,[18–20] these ideas also became experimentally accessible and the first "molecular clutch" model postulated that FA link F-actin to the ECM substrate and mechanically resist NM II-driven retrograde actin flow to allow net protrusion extension.[15]

Since then, many studies followed up on these pioneering experiments and it became increasingly clear that actomyosin contractility itself is an essential component to integrate the protrusive, adhesive, and contractile machineries.[10,12–14,16,22] For a cell to move directional, traction forces on the substrate emerge behind the leading edge and along the trailing edge, with the contractile energy being directed to the cell center each, allowing to pull the cell forward while retracting the rear upon FA detachment (Figure 1B). Consequently, actomyosin contractility not only accumulates at the cell center to facilitate cell body retraction, but also exerts considerable traction forces on the cell front.[16,22] To facilitate coherent locomotion, actomyosin contractility behind the leading edge generates dynamic, fluctuating "tugging" trac-

tion forces on the substrate, while contractility in the center and rear generates steady "holding" traction forces, allowing cell body shortening rather than extension.[23] This pattern of traction force generation enables cells to sense the mechanical properties of the ECM[24–28] and, depending on the ECM's mechanical properties, subsequently steer the cell body or remodel the migratory path.[29–33] Therefore, actomyosin contractility plays an integral role throughout all the steps of the migratory cycle, including active mechanosensing.[26]

In this review, we will highlight how the actomyosin system assembles into a steady gradient of contractile energy along the front-rear axis of migratory cells, exhibiting explorative forces at the front and stable forces at the rear that allow coherent cellular locomotion and highly flexible cell body steering at the same time. We will explain the hierarchic assembly and self-regulatory network structure of the actomyosin system and emphasize how the kinetics of different NM II paralogs synergistically tune the contractile output[34–39] along the front-rear axis of migrating cells, especially during chemotaxis and durotaxis. Our goal is to illustrate a conceptual basis that will deepen our understanding of how actomyosin forces shape morphogenetic movements in development, homeostasis, and disease. To this end, we hypothesize how expression ratios of the three different NM II paralogs might tune aspects of migration in vivo, illustrating possible directions for future research.

2 | MAIN

2.1 | Assembly of actomyosin filaments and distribution of traction forces in migrating cells

To adapt to the continuous changes in cell shape during migration, the actomyosin cytoskeleton undergoes continuous assembly-disassembly cycles. As several comprehensive reviews describe the molecular pathways and structural hierarchies of actomyosin assembly in detail,[2,3,9,40–44] we will only highlight key aspects here (Text Box 1).

In polarized mesenchymal cells with a defined front-rear axis, NM II molecules nucleate new filaments along the lamella region, right behind the leading edge.[14,45,46] Depending on the lifetime of individual NM II motors in the filamentous state, these pioneer filaments grow and travel with the retrograde actin flow centripetally through the cell body, until they accumulate in the cell center, where they eventually disassemble (Figure 1C). It should be noted that processive anterograde movement of some NM II filaments was also demonstrated recently,[47] however, combined biochemical and biomechanical signals lead to spatially primed growth of actomyosin filaments in retrograde direction.[48] The best-known biochemical example is the mutual inhibition of Rac1 and RhoA.[49–51] While Rac1 is active at the leading edge of polarized mesenchymal cells and favors protrusive actin polymerization, RhoA is active in the cell center and rear, and promotes actomyosin activation.[52,53] Thus, NM II activation is reinforced towards the cell center rather than the leading edge. Biomechanically, NM II filament formation is also reinforced with increasing distance from the leading edge. Individual NM II molecules preferentially fuse with existing clusters rather than forming filaments de novo,[45,54,55] and new filaments can nucleate from pre-existing ones.[46,56,57] Additionally, the cross-linking properties of the NM II motors reinforce F-actin bundling, creating new binding sites for additional NM II filaments.[45,54,58] Together, these strategies lead to a gradual increase in actomyosin filament abundance along the front-rear axis of migrating cells, with NM II clusters being small right behind the leading edge but growing towards the cell center (Figure 1C). Consequently, contractility also gradually increases and peak forces are reached in the cell center.[59]

Box 1: Hierarchical NM II self-assembly into higher-ordered structures

The assembly of actomyosin structures follows a hierarchic sequence of events. The basic molecular unit comprises a hexamer consisting of two nonmuscle myosin II heavy chains (NMHC II), two essential light chains (ELC), and two regulatory light chains (RLC). While the NMHC II determines the kinetic properties of the NM II hexamer, ELC and RLC fulfill stabilizing and regulatory functions. Three different NMHC II paralogs, termed NMHC IIA, NMHC IIB, and NMHC IIC, are encoded by the genes *Myh9*, *Myh10*, and *Myh14*, respectively. Moreover, several RLC and ELC paralogs exist, but it is not known if there is any isoform-specificity to a given NMHC II.[2] NM II hexamers exist in an assembly-incompetent (10S) and assembly-competent (6S) conformation.[60,61] Relieving the autoinhibitory 10S conformation occurs through phosphorylation of the RLCs at Ser19, with the most prominent kinases being the Ca^{2+}/Calmodulin-dependent myosin light chain kinase (MLCK) and the Rho-associated kinase (ROCK). While MLCK directly acts on RLC phosphorylation, the function of ROCK is two-fold: Phosphorylating RLCs and inhibiting Myosin light chain phosphatase (MLCP).[62] Up to 30 NM II hexamers assemble into NM II minifilaments (named for their small size compared to the sarcomere counterpart) via parallel and anti-parallel electrostatic interactions between their coiled-coil domains.[63] New filaments can either assemble de novo or nucleate from pre-existing filaments.[46,45,54,56] An additional higher-ordered NM II structure was recently described, comprising several minifilaments that concatenate into parallel filament stacks through molecular co-attraction of NM II filaments.[56,57,55] The precise function of these stacks remains to be solved, as well as if similar structures exist in vivo. In contrast to the assembly, comparably less is known about filament disassembly, but it was suggested that the disassembly of NM II hexamers could be regulated in an isoform-specific fashion by phosphorylating the NMHC II tail regions.[2,43,64,65]

2.2 | From intracellular contractility to extracellular traction forces via molecular clutches

The centripetal actomyosin flow is an intrinsic feature of cells, influencing the magnitude of cell contraction in the respective subcellular regions of the actomyosin cortex. For cellular locomotion, however, cellular contraction forces need to be transmitted into traction forces on the ECM substrate. Cells generate these traction forces via bundled actomyosin cables, termed stress fibers (SF), that either form de novo via two distinct pathways,[66] or assemble from the actin cortex in an actomyosin-dependent fashion.[58] SFs are directly or indirectly connected to FA,[66,67] which in turn bind to the extracellular ECM (Figure 1C). During cell migration, traction forces emerge behind the leading edge and along the trailing edge, with the contractile energy being directed to the cell center each, allowing to pull the cell forward while retracting the rear upon FA detachment (Figure 1B). The amount of traction force transmitted to the ECM depends on the number and kinetic properties of NM II motors, the distribution of actin bundles, the amount and distribution of FA, and the stiffness as well as ligand density of the ECM.[15,16,68] This interdependence has been described in the generalized framework of the "molecular clutch" model[15,16,68] and was reviewed in detail in several comprehensive articles.[6,69]

Aiming to explain the rate of force transmission from the actomyosin cytoskeleton to the ECM, the molecular clutch model assumes a loading rate, which is defined as the speed by which forces build up in the engaged clutch. The clutches are represented by FA, while the speed of the retrograde actomyosin flow depends on the number and kinetic properties of NM II motors that pull versus the resistance that these motors experience from the ECM. The classic model predicts an optimal ECM stiffness and ECM ligand density at which force transmission and subsequent migration velocity are maximal.[15] Assuming an identical number of NM II motors pulling with the same velocity on a FA, the retrograde actomyosin flow will be high, if stiffness and/or ligand density of the ECM (i.e. the resistance) are low, and vice versa.[15] In terms of cell migration, this means that if the resistance from the ECM is very low, less actomyosin contraction will be transmitted to the ECM and the amount of traction force is also very low (Figure 1D). Consequently, the cell "slips", rather than translocating the cell body into a distinct direction. On very rigid substrates in contrast, engagement of a high number of clutches should lead to repeated cycles of force buildup and release due to clutch breakage, rendering the cell immobile. This latter model prediction, however, becomes complicated by the positive reinforcement of FA maturation and migration velocity that can be experimentally observed on stiff substrates or substrates with high ligand density, if actomyosin concentration increases accordingly.[10,16,26,68,70,71] Consequently, stiff ECM substrates or substrates with high ligand density often cause high migration velocities due to stronger actomyosin assembly, FA maturation, and increased force transmission (Figure 1D,E).

In a very simplistic view, the above-described scenarios can explain cell migration by an asymmetric distribution of FA along the cell axis, allowing actomyosin contraction to be translated into asymmetric substrate traction. If more clutches are engaged behind the leading edge, overall traction forces at the front will win over the rear, allowing to translocate the cell body into a distinct direction. In addition, however, coherent locomotion requires to continuously convert dynamic "tugging" traction at the front into stable "slipping" traction at the rear. At the leading edge, FA and traction forces need to remain short-lived, allowing to probe, explore, and react to variations in guidance cues like the ECM's mechanical properties.[26] Once a new cycle of leading edge extension and cell adhesion starts, FA and traction forces that were previously located at the very front now move towards the cell center and consequently need to be stabilized, to maintain constant tension on the cell body. As the contractile actin cortex and the SFs are directly or indirectly interconnected,[58,67] this guarantees rapid cell body recoil upon FA detachment. While the force quantity that acts on the clutch is tuned by the retrograde flow and accumulation of actomyosin in the rear, the force quality is additionally regulated via the expression of up to three different NM II paralogs, each with specific kinetic features to modulate the contractile output.

2.3 | Modulation of contractile force generation by different NM II paralogs

In vertebrates, the genes *Myh9*, *Myh10*, and *Myh14* encode for three different NM II paralogs NMHC IIA, NMHC IIB, and NMHC IIC, respectively. Together with their RLCs and ELCs, the NMHC IIs form the holoenzymes NM IIA, NM IIB, and NM IIC (Box 1). Individual cells usually express two or even all three paralogs simultaneously, however, in different ratios to each other. In most cases, NM IIA is the highest abundant paralog, followed by NM IIB.[37,72,73] Moreover, although large fractions of the individual NM II paralogs overlap and co-localize intracellularly, each paralog has a distinct localization pattern and impact on the contractile force generation, affecting the cellular morphology, SF, and FA architecture (Box 2 with Figure 2).

To tune the contractile load during cell migration, especially NM IIA and B have been shown to be of critical importance. More precisely, both paralogs synergize to achieve a transition from tugging to holding contractility along the front-rear axis of migrating cells. Evidence for this can be found in the kinetic features, subcellular distribution, and functional synergy of NM IIA and NM IIB.[34–39,74]

2.4 | Complementary traction force generation by NM IIA and B during cell migration

Although NM IIA and B share 80% amino acid sequence identity,[79] they possess different mechanoenzymatic features. Kinetically, NM IIA propels actin filaments significantly faster than NM IIB, while the duty ratio (the time during the enzymatic cycle where myosin heads are stably bound to actin) is significantly higher for NM IIB.[4,80,81] These features make NM IIA more suitable to produce rapid contractility and NM IIB prone to bear tension on longer timescales. Strikingly, in polarized migrating cells, NM IIA and B segregate into distinct subcellular localization patterns along the front-rear axis. While NM IIA is

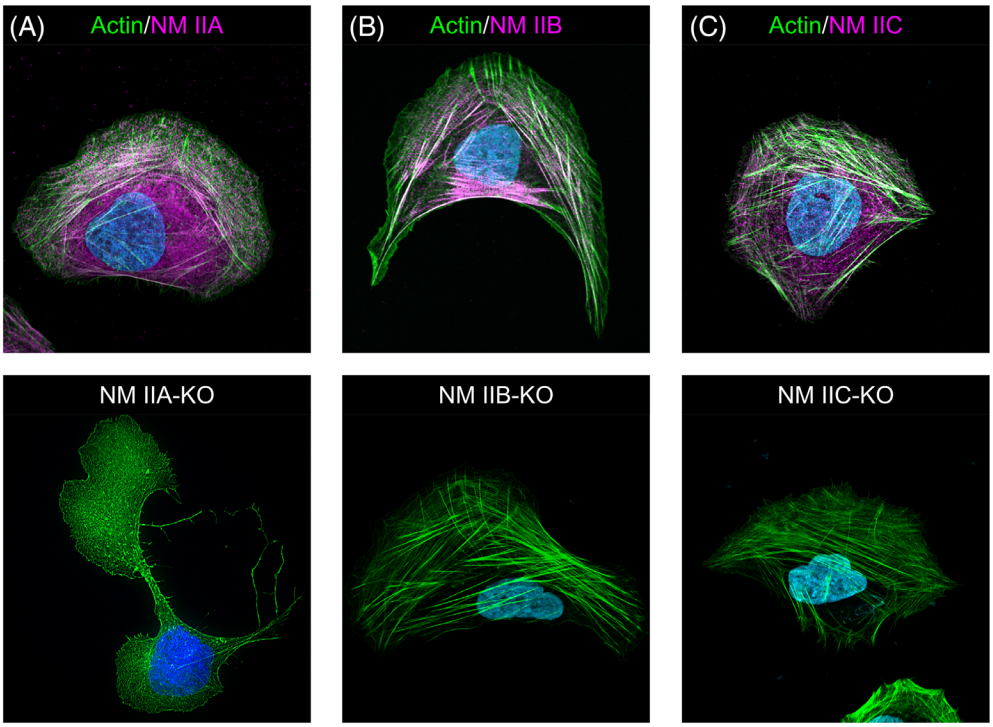

FIGURE 2 (Corresponding to text Box 2): Expression of nonmuscle myosin II (NM II) paralogs in U2OS cells and their impact on the cellular phenotype. Originally published under the Creative Commons Attribution-Non Commercial 4.0 International Public License (CC BY-NC 4.0) (https://doi.org/10.7554/eLife.71888).

homogeneously distributed throughout the cell body, NM IIB is gradually enriched towards the cell rear[38,82–84] (Figure 3A). Connecting this pattern with the distribution of contraction forces along the front-rear axis of migrating cells suggests that NM IIA-enriched filaments generate dynamic but short-lived traction forces behind the leading edge, while NM IIB-enriched filaments form stable and long-lived traction forces in the cell rear. In line with this, local pulsatile contractions are an intrinsic feature of NM IIA but not NM IIB,[85] while NM IIB is more mechanosensitive, influencing the period and amplitude of NM IIA-induced contractions in a catch bond manner through elastic stabilization.[38,74,86]

Given this functional synergy, it is not surprising that super-resolution microscopy revealed the co-assembly of NM IIA and B into heterotypic filaments,[34,36] with their formation following akin principles of an excitable and self-limiting system[87–92] (Figure 3B). Excitable systems are defined by two criteria: a fast excitation that reflects a local activating stimulus and a delayed inhibition that globally suppresses the activator. Due to higher abundance and favored electrostatic interaction, NM IIA preferentially nucleates into "pioneer" filaments, while it is more likely for NM IIB to co-assemble in preformed NM IIA filaments[34,38,63,72] (Figure 3C). NM IIB co-assembles with a delay but subsequently blocks the incorporation of additional NM IIA molecules, preventing contractile overshoots[38,45,54] (Figure 3B). The contractile kinetics of heterotypic filaments shift with the ratio of the paralogs, either towards fast but weak if more NM IIA is present, or slow but strong if more NM IIB is present (Figure 3D). This way, NM IIA and NM IIB create a synergistic gradient of contractility in migrating cells. NM IIA is the "first responder" that initiates dynamic and fast contractile forces behind the leading edge, while NM IIB consolidates these pre-initiated contraction forces with increasing distance from the leading edge.

In migrating cells, this pattern of complementary force generation enables the transition from tugging to holding traction forces along the front-rear axis, integrating polarization, FA maturation, and cell body contraction/retraction via a hierarchic self-sorting mechanism. In the following, we discuss how this enables coherent cellular locomotion during chemotaxis and durotaxis, as the best characterized examples. However, similar mechanisms likely operate in haptotaxis, ratchetaxis, and possibly electrotaxis, although these modes are comparably less well studied.

2.5 | Cell polarization, FA stability, and cell body translocation during chemotaxis

During chemotaxis, initial symmetry breaking, cell polarization, and directionality are established by detecting a local source of chemoattractant in the extracellular space.[93] While the signal detection is achieved in an actomyosin-independent fashion, in most cases either through G-protein coupled receptors[94] or receptor tyrosine kinases,[95] synergistic force generation by NM IIA and B integrates leading edge protrusion, FA formation, maintenance of front-rear cell polarity, cell contraction, and rear retraction, leading to coherent motion with persistence in speed and direction.[11]

Box 2: Expression and impact of NM II paralogs on the cellular phenotype

NM IIA is homogeneously distributed throughout the cell body and its depletion of NM IIA has the most drastic impact (Figure 2A). Available methods are barely sensitive enough to measure remaining traction forces in most NM IIA-KO cells,[35,74,75] showing that NM IIA is the initiator and main contributor of contractile energy. Consequently, NM IIA deficient cells are characterized by aberrant cell morphology, loss of bundled actin SF, and lack of mature FA[13,35,39,72,75–77] (Figure 2A). Instead, the protrusive area is often increased, and cells form several protrusions in different directions. None of the observed effects can be rescued by overexpressing NM IIB or NM IIC,[35,38,74] linking these phenotypes to the loss of NM IIA's specific features rather than the overall reduction of NM II concentration.

While NM IIB mainly accumulates in the cell center and rear of polarized cells, its depletion has only a subtle impact on the cellular phenotype, SF, and FA structures (Figure 2B), and the magnitude of traction force generation is not reduced.[35,74,75] However, although initiation of traction forces remains unaffected in NM IIB-KO cells, differences in the spatiotemporal dynamics emerge[74] and these can impact the balance between front and rear traction forces in migrating cells, as explained in detail in the following chapter.

Knowledge about the impact of NM IIC on contractile force generation is sparse. In U2OS cells, depletion of NM IIC has no obvious impact on polarization, SF and FA assembly, or traction force generation[74] (Figure 2C). Moreover, although NM IIC is homogeneously distributed throughout the cell body, filaments are less strictly localized to SF than its paralogs, possibly reflecting localization in the actin cortex.[78] It should be noted that NM IIC is by far the least abundant paralog in U2OS cells[72] and these findings should be confirmed in cells expressing high levels of NM IIC.

In combination with the retrograde actin flow, the described principle of synergistic NM IIA/B activity ensures the continuous polarization of the actomyosin cytoskeleton[35] (Figure 4A). NM IIA not only propels actin filaments much faster, but it also possesses a higher turnover (assembly/disassembly kinetics of monomers into filaments) than NM IIB.[96,97] New NM IIA filaments arise along the lamella, right behind the actomyosin-free zone of the leading edge (Figure 4B). However, the lifetime of individual NM IIA molecules in filaments is short and as some disassemble, they may, although less likely, be replaced by a NM IIB molecule. If a NM IIB molecule takes the place, it will stay bound in the filament for a longer time due to lower turnover[38,83,84] (Figure 4B). With progressing distance from the leading edge, the filaments are gradually enriched in NM IIB, as it moves with the retrograde flow towards the cell center[35,98] (Figure 4A).

This self-sorting allows the emergence of self-limiting subcellular pulses and propagating waves of cell contractions,[85,89,91] enabling cells to maintain their shape and explore their surrounding at the same time.[10,24,35] NM IIA is always more abundant at the front and contractions are pulsatile and short-lived,[85] allowing to quickly adjust the cell body to changing extracellular conditions (Figure 4C). NM IIB rich filaments rather generate steady and elastic pulling forces, creating a stable cell rear, which maintains its shape. As the rear part is under more load due to the load-bearing capacities of NM IIB, a quick recoil of the cell body is guaranteed, once detached from the substrate (Figure 4C).

A similar principle also accounts for FA. Newly formed FA arise along the leading edge, where they promote Rac1 signaling, leading to protrusive activity. Simultaneously, NM IIA forms filaments along the leading edge that are incorporated in newly formed SF, which connect to nascent FA, promoting their maturation in a force-dependent manner.[70] Upon progressive incorporation of NM IIB, FA gets stabilized, do not turnover, and do not signal to Rac1 anymore.[84,99] Instead, they remain during cell body translocation and move centripetally to create a stable rear that is devoid of protrusive signaling[99] (Figure 4C).

Exploiting this system, cell polarity can be steadily maintained over long distances. Without NM IIA, cells initially polarize after plating but cannot coherently translocate due to lacking force generation and deficient tail retraction, as suggested by long retraction fibers that remain as remnants of the cell rear (Figure 4D). Numerous nascent FA form but do not mature, leading to increased protrusive activity at the cell margin.[99] In NM IIA-KO cells, the direction of protrusion formation is changed repeatedly, and cells form independent protrusions in various directions[100] (Figure 4D). In the absence of NM IIB, cells show increased speed due to rapid force production by NM IIA but lose directionality, as no stable cell rear is created. Although FA mature, they are not stabilized on longer time scales and still signal to Rac1, leading to the spontaneous generation of protrusions at the trailing edge (Figure 4E).[99] Thus, NM IIB knockdown cells show an increased posterior region and spontaneously protrude and reverse direction.[100]

Compared to the extensive knowledge about the intersection of actomyosin contractility and FA maturation during protrusion stabilization, much less is known about the intersection of actomyosin contractility and FA disassembly during the rear retraction phase. Considering the continuous self-sorting and polarized distribution of NM IIA/B along the front-rear axis, it is a interesting question, if this gradient of contractile energy not only leads to a biased maturation of FA at the leading edge, but also primes FA disassembly at the cell rear. A possible mechanism comprises feedback between actomyosin and membrane tension. Lower membrane tension in the cell rear leads to the formation of caveolae, which enhance RhoA signaling, leading to rapid retraction.[101] NM IIB-KO cells were shown to possess a overall higher actin cortex tension compared to WT cells.[37,74] Given the gradual enrichment of NM IIB at the rear of migrating cells, it

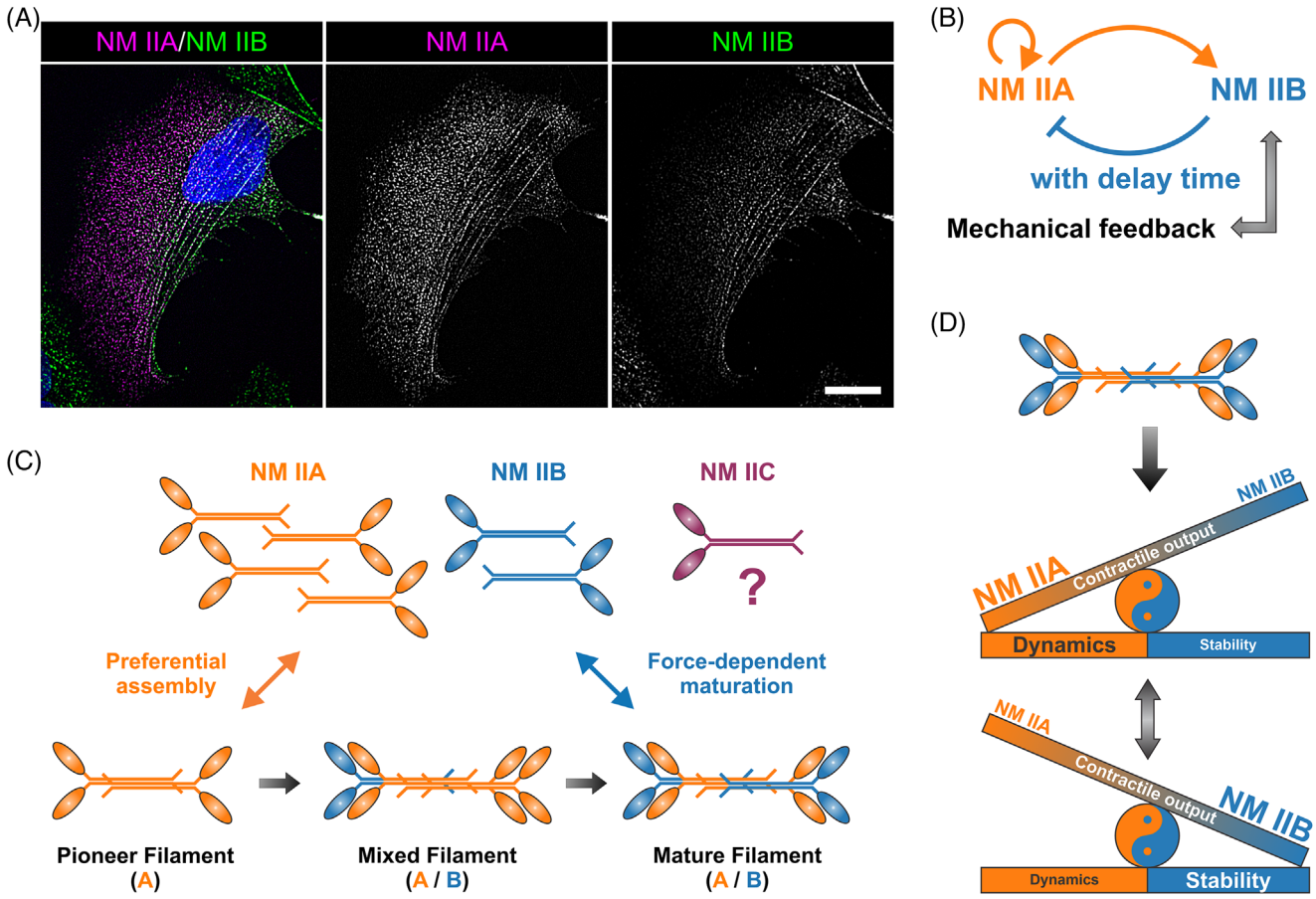

FIGURE 3 Induction and consolidation of actomyosin forces by NM IIA and NM IIB. (A) Immunofluorescent micrograph, showing the segregation of NM IIA and NM IIB into distinct subcellular localization patterns along the front-rear axis of a polarized U2OS cell. NM IIA is homogeneously distributed throughout the cell body, while NM IIB is gradually enriched towards the cell rear. Originally published under the Creative Commons Attribution-Non Commercial 4.0 International Public License (CC BY-NC 4.0) (https://doi.org/10.1016/j.ejcb.2022.151213). (B) Network structure of an excitable and self-limiting NM IIA/B system, consisting of a fast local activator (NM IIA) that amplifies its own activity and a delayed inhibitor (NM IIB) that suppresses the activator with a temporal delay. The dynamics of NM IIB can be further tuned by mechanical feedback, e.g., via the elasticity in the ECM. (C) The co-assembly of the heterotypic actomyosin system follows a hierarchical order, relying on the paralog-specific kinetic properties. NM IIA preferentially assembles due to higher molar abundancy and favored electrostatic interactions. NM IIB is more likely to co-assemble into preformed NM IIA filaments, but it stays longer bound in the filament and its lifetime can be increased by mechanical feedback. The role of NM IIC remains unclear in this context. (D) The ratio of NM IIA to NM IIB in heterotypic filaments tunes the overall contractile output, either becoming more dynamic but weak or stable but slow. ECM, extracellular matrix; NM II, nonmuscle myosin II.

seems therefore plausible that localized NM IIB accumulation promotes cortex softening, leading to lower membrane tension at the cell rear (Figure 4A). Future research might follow up on this direction, dissecting the intersection of NM IIB, membrane tension, and the disassembly of FA. For example, activation of Ca^{2+}-sensitive proteins like S100A11 was recently suggested to mediate FA disassembly in a Piezo1- and actomyosin-dependent manner.[102] Elevated RhoA signaling, mediated by low membrane tension, might promote stretch-activation of Piezo1 ion channels and Ca^{2+} influx at the cell rear, favoring FA disassembly via localized activation of S100A11.

Altogether, a precise interplay between actomyosin contraction and FA dynamics arises due to the self-sorting of contractile NM IIA/B bundles along the cell axis, allowing maximal migration velocity while simultaneously preserving cell body integrity and polarization.[10,35]

2.6 | FA tugging and mechanosensing during durotaxis

In the above-described scenario, the sorting of NM IIA and B is cell autonomous and uncoupled from extracellular cues, once symmetry is broken by a chemotactic guidance cue. In vivo, however, cells adhere and migrate in a complex ECM, where heterogeneities in ECM fiber stiffness or topography can be interpreted as mechanical guidance cues.[24–28] Moreover, depending on the mechanical properties, cells may (re-)polarize or remodel their migratory path.[29–33] In these cases, actomyosin forces are required to actively sense the mechanical properties of the ECM.

Most of the actomyosin-related work in this regard has focused on durotaxis, where cells follow stiffness gradients, typically towards the

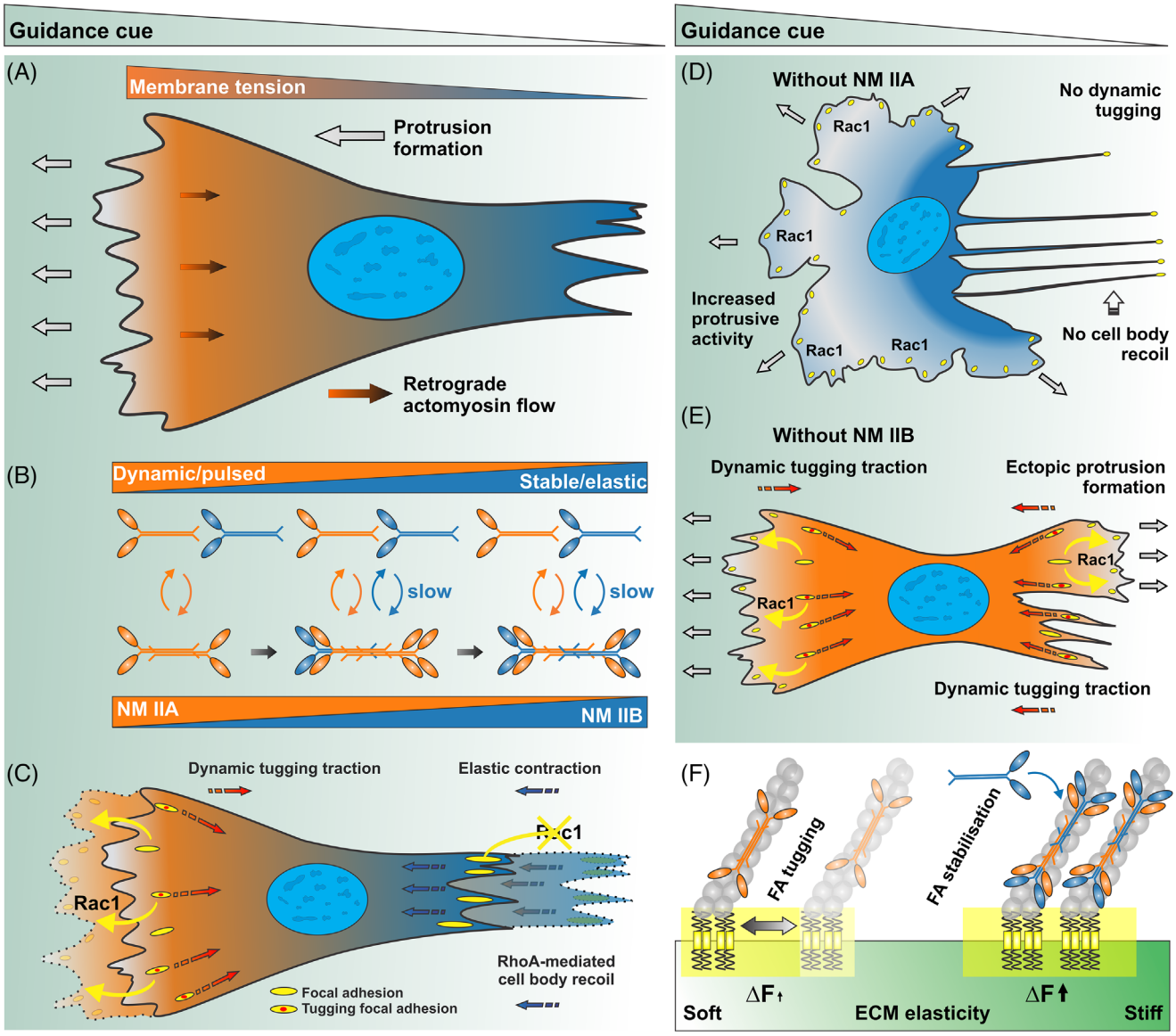

FIGURE 4 Nonmuscle myosin II (NM II) self-sorting regulates polarization and migration during chemotaxis and durotaxis. (A) Self-sorting of heterotypic NM II filaments in migrating cells arises from a combination of hierarchic NM II assembly and retrograde actin flow. The cell front is enriched with NM IIA, while the rear is enriched with NM IIB. (B) The underlying mechanism of this self-sorting relies on the assembly and turnover kinetics of NM IIA and NM IIB. NM IIA preferentially assembles but also detaches faster than NM IIB. NM IIB preferentially co-assembles but stays longer bound and consequently accumulates at the rear, as it travels with the retrograde actin flow over longer distances. (C) The NM IIA-rich cell front generates dynamic and pulsatile contractions, allowing rapid cell shape adjustments to changing extracellular conditions. Tugging focal adhesion (FA) connected to NM IIA-rich actomyosin bundles locally probe the substrate rigidity during durotaxis and more likely promote Rac1 signaling due to higher turnover. NM IIB rich filaments generate steady pulling forces, establishing a stable cell rear. Progressive incorporation of NM IIB stabilizes FA, preventing Rac1 signaling and FA tugging. The elastic capacities of NM IIB guarantee a quick cell body recoil upon FA detachment from the substrate. (D) In absence of NM IIA, cells initially polarize but fail to translocate due to lacking force generation. Increased protrusive activity is present at the cell margin, as nascent adhesions do not mature. (E) In absence of NM IIB, rapid force production by NM IIA is sufficient to translocate the cell body. However, the cell lacks a stable rear, leading to ectopic protrusion formation via excessive Rac1 signaling and FA tugging, ultimately reducing cell directionality. (F) Schematic of FA tugging during Durotaxis. Softer substrate regions promote FA tugging, while stiffer substrate regions reciprocally stabilize FA and prevent tugging, possibly facilitated by increased NM IIB incorporation.

regions of higher stiffness,[103–105] as these stiffness regimes allow cells to exert the highest traction forces due to adhesion reinforcement, as described above.[16] It should be noted that "negative" durotaxis and polarization of axonal growth cones towards softer regions have been reported as well,[106–109] however, the molecular mechanism is comparably well investigated in these cases.[103,110,111] One possible scenario is that cells, which lack the described FA reinforcement on stiff substrates, migrate towards softer regions to experience intermediate substrate rigidities that allow them to exert maximal traction force, in accordance with the predicted stiffness optimum in the

classic molecular clutch model.[106] In any of these cases, however, it is believed that the level of actomyosin contractility downstream of FA, is crucial for sensing these differences in substrate rigidity.[25] Moreover, it is likely that actomyosin forces might play essential roles during substrate sensing in other migration modii as well.[112] Nevertheless, we will focus on "positive" durotaxis in the following, given that the actomyosin-related mechanisms are best described for this mode.

During durotaxis, the same differential polarization of NM IIA/B is observed and like during chemotaxis, NM IIA possesses the key upstream role in force generation, while polarized NM IIB localization at the rear is necessary for directionality, by stabilizing cell polarity[24,112] (Figure 4C–F). Strikingly, even mild reductions of NM IIB levels significantly reduce durotaxis efficiency and overexpression reduces durotactic efficiency as well, whereas overexpressing NM IIA has no effect.[24] This shows that i) the less abundant paralog NM IIB is equally important for durotaxis than the main force generator NM IIA, and ii) a tight regulation of the expression ratio between NM IIA and B is necessary for optimal durotactic efficiency. The significance of NM IIB in mesenchymal durotaxis is further highlighted by its absence in many amoeboid cells,[113] which primarily migrate without FA engagement and therefore don't use the classic mechanism for durotaxis.[114,115] Unlike mesenchymal cells, amoeboid cells navigate through ECM meshworks by squeezing along paths of least resistance rather than sensing and remodeling the ECM.[116] As the sole force generator, NM IIA enhances the viscosity of the actomyosin cytoskeleton,[74] which is a necessary feature when squeezing the cell body through narrow pores. Since NM IIB rather promotes a viscoelastic behavior, it seems dispensable for amoeboid migration,[115] but crucial for FA-dependent durotaxis.

The self-sorting of NM IIA/B enables the system to be receptive and reactive at the same time, favoring a picture in which both, local substrate sensing and polarization reinforcement for migratory persistence, happens simultaneously. How this is facilitated on a molecular scale is ongoing research, but strong hints point again towards a precisely regulated balance of FA-mediated adhesion and actomyosin-generated traction.[25] It was shown that cells use FA to locally probe their underlying substrate by applying fluctuating tugging actomyosin forces[26] (Figure 4C). However, only a fraction of FA showed this tugging behavior, and stiff substrates generally favored the stabilization of the FA/abolished force fluctuations. Therefore, a positive feedback loop between substrate stiffness and NM IIB co-assembly might exist (Figure 4F). FA dynamically sample the substrate rigidity via oscillating force patterns, which is an intrinsic feature of NM IIA but not NM IIB.[85] Upon attachment to stiff substrate regions, the mechanical feedback from the substrate is stronger, possibly reinforcing the NM IIB catch bond that subsequently stabilizes FA and increases the adhesion strength.[10] Conformingly, locally self-amplifying actomyosin force patterns emerge from an excitable system in response to matrix elasticity,[89] and NM IIB accumulation is mechanosensitive and dependent on its motor activity.[38,86]

An interesting research direction comprises the recent finding that amoeboid cells can also undergo durotaxis, even in the absence of FA.[114,115] As it was suggested that this specific type of durotaxis does not require NM IIB but still relies on retrograde actomyosin flow and rear contraction,[115] it will be interesting to delineate the mechanism, by which amoeboid cells translate stiffness differences into retrograde actomyosin flow, in the absence of FA.

Additionally, it was shown that FA-dependent durotaxis only emerges on certain ECM conditions. While fibronectin promotes durotaxis, laminin does not.[117,118] In contrast to laminin, fibronectin fibrils can be stretched significantly and expose cryptic binding sites upon fibril extension.[119,120] This suggests that FA-dependent durotaxis depends not only on the elastic nature of the actomyosin cytoskeleton, but also requires an elastic counterpart in the ECM. Mechanistically, actomyosin pulling forces that are transmitted to the ECM via FA expose synergistic binding sites for integrins on fibronectin fibrils.[31,32] The result of this is two-fold, i) cell adhesion is reinforced on tensed (and thus stiffer) fibronectin fibrils, and ii) tension on the ECM fibrils locally increases, reinforcing their alignment, clustering, and the formation of prestrain along the migratory path.[29,30,33,121–123] These findings open exciting avenues for future research, exploring the possibility that cells not only follow pre-defined gradients of stiffness, but also self-generate such mechanical gradients for follower cells, while migrating.[33,124,125] Self-generation of gradients has recently been acknowledged for durotactic[124] and haptotactic[33] gradients, and at least in one case relies mechanistically on actomyosin forces.[33] Strikingly, also in these cases, the less abundant NM IIB might be equally important to NM IIA. NM IIB-KO fibroblasts translocate Collagen I fibers significantly slower than WT cells,[123] and ECM remodeling is altered in NM IIB-deficient tissues.[126] It will be interesting to see if NM II paralogs are differentially involved in self-generating mechanical gradients.

2.7 | Force modulation by NM II paralogs during migration in vivo: Should I stay, or should I go?

While the previous sections highlight the vast mechanoreciprocity between NM II paralogs, FA, and the ECM, as observed during migration in vitro,[127] it was always acknowledged that in vitro culture conditions only poorly reflect the complexity in vivo. While a reduction in complexity is necessary to unravel the molecular details of novel discoveries, the reciprocal experiment, that is, to investigate if predictions from in vitro experiments also hold true in vivo, might open avenues for novel ideas and new ways of thinking about problems. When monitoring the impact of the NM II paralogs on contractile force generation in vivo, novel ideas might evolve and lead to hypothesis that can be tested in molecular detail in vitro. In this last chapter, we want to briefly highlight possible future perspectives for research on the plasticity of the actomyosin system, especially regarding the expression and interaction of different NM II paralogs in vivo.

An interesting aspect concerns the expression ratio and relative abundance of the various NM II paralogs in the respective tissues. NM IIA and NM IIB are considered ubiquitously expressed from early development on, but NM IIA is predominantly expressed, outnumbering NM IIB and NM IIC by far in most cases (except for some neuronal

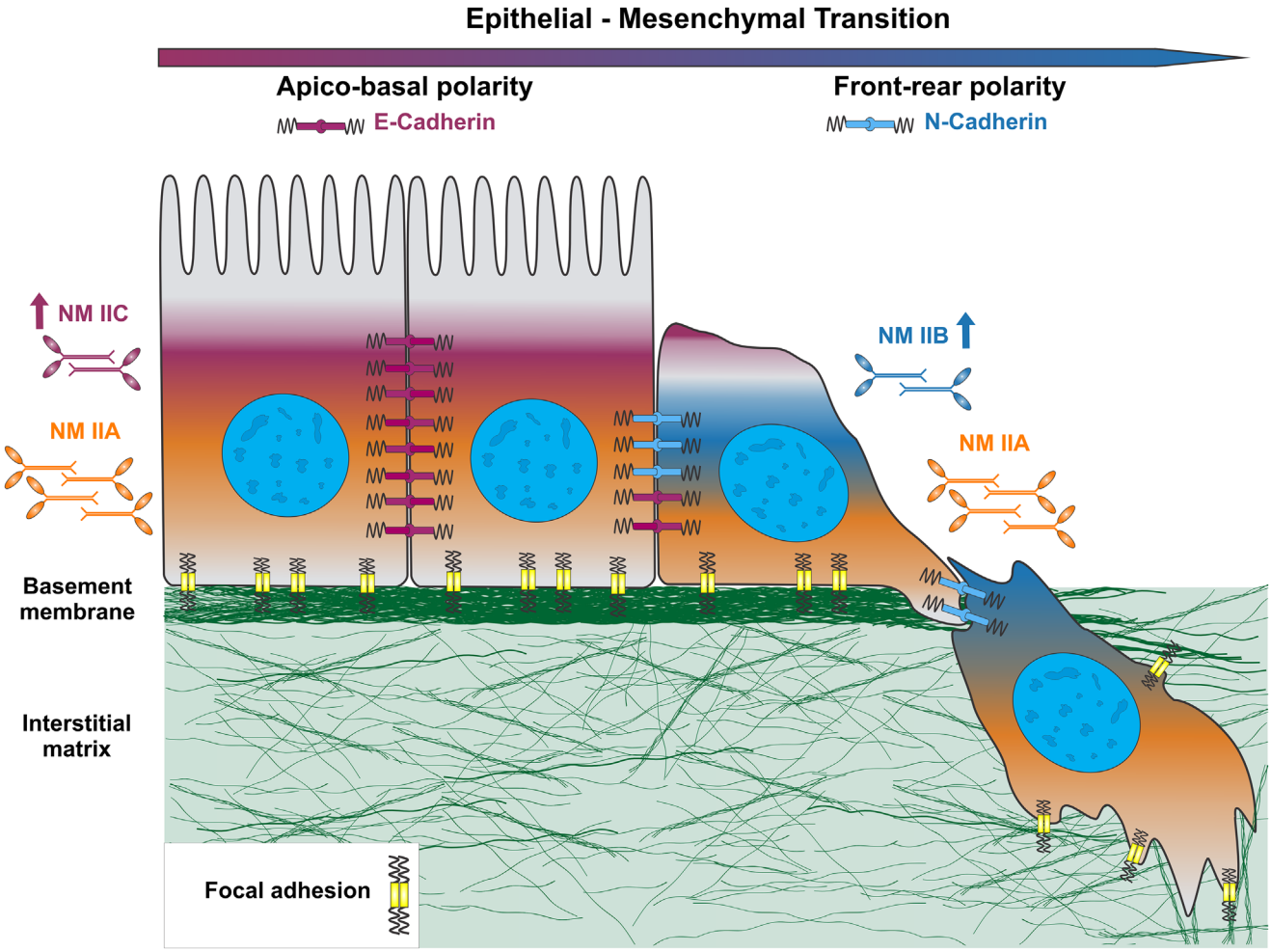

FIGURE 5 Actomyosin contractility from in vitro to in vivo. Nonmuscle myosin II (NM II) paralog abundancies switch during cell delamination, reminiscent of the Cadherin switch in EMT. In epithelial tissues with apico-basal polarization, NM IIA and NM IIC are highly expressed, while NM IIB is often less abundant. Upon delamination and invasion, the expression shifts, and NM IIC is downregulated while NM IIB is upregulated. Notably, NM IIA is always the most abundant, highlighting its initiating role in force generation. Once delaminated from a coherent epithelial tissue, increased expression of NM IIB might correlate with extracellular matrix (ECM) fiber alignment and self-generation of mechanical gradients during 3D invasion in vivo.

tissues, where NM IIB is more abundant).[72,73,79] This is in line with the role of NM IIA as the canonical initiator and generator of contractile forces. In striking contrast, however, expression of NM IIC is not only absent during embryonic development until E11.5 in mice,[79] its expression pattern is also more restricted,[79] with the highest expression found in epithelial tissues.

In contrast to mesenchymal cells, epithelial cells of physiologically healthy tissues do not migrate as individual cells but collectively as a coherent sheet.[128–130] Often, polarized epithelial cells even reside statically in the tissue, forming a coherent epithelium.[131] For epithelial cells to become individually motile, they first need to undergo large-scale changes in their adhesive and contractile apparatus, a process usually described as epithelial-mesenchymal transition (EMT).[130,132–134] With onset of EMT, epithelial cells delaminate by breaking down E-Cadherin mediated adherens junctions, re-polarizing from apico-basal to front-rear, and acquiring an invasive phenotype.[134] Strikingly, Beach and colleagues proposed that akin to the switch from E-Cadherin to N-Cadherin expression during EMT, an NM II paralog switch from NM IIA/C to NM IIA/B occurs downstream of TGF-β signaling in murine mammary glands[135] (Figure 5). They show that NM IIC is expressed in luminal epithelial cells, while NM IIB is expressed in more contractile myoepithelial cells.[136] Moreover, induction of EMT in mammary gland epithelial cells in vitro leads to the same NM II paralog switch, and upon induction of NM IIB expression/downregulation of NM IIC via the EMT program, these cells become more individually invasive and migratory.[135]

These findings suggest that, in striking contrast to the synergistic activity of NM IIA/B during mesenchymal cell migration, increased expression of NM IIC maybe rather correlates with the opposite and favors a jammed state in which cell remain static in an epithelium with apico-basal polarity (Figure 5). In many cell types and tissues, one NM II paralog is significantly less abundant, and while NM IIA is usually the most abundant, expression of NM IIB and NM IIC are often inversely proportional to each other.[73,79] Moreover, NM IIB is more abundant

in mesenchymal tissues and essential for nuclear translocation during 3D invasion,[137] while NM IIC is higher expressed in static tissues with epithelial characteristics.[73,79,135] NM IIC is enriched along the apical side of mouse epithelia[79] and along the sarcomeric belt in the Organ of corti,[138] and it limits the length of epithelial brush border microvilli by counteracting actin polymerization in the villus tip.[78] Strikingly, pharmacological activation of NM IIC decreases cancer dissemination and metastasis formation, while reinforcing the formation of sarcomeric actin belts,[139,140] a structural feature of jammed epithelial tissues with strong cell-cell adhesions.

How is it regulated on the mechanistic scale, that the combination of two NM II motor paralogs (A/B) leads to a contractile output which favors migration and invasion of single cells, while the combined contractile output of two others (A/C) blocks invasion and favors a static phenotype? In contrast to NM IIA and B, we still lack a detailed understanding of how NM IIC is involved in contractile force generation on the subcellular scale, and how it interacts with its paralogs. NM IIC differs structurally and kinetically from its paralogs, sharing only 64% amino acid identity.[79] Ultrastructural analysis showed that NM IIA and NM IIB minifilaments each consist of 28–30 hexamers, while NM IIC minifilaments only contain around 14. Since actin-crosslinking properties depend on both, duty ratio and the number of myosin heads, NM IIC has a much lower power to cross-link actin than NM IIA and NM IIB.[141] This makes NM IIC neither suitable for rapid actin translocation, nor for stable actin cross-linking, pointing towards diverging functions from its paralogs. Moreover, NM IIC filaments are less strictly localized along SF than its paralogs in U2OS cells. The small size and rather diffuse localization could suggest that NM IIC is capable of binding to dense actin meshworks along the circumferential actin belt of epithelial cells, possibly reflecting the suggested function in maintaining apico-basal polarity.[78] As heterotypic NM IIA/C filaments were reported as well,[34] NM IIC could mechanistically serve as a mechanosensitive dampener of NM IIA-induced contractility,[74] facilitating tensional homeostasis and structural integrity in mechanically loaded and stressed epithelial tissues.[142,143]

Importantly, however, it should be noted that NM IIB is also involved in adherens junction biogenesis in many epithelia and co-localizes with NM IIC in some epithelial tissues.[39,73,79,144] This suggests that there might be a certain plasticity, rather than a complete switch of NM II paralog expression, akin to the suggestion that E-Cadherin is not completely switched off during EMT, but rather that the ratio of E- to N-Cadherin is shifted towards the latter.[133] EMT basically consists of three consecutive steps: (i) reduction of cell-cell adhesion, (ii) reduction of apico-basal polarity, and (iii) acquisition of front-rear polarity and cell motility. While the simplistic view is that these steps happen sequentially, growing evidence suggests that they could take place simultaneously.[133] The ratio between NM IIA, B, and C could shape the balance between cell-cell adhesion and cell motility during EMT, with NM IIA/B being more important to control cell motility, while NM IIA/C could control cell-cell junctional dynamics. This might be of specific interest during collective cell migration. For example, if all three paralogs are expressed simultaneously, the ratio between NM IIB and NM IIC could control the level of cohesiveness in a migrating cluster.

Box 3: Glossary of terms

Actin cortex: A contractile layer of actin mesh that is attached to the inner side of the plasma membrane.

Catch bond: A noncovalent bond whose dissociation lifetime increases upon applying mechanical load.

Chemotaxis: Migration along gradients of soluble chemoattractants.

Contraction forces: Actomyosin forces are generated in the actin cortex and actin bundles that basically minimize the cell surface via the generation of isometric tension to the middle.

Durotaxis: Migration along stiffness gradients in the ECM or adjacent cell sheets.

Electrotaxis: Migration along an electric field. Sometimes also referred to as Galvanotaxis.

Focal adhesion: Adhesive structure consisting of transmembrane integrin receptors and adaptor proteins that link the actomyosin cytoskeleton to the extracellular matrix-binding integrins.

Haptotaxis: Migration along gradients of bound chemoattractants.

Ratchetaxis: Migration along gradients of adhesive molecules, as present on certain ECM fibers topographies.

Retraction forces: Forces to retract the cell body asymmetrically, usually describe the shortening of the cell rear during migration.

Stress fiber: Contractile actomyosin bundle that is connected to focal adhesions on one or both ends.

Traction forces: Actomyosin contraction forces that are transmitted to the extracellular environment (the ECM or neighboring cells), via the connection of actomyosin bundles to cell adhesions.

Future research focusing on physiological tissue models that express different ratios of the three NM II paralogs might reveal in which scenarios NM IIC is crucial, and how it interferes with its paralogs to modulate junction formation and cell motility on the mechanistic scale.

3 | CONCLUSION

With the advent of mechanobiology, it has been revealed that the functions of actomyosin contractility in terms of cell migration cover way more than just cell body translocation. Extensive synergistic and possibly also antagonistic feedback loops between the different NM II paralogs and their upstream regulators converge in precisely tuned waves of actomyosin assembly and contraction, allowing cells to sense their physical environment, remodel their ECM, maintain their polarity, and translocate their cell body simultaneously. The next steps should be to transfer this knowledge to higher-ordered systems, trying to

derive a system-level understanding of how actomyosin contractility is coordinated and synchronized in collective and supracellular migration in vivo.[94,132,145,146] This includes the synergistic activities of NM IIA and NM IIB during the invasion, ECM remodeling, and gradient formation, but also possible diverging functions of NM IIC in tensional homeostasis and maintenance of epithelial apico-basal polarity. Additional in vivo migration modii such as amoeboid migration of immune cells, and the regulation of migratory plasticity[7] (i.e. mesenchymal-amoeboid transition[147,148]) have not been covered here in detail, but are also of high interest due to their physiological relevance.[7,113]

Today, methods and toolboxes necessary to tackle these questions in vivo are largely established. Improvements in imaging techniques enable better resolution, optogenetics, and laser ablation allow to spatiotemporally control or disturb actomyosin with unprecedented precision,[94] methods to monitor force generation in situ have been established,[149,150] and gene editing via the CRISPR-Cas toolbox allows much more refined ways to manipulate the contractile machinery, beyond the problems and caveats of lethal loss-of-function studies. Thus, it will be exciting to see the field shifting from single cells in vitro to tissues and multicellular assemblies ex vivo and in vivo.

AUTHOR CONTRIBUTIONS

Kai Weißenbruch and Roberto Mayor conceptualised and edited the manuscript. Kai Weißenbruch wrote the initial draft and created illustrations.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Images in Figures 2 and 3A that support the conclusions in this manuscript are openly available at https://doi.org/10.7554/eLife.71888 and https://doi.org/10.1016/j.ejcb.2022.151213.

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REFERENCES

1. Ma, X., & Adelstein, R. S. (2014). The role of vertebrate nonmuscle Myosin II in development and human disease. *Bioarchitecture*, *4*(3), 88–102. https://doi.org/10.4161/bioa.29766
2. Heissler, S. M., & Manstein, D. J. (2013). Nonmuscle myosin-2: Mix and match. *Cellular and Molecular Life Sciences*, *70*(1), 1–21. https://doi.org/10.1007/s00018-012-1002-9
3. Shutova, M. S., & Svitkina, T. M. (2018). Mammalian nonmuscle myosin II comes in three flavors. *Biochemical and Biophysical Research Communications*, *506*(2), 394–402. https://doi.org/10.1016/j.bbrc.2018.03.103
4. Kovacs, M., Thirumurugan, K., Knight, P. J., & Sellers, J. R. (2007). Load-dependent mechanism of nonmuscle myosin 2. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(24), 9994–9999. https://doi.org/10.1073/pnas.0701181104
5. Mayor, R., & Etienne-Manneville, S. (2016). The front and rear of collective cell migration. *Nature Reviews. Molecular Cell Biology*, *17*(2), 97–109. https://doi.org/10.1038/nrm.2015.14
6. Fortunato, I. C., & Sunyer, R. (2022). The forces behind directed cell migration. *Biophysica*, *2*(4), 548–563.
7. Cowan, J. M., Duggan, J. J., Hewitt, B. R., & Petrie, R. J. (2022). Non-muscle myosin II and the plasticity of 3D cell migration. *Frontiers in Cell and Developmental Biology*, *10*, 1047256. https://doi.org/10.3389/fcell.2022.1047256
8. Agarwal, P., & Zaidel-Bar, R. (2019). Diverse roles of non-muscle myosin II contractility in 3D cell migration. *Essays Biochemistry*, *63*(5), 497–508. https://doi.org/10.1042/EBC20190026
9. Vicente-Manzanares, M., Ma, X., Adelstein, R. S., & Horwitz, A. R. (2009). Non-muscle myosin II takes centre stage in cell adhesion and migration. *Nature Reviews. Molecular Cell Biology*, *10*(11), 778–790. https://doi.org/10.1038/nrm2786
10. Gupton, S. L., & Waterman-Storer, C. M. (2006). Spatiotemporal feedback between actomyosin and focal-adhesion systems optimizes rapid cell migration. *Cell*, *125*(7), 1361–1374. https://doi.org/10.1016/j.cell.2006.05.029
11. Yolland, L., Burki, M., Marcotti, S., Luchici, A., Kenny, F. N., Davis, J. R., & Stramer, B. M. (2019). Persistent and polarized global actin flow is essential for directionality during cell migration. *Nature Cell Biology*, *21*(11), 1370–1381. https://doi.org/10.1038/s41556-019-0411-5
12. Giannone, G., Dubin-Thaler, B. J., Rossier, O., Cai, Y., Chaga, O., Jiang, G., & Sheetz, M. P. (2007). Lamellipodial actin mechanically links myosin activity with adhesion-site formation. *Cell*, *128*(3), 561–575. https://doi.org/10.1016/j.cell.2006.12.039
13. Cai, Y., Biais, N., Giannone, G., Tanase, M., Jiang, G., Hofman, J. M., & Sheetz, M. P. (2006). Nonmuscle myosin IIA-dependent force inhibits cell spreading and drives F-actin flow. *Biophysical Journal*, *91*(10), 3907–3920. https://doi.org/10.1529/biophysj.106.084806
14. Burnette, D. T., Manley, S., Sengupta, P., Sougrat, R., Davidson, M. W., Kachar, B., & Lippincott-Schwartz, J. (2011). A role for actin arcs in the leading-edge advance of migrating cells. *Nature Cell Biology*, *13*(4), 371–382. https://doi.org/10.1038/ncb2205
15. Chan, C. E., & Odde, D. J. (2008). Traction dynamics of filopodia on compliant substrates. *Science*, *322*(5908), 1687–1691. https://doi.org/10.1126/science.1163595
16. Elosegui-Artola, A., Oria, R., Chen, Y., Kosmalska, A., Perez-Gonzalez, C., Castro, N., & Roca-Cusachs, P. (2016). Mechanical regulation of a molecular clutch defines force transmission and transduction in response to matrix rigidity. *Nature Cell Biology*, *18*(5), 540–548. https://doi.org/10.1038/ncb3336
17. Mitchison, T., & Kirschner, M. (1988). Cytoskeletal dynamics and nerve growth. *Neuron*, *1*(9), 761–772. https://doi.org/10.1016/0896-6273(88)90124-9
18. Dembo, M., & Wang, Y. L. (1999). Stresses at the cell-to-substrate interface during locomotion of fibroblasts. *Biophysical Journal*, *76*(4), 2307–2316. https://doi.org/10.1016/S0006-3495(99)77386-8
19. Oliver, T., Dembo, M., & Jacobson, K. (1995). Traction forces in locomoting cells. *Cell Motility and the Cytoskeleton*, *31*(3), 225–240. https://doi.org/10.1002/cm.970310306
20. Harris, A. K., Wild, P., & Stopak, D. (1980). Silicone rubber substrata: A new wrinkle in the study of cell locomotion. *Science*, *208*(4440), 177–179. https://doi.org/10.1126/science.6987736
21. Prager-Khoutorsky, M., Lichtenstein, A., Krishnan, R., Rajendran, K., Mayo, A., Kam, Z., & Bershadsky, A. D. (2011). Fibroblast polarization is a matrix-rigidity-dependent process controlled by focal adhesion

mechanosensing. *Nature Cell Biology*, *13*(12), 1457–1465. https://doi.org/10.1038/ncb2370
22. Balaban, N. Q., Schwarz, U. S., Riveline, D., Goichberg, P., Tzur, G., Sabanay, I., & Geiger, B. (2001). Force and focal adhesion assembly: A close relationship studied using elastic micropatterned substrates. *Nature Cell Biology*, *3*(5), 466–472. https://doi.org/10.1038/35074532
23. Fournier, M. F., Sauser, R., Ambrosi, D., Meister, J. J., & Verkhovsky, A. B. (2010). Force transmission in migrating cells. *Journal of Cell Biology*, *188*(2), 287–297. https://doi.org/10.1083/jcb.200906139
24. Raab, M., Swift, J., Dingal, P. C., Shah, P., Shin, J. W., & Discher, D. E. (2012). Crawling from soft to stiff matrix polarizes the cytoskeleton and phosphoregulates myosin-II heavy chain. *Journal of Cell Biology*, *199*(4), 669–683. https://doi.org/10.1083/jcb.201205056
25. Yeoman, B., Shatkin, G., Beri, P., Banisadr, A., Katira, P., & Engler, A. J. (2021). Adhesion strength and contractility enable metastatic cells to become adurotactic. *Cell Reports*, *34*(10), 108816. https://doi.org/10.1016/j.celrep.2021.108816
26. Plotnikov, S. V., Pasapera, A. M., Sabass, B., & Waterman, C. M. (2012). Force fluctuations within focal adhesions mediate ECM-rigidity sensing to guide directed cell migration. *Cell*, *151*(7), 1513–1527. https://doi.org/10.1016/j.cell.2012.11.034
27. Vishwakarma, M., Di Russo, J., Probst, D., Schwarz, U. S., Das, T., & Spatz, J. P. (2018). Mechanical interactions among followers determine the emergence of leaders in migrating epithelial cell collectives. *Nature Communications*, *9*(1), 3469. https://doi.org/10.1038/s41467-018-05927-6
28. Ruppel, A., Worthmuller, D., Misiak, V., Kelkar, M., Wang, I., Moreau, P., & Balland, M. (2023). Force propagation between epithelial cells depends on active coupling and mechano-structural polarization. *Elife*, *12*, . https://doi.org/10.7554/eLife.83588
29. Lickert, S., Kenny, M., Selcuk, K., Mehl, J. L., Bender, M., Fruh, S. M., & Vogel, V. (2022). Platelets drive fibronectin fibrillogenesis using integrin alphaIIbbeta3. *Science Advances*, *8*(10), eabj8331. https://doi.org/10.1126/sciadv.abj8331
30. Lu, J., Doyle, A. D., Shinsato, Y., Wang, S., Bodendorfer, M. A., Zheng, M., & Yamada, K. M. (2020). Basement membrane regulates fibronectin organization using sliding focal adhesions driven by a contractile winch. *Developmental Cell*, *52*(5), 631–646.e4 e634. https://doi.org/10.1016/j.devcel.2020.01.007
31. Zhong, C., Chrzanowska-Wodnicka, M., Brown, J., Shaub, A., Belkin, A. M., & Burridge, K. (1998). Rho-mediated contractility exposes a cryptic site in fibronectin and induces fibronectin matrix assembly. *Journal of Cell Biology*, *141*(2), 539–551. https://doi.org/10.1083/jcb.141.2.539
32. Zhang, Q., Magnusson, M. K., & Mosher, D. F. (1997). Lysophosphatidic acid and microtubule-destabilizing agents stimulate fibronectin matrix assembly through Rho-dependent actin stress fiber formation and cell contraction. *Molecular Biology of the Cell*, *8*(8), 1415–1425. https://doi.org/10.1091/mbc.8.8.1415
33. Clark, A. G., Maitra, A., Jacques, C., Bergert, M., Perez-Gonzalez, C., Simon, A., & Vignjevic, D. M. (2022). Self-generated gradients steer collective migration on viscoelastic collagen networks. *Nature Materials*, *21*(10), 1200–1210. https://doi.org/10.1038/s41563-022-01259-5
34. Beach, J. R., Shao, L., Remmert, K., Li, D., Betzig, E., & Hammer, J. A., 3rd. (2014). Nonmuscle myosin II isoforms coassemble in living cells. *Current Biology*, *24*(10), 1160–1166. https://doi.org/10.1016/j.cub.2014.03.071
35. Shutova, M. S., Asokan, S. B., Talwar, S., Assoian, R. K., Bear, J. E., & Svitkina, T. M. (2017). Self-sorting of nonmuscle myosins IIA and IIB polarizes the cytoskeleton and modulates cell motility. *Journal of Cell Biology*, *216*(9), 2877–2889. https://doi.org/10.1083/jcb.201705167
36. Shutova, M. S., Spessott, W. A., Giraudo, C. G., & Svitkina, T. (2014). Endogenous species of mammalian nonmuscle myosin IIA and IIB include activated monomers and heteropolymers. *Current Biology*, *24*(17), 1958–1968. https://doi.org/10.1016/j.cub.2014.07.070
37. Taneja, N., Bersi, M. R., Baillargeon, S. M., Fenix, A. M., Cooper, J. A., Ohi, R., & Burnette, D. T. (2020). Precise tuning of cortical contractility regulates cell shape during cytokinesis. *Cell Reports*, *31*(1), 107477. https://doi.org/10.1016/j.celrep.2020.03.041
38. Weissenbruch, K., Fladung, M., Grewe, J., Baulesch, L., Schwarz, U. S., & Bastmeyer, M. (2022). Nonmuscle myosin IIA dynamically guides regulatory light chain phosphorylation and assembly of nonmuscle myosin IIB. *European Journal of Cell Biology*, *101*(2), 151213. https://doi.org/10.1016/j.ejcb.2022.151213
39. Heuze, M. L., Sankara Narayana, G. H. N., D'Alessandro, J., Cellerin, V., Dang, T., Williams, D. S., & Ladoux, B. (2019). Myosin II isoforms play distinct roles in adherens junction biogenesis. *Elife*, *8*, https://doi.org/10.7554/eLife.46599
40. Quintanilla, M. A., Hammer, J. A., & Beach, J. R. (2023). Non-muscle myosin 2 at a glance. *Journal of Cell Science*, *136*(5), https://doi.org/10.1242/jcs.260890
41. Garrido-Casado, M., Asensio-Juarez, G., & Vicente-Manzanares, M. (2021). Nonmuscle myosin II regulation directs its multiple roles in cell migration and division. *Annual Review of Cell and Developmental Biology*, *37*, 285–310. https://doi.org/10.1146/annurev-cellbio-042721-105528
42. Garrido-Casado, M., Asensio-Juarez, G., Talayero, V. C., & Vicente-Manzanares, M. (2024). Engines of change: Nonmuscle myosin II in mechanobiology. *Current Opinion in Cell Biology*, *87*, 102344. https://doi.org/10.1016/j.ceb.2024.102344
43. Dulyaninova, N. G., & Bresnick, A. R. (2013). The heavy chain has its day: Regulation of myosin-II assembly. *Bioarchitecture*, *3*(4), 77–85. https://doi.org/10.4161/bioa.26133
44. Chinthalapudi, K., & Heissler, S. M. (2024). Structure, regulation, and mechanisms of nonmuscle myosin-2. *Cellular and Molecular Life Sciences*, *81*(1), 263. https://doi.org/10.1007/s00018-024-05264-6
45. Quintanilla, M. A., Patel, H., Wu, H., Sochacki, K. A., Chandrasekar, S., Akamatsu, M., & Beach, J. R. (2024). Local monomer levels and established filaments potentiate non-muscle myosin 2 assembly. *Journal of Cell Biology*, *223*(4). https://doi.org/10.1083/jcb.202305023
46. Beach, J. R., Bruun, K. S., Shao, L., Li, D., Swider, Z., Remmert, K., & Hammer, J. A. (2017). Actin dynamics and competition for myosin monomer govern the sequential amplification of myosin filaments. *Nature Cell Biology*, *19*(2), 85–93. https://doi.org/10.1038/ncb3463
47. Vitriol, E. A., Quintanilla, M. A., Tidei, J. J., Troughton, L. D., Cody, A., Cisterna, B. A., & Beach, J. R. (2023). Nonmuscle myosin 2 filaments are processive in cells. *Biophysical Journal*, *122*(18), 3678–3689. https://doi.org/10.1016/j.bpj.2023.05.014
48. Asokan, S. B., Johnson, H. E., Rahman, A., King, S. J., Rotty, J. D., Lebedeva, I. P., & Bear, J. E. (2014). Mesenchymal chemotaxis requires selective inactivation of myosin II at the leading edge via a noncanonical PLCgamma/PKCalpha pathway. *Developmental Cell*, *31*(6), 747–760. https://doi.org/10.1016/j.devcel.2014.10.024
49. Machacek, M., Hodgson, L., Welch, C., Elliott, H., Pertz, O., Nalbant, P., & Danuser, G. (2009). Coordination of Rho GTPase activities during cell protrusion. *Nature*, *461*(7260), 99–103. https://doi.org/10.1038/nature08242
50. Xu, J., Wang, F., Van Keymeulen, A., Herzmark, P., Straight, A., Kelly, K., & Bourne, H. R. (2003). Divergent signals and cytoskeletal assemblies regulate self-organizing polarity in neutrophils. *Cell*, *114*(2), 201–214. https://doi.org/10.1016/s0092-8674(03)00555-5
51. Wang, Y., Ku, C. J., Zhang, E. R., Artyukhin, A. B., Weiner, O. D., Wu, L. F., & Altschuler, S. J. (2013). Identifying network motifs that buffer front-to-back signaling in polarized neutrophils. *Cell Reports*, *3*(5), 1607–1616. https://doi.org/10.1016/j.celrep.2013.04.009
52. Byrne, K. M., Monsefi, N., Dawson, J. C., Degasperi, A., Bukowski-Wills, J. C., Volinsky, N., & Kholodenko, B. N. (2016). Bistability in the Rac1, PAK, and RhoA signaling network drives actin cytoskele-

ton dynamics and cell motility switches. *Cell Systems*, *2*(1), 38–48. https://doi.org/10.1016/j.cels.2016.01.003
53. Nguyen, L. K., Kholodenko, B. N., & von Kriegsheim, A. (2018). Rac1 and RhoA: Networks, loops and bistability. *Small GTPases*, *9*(4), 316–321. https://doi.org/10.1080/21541248.2016.1224399
54. Chou, W. H., Molaei, M., Wu, H., Oakes, P. W., Beach, J. R., & Gardel, M. L. (2023). Limiting pool and actin architecture controls myosin cluster sizes in adherent cells. *Biophysical Journal*, 157–171. https://doi.org/10.1016/j.bpj.2023.12.004
55. Hu, S., Grobe, H., Guo, Z., Wang, Y. H., Doss, B. L., Pan, M., & Zaidel-Bar, R. (2019). Reciprocal regulation of actomyosin organization and contractility in nonmuscle cells by tropomyosins and alpha-actinins. *Molecular Biology of the Cell*, *30*(16), 2025–2036. https://doi.org/10.1091/mbc.E19-02-0082
56. Fenix, A. M., Taneja, N., Buttler, C. A., Lewis, J., Van Engelenburg, S. B., Ohi, R., & Burnette, D. T. (2016). Expansion and concatenation of non-muscle myosin IIA filaments drive cellular contractile system formation during interphase and mitosis. *Molecular Biology of the Cell*, *27*(9), 1465–1478. https://doi.org/10.1091/mbc.E15-10-0725
57. Hu, S., Dasbiswas, K., Guo, Z., Tee, Y. H., Thiagarajan, V., Hersen, P., & Bershadsky, A. D. (2017). Long-range self-organization of cytoskeletal myosin II filament stacks. *Nature Cell Biology*, *19*(2), 133–141. https://doi.org/10.1038/ncb3466
58. Lehtimaki, J. I., Rajakyla, E. K., Tojkander, S., & Lappalainen, P. (2021). Generation of stress fibers through myosin-driven reorganization of the actin cortex. *Elife*, *10*, https://doi.org/10.7554/eLife.60710
59. Mohl, C., Kirchgessner, N., Schafer, C., Hoffmann, B., & Merkel, R. (2012). Quantitative mapping of averaged focal adhesion dynamics in migrating cells by shape normalization. *Journal of Cell Science*, *125*(Pt 1), 155–165. https://doi.org/10.1242/jcs.090746
60. Heissler, S. M., Arora, A. S., Billington, N., Sellers, J. R., & Chinthalapudi, K. (2021). Cryo-EM structure of the autoinhibited state of myosin-2. *Science Advances*, *7*(52), eabk3273. https://doi.org/10.1126/sciadv.abk3273
61. Yang, S., Tiwari, P., Lee, K. H., Sato, O., Ikebe, M., Padron, R., & Craig, R. (2020). Cryo-EM structure of the inhibited (10S) form of myosin II. *Nature*, *588*(7838), 521–525. https://doi.org/10.1038/s41586-020-3007-0
62. Totsukawa, G., Yamakita, Y., Yamashiro, S., Hartshorne, D. J., Sasaki, Y., & Matsumura, F. (2000). 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. *Journal of Cell Biology*, *150*(4), 797–806. https://doi.org/10.1083/jcb.150.4.797
63. Kaufmann, T. L., & Schwarz, U. S. (2020). Electrostatic and bending energies predict staggering and splaying in nonmuscle myosin II minifilaments. *PLoS Computational Biology*, *16*(7), e1007801. https://doi.org/10.1371/journal.pcbi.1007801
64. Dulyaninova, N. G., House, R. P., Betapudi, V., & Bresnick, A. R. (2007). Myosin-IIA heavy-chain phosphorylation regulates the motility of MDA-MB-231 carcinoma cells. *Molecular Biology of the Cell*, *18*(8), 3144–3155. https://doi.org/10.1091/mbc.e06-11-1056
65. van Leeuwen, F. N., van Delft, S., Kain, H. E., van der Kammen, R. A., & Collard, J. G. (1999). Rac regulates phosphorylation of the myosin-II heavy chain, actinomyosin disassembly and cell spreading. *Nature Cell Biology*, *1*(4), 242–248. https://doi.org/10.1038/12068
66. Hotulainen, P., & Lappalainen, P. (2006). Stress fibers are generated by two distinct actin assembly mechanisms in motile cells. *Journal of Cell Biology*, *173*(3), 383–394. https://doi.org/10.1083/jcb.200511093
67. Vignaud, T., Copos, C., Leterrier, C., Toro-Nahuelpan, M., Tseng, Q., Mahamid, J., & Kurzawa, L. (2021). Stress fibres are embedded in a contractile cortical network. *Nature Materials*, *20*(3), 410–420. https://doi.org/10.1038/s41563-020-00825-z
68. Oria, R., Wiegand, T., Escribano, J., Elosegui-Artola, A., Uriarte, J. J., Moreno-Pulido, C., & Roca-Cusachs, P. (2017). Force loading explains spatial sensing of ligands by cells. *Nature*, *552*(7684), 219–224. https://doi.org/10.1038/nature24662
69. Elosegui-Artola, A., Trepat, X., & Roca-Cusachs, P. (2018). Control of mechanotransduction by molecular clutch dynamics. *Trends in Cell Biology*, *28*(5), 356–367. https://doi.org/10.1016/j.tcb.2018.01.008
70. Chrzanowska-Wodnicka, M., & Burridge, K. (1996). Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. *Journal of Cell Biology*, *133*(6), 1403–1415. https://doi.org/10.1083/jcb.133.6.1403
71. Bangasser, B. L., Shamsan, G. A., Chan, C. E., Opoku, K. N., Tuzel, E., Schlichtmann, B. W., & Odde, D. J. (2017). Shifting the optimal stiffness for cell migration. *Nature Communications*, *8*, 15313. https://doi.org/10.1038/ncomms15313
72. Kage, F., Vicente-Manzanares, M., McEwan, B. C., Kettenbach, A. N., & Higgs, H. N. (2022). Myosin II proteins are required for organization of calcium-induced actin networks upstream of mitochondrial division. *Molecular Biology of the Cell*, *33*(7), ar63. https://doi.org/10.1091/mbc.E22-01-0005
73. Ma, X., Jana, S. S., Conti, M. A., Kawamoto, S., Claycomb, W. C., & Adelstein, R. S. (2010). Ablation of nonmuscle myosin II-B and II-C reveals a role for nonmuscle myosin II in cardiac myocyte karyokinesis. *Molecular Biology of the Cell*, *21*(22), 3952–3962. https://doi.org/10.1091/mbc.E10-04-0293
74. Weissenbruch, K., Grewe, J., Hippler, M., Fladung, M., Tremmel, M., Stricker, K., & Bastmeyer, M. (2021). Distinct roles of nonmuscle myosin II isoforms for establishing tension and elasticity during cell morphodynamics. *Elife*, *10*, https://doi.org/10.7554/eLife.71888
75. Even-Ram, S., Doyle, A. D., Conti, M. A., Matsumoto, K., Adelstein, R. S., & Yamada, K. M. (2007). Myosin IIA regulates cell motility and actomyosin-microtubule crosstalk. *Nature Cell Biology*, *9*(3), 299–309. https://doi.org/10.1038/ncb1540
76. Sandquist, J. C., Swenson, K. I., Demali, K. A., Burridge, K., & Means, A. R. (2006). Rho kinase differentially regulates phosphorylation of nonmuscle myosin II isoforms A and B during cell rounding and migration. *Journal of Biological Chemistry*, *281*(47), 35873–35883. https://doi.org/10.1074/jbc.M605343200
77. Vicente-Manzanares, M., Zareno, J., Whitmore, L., Choi, C. K., & Horwitz, A. F. (2007). Regulation of protrusion, adhesion dynamics, and polarity by myosins IIA and IIB in migrating cells. *Journal of Cell Biology*, *176*(5), 573–580. https://doi.org/10.1083/jcb.200612043
78. Chinowsky, C. R., Pinette, J. A., Meenderink, L. M., Lau, K. S., & Tyska, M. J. (2020). Nonmuscle myosin-2 contractility-dependent actin turnover limits the length of epithelial microvilli. *Molecular Biology of the Cell*, *31*(25), 2803–2815. https://doi.org/10.1091/mbc.E20-09-0582
79. Golomb, E., Ma, X., Jana, S. S., Preston, Y. A., Kawamoto, S., Shoham, N. G., & Adelstein, R. S. (2004). Identification and characterization of nonmuscle myosin II-C, a new member of the myosin II family. *Journal of Biological Chemistry*, *279*(4), 2800–2808. https://doi.org/10.1074/jbc.M309981200
80. Wang, F., Kovacs, M., Hu, A., Limouze, J., Harvey, E. V., & Sellers, J. R. (2003). Kinetic mechanism of non-muscle myosin IIB: Functional adaptations for tension generation and maintenance. *Journal of Biological Chemistry*, *278*(30), 27439–27448. https://doi.org/10.1074/jbc.M302510200
81. Kovacs, M., Wang, F., Hu, A., Zhang, Y., & Sellers, J. R. (2003). Functional divergence of human cytoplasmic myosin II: Kinetic characterization of the non-muscle IIA isoform. *Journal of Biological Chemistry*, *278*(40), 38132–38140. https://doi.org/10.1074/jbc.M305453200
82. Kolega, J. (2003). Asymmetric distribution of myosin IIB in migrating endothelial cells is regulated by a rho-dependent kinase and contributes to tail retraction. *Molecular Biology of the Cell*, *14*(12), 4745–4757. https://doi.org/10.1091/mbc.e03-04-0205
83. Juanes-Garcia, A., Chapman, J. R., Aguilar-Cuenca, R., Delgado-Arevalo, C., Hodges, J., Whitmore, L. A., & Vicente-Manzanares, M.

(2015). A regulatory motif in nonmuscle myosin II-B regulates its role in migratory front-back polarity. *Journal of Cell Biology*, *209*(1), 23–32. https://doi.org/10.1083/jcb.201407059

84. Vicente-Manzanares, M., Koach, M. A., Whitmore, L., Lamers, M. L., & Horwitz, A. F. (2008). Segregation and activation of myosin IIB creates a rear in migrating cells. *Journal of Cell Biology*, *183*(3), 543–554. https://doi.org/10.1083/jcb.200806030
85. Baird, M. A., Billington, N., Wang, A., Adelstein, R. S., Sellers, J. R., Fischer, R. S., & Waterman, C. M. (2017). Local pulsatile contractions are an intrinsic property of the myosin 2A motor in the cortical cytoskeleton of adherent cells. *Molecular Biology of the Cell*, *28*(2), 240–251. https://doi.org/10.1091/mbc.E16-05-0335
86. Schiffhauer, E. S., Ren, Y., Iglesias, V. A., Kothari, P., Iglesias, P. A., & Robinson, D. N. (2019). Myosin IIB assembly state determines its mechanosensitive dynamics. *Journal of Cell Biology*, *218*(3), 895–908. https://doi.org/10.1083/jcb.201806058
87. Werner, M. E., Ray, D. D., Breen, C., Staddon, M. F., Jug, F., Banerjee, S., & Maddox, A. S. (2023). Mechanical positive feedback and biochemical negative feedback combine to generate complex contractile oscillations in cytokinesis. *BioRxiv*. https://doi.org/10.1101/2023.12.01.569672
88. Kamps, D., Koch, J., Juma, V. O., Campillo-Funollet, E., Graessl, M., Banerjee, S., & Dehmelt, L. (2020). Optogenetic tuning reveals rho amplification-dependent dynamics of a cell contraction signal network. *Cell Reports*, *33*(9), 108467. https://doi.org/10.1016/j.celrep.2020.108467
89. Graessl, M., Koch, J., Calderon, A., Kamps, D., Banerjee, S., Mazel, T., & Nalbant, P. (2017). An excitable Rho GTPase signaling network generates dynamic subcellular contraction patterns. *Journal of Cell Biology*, *216*(12), 4271–4285. https://doi.org/10.1083/jcb.201706052
90. Bement, W. M., Leda, M., Moe, A. M., Kita, A. M., Larson, M. E., Golding, A. E., & von Dassow, G. (2015). Activator-inhibitor coupling between Rho signalling and actin assembly makes the cell cortex an excitable medium. *Nature Cell Biology*, *17*(11), 1471–1483. https://doi.org/10.1038/ncb3251
91. Michaux, J. B., Robin, F. B., McFadden, W. M., & Munro, E. M. (2018). Excitable RhoA dynamics drive pulsed contractions in the early C. elegans embryo. *Journal of Cell Biology*, *217*(12), 4230–4252. https://doi.org/10.1083/jcb.201806161
92. Marshall-Burghardt, S., Migueles-Ramírez, R. A., Lin, Q., Baba, N. E., Saada, R., Umar, M., & Hayer, A. (2023). Excitable Rho dynamics drive cell contractions by sequentially inducing ERM protein-mediated actin-membrane attachment and actomyosin contractility. *BioRxiv*. https://doi.org/10.1101/2023.12.19.572346
93. Bagorda, A., & Parent, C. A. (2008). Eukaryotic chemotaxis at a glance. *Journal of Cell Science*, *121*(Pt 16), 2621–2624. https://doi.org/10.1242/jcs.018077
94. Shellard, A., Szabo, A., Trepat, X., & Mayor, R. (2018). Supracellular contraction at the rear of neural crest cell groups drives collective chemotaxis. *Science*, *362*(6412), 339–343. https://doi.org/10.1126/science.aau3301
95. Desmarais, V., Yamaguchi, H., Oser, M., Soon, L., Mouneimne, G., Sarmiento, C., & Condeelis, J. (2009). N-WASP and cortactin are involved in invadopodium-dependent chemotaxis to EGF in breast tumor cells. *Cell Motility and the Cytoskeleton*, *66*(6), 303–316. https://doi.org/10.1002/cm.20361
96. Sandquist, J. C., & Means, A. R. (2008). The C-terminal tail region of nonmuscle myosin II directs isoform-specific distribution in migrating cells. *Molecular Biology of the Cell*, *19*(12), 5156–5167. https://doi.org/10.1091/mbc.e08-05-0533
97. Wang, A., Ma, X., Conti, M. A., Liu, C., Kawamoto, S., & Adelstein, R. S. (2010). Nonmuscle myosin II isoform and domain specificity during early mouse development. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(33), 14645–14650. https://doi.org/10.1073/pnas.1004023107
98. Newman, D., Young, L. E., Waring, T., Brown, L., Wolanska, K. I., MacDonald, E., & Zech, T. (2023). 3D matrix adhesion feedback controls nuclear force coupling to drive invasive cell migration. *Cell Reports*, *42*(12), 113554. https://doi.org/10.1016/j.celrep.2023.113554
99. Vicente-Manzanares, M., Newell-Litwa, K., Bachir, A. I., Whitmore, L. A., & Horwitz, A. R. (2011). Myosin IIA/IIB restrict adhesive and protrusive signaling to generate front-back polarity in migrating cells. *Journal of Cell Biology*, *193*(2), 381–396. https://doi.org/10.1083/jcb.201012159
100. Kuragano, M., Murakami, Y., & Takahashi, M. (2018). Nonmuscle myosin IIA and IIB differentially contribute to intrinsic and directed migration of human embryonic lung fibroblasts. *Biochemical and Biophysical Research Communications*, *498*(1), 25–31. https://doi.org/10.1016/j.bbrc.2018.02.171
101. Hetmanski, J. H. R., de Belly, H., Busnelli, I., Waring, T., Nair, R. V., Sokleva, V., & Caswell, P. T. (2019). Membrane tension orchestrates rear retraction in matrix-directed cell migration. *Developmental Cell*, *51*(4), 460–475.e410. https://doi.org/10.1016/j.devcel.2019.09.006
102. Mohammed, T. O., Lin, Y. R., Akter, L., Weissenbruch, K., Ngo, K. X., Zhang, Y., & Franz, C. M. (2024). S100A11 promotes focal adhesion disassembly via myosin II-driven contractility and Piezo1-mediated Ca2+ entry. *Journal of Cell Science*, *137*(2), https://doi.org/10.1242/jcs.261492
103. Saez, P., & Venturini, C. (2023). Positive, negative and controlled durotaxis. *Soft Matter*, *19*(16), 2993–3001. https://doi.org/10.1039/d2sm01326f
104. Espina, J. A., Marchant, C. L., & Barriga, E. H. (2022). Durotaxis: The mechanical control of directed cell migration. *Febs Journal*, *289*(10), 2736–2754. https://doi.org/10.1111/febs.15862
105. Shellard, A., & Mayor, R. (2021). Durotaxis: The hard path from in vitro to in vivo. *Developmental Cell*, *56*(2), 227–239. https://doi.org/10.1016/j.devcel.2020.11.019
106. Isomursu, A., Park, K. Y., Hou, J., Cheng, B., Mathieu, M., Shamsan, G. A., & Odde, D. J. (2022). Directed cell migration towards softer environments. *Nature Materials*, *21*(9), 1081–1090. https://doi.org/10.1038/s41563-022-01294-2
107. Huang, Y., Su, J., Liu, J., Yi, X., Zhou, F., Zhang, J., & Wu, C. (2022). YAP activation in promoting negative durotaxis and acral melanoma progression. *Cells*, *11*(22), 3543. https://doi.org/10.3390/cells11223543
108. Thompson, A. J., Pillai, E. K., Dimov, I. B., Foster, S. K., Holt, C. E., & Franze, K. (2019). Rapid changes in tissue mechanics regulate cell behaviour in the developing embryonic brain. *Elife*, *8*, https://doi.org/10.7554/eLife.39356
109. Koser, D. E., Thompson, A. J., Foster, S. K., Dwivedy, A., Pillai, E. K., Sheridan, G. K., & Franze, K. (2016). Mechanosensing is critical for axon growth in the developing brain. *Nature Neuroscience*, *19*(12), 1592–1598. https://doi.org/10.1038/nn.4394
110. Mathieu, M., Isomursu, A., & Ivaska, J. (2024). Positive and negative durotaxis—mechanisms and emerging concepts. *Journal of Cell Science*, 137(8). https://doi.org/10.1242/jcs.261919
111. Ji, C., & Huang, Y. (2023). Durotaxis and negative durotaxis: Where should cells go? *Communications Biology*, *6*(1), 1169. https://doi.org/10.1038/s42003-023-05554-y
112. Lo, C. M., Buxton, D. B., Chua, G. C., Dembo, M., Adelstein, R. S., & Wang, Y. L. (2004). Nonmuscle myosin IIb is involved in the guidance of fibroblast migration. *Molecular Biology of the Cell*, *15*(3), 982–989. https://doi.org/10.1091/mbc.e03-06-0359
113. Jacobelli, J., Chmura, S. A., Buxton, D. B., Davis, M. M., & Krummel, M. F. (2004). A single class II myosin modulates T cell motility and stopping, but not synapse formation. *Nature Immunology*, *5*(5), 531–538. https://doi.org/10.1038/ni1065
114. Shellard, A., Weißenbruch, K., Hampshire, P. A. E., Stillman, N. R., Dix, C. L., Thorogate, R., & Mayor, R. (2024). Frictiotaxis underlies

adhesion-independent durotaxis. *BioRxiv*. https://doi.org/10.1101/2023.06.01.543217
115. Kang, C., Chen, P., Yi, X., Li, D., Hu, Y., Yang, Y., & Wu, C. (2024). Amoeboid cells undergo durotaxis with soft end polarized NMIIA. *Elife*, *13:RP96821*. https://doi.org/10.7554/eLife.96821.1
116. Renkawitz, J., Kopf, A., Stopp, J., de Vries, I., Driscoll, M. K., Merrin, J., & Sixt, M. (2019). Nuclear positioning facilitates amoeboid migration along the path of least resistance. *Nature*, *568*(7753), 546–550. https://doi.org/10.1038/s41586-019-1087-5
117. Hartman, C. D., Isenberg, B. C., Chua, S. G., & Wong, J. Y. (2017). Extracellular matrix type modulates cell migration on mechanical gradients. *Experimental Cell Research*, *359*(2), 361–366. https://doi.org/10.1016/j.yexcr.2017.08.018
118. Hartman, C. D., Isenberg, B. C., Chua, S. G., & Wong, J. Y. (2016). Vascular smooth muscle cell durotaxis depends on extracellular matrix composition. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(40), 11190–11195. https://doi.org/10.1073/pnas.1611324113
119. Vogel, V. (2018). Unraveling the Mechanobiology of Extracellular Matrix. *Annual Review of Physiology*, *80*, 353–387. https://doi.org/10.1146/annurev-physiol-021317-121312
120. Klotzsch, E., Smith, M. L., Kubow, K. E., Muntwyler, S., Little, W. C., Beyeler, F., & Vogel, V. (2009). Fibronectin forms the most extensible biological fibers displaying switchable force-exposed cryptic binding sites. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(43), 18267–18272. https://doi.org/10.1073/pnas.0907518106
121. Doyle, A. D., Sykora, D. J., Pacheco, G. G., Kutys, M. L., & Yamada, K. M. (2021). 3D mesenchymal cell migration is driven by anterior cellular contraction that generates an extracellular matrix prestrain. *Developmental Cell*, *56*(6), 826–841,e824. https://doi.org/10.1016/j.devcel.2021.02.017
122. Palmquist, K. H., Tiemann, S. F., Ezzeddine, F. L., Yang, S., Pfeifer, C. R., Erzberger, A., & Shyer, A. E. (2022). Reciprocal cell-ECM dynamics generate supracellular fluidity underlying spontaneous follicle patterning. *Cell*, *185*(11), 1960–1973,e1911. https://doi.org/10.1016/j.cell.2022.04.023
123. Meshel, A. S., Wei, Q., Adelstein, R. S., & Sheetz, M. P. (2005). Basic mechanism of three-dimensional collagen fibre transport by fibroblasts. *Nature Cell Biology*, *7*(2), 157–164. https://doi.org/10.1038/ncb1216
124. Shellard, A., & Mayor, R. (2021). Collective durotaxis along a self-generated stiffness gradient in vivo. *Nature*, *600*(7890), 690–694. https://doi.org/10.1038/s41586-021-04210-x
125. Alexander, S., Koehl, G. E., Hirschberg, M., Geissler, E. K., & Friedl, P. (2008). Dynamic imaging of cancer growth and invasion: A modified skin-fold chamber model. *Histochemistry and Cell Biology*, *130*(6), 1147–1154. https://doi.org/10.1007/s00418-008-0529-1
126. Kim, H. T., Yin, W., Jin, Y. J., Panza, P., Gunawan, F., Grohmann, B., & Stainier, D. Y. R. (2018). Myh10 deficiency leads to defective extracellular matrix remodeling and pulmonary disease. *Nature Communications*, *9*(1), 4600. https://doi.org/10.1038/s41467-018-06833-7
127. van Helvert, S., Storm, C., & Friedl, P. (2018). Mechanoreciprocity in cell migration. *Nature Cell Biology*, *20*(1), 8–20. https://doi.org/10.1038/s41556-017-0012-0
128. Ewald, A. J., Brenot, A., Duong, M., Chan, B. S., & Werb, Z. (2008). Collective epithelial migration and cell rearrangements drive mammary branching morphogenesis. *Developmental Cell*, *14*(4), 570–581. https://doi.org/10.1016/j.devcel.2008.03.003
129. Poujade, M., Grasland-Mongrain, E., Hertzog, A., Jouanneau, J., Chavrier, P., Ladoux, B., & Silberzan, P. (2007). Collective migration of an epithelial monolayer in response to a model wound. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(41), 15988–15993. https://doi.org/10.1073/pnas.0705062104
130. Mitchel, J. A., Das, A., O'Sullivan, M. J., Stancil, I. T., DeCamp, S. J., Koehler, S., & Park, J. A. (2020). In primary airway epithelial cells, the unjamming transition is distinct from the epithelial-to-mesenchymal transition. *Nature Communications*, *11*(1), 5053. https://doi.org/10.1038/s41467-020-18841-7
131. Park, J. A., Atia, L., Mitchel, J. A., Fredberg, J. J., & Butler, J. P. (2016). Collective migration and cell jamming in asthma, cancer and development. *Journal of Cell Science*, *129*(18), 3375–3383. https://doi.org/10.1242/jcs.187922
132. Scarpa, E., & Mayor, R. (2016). Collective cell migration in development. *Journal of Cell Biology*, *212*(2), 143–155. https://doi.org/10.1083/jcb.201508047
133. Yang, J., Antin, P., Berx, G., Blanpain, C., Brabletz, T., Bronner, M., & Association, E. M. T. I. (2020). Guidelines and definitions for research on epithelial-mesenchymal transition. *Nature Reviews. Molecular Cell Biology*, *21*(6), 341–352. https://doi.org/10.1038/s41580-020-0237-9
134. Lamouille, S., Xu, J., & Derynck, R. (2014). Molecular mechanisms of epithelial-mesenchymal transition. *Nature Reviews. Molecular Cell Biology*, *15*(3), 178–196. https://doi.org/10.1038/nrm3758
135. Beach, J. R., Hussey, G. S., Miller, T. E., Chaudhury, A., Patel, P., Monslow, J., & Egelhoff, T. T. (2011). Myosin II isoform switching mediates invasiveness after TGF-beta-induced epithelial-mesenchymal transition. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(44), 17991–17996. https://doi.org/10.1073/pnas.1106499108
136. Sirka, O. K., Shamir, E. R., & Ewald, A. J. (2018). Myoepithelial cells are a dynamic barrier to epithelial dissemination. *Journal of Cell Biology*, *217*(10), 3368–3381. https://doi.org/10.1083/jcb.201802144
137. Thomas, D. G., Yenepalli, A., Denais, C. M., Rape, A., Beach, J. R., Wang, Y. L., & Egelhoff, T. T. (2015). Non-muscle myosin IIB is critical for nuclear translocation during 3D invasion. *Journal of Cell Biology*, *210*(4), 583–594. https://doi.org/10.1083/jcb.201502039
138. Ebrahim, S., Fujita, T., Millis, B. A., Kozin, E., Ma, X., Kawamoto, S., & Kachar, B. (2013). NMII forms a contractile transcellular sarcomeric network to regulate apical cell junctions and tissue geometry. *Current Biology*, *23*(8), 731–736. https://doi.org/10.1016/j.cub.2013.03.039
139. Surcel, A., Schiffhauer, E. S., Thomas, D. G., Zhu, Q., DiNapoli, K. T., Herbig, M., & Robinson, D. N. (2019). Targeting mechanoresponsive proteins in pancreatic cancer: 4-hydroxyacetophenone blocks dissemination and invasion by activating MYH14. *Cancer Research*, *79*(18), 4665–4678. https://doi.org/10.1158/0008-5472.CAN-18-3131
140. Bryan, D. S., Stack, M., Krysztofiak, K., Cichon, U., Thomas, D. G., Surcel, A., & Weichselbaum, R. R. (2020). 4-Hydroxyacetophenone modulates the actomyosin cytoskeleton to reduce metastasis. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(36), 22423–22429. https://doi.org/10.1073/pnas.2014639117
141. Billington, N., Wang, A., Mao, J., Adelstein, R. S., & Sellers, J. R. (2013). Characterization of three full-length human nonmuscle myosin II paralogs. *Journal of Biological Chemistry*, *288*(46), 33398–33410. https://doi.org/10.1074/jbc.M113.499848
142. Webster, K. D., Ng, W. P., & Fletcher, D. A. (2014). Tensional homeostasis in single fibroblasts. *Biophysical Journal*, *107*(1), 146–155. https://doi.org/10.1016/j.bpj.2014.04.051
143. Stamenovic, D., & Smith, M. L. (2020). Tensional homeostasis at different length scales. *Soft Matter*, *16*(30), 6946–6963. https://doi.org/10.1039/d0sm00763c
144. Yamamoto, N., Okano, T., Ma, X., Adelstein, R. S., & Kelley, M. W. (2009). Myosin II regulates extension, growth and patterning in the mammalian cochlear duct. *Development (Cambridge, England)*, *136*(12), 1977–1986. https://doi.org/10.1242/dev.030718

145. Shellard, A., & Mayor, R. (2019). Supracellular migration—beyond collective cell migration. *Journal of Cell Science*, *132*(8). https://doi.org/10.1242/jcs.226142
146. Schumacher, L. (2019). Collective cell migration in development. *Advances in Experimental Medicine and Biology*, *1146*, 105–116. https://doi.org/10.1007/978-3-030-17593-1_7
147. Liu, Y. J., Le Berre, M., Lautenschlaeger, F., Maiuri, P., Callan-Jones, A., Heuze, M., & Piel, M. (2015). Confinement and low adhesion induce fast amoeboid migration of slow mesenchymal cells. *Cell*, *160*(4), 659–672. https://doi.org/10.1016/j.cell.2015.01.007
148. Ruprecht, V., Wieser, S., Callan-Jones, A., Smutny, M., Morita, H., Sako, K., & Heisenberg, C. P. (2015). Cortical contractility triggers a stochastic switch to fast amoeboid cell motility. *Cell*, *160*(4), 673–685. https://doi.org/10.1016/j.cell.2015.01.008
149. Serwane, F., Mongera, A., Rowghanian, P., Kealhofer, D. A., Lucio, A. A., Hockenbery, Z. M., & Campas, O. (2017). In vivo quantification of spatially varying mechanical properties in developing tissues. *Nature Methods*, *14*(2), 181–186. https://doi.org/10.1038/nmeth.4101
150. Yamaguchi, N., Zhang, Z., Schneider, T., Wang, B., Panozzo, D., & Knaut, H. (2022). Rear traction forces drive adherent tissue migration in vivo. *Nature Cell Biology*, *24*(2), 194–204. https://doi.org/10.1038/s41556-022-00844-9

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