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Cytoskeletal crosstalk: when three different personalities team up

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Cell shape and mechanics are determined by the interplay of three distinct cytoskeletal networks, made of actin filaments, microtubules, and intermediate filaments. These three types of cytoskeletal polymers have rather different structural and physical properties, enabling specific cellular functions. However, there is growing evidence that the three cytoskeletal subsystems also exhibit strongly coupled functions necessary for polarization, cell migration, and mechano-responsiveness. Here we summarize this evidence from a biophysical point of view, focusing on physical (direct) interactions between the cytoskeletal elements and their influence on cell mechanics and cell migration.

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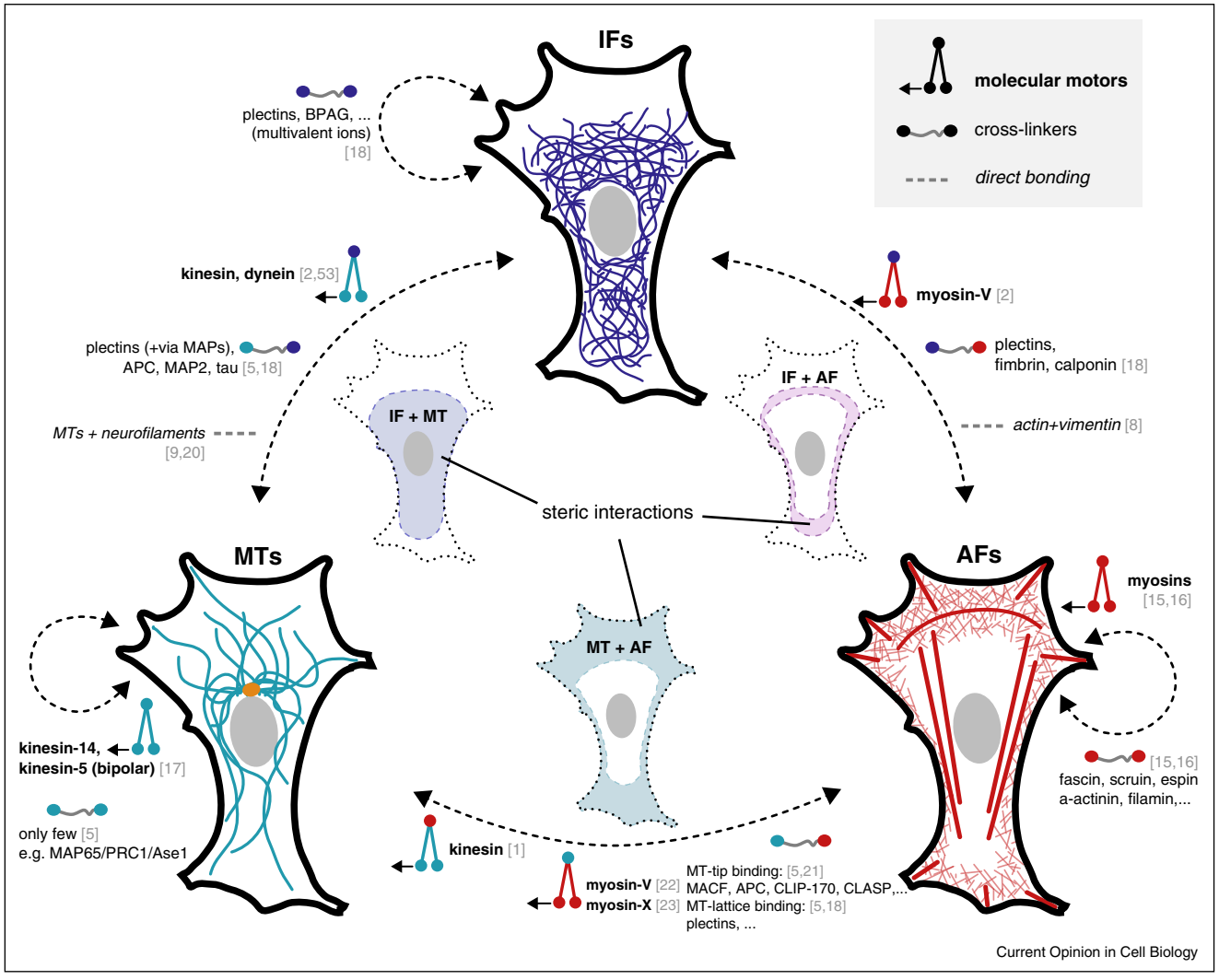
Introduction

Animal cells undergo substantial changes in shape as they grow, divide, and move. Yet, they also have an extraordinary ability to maintain their characteristic shape upon exposure to mechanical forces. Understanding how cells can combine dynamic shape control with mechanical stability is a shared goal of cell biologists and biophysicists. The principal molecular machinery responsible for cell shape and mechanics is the cytoskeleton. Given its enormous molecular complexity, it is common to study it in terms of distinct functional subsystems: actin filaments (AFs), microtubules (MTs), and intermediate filaments (IFs). There has been extensive experimental and theoretical work to understand the mechanisms that regulate the turnover dynamics and spatial organization of each subsystem in cells. AFs and MTs are both polar filaments, which exhibit fast out-of-equilibrium polymerization dynamics, and network-wide turnover rates on the order of minutes. By contrast, IFs have no structural polarity, polymerize in the minute time-scale, and have network-wide remodeling rates in the time-scale of hours. As powerful as the division into cytoskeleton subsystems has been in the past, it partly obstructed the view of the cytoskeleton as a highly intertwined entity. The last decade has brought growing evidence for strong coupling between all three cytoskeletal subsystems during key cellular functions ranging from cell motility and division to mechano-responsiveness [1–3]. In this review we summarize these findings from a biophysical point of view, focusing on physical (direct) interactions between the cytoskeletal elements and their implications for the mechanical properties and migratory behavior of animal cells. Since the human IF family encompasses more than 65 different members, with cell-specific and tissue-specific functions, and given that we wish to focus on general physical concepts applicable to different cellular settings, we mainly consider the two most ubiquitous IFs: vimentin and keratin.

Evidence for three-way cytoskeletal crosstalk

There is a wealth of evidence that the three cytoskeletal subsystems interact indirectly via biochemical signaling [2,4•,5] and gene regulation [6,7]. In addition, they also interact through direct physical contact, mediated by direct binding, cross-linkers, or through steric effects (as summarized in Figure 1). *In vivo* and *in vitro* studies have highlighted direct binding between filamentous actin and vimentin [8] and between dephosphorylated neurofilaments and MTs [9]. More established is a variety of cross-linking protein complexes which include both active (i.e. AF-based and MT-based motor proteins) and passive components (i.e. plectins, members of the plakin family, [10]). There are also interconnections mediated by protein complexes situated at cell–matrix and cell–cell junctions and at the nuclear envelope [11,12•]. Finally, the three cytoskeletal subsystems can also interact through nonspecific steric interactions. A recent study of mouse fibroblasts on micropatterns [13] for instance clearly showed how MTs interpenetrate a dense network of AFs near the cell periphery, and a dense network of vimentin in the cytoplasm (Figure 2a–c). Steric interactions, while often ignored, can contribute importantly to cell mechanics and shape control by influencing the mobility of cytoskeletal filaments [14] and by synergistically reinforcing the cytoskeleton, as reviewed below.

Figure 1



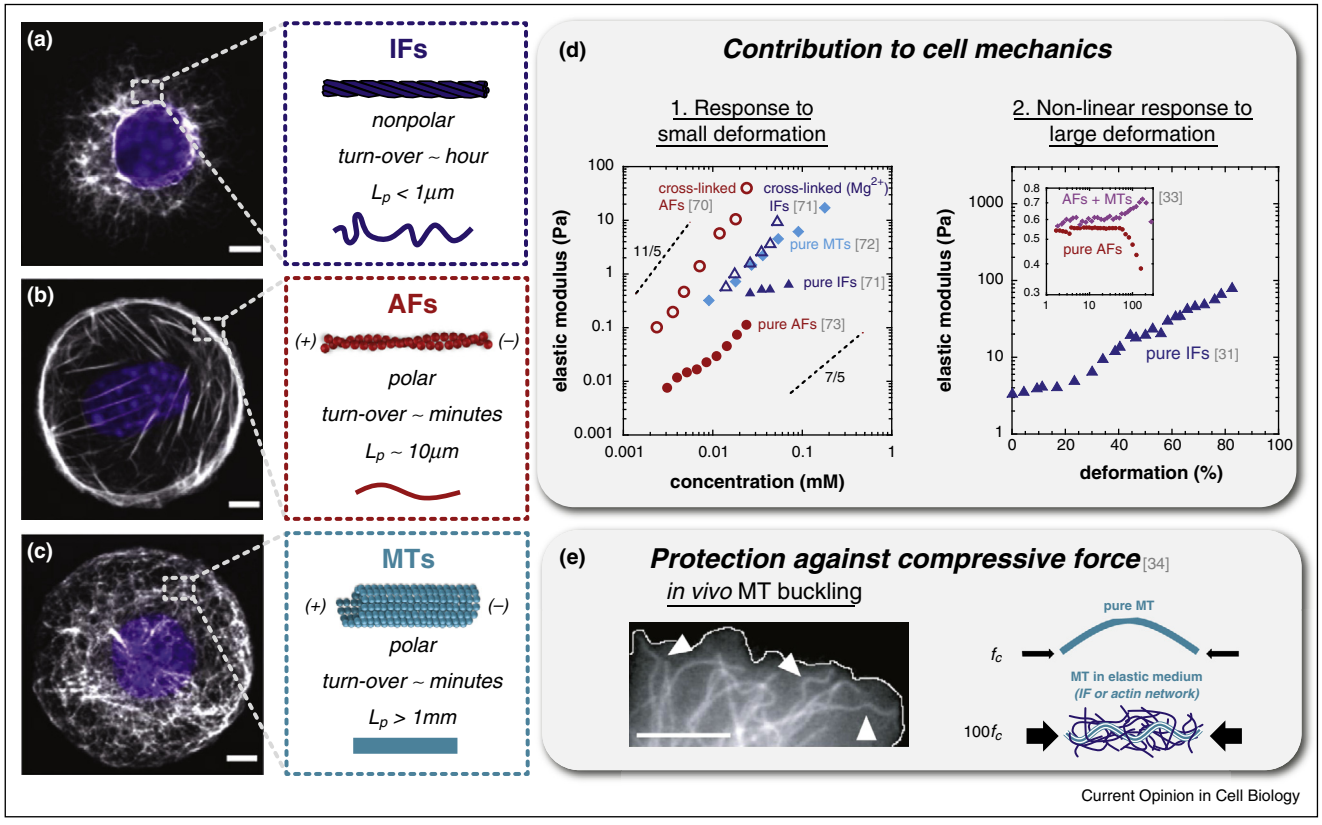
Multiple physical interactions exist within and in between the three cytoskeletal subsystems: intermediate filaments (IFs), microtubules (MTs) and actin filaments (AFs). In regions of spatial overlap (center), the subsystems interact via steric effects between IFs and MTs (mainly in the cell interior), MTs and AFs (mainly in the cell periphery), and IFs and AFs (mainly at the periphery of the IF network). For each individual cytoskeleton subsystem, there are cross-linkers and motors for AFs [15,16] and for MTs [5,17], while IFs are only connected by cross-linkers [18]. Crosstalk between AFs and IFs is facilitated by cross-linkers and motors [2,18] and also by direct binding in the case of vimentin [8]. Crosstalk between MTs and IFs is also mediated by cross-linkers and motors [2,5], and direct binding in the case of neurofilaments (NFs) [9,20]. Crosstalk between AFs and MTs is mediated by numerous cross-linkers [1,5,21] as well as AF-based [1,22,23] and MT-based motors [1].

Role of cytoskeletal crosstalk in cell mechanics

A plethora of biophysical techniques is now available to probe the mechanical properties of single cells, usually by inducing local or whole-cell deformations through contact-based, hydrodynamic, magnetic, or optical forces [15,24]. Cells show large variations in mechanical properties [25] depending on intrinsic factors such as cell type and cycle, and on external factors such as substrate stiffness. It is still an unresolved question how the three cytoskeletal elements contribute individually to the mechanical behavior of cells. Usually, cells are perturbed with drugs or genetic methods to manipulate one of the cytoskeletal subsystems. However, this often leads to secondary changes in the other two subsystems, such as keratin gene up-regulation following actin depolymerization [7].

AFs and IFs are generally considered the main determinants of cell stiffness and strength [26••,27,28]. Their relative contributions seem to depend on external cues such as substrate stiffness [28] and applied mechanical

Figure 2



Contribution of the cytoskeleton to the mechanical properties of cells, highlighted by *in vitro* studies. IFs, AF and MTs markedly differ in their polarity, turn-over dynamics and persistence length (a, b, c from [13]). Cytoskeletal networks display a wide range of elastic moduli at small deformations (d), spanning about five orders of magnitude, depending on their concentration and on the extent of cross-linking. Data were taken from [31,33,70–73]. The elastic modulus increases as a power law with concentration with exponents of 7/5 for entangled networks and 11/5 for cross-linked networks (dashed lines). A particularity of IF network is their non-linear response to large strain (d) [31]. MTs promote strain-stiffening of actin networks by suppressing inhomogeneous AF deformations (d, inset) [33]. Actin and IF networks provide an elastic background network that reinforces MTs against compressive forces (e), increasing the critical buckling force f_c [34••]. All scale bars shown are 5μm.

stress [29]. Local mechanical probing has shown that vimentin contributes mainly to cytoplasmic stiffness, whereas actin dominates cortical stiffness [30]. Since the complex interlacing of the three cytoskeletal subsystems often hampers an unambiguous conclusion, quantitative rheological measurements on *in vitro* reconstituted networks have become a popular complementary approach [24,25]. Such *in vitro* studies have led to detailed predictive models of the mechanical properties of one-component cytoskeletal networks in terms of network architecture and the intrinsic mechanical properties of the filaments and cross-linking proteins [29]. The three cytoskeletal subsystems differ markedly in their mechanical behavior (Figure 2). At the single filament level, IFs are the softest, with a persistence length (roughly a measure of the distance over which a filament is straight) in the 1 μm range [24]. AFs have an intermediate persistence length of about 10 μm, and MTs are the stiffest, with a persistence length on the order of millimeters [15,24]. Furthermore, IFs can be stretched by up to 3 times their original length [19], whereas AFs and MTs tend to break at strains of less than 50% [31]. These differences at the single filament level cause marked differences in stiffness and strength at the whole-network level (Figure 2d). Concomitantly, IF networks tend to be softer than MT and actin networks at low strain, but they can withstand much larger deformations [31]. Thus, IFs are generally believed to dominate the mechanical response of cells at large deformations, which is supported by recent coarse-grained simulations [32].

Recent studies have revealed that the composite nature of the cytoskeleton promotes surprising emergent mechanical behavior that cannot be expected simply by a sum of the parts. For instance, the elastic filamentous background provided by AFs and IFs stabilizes MTs against buckling. In cells, MTs usually display multiple short-wavelength

bends (Figure 2e) and can withstand compressive forces that are more than a 100-fold larger than the critical buckling force of an isolated microtubule [34••]. Theoretical modeling revealed that this reinforcement is a generic consequence of embedding stiff MTs in an elastic medium comprised of more flexible filaments [35]. Conversely, MTs were found to promote stiffening of actin networks (inset in Figure 2d) under an applied mechanical shear [33]. At low cross-linker density, actin networks tend to deform inhomogeneously by non-affine deformation modes that are sensitive to the local architecture of the network. Computational modeling has shown that inclusion of stiff MTs can suppress these soft modes and thereby favor strain-stiffening [36]. Simulations and experiments have further shown that the inclusion of stiff MTs in an otherwise incompressible actin matrix can make the network compressible as a whole [37,38]. Thus, the interpenetration and steric interactions among different cytoskeletal networks combined with specific cross-linking (i.e. by plectins) are likely to strongly influence the overall mechanical response of cells [39]. It may be fruitful to compare observations from *in vitro* experiments and single cell rheological measurements with findings from materials science, which already widely exploits the synergistic interplay that emerges from combining polymers with disparate stiffness to design stronger materials [40].

Role of cytoskeletal crosstalk in cell shape control

Three-way cytoskeletal crosstalk also plays an important role in orchestrating cellular shape changes. A prominent example is cell migration, which is realized through a series of cytoskeletal remodeling processes which depend on cell type and extra-cellular environment [41]. Here, we focus on crawling (or mesenchymal) cell migration on flat substrates, since cytoskeletal crosstalk has been studied most extensively in this context.

Functional modules for cell migration

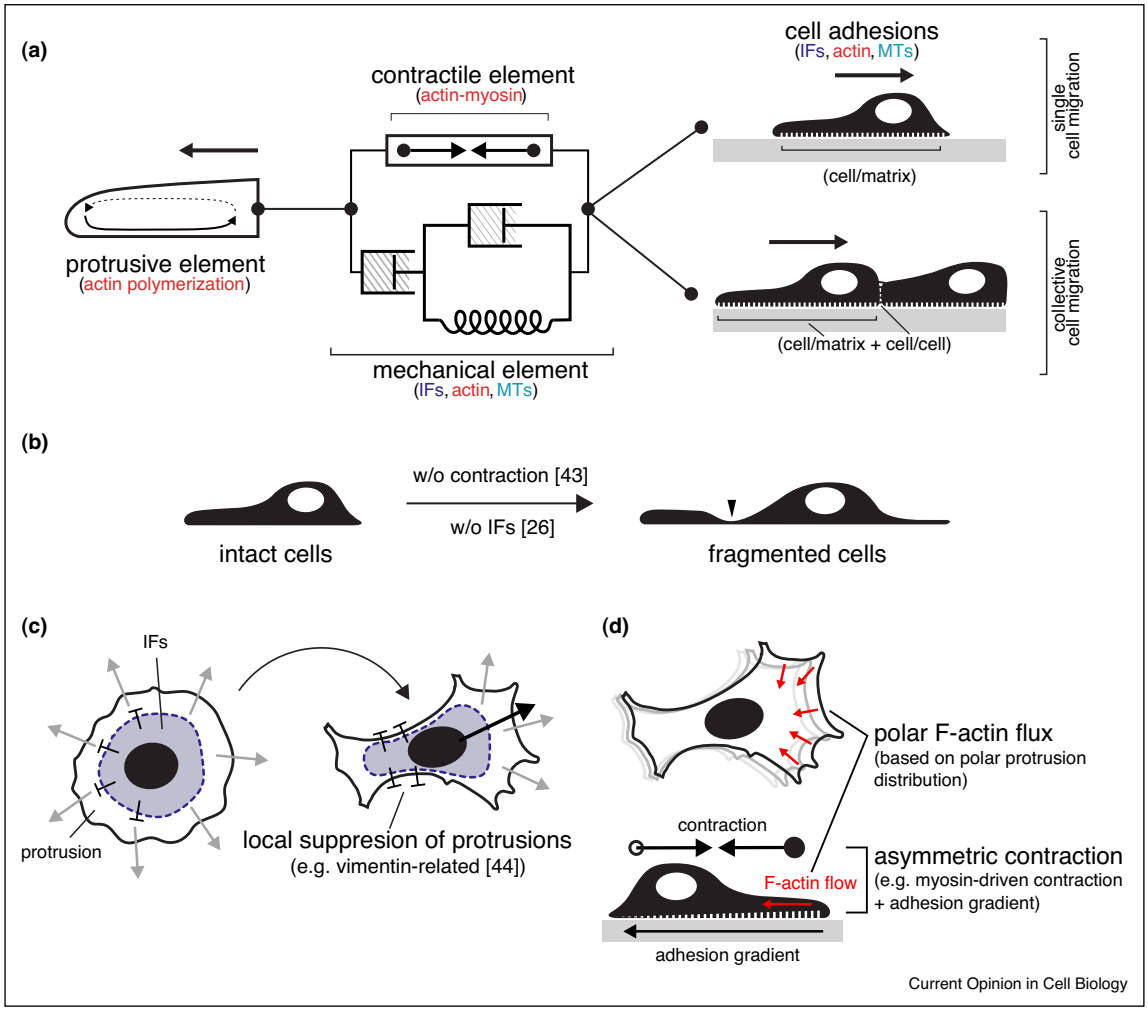
From a functional perspective, cell crawling can be dissected into three largely autonomous modules (Figure 3a). The first module, responsible for force generation, comprises a protrusive element that induces expansion and a contractile element that counteracts it. With few known exceptions [5,15], the acto-myosin cytoskeleton serves as the major protrusive and contractile element. Protrusion is driven by a dense actin meshwork in the lamellipodium which is interspersed with parallel bundles of AFs in filopodia [16,42]. AF nucleation and polymerization at the cell edge drive actin network expansion, which is followed by delayed network disassembly at the lamellum, and consequent directed flow of actin structures away from the leading edge. Contraction is driven by myosin motors that exert pulling forces on AFs organized in anti-parallel bundles in the lamellum [16], as well as in stress-fibers which span the length of the cell. The second functional module required for cell crawling is comprised of cell-to-matrix adhesions, which transmit cell-generated forces to the substrate. The third functional module is a mechanical element, that is, the entire cytoskeleton as a compound material, which ensures mechanical integration between the force-generating and adhesion modules. Although the functional modules contain the ability to drive cell migration, controlled and directed motion requires their tight coordination. Perturbations of any of the three functional elements can be deleterious for proper migration. For instance, weakening the central mechanical element by removing keratins [26••] or reducing contractile activity by impairing myosin motor activity [43] can lead to unbalanced motion of the front and rear of the cell, resulting in cell fragmentation (Figure 3b).

From autonomous functional modules to cell movement via cytoskeletal crosstalk

Coordination between the three functional modules involved in cell crawling requires that cells integrate the functions of all three cytoskeletal subsystems. This, however, makes it difficult to dissect their individual contributions. In the context of cell migration, the actin cytoskeleton has been the most extensively studied cytoskeletal component [15,16]. However, there is increasing appreciation of the crucial contributions of MTs [1,5] and IFs [3] to cell migration based on a growing list of observations on cytoskeletal interactions (Figure 4). We note, though, that the precise behaviors that stem from such instances of cytoskeletal crosstalk are generally not yet understood on a mechanistic level.

The most fundamental requirement for net cell motion is a polarized organization of the cytoskeleton (Figure 3c). Until recently, polarity establishment was mostly ascribed to the actin and MT cytoskeleton and their interactions with cell-to-matrix adhesions and with the nucleus. There is, however, growing evidence that IFs are also decisively involved in cell motion [44••,45•] and mechano-sensing [46]. Figure 4 summarizes some of the main cytoskeletal crosstalk instances in the cell polarization pathway, including those in which vimentin has been implicated. The first important instance controls the selective disassembly of cell-to-matrix adhesion sites. It is well-known that adhesion formation and maturation are tightly coupled to the dynamics of the actin network at the leading edge of cells and to stress-fiber contractility [42,47]. Focal adhesions promote stress-fiber maturation [47,48], which in turn promotes focal adhesion maturation via contractile forces. There is evidence that the polarized disassembly of focal adhesions relies on crosstalk between AFs and MTs [5,49]. *In vivo* and *in vitro* work has demonstrated that actin stress fibers serve as guiding tracks for growing MTs, which can thus target adhesion sites with their growing ends [49,50], an essential step in triggering focal adhesion disassembly. Several IFs have now also been shown to interact with cell–matrix

Figure 3

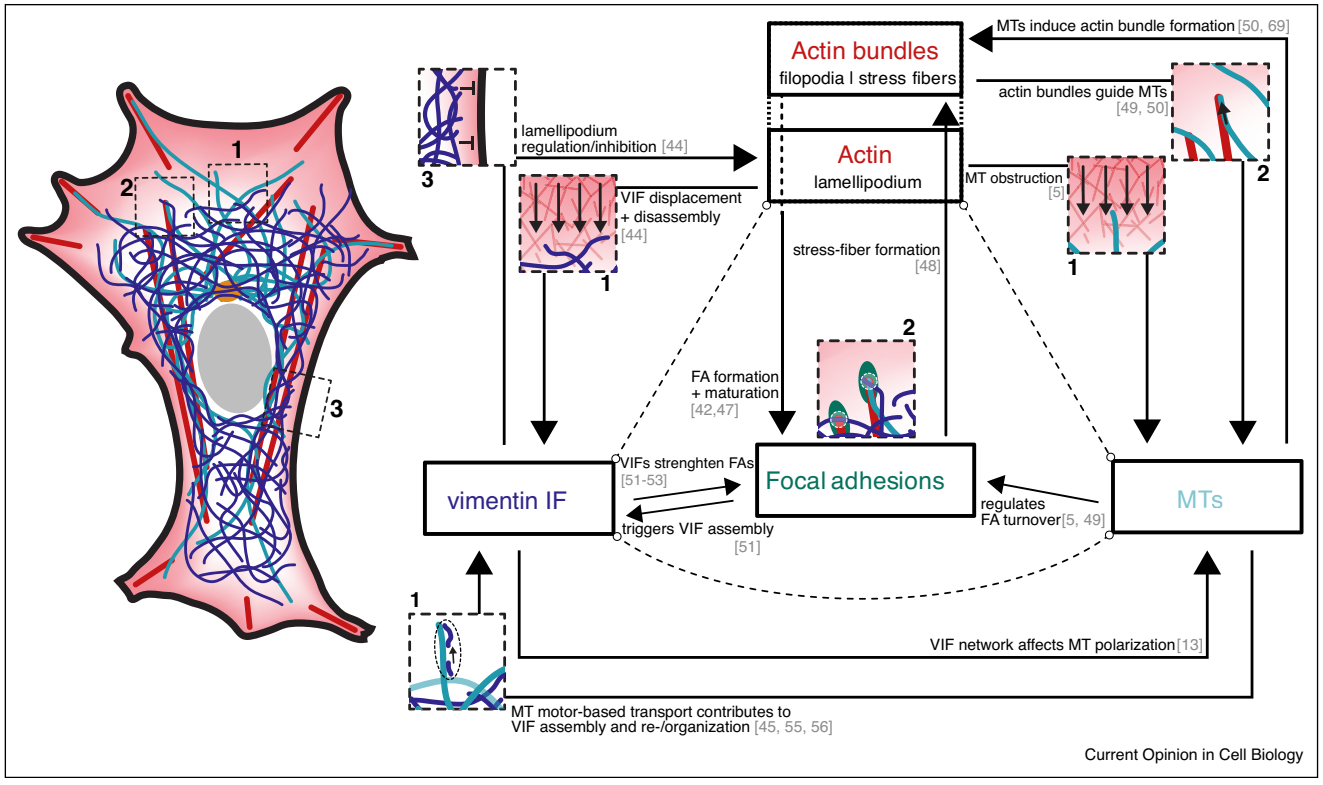


Crawling cell migration requires close coordination between three distinct functional modules. **(a)** Forward forces are generated by protrusive elements (lamellipodium/lamellum, filopodia), which are opposed by contractile elements (actin-myosin assemblies such as stress fibers). Movement is generated by transmitting forces to the substrate via cell adhesions that interact with all three cytoskeletal subsystems. A visco-elastic mechanical module (represented in terms of springs and dashpots in A), provides physical coupling between the other two modules. **(b)** Perturbations of one module can cause cell fragmentation [26••,43]. **(c)** Net cell movement requires a polarized organization of the cytoskeleton. One way to break symmetry is by suppressing actin-based protrusions via an as yet unknown pathway involving vimentin [44••]. Once polarized, the asymmetrical distribution of protrusions produces a polar AF flux and thereby a net force **(d)**. Acto-myosin driven contraction along the polar axis can also result in overall movement if combined with a gradient in adhesion strength.

adhesions. In the case of vimentin, this interaction is mediated by plectin [51]. Interestingly, this interaction leads to a reciprocal crosstalk where vimentin causes strengthening of the adhesion sites [51–53], while the adhesions serve as sites of *de novo* vimentin network formation via as yet unknown pathways [51]. Finally, vimentin also influences the selective disassembly of cell-to-matrix adhesions by mediating the recycling of integrins, together with filamin [54].

A second important instance of cytoskeletal crosstalk controls the maintenance of a polarized cell shape. Recent work shows that vimentin can have a pro-migratory function through interactions with actin networks at the cell periphery. The strong observed correlation between lamellipodia formation and vimentin network disassembly [44••] suggests that vimentin locally suppresses the formation of actin-based lamellipodia, leading to an asymmetrical distribution of protrusions and thereby a net force (Figure 3c,d). Furthermore, it has been shown that upon vimentin removal, motile cells adopt epithelial-like shapes [44••,45•], an effect that can be reversed by vimentin micro-injection. Vimentin network assembly [45•,55] and spatial organization [53,56•] are also tightly coupled to the MT cytoskeleton, for instance through transport by kinesin motors [55] or regulated polymerization along MTs

Figure 4



On the left, a sketch of the three cytoskeletal networks in an adherent cell crawling on a flat substrate. The three boxed regions (labeled 1, 2, 3 on the left panel) illustrate areas of intense cytoskeletal crosstalk. On the right, an overview of experimentally observed interactions between IFs (shown here: vimentin), MTs, and AFs, which take place within the lamellipodium (Box 1), at cell–matrix adhesion sites (Box 2), and in the more interior cytoplasm (Box 3). Although some of the drawn interactions are known to be linked to specific molecular mediators (e.g. MT guidance along actin bundles by spectraplakins such as MACF [50]), others remain mostly descriptive. We also expect the number of relevant interactions to increase fast with future *in vivo* observations (See reference [69]).

controlled by MT plus-end trackers [56•]. Conversely, a recent study on micro-patterned cells showed that the polar organization of MTs in turn relies partly on the presence of vimentin (Figure 4) [13]. Given that the MT cytoskeleton plays a crucial role in cell polarity through its control of focal adhesion turnover and nuclear positioning [5], this further implicates vimentin in cell polarity establishment and maintenance. Finally, single-molecule tracking of vimentin subunits along the MT cytoskeleton showed that the actin cytoskeleton can restrict IF transport [55], pointing to a complex three-way cytoskeletal crosstalk.

Unlike vimentin, keratin is usually associated with inhibition of cell migration since its expression is generally associated with epithelial-to-mesenchymal transitions [3]. Different from vimentin, keratin organization seems to depend mostly on interactions with AFs [3,45•]. Cell-to-matrix adhesions for instance contribute to keratin nucleation [57,58]. However, recent observations suggest that keratin-MT crosstalk also occurs, by destabilization of MTs via an unresolved mechanism that involves plectin [59].

How cytoskeleton crosstalk contributes to 3D cell motility strategies, such as bleb-based motion in tissues [60] or cancer cell invasion remains largely unknown. Interestingly, both vimentin and microtubules were recently shown to be necessary for elongation of the actin-based invadopodia of metastatic cancer cells [30]. Likewise, it remains poorly understood how cytoskeletal crosstalk contributes to collective cell migration during tissue morphogenesis in developing embryos and in wound healing of epithelial tissues [12•,61]. IFs seem to play a key role in the response of cell–cell junctions to inter-cellular tension and in maintaining the cell sheet's integrity. Pulling on cadherins of *Xenopus laevis* embryo cells by magnetic tweezers was recently observed to trigger recruitment of keratin, which was shown to be required for persistent migration [12•]. Wound healing assays using different cell types further showed faster wound closure upon keratin down-regulation [62] or knockout [63]. Depending on cell type, this was accompanied by a loss of cell–cell contacts [62]. This phenomenon probably contributes to keratin's reported involvement in decreasing cell invasiveness [26••]. Vimentin crosstalk with integrin-based focal adhesions was shown

to be involved in migration of repair cells at the leading edge of wounded lens epithelia, contributing essentially to wound closure [64].

Future directions

It is now clear that reciprocal regulation and physical interactions between all three cytoskeletal subsystems (AFs, MTs, and IFs) are essential to ensure both the mechanical stability and dynamic shape changes of cells. Indeed, many of the basic components are highly conserved and hence co-evolved over long periods of time [65]. The different cytoskeletal systems strongly differ at the single-filament level, in terms of structure and dynamics, as well as at the network level, in terms of architecture and mechanics. Cytoskeletal crosstalk provides a powerful strategy to combine those disparate properties without having to reinvent entirely new materials for each task. A variety of interactions involving structural links based on cross-linkers and motor proteins, as well as regulatory pathways, makes diverse mechanical and functional settings feasible. Future cell and developmental biology studies will have to decipher the key molecular elements that mediate this inter-cytoskeletal regulation in different tissue contexts. An important technical challenge remains to simultaneously image all three cytoskeletal subsystems in live cells and model organisms with comparative levels of spatio-temporal accuracy. We expect that advances in fluorescent labeling, super-resolution fluorescence microscopy, and electron microscopy will greatly accelerate this research. In addition, a precise dissection of the molecular mechanisms of cytoskeletal crosstalk will also require complementary *in vitro* experiments. Advanced methods in microfabrication such as high-resolution surface patterning [13,66,67] and confinement in microfluidic devices [68] are likely to play an important role in bridging the gap between *in vivo* and *in vitro* observations by offering the possibility to 'standardize' live cell experiments.

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