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## Focal adhesions, stress fibers and mechanical tension

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### Abstract

Stress fibers and focal adhesions are complex protein arrays that produce, transmit and sense mechanical tension. Evidence accumulated over many years led to the conclusion that mechanical tension generated within stress fibers contributes to the assembly of both stress fibers themselves and their associated focal adhesions. However, several lines of evidence have recently been presented against this model. Here we discuss the evidence for and against the role of mechanical tension in driving the assembly of these structures. We also consider how their assembly is influenced by the rigidity of the substratum to which cells are adhering. Finally, we discuss the recently identified connections between stress fibers and the nucleus, and the roles that these may play, both in cell migration and regulating nuclear function.

### Keywords

Myosin II; RhoA; Integrins; Substratum rigidity

## 1. Introduction

Growing in culture many cell types, particularly those of mesenchymal origin, display prominent bundles of filamentous actin (F-actin) associated with myosin II,  $\alpha$ -actinin and several other cytoskeletal proteins. These structures, known as stress fibers (SFs) occur in several distinct forms. Frequently, they are associated at one or both ends with adhesions to the underlying matrix, known most commonly as focal adhesions (FAs). For over 40 years there has been considerable interest in the functions of these structures, their role in cell migration, and how they assemble and disassemble. Much evidence has indicated that these structures are mechanosensitive [1–5] and it was concluded earlier that mechanical tension contributes to their assembly [1,6,7]. However, several recent studies have challenged this view and demonstrated a more complex situation [8–11]. Here, we consider the role of mechanical force in the assembly of SFs and FAs.

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It is often forgotten, even by those who study FAs and SFs, that these structures are not needed for cell migration [1,12]. Many cells (e.g. leukocytes) do not develop FAs or SFs but migrate highly effectively. Indeed, the presence of FAs can hinder cell migration due to excessive adhesion. Nevertheless, many migratory cells do display FAs and SFs. In these cells there must be a dynamic coupling of adhesion strength and traction force for cells to move forward. It is important that both adhesion and traction at the front are stronger than at the rear, and so mechanisms must exist for modulating these. Force at the front of migrating cells derives from both retrograde actin flow and myosin-generated tension [4].

For many years, the term stress fiber was most commonly applied to the large bundles of F-actin that traverse much of the cell and that are anchored at both ends by FAs. However, it was readily apparent that a variety of structures were often referred to as stress fibers. In 1998, Small and colleagues distinguished 2 types of SF: ventral SFs, which are anchored at each end by a FA, and dorsal SFs, which are anchored only at one end by a FA close to the cell front. Dorsal SFs extend back toward the nucleus and upwards toward the dorsal cell surface. They also discussed “arcs”, convex bundles of F-actin that form behind the leading edge of migrating or spreading cells and which move rearwards below the dorsal surface [13]. Subsequent studies have included arcs, often referred to as transverse arcs, as a form of SF [14]. Arcs contain many of the same proteins, but unlike other types of SF, they are not directly anchored by adhesions to the matrix. However, arcs can give rise to ventral SFs and will be considered here as a form of SF.

One complicating factor in the relationship between mechanical tension and the assembly of FAs and SF is that the three types of filament bundle collectively referred to as SFs differ in their genesis, behavior, and relationship to FAs. Additionally, different models have been used to study how SFs and FAs assemble.

## 2. Systems for analyzing SF and FA assembly

Most studies have examined the assembly and disassembly of these structures as cells spread and migrate on coverslips coated with extracellular matrix (ECM) (most commonly fibronectin) [4,14–17]. This system is well suited to analyzing adhesion dynamics and actin organization as cells migrate. The second experimental model for examining FA and SF assembly was pioneered by Ridley and Hall [18]. They exploited the observation that some cells lose their SFs and FAs when deprived of serum to become quiescent. Upon re-addition of serum or other factors that activate RhoA, FAs and SFs rapidly reassemble. It should be noted that these cells are usually in a non-migratory state and often confluent. This system was used to identify RhoA as a key regulatory protein controlling the assembly of these structures. It was also the system used to show that RhoA-induced assembly of FAs and SFs was blocked by a variety of inhibitors of myosin activity and contractility. This led to the conclusion that RhoA-stimulated myosin activity drives the assembly of SFs and FAs [7]. The bundling of F-actin to form a SF was attributed not only to the tension generated by myosin but also to myosin’s crosslinking of F-actin [7,19]. Contractility inhibitors available at the time of these experiments were relatively non-specific. However, subsequent studies using blebbistatin and Y27632, which inhibit the activities of myosin II and ROCK respectively, have also shown that inhibiting myosin activity blocks the formation of most

FAs and SFs [20,21]. Similarly, knockout or knockdown of myosin II expression prevented maturation of nascent adhesions into FAs [21,22]. In one study this was found for both myosin IIA and IIB [22], whereas in another this was dependent on knockout of myosin IIA but not IIB [21]

This second model system (stimulation of quiescent cells with serum or Rho-activating factors) was well suited to microinjection of constitutively active or dominant negative constructs. In addition, it has the advantage of allowing synchronous assembly of FAs and SFs to be studied in many cells. However, many cell types are resistant to serum-starvation; they either maintain FAs and SFs, or show only a slight decrease in these structures when deprived of serum. Another disadvantage is that this system does not recapitulate the events that occur as cells migrate and engage the ECM at new sites.

Cell migration involves a series of transitions that affect both the adhesions and organization of the actin cytoskeleton. As the lamellipodium extends, driven by Arp2/3 complex-mediated actin polymerization, initial adhesions form as integrin receptors engage the underlying matrix [4,23]. Such “nascent adhesions” are often transient and many rapidly disassemble. Maturation of adhesions that are not disassembled occurs at the transition between the lamellipodium and lamella, where retrograde actin flow changes from being driven by actin polymerization to myosin-based contraction. Whereas actin is organized in the lamellipodium as a branching dendritic network, in the lamella it is often bundled into the different SF types [24]. The maturing adhesions elongate in the direction of retrograde actin flow and retard the rate of rearward movement of actin [25]. They act as “molecular clutches” that couple the force of retrograde flow into forward extension of the lamellipodium [4]. Consistent with this clutch-like function, it was observed that in stationary cells FAs are often pulled toward the nucleus, whereas in migrating cells they are stationary [26]. The small maturing adhesions are often referred to as “focal complexes” [27]. Some continue enlarging to become classical “focal adhesions”. However, the different types of adhesion are poorly defined and it is often difficult to distinguish one type from another. In general, FAs are dependent on RhoA activity, whereas focal complexes are dependent on active Rac1 or Cdc42 [27].

Hotulainen and Lappalainen used live cell imaging to analyze assembly of the different SF types in migrating osteosarcoma cells [14]. They observed dorsal SFs initiating at small adhesions forming behind the leading edge. As the cell front extended away from the adhesion, dorsal SFs elongated. This SF growth was inhibited by depleting cells of the formin mDia1. Alpha-actinin was incorporated into the growing SF. Recruitment of myosin II into the dorsal SF was a relatively late event. A subsequent study using higher resolution imaging concluded that little if any myosin II is incorporated into dorsal SFs [16]. Transverse arcs arose behind the lamellipodium from the combination of short myosin filaments plus actin filaments generated at the leading edge by the Arp2/3 complex. In this system, ventral SFs developed most commonly from the fusion of each end of an arc with a dorsal SF. The annealing of two dorsal SFs growing from opposite sides of a cell also gave rise to ventral SFs, again anchored at each end by FAs. A subtype of ventral SFs (discussed later) is the perinuclear “actin cap”, where ventral SFs wrap over nuclei and anchor to elongated FAs [17]. The different SF types are illustrated in Fig. 1.

Burnette and colleagues used the same cells to explore the factors that maintain the lamella flat as cells migrate [16]. They determined that dorsal SFs, which they found contained little to no myosin II, acted as struts connecting the ventral adhesions with the dorsal contractile actin meshwork. Their analysis revealed that contraction of transverse arcs generated tension on dorsal SFs, and this caused the dorsal SFs to pivot, thereby flattening the lamella [16].

### 3. The role of myosin and tension in the development of stress fibers and focal adhesions

Besides the evidence that blocking Rho-mediated myosin activity inhibited ventral SF and FA assembly in quiescent cells [7], support for mechanical tension stimulating assembly comes from several observations. For example, shear stress at levels equivalent to that experienced in arteries induced endothelial cells in culture to develop SFs [28]. Direct evidence for mechanical tension stimulating growth of FAs came from Riveline and colleagues who applied force directly to individual cells with a glass rod. They observed growth of adhesions by IRM optics and incorporation of fluorescently labeled FA proteins [6]. Adhesion growth was blocked by inhibiting RhoA activity, but this could be rescued by expression of the constitutively active formin mDia1, which is normally activated by RhoA. It was concluded that FA growth requires both tension and actin polymerization, with each being driven by active RhoA. Supporting this idea that growth of FAs reflects the tension applied to them, Geiger's group used traction force microscopy to reveal a close correlation between the size of FAs and the force that is transmitted across them [29,30].

If mechanical force promotes the growth and maturation of FAs, one prediction is that new components will be recruited to FAs in response to force. This is indeed what was seen both when individual components such as vinculin were examined [31], as well as when the FA proteome was analyzed [32,33]. Examining the composition of isolated FAs, many proteins were found to be recruited to FAs in the presence, but not the absence of active myosin [32,33]. The idea that tension stimulates FA growth and maturation is also supported by experiments using beads coated with integrin ligands to apply force to cells. Reinforcement of the adhesion made to beads has been observed in multiple studies [31,34–37]. FA proteins are recruited to the bead adhesion sites under tension [31], again consistent with force contributing to adhesion assembly.

What is the basis for the stiffening or reinforcement that occurs when tension is applied exogenously on integrins? One of the signaling pathways activated in response to tension on integrins is the RhoA pathway [36–38]. Not only will this lead to increased actin polymerization via the RhoA-activated formin mDia1 [39], but it will also promote the stability of actin filaments by inhibiting the severing activity of cofilin (via ROCK4 LIMK4 phosphorylation of cofilin). Additionally, the activation of myosin II by ROCK [40,41] will lead to a positive feedback loop increasing tension. Just as importantly, activated myosin II that has assembled into bipolar filaments is a multivalent cross-linker and bundler of F-actin [8,19]. Additionally, F-actin reveals enhanced affinity for myosin II when under tension [42]. Similarly, in response to tension, F-actin binds less cofilin and is more resistant to severing by cofilin [43].

How might force on a FA lead to recruitment of new components? One way may involve exposure of tension-sensitive cryptic protein binding sites (illustrated in Fig. 2A). Originally demonstrated for fibronectin [44], this has also been shown for proteins at the cytoplasmic face of FAs, such as talin. Applying tension to single talin molecules exposed previously buried vinculin binding sites [45]. Similarly, other proteins may expose sites that become modified, thereby facilitating new interactions [46]. For example, mechanically stretching p130cas exposed multiple tyrosines that could be phosphorylated by Src [47].

SFs have also been shown to undergo reinforcement in response to strain. First demonstrated when cells experience cyclic stretch or shear stress [48], zyxin shuttles from FAs to zones of tension within SFs and triggers local recruitment of  $\alpha$ -actinin and vasodilator-stimulated phosphoprotein (VASP) that thickens and reinforces SFs [48,49]. Zyxin-dependent SF reinforcement has also been observed in cells that are not subjected to external mechanical stress. This repair mechanism serves to limit SF elongation and breakage at sites of excessive strain [50]. Interestingly, zyxin targeting to SFs depends on MAPK activation. Although the mechanism involved is not fully understood, multiple models have been proposed and hinge on tension-dependent conformational changes in SF-associated proteins to expose new docking sites for zyxin [50].

Little is known about how the bonds between particular proteins in FAs respond to mechanical tension. However, in some cases “catch” bonds, which strengthen in response to tension [51], have been identified, for example, for the fibronectin-binding integrin  $\alpha 5\beta 1$  [52,53]. We suspect that more catch bonds will be identified operating in FAs. We anticipate that some of the signaling associated with mechanical tension will modify protein interactions, converting slip bonds into catch bonds, thereby facilitating tension-induced stabilization. Conversely, the conversion of catch bonds into slip bonds may contribute to the disassembly of adhesions at the rear of migrating cells.

#### **4. Evidence against tension playing a dominant role in FA and SF assembly**

In contrast to the above studies, several have challenged the importance of myosin-generated tension in FA and SF assembly. Some of the different conclusions may reflect that different types of SF and FAs assemble through different mechanisms. So, for example, in the study by Hotulainen and Lappalainen, inhibiting myosin activity rapidly blocked formation of transverse arcs, but had little initial effect on dorsal SFs and the small adhesions that anchor them [14]. Tension was also important in the conversion of transverse arcs into ventral SFs, following their fusion with dorsal SFs. The role of myosin in dorsal SF assembly seems minimal. In several ways, dorsal SFs resemble filopodia. Both structures consist of narrow bundles of F-actin anchored either in a tip complex (filopodium) or nascent adhesion (dorsal SF). The filaments within these bundles all appear initially to have a single polarity, with polymerization occurring at the membrane attachment site, i.e. the filopodial tip or matrix adhesion. Because myosin II cannot generate contractile force on bundles of F-actin with a single polarity, whenever myosin II incorporates into these structures (such as when dorsal SFs are fusing with transverse arcs), then the polarity of the actin filaments must be broken

so that the bundle contains F-actin of opposite polarities. Little is known about how this polarity transition occurs, although some possibilities have been suggested [5,14].

Tension generated within stress fibers is transmitted across FAs as demonstrated by studying traction forces at a subcellular scale [29,54–57] and shown most directly using a vinculin tension sensor [58]. As mentioned above, Geiger and his colleagues correlated the magnitude of force generated at a FA with the cross-sectional area of the FA [29]. A very different conclusion was reached by Beningo and colleagues who found that small adhesions at the front of a migrating cell developed greater tractional force than the larger adhesions further away from the front [55]. Similar results were reported by Stricker et al. [9]. A key difference in these studies is that these last two groups examined FAs in migrating cells, whereas the study reporting a positive correlation between FA size and tension used stationary cells [29]. Another study found that for larger FAs their size was proportional to the force being transmitted, but for small adhesions the correlation broke down [56]. Since small adhesions are usually at the cell front, a potential explanation is that the additional force experienced by these small adhesions arises from retrograde actin flow and from transverse arcs that transmit tension to the matrix via dorsal SFs. However, this does not explain why these small adhesions do not enlarge in response to the high force being applied, which would be predicted by the Riveline study [6].

Choi and colleagues investigated the role of myosin II in nascent adhesion maturation [8]. Initial adhesion assembly occurred within the lamellipodium independently of myosin II on a template, the dorsal SF. The rate of assembly was proportional to the rate of protrusion of the lamellipodium. Template formation required  $\alpha$ -actinin, indicating the importance of F-actin crosslinking. The subsequent maturation of these nascent adhesions into FAs was blocked by knockdown of myosin IIA expression. Strikingly, the cells depleted of myosin IIA could be rescued by inactive motor mutants of myosin IIA that were unable to generate force. However, these mutants maintained their actin-binding ability. It was concluded that myosin's crosslinking function is more important than its tension-generating function for maturation of nascent adhesions [8].

Some of the strongest arguments against tension driving assembly of FAs have come from Gardel and her colleagues. They demonstrated that in migrating cells up to 60% of the force exerted on the substratum is generated by the lamellar actin network rather than by SFs [59]. Using blebbistatin wash-out as a trigger for inducing myosin activity, they showed that the assembly of SFs lagged temporally behind the development of traction forces. They also noted that FA growth rate remained constant under a range of different tensions [60]. Titrating the level of myosin activity by varying the concentration of inhibitors led them to conclude that tension is necessary but not sufficient for FA maturation [10]. They also concluded that the dorsal SF was critical as a structural template [10].

Waterman's group found that FA growth was fast in cells lacking vinculin but that these cells exerted low traction on the substratum [11]. They concluded that vinculin when present couples retrograde actin flow to integrins and acts as a molecular clutch. Their finding that vinculin promotes the transmission of force to the substratum but results in slower growth of FAs runs counter to the idea that force itself contributes to FA growth.

## 5. Reconciling the differences

How can these seemingly different conclusions be reconciled? Some of the differences may come from the use of the different systems, migrating cells versus quiescent stationary cells. Other differences surely derive from grouping together the different types of SF and adhesion that are seen in migrating cells. The organization and behavior of dorsal SFs is different from transverse arcs and ventral SFs. The former seem largely independent of myosin-generated contractility whereas myosin is important for both transverse arcs and ventral SFs. However, myosin is needed for the maturation of adhesions, particularly its crosslinking function as shown by Choi and colleagues [8].

In the original model of tension driving SF and FA assembly, actin polymerization was relatively minor [7]. The pulling together and alignment of pre-existing filaments, already attached to integrins, was more important than nucleation of new filaments. This was supported by limited actin polymerization as SFs and FAs assembled in the quiescent cell model [61]. However, it is clear that actin polymerization is essential for the development of SFs and FAs in migrating cells [10,14]. One possibility is that mechanical tension may stimulate formin-mediated actin polymerization at FAs. Such a mechanism was suggested in the Rive-line study [6], in which they observed that tension-dependent adhesion growth was dependent on mDia. Consistent with this, Schiller and colleagues recently observed that actomyosin contractility was necessary to recruit mDia to adhesions [62]. Using a proteomic approach, they discovered that  $\beta 1$  and  $\alpha v$  integrins cooperate to activate RhoA during adhesion to fibronectin but that downstream of RhoA the pathways diverged. Unexpectedly,  $\beta 1$  integrins were coupled to ROCK and activation of myosin II, whereas  $\alpha v$  was coupled to activation of mDia and actin polymerization. However, in response to myosin II activity and high tension,  $\alpha v$  integrin was recruited along with mDia to the adhesions. They concluded that cooperation between these fibronectin-binding integrins and their RhoA signaling pathways (Fig. 2B) contributed to adhesion maturation [62].

## 6. Stress fibers, focal adhesions and rigidity of the substratum

It has long been recognized that SFs are more prominent in cells growing on plastic or glass surfaces than they are in the same cells in their native tissue environment or growing in 3D systems [1]. Is the prominence of SFs and FAs in cells growing *in vitro* due to the two dimensional nature of the substratum, to its rigidity, or both? Fibroblasts cultured in three dimensional collagen gels rarely develop SFs. Fibroblasts will actively contract free-floating gels, but if the gels are anchored to prevent contraction to a smaller volume, the cells develop isometric tension and develop SFs [63–65]. Release of the gels from their attachments to the culture dish results in rapid contraction of the gels and disassembly of the SFs [63–65].

The role of substrate rigidity in SF and FA assembly was examined directly by Pelham and Wang, who discovered that assembly of these structures was inhibited on soft substrata [66]. How might this occur? With the recognition that active RhoA drives formation of these structures [18], this raised the question whether adhesion to a rigid substratum stimulates RhoA activity. Measuring RhoA activity revealed that RhoA is activated in cells grown on



rigid substrata [67,68]. Experimentally applying tension to engaged integrins was also found to activate RhoA [37,38]. Dissecting the pathway, Guilluy and colleagues identified both GEF-H1 and LARG as guanine nucleotide exchange factors (GEFs) responding to tension applied to fibronectin-coated beads [37]. GEF-H1 was activated downstream from FAK, Ras and the MEK/ERK pathway, whereas LARG was activated via the tyrosine kinase Fyn. Keely's group also found GEF-H1 was activated in cells adhering to rigid substrata, but in their case this was attributed to the release of GEF-H1 from microtubules [69]. Using proteomic approaches two groups have observed both GEF-H1 and mDia being recruited to adhesions in response to actomyosin contractility, suggesting that mechanical tension activates a GEF-H1/RhoA/mDia pathway that may promote adhesion growth [32,62].

High resolution traction force microscopy revealed that traction generated at one FA was often independent of the traction being generated by an adjacent FA within the same cell [54]. The force transmitted by an adhesion was either stable over time or it fluctuated. In situations where it fluctuated, this "tugging" at FAs was dispensable for their maturation, as well as for directional migration in response to chemotactic or haptotactic cues. However, it was needed for durotaxis, the migration up a stiffness gradient [54]. The authors concluded that fluctuating tension at FAs provides a mechanism by which a cell senses the stiffness of the substratum.

## 7. Connections to the nucleus

It has long been known that tension exerted on the cell surface is transmitted to the nucleus and that multiple connections link the nucleus to the cytoskeleton [70]. Indeed, mechanical force exerted on cells has profound effects on gene transcription [71–74], and tension exerted via SFs impacts nuclear structure and function [75,76]. The nesprin family of proteins span the outer nuclear membrane, connecting the different filament systems to proteins spanning the inner nuclear membrane such as SUN 1 and 2 [74,77,78]. These proteins connecting the nucleus with the cytoskeleton have been referred to as the Linker of nucleoskeleton and cytoskeleton (LINC) complex [79].

Investigating the relationship of the centrosome and nucleus to signals driving directional fibroblast migration, Gundersen's group observed that the nucleus was pulled rearwards by actin filaments, such that the centrosome came lie in front of it [80]. Pursuing this further, they identified bundles of actin filaments (dorsal actin cables) moving rearward over the nucleus and forming aligned arrays with the LINC complex. These transmembrane actin-associated nuclear lines (TAN lines) appear closely related to arcs that arise near the cell front and move backwards via retrograde flow [81]. However, once these arcs pass over the nucleus, they engage and align the LINC complex. The rearward movement of nuclei and TAN lines is closely correlated. Disrupting the LINC complex disrupts TAN lines and prevents nuclear movement in response to migration signals [81]. Depletion of emerin, an inner nuclear membrane protein involved in the interaction of the LINC complex with the nuclear lamins, caused the TAN lines to slip over immobile nuclei [82].

Examining the relationship between cell and nuclear shape, Wirtz's group identified a subset of ventral SFs that wrap over the nucleus. These SFs connect to the nucleus via the LINC

complex and form a perinuclear “actin cap”. These SFs were implicated in determining nuclear shape, which was observed to be elongated in the direction of cell migration in moving cells, but round in stationary cells [17]. Like other ventral SFs, the SFs of the actin cap are anchored by FAs at each end. However, these FAs and their associated SFs are distinctive in several respects. The FAs have a larger area and are more elongated than the FAs associated with other ventral SFs [83]. Together with the SFs of the actin cap, they are more susceptible to disassembly by the actin depolymerizing drug Latrunculin B. They are also more dynamic, with several FA components having shorter half-lives. Strikingly, the FAs associated with the actin cap were the first to disassemble after adhesion to soft substrata, leading to the conclusion that these FAs are more mechanosensitive than most FAs [83]. One has to wonder whether these actin cap-associated FAs are the same as those described by Waterman’s group, i.e. fluctuating in tension to “sense” the stiffness of the substratum [54]. Persistent migration was associated with an actin cap, whereas this disassembled when cells paused to change direction. Experimentally disrupting the actin cap inhibited persistent cell migration [84].

The above results raise the question of what the role of the nucleus is in cell migration, a topic beyond the scope of this brief review. However, numerous studies have observed that disrupting the LINC complex and hence the connections between the nucleus and the cytoskeleton have major effects on cell migration [78,85]. Whereas the nucleus appears important in cell migration, it should also be remembered that cells lacking a nucleus are capable of migrating [86,87].

## 8. Concluding remarks

Our knowledge of the structure and organization of SFs and FAs has increased greatly in the last few years. Although there are still components that need to be identified, much of the analysis is moving toward the biophysical and attempting to understand how these structures that generate and transmit tension are organized by the forces that they are experiencing. One area that will be important in the future is characterizing which protein interactions within SFs and FAs are governed by catch bonds. Another area concerns how cells respond to different strain rates and the effect of different force regimes. Already some important results have been obtained. For example, Fletcher’s group found that the rate of application of force impacts a cell’s response [88]. A step displacement causing the height of a 12 mm tall cell to increase by 1 mm resulted in immediate increase in tension followed by a rapid viscoelastic relaxation to a new baseline above the previous steady-state level. However, when the same change in height was imposed at a rate of 0.1 mm/min, there was just a gradual increase in steady-state tension to a new level that was below the value eventually achieved by the step increase in height. There was also no viscoelastic relaxation. When the height was increased ten times faster (by 1 mm/min), there was a much greater increase in steady-state tension, but again no viscoelastic relaxation [88]. High rates of strain have also been found to lead to fluidization of the cytoskeleton [89,90]. Reconciling the different responses to force, i.e. reinforcement versus fluidization will be important. Although this may partly reflect differences in experimental conditions, ultimately it will involve understanding how the different protein interactions respond to different force regimes and whether slip or catch bonds are involved.

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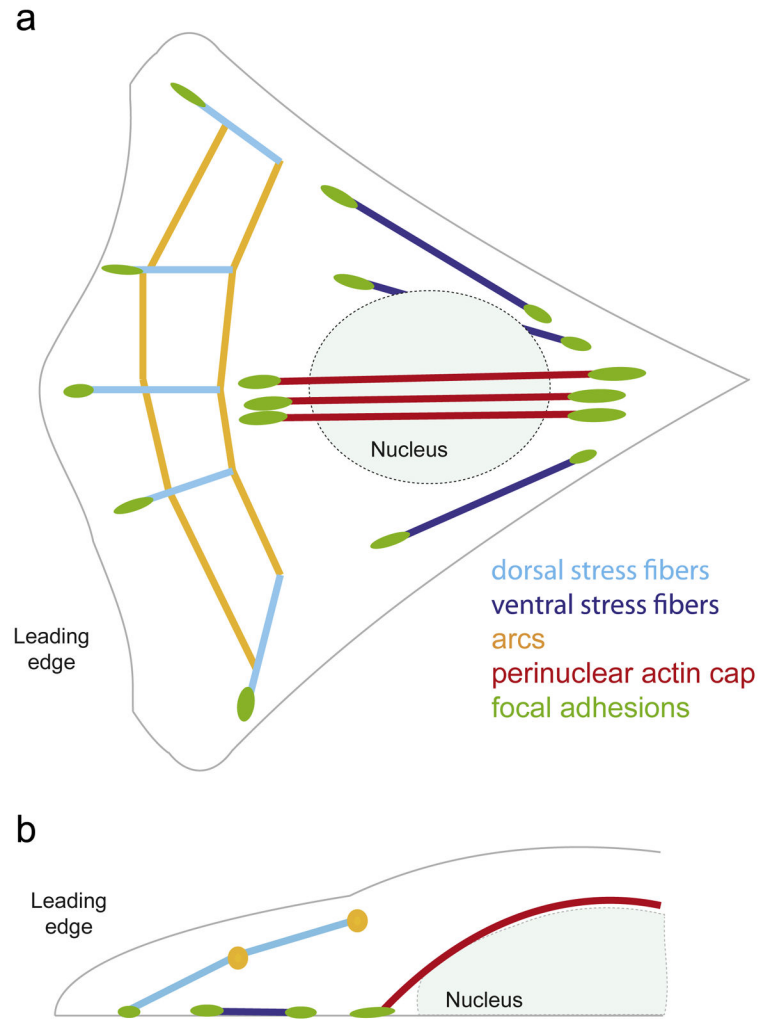
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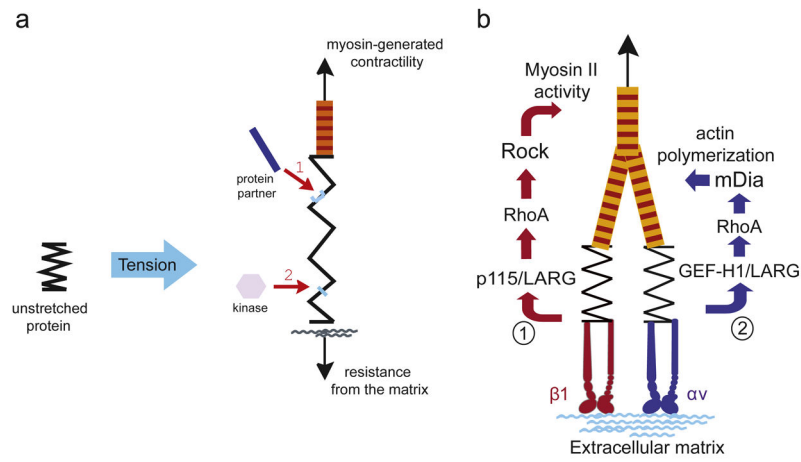
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**Fig. 1.** Types of stress fiber in migrating cells. Schematic representation of SFs in motile cells, (a) top and (b) side views. Four categories of SFs are observed: dorsal SFs, transverse arcs, ventral SFs and the perinuclear actin cap.





**Fig. 2.** Mechanical tension activates signaling pathways at adhesion sites. a. Exposure of cryptic sites. Tension can affect protein conformation to expose protein binding sites (e.g. talin) or sites for kinases or other protein modifications (e.g. p130cas). b. RhoA regulation of adhesion maturation through Rock and mDia. Adhesion to fibronectin activates RhoA through p115 RhoGEF and LARG [91], leading to Rock-mediated myosin stimulation. In response to tension, GEF-H1, LARG and mDia are recruited [32,37] and promote actin polymerization. B1 (1) and  $\alpha v$  (2) integrin subtypes cooperate to regulate RhoA signaling and adhesion maturation [62].