Actin stress fibers – assembly, dynamics and biological roles

Sari Tojkander, Gergana Gateva and Pekka Lappalainen*
Institute of Biotechnology, P.O. Box 56, University of Helsinki, 00014, Finland
*Author for correspondence (pekka.lappalainen@helsinki.fi)

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Summary
Actin filaments assemble into diverse protrusive and contractile structures to provide force for a number of vital cellular processes. Stress fibers are contractile actomyosin bundles found in many cultured non-muscle cells, where they have a central role in cell adhesion and morphogenesis. Focal-adhesion-anchored stress fibers also have an important role in mechanotransduction. In animal tissues, stress fibers are especially abundant in endothelial cells, myofibroblasts and epithelial cells. Importantly, recent live-cell imaging studies have provided new information regarding the mechanisms of stress fiber assembly and how their contractility is regulated in cells. In addition, these studies might elucidate the general mechanisms by which contractile actomyosin arrays, including muscle cell myofibrils and cytokinetic contractile ring, can be generated in cells. In this Commentary, we discuss recent findings concerning the physiological roles of stress fibers and the mechanism by which these structures are generated in cells.

Key words: Actin, Adhesion, Assembly, Mechanotransduction, Stress fibers

Introduction
The actin cytoskeleton has a fundamental role in various cellular processes such as migration, morphogenesis, cytokinesis, endocytosis and phagocytosis. Consequently, the precise regulation of the structure and dynamics of the actin cytoskeleton is essential for many developmental and physiological processes in multicellular organisms, and abnormalities in actin dynamics are associated with many pathological disorders such as cancer, neurological disorders and myofibrillar myopathies (Pollard and Cooper, 2009).

The most important physiological function of actin filaments in cells is to produce force for the above-mentioned cellular processes. Actin filaments achieve this function by two distinct mechanisms. First, coordinated polymerization of actin filaments against cellular membranes provides force, for example, for the generation of plasma membrane protrusions during cell migration and morphogenesis and for the formation of plasma membrane invaginations in endocytosis. During these processes, the structure and dynamics of actin filament networks are precisely regulated by a large array of actin-binding proteins, which control the nucleation, elongation and disassembly of actin filaments as well as their organization into desired three-dimensional arrays (Kaksonen et al., 2006; Pollard and Cooper, 2009; Bugyi and Carlier, 2010).

In addition to protrusive actin filament networks, in which the force is produced through actin polymerization, actin filaments together with myosin II filaments form contractile structures in cells. Here, the force is produced by ATP-driven movement of the myosin II motor domains along the actin filaments. Because myosin II assembles into bi-polar bundles, and the actin filaments in these structures are arranged in bi-polar arrays, the motor activity of myosin II bundles results in the contraction of the actomyosin bundle. In animal cells, contractile actomyosin structures include the cytokinetic contractile ring, myofibrils of muscle cells and stress fibers of non-muscle cells. Whereas the assembly mechanisms of protrusive actin filament networks are relatively well understood, the molecular mechanisms underlying the assembly of myosin-II-containing contractile actin filament structures, such as stress fibers and muscle myofibrils, are still largely unknown (Ono, 2010; Michelot and Drubin, 2011). In this Commentary, we discuss the assembly, organization and physiological roles of stress fibers, as well as possible similarities in the pathways generating diverse contratile actomyosin structures in cells.

Different stress fiber types in animal cells
Stress fibers are the major contractile structures in many cultured animal cells. These actomyosin bundles are especially prominent in fibroblasts, smooth muscle, endothelial and some cancer cell lines. Stress fibers in non-motile cells are usually thick and relatively stable. By contrast, highly motile cells typically contain fewer, thinner and more dynamic stress fibers (Pellegrin and Mellor, 2007).

Actin filaments are polar helical structures, with a rapidly growing barbed end and a slowly growing pointed end (Pollard and Cooper, 2009). Stress fibers are composed of bundles of ~10–30 actin filaments, which are crosslinked together by α-actinin, typically in a bi-polar arrangement. These contractile actomyosin bundles are often anchored to focal adhesions, which connect the extracellular matrix (ECM) to the actin cytoskeleton (Cramer et al., 1997; Pellegrin and Mellor, 2007; Naumanen et al., 2008). However, it is important to note that stress fibers vary in their morphology and association with focal adhesions. Therefore, stress fibers can be divided into at least four different categories: dorsal and ventral stress fibers, transverse arcs and the perinuclear actin cap (Heath, 1983; Small et al., 1998; Khatau et al., 2009) (Fig. 1A,B).

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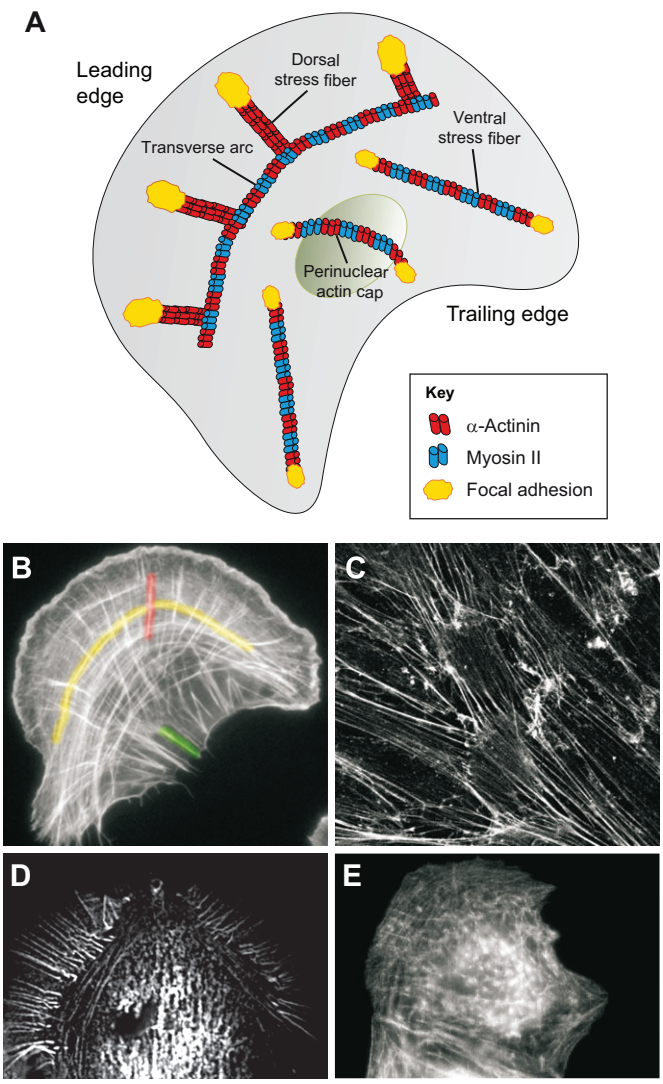

Fig. 1. Different types of stress fibers in cultured animal cells. (**A**) Schematic presentation of the stress fiber network of motile mesenchymal cells. These cells can contain at least four discrete categories of stress fibers; (i) dorsal stress fibers, which are anchored to focal adhesions at their distal end, (ii) transverse arcs, which are curved actomyosin bundles that flow towards the cell center and are typically connected to focal adhesions through interactions with dorsal stress fibers; (iii) ventral stress fibers, which are actomyosin bundles anchored to focal adhesions at both ends, and (iv) perinuclear actin cap bundles, which resemble ventral stress fibers but their central parts are located above the nucleus. (**B**) The stress fiber network of a motile U2OS cells. Shown here are examples of dorsal stress fibers, arcs and ventral stress fibers, indicated with red (dorsal), yellow (arcs) and green (ventral) lines, respectively. (**C**) Stress fibers in human umbilical vein endothelial cells (HUVECs). In these cells, stress fibers from neighboring cells are physically connected through discontinuous adherens junctions. (**D**) Visualization of actin arcs in a living neuron isolated from *Aplysia*. (**E**) Stress-fiber-like precursors of myofibrils in cultured rat neonatal cardiomyocytes. The actin filaments in panels shown in B–E are visualized by phalloidin staining. The images shown in panels B, C and D are from (Hotulainen and Lappalainen, 2006; Millan et al., 2010; Schaefer et al., 2008), respectively, and reproduced with permissions from the Rockefeller University Press and Elsevier.

Dorsal stress fibers are anchored to focal adhesions at their distal ends. These actin filament bundles do not typically contain myosin II (Tojkander et al., 2011). Therefore, unlike the other types of stress fibers discussed below, they cannot contract. The exact organization of actin filaments in dorsal stress fibers is not known, but in structurally related ‘graded polarity bundles’, the distal ends are composed of unipolar actin filaments with rapidly growing barbed ends that face the cell periphery, whereas the more proximal parts of the bundle are composed of actin filaments with mixed polarity (Cramer et al., 1997; Pellegrin and Mellor, 2007). Although they do not possess the ability to contract, dorsal stress fibers appear to serve as a platform for the assembly of other types of stress fibers, as well as to link them to focal adhesions (Hotulainen and Lappalainen, 2006; Tojkander et al., 2011).

Transverse arcs are curved actin filament bundles, which display a periodic α-actinin–myosin pattern that is typical for contractile actomyosin bundles. Arcs do not directly attach to focal adhesions, but convey contractile force to the surrounding environment through their connections with dorsal stress fibers. An important feature of transverse arcs in migrating cells is their ability to flow from the leading cell edge towards the cell center (Heath, 1983; Small et al., 1998; Hotulainen and Lappalainen, 2006). This process, known as retrograde flow, is believed to be driven by the continuous contraction of arcs (Zhang et al., 2003).

Ventral stress fibers are contractile actomyosin bundles that are attached to focal adhesions at both ends, and they represent the major contractile machinery in many interphase cells (Small et al., 1998). Ventral stress fibers are often located at the posterior parts of the cell, where occasional contraction cycles promote rear constriction and facilitate cell movement (Chen, 1981; Mitchison and Cramer, 1996).

The perinuclear actin cap is a recently identified actin structure, which consists of stress fibers positioned above the nucleus. The key function of the perinuclear actin cap is to regulate the shape of the nucleus in interphase cells. Furthermore, the perinuclear actomyosin fibers might act as mechanotransducers to convey force from the cell environment to the nucleus (Khatau et al., 2009). It is also important to note that certain stress-fiber-like structures also associate with the nuclear membrane through specific membrane proteins (Luxton et al., 2010) and stabilize the position of the nucleus (Nagayama et al., 2011). Thus, similar to the connections that canonical stress fibers make with the ECM through focal adhesions, a subset of stress fibers appears to be mechanically connected to nuclear membrane proteins to regulate nuclear movement.

Lamella are composed of a network of contractile transverse arcs

Two distinct actin filament networks, lamellipodium and lamella, have been proposed to contribute to cell migration. Although it is well established that lamellipodium is composed of a branched network of actin filaments, elucidation of the origin and organization of actin filaments in the lamellum has been more elusive (Vallotton and Small, 2009; Ydenberg et al., 2011). However, several recent studies provide evidence that, in migrating cells, the lamellum corresponds to the transverse arc network, as both structures are composed of condensed actin bundles and undergo retrograde flow towards the cell center (Ponti et al., 2004; Hotulainen and Lappalainen, 2006). Lamella and arcs also display similar protein compositions. For example,

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tropomyosins, which are nearly absent from the lamellipodium, are highly enriched in the lamellum (DesMarais et al., 2002) and in transverse arcs (Tojkander et al., 2011). Importantly, both arcs and the lamellum are generated through the condensation of lamellipodial actin filaments into arc-shaped actin bundles that run parallel to the cell edge (Hotulainen and Lappalainen, 2006; Tojkander et al., 2011; Burnette et al., 2011).

Stress fibers in cell adhesion, migration and mechanotransduction

An important feature of many cells is their ability to migrate towards particular chemical or mechanical stimuli. This is crucial, for example, during development and wound healing (Gilbert, 2003; Ridley et al., 2003). Focal adhesions are complex structures that ensure the proper communication between the cell and the ECM during adhesion and migration. Focal adhesions are often connected to actin stress fibers, which thus appear to play an important role in cell adhesion and migration (Geiger et al., 2009; Parsons et al., 2010). The assembly, growth and maintenance of focal adhesions depend on mechanical stress. Inhibition of myosin-II-promoted contractility leads to a decrease in focal adhesion size (Balaban et al., 2001; Chrzanowska-Wodnicka and Burridge, 1996; Helfman et al., 1999), whereas applying mechanical force to the adhesions increases their size in a myosin-II-independent manner (Riveline et al., 2001). The mechanical force that is transmitted to focal adhesions by stress fibers can alter the conformation of mechanosensitive focal adhesion proteins, including that of p130CAS (also known as BCAR1) (Sawada et al., 2006), β-integrin (Puklin-Faucher et al., 2006) and talin (Gingras et al., 2006; Papagrigoriou et al., 2004; del Rio et al., 2009). This suggests that stress fiber tension or contractility can convert mechanical signals into biochemical cues, and thus has an important role in focal adhesion maturation and dynamics (Johnson et al., 2007; Vogel, 2006).

Stress fibers are also dependent on tension and myosin-II-mediated contractility because myosin II inhibition leads to the disassembly of stress fibers (Bershadsky et al., 2006; Chrzanowska-Wodnicka and Burridge, 1996). Furthermore, many focal-adhesion-associated proteins are involved in stress fiber regulation. For example, zyxin has been implicated in force-dependent actin polymerization (Hirata et al., 2008), stress fiber mechanosensing (Colombelli et al., 2009) and stress fiber repair (Smith et al., 2010).

Although stress fibers contribute to cell adhesion, their exact role in cell migration has been more elusive. Stress fibers are absent from many highly motile cells, such as leukocytes (Valerius et al., 1981) and *Dictyostelium discoideum* amoeba (Rubino et al., 1984). These observations, together with the apparent lack of stress fibers in cells that have been embedded in a three-dimensional environment led to the suggestion that they are not essential for cell migration (Burridge et al., 1988). Indeed, it is possible that, under many conditions, stress fibers instead inhibit cell motility because the reorganization of stable actin bundles and focal adhesions can be a relatively slow process. The physiological significance of stress fibers in cell migration might thus be linked to their role in constricting the ECM and deforming the substrate through the generation of tension (Castella et al., 2010). This could be important in wound healing processes and in cell migration on stiff matrices. Recent data, which reveal focal adhesions and stress-fiber-like actomyosin bundles in cells cultured in a three-dimensional matrix, should therefore encourage more thorough studies that investigate the presence and function of stress fibers in cells in the context of a three-dimensional milieu (Kubow and Horwitz, 2011; Fischer et al., 2009).

Stress fibers also have an important role in mechanosensing. First, as described above, the contractile force generated by stress fibers regulates the assembly and dynamics of focal adhesions. This has also been demonstrated by recent laser nanosurgery and drug treatment experiments showing that the localization of zyxin to focal adhesions is rapidly regulated by the force that is generated by stress fibers (Colombelli et al., 2009). Second, direct mechanical stimulation (stretching) of an actin stress fiber using optical tweezers can activate mechanosensitive channels in cultured human umbilical vein endothelial cells (Hayakawa et al., 2008). Thus, the force generated by stress fibers might regulate many different biochemical or signaling pathways in cells. The ability of cells to sense the mechanical aspects of the environment is important for cell differentiation and cell fate determination. Substrate stiffness controls the organization and prominence of stress fibers and the maturation of actomyosin bundles in myotubes (Discher et al., 2005; Geiger et al., 2009; Walcott and Sun, 2010). Furthermore, environmental mechanosensing is a crucial factor in stress fiber organization and the lineage determination of stem cells (Engler et al., 2006; Zemel et al., 2010).

Physiological roles of stress fibers

Biochemical and mechanical interactions between cells and the environment modulate stress fiber abundance, structure and organization. Therefore, it is not surprising that only a fraction of cells in our bodies contain these contractile actomyosin bundles. Cells assemble stress fibers only when they encounter mechanical stress (force). Thus, most animal cell types that are grown on rigid substrates, such as glass or plastic, display thick stress fibers, whereas these structures are typically absent or only very thin in the same cells grown on soft substrates. Furthermore, both stress fibers and focal adhesions are aligned along the major cell axis when cells are grown on a rigid matrix, whereas, on a compliant matrix, focal adhesions are smaller and stress fibers are poorly aligned (Discher et al., 2005; Prager-Khoutorsky et al., 2011) (Fig. 2). Consequently, the most prominent examples of stress fibers in tissues are found under conditions in which

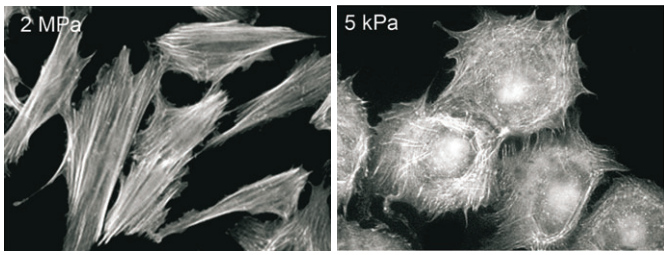

Fig. 2. Effect of substrate stiffness on stress fibers. Stress fibers are more prominent in cells that are grown on rigid than on soft substrates. Shown here are fibroblasts that have been plated on substrates with various rigidities that are made of poly(dimethylsiloxane) (PDMS). As shown in the image on the left, cells plated on a rigid (2 MPa) substrate display thick and well-aligned stress fibers. By contrast, as shown on the right, cells plated on a compliant (5 kPa) substrate display thinner and poorly oriented stress fibers. Images are from (Prager-Khoutorsky et al., 2011) and reproduced with permission from Nature Publishing Group.

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cells are confronted with considerable mechanical stresses. For example, during their development to myofibroblasts in dermal wound tissue, fibroblasts develop prominent stress fibers, which allow wound closure through the generation of tension and ECM remodelling (Sandbo and Dulin, 2011). However, in contrast to stress fibers of non-muscle cells, in which β-actin is the main actin isoform, myofibroblasts express smooth muscle α-actin that is incorporated into stress fibers (Hintz et al., 2002). Mechanical tension during wound closure also induces stress fiber assembly in epithelial cells, which then differentiate into myoepithelial cells (Pellegrin and Mellor, 2007). In developing animals, stress fibers are also present, for example, in epithelial cells during dorsal closure in *Drosophila* embryos (Jacinto et al., 2002).

The hydrostatic pressure and cyclic strain in the vasculature represent a major stress on the endothelial cells lining the blood vessels. Therefore, it is not surprising that these cells assemble prominent stress fibers (Wong et al., 1983). Interestingly, in cultures of endothelial cells, stress fibers in adjacent cells can become linked with each other through adherens junctions, suggesting that stress fibers can be also stabilized by multi-protein complexes associated with adherens junctions that are distinct from focal adhesions (Millan et al., 2010) (Fig. 1C). After applying fluid shear stress, cultured endothelial cells show marked elongation and orientation in the flow direction. In addition, thick stress fibers appear and align along the long axis of the cell. Thus, it is believed that stress fibers also contribute to the resistance of endothelial cells against fluid shear (Sato and Ohashi, 2005).

Contractile stress fibers are also typical for certain other types of specialized animal cells. For example, stress-fiber-like structures serve as templates for the assembly of myofibrils during the development of striated muscle cells, and transverse arcs that undergo typical retrograde flow are also present in the neuronal growth cones (Sanger et al., 2005; Schaefer et al., 2008) (Fig. 1D,E). Contractile transverse arcs are also present in the immunological synapses of T lymphocytes, where they regulate the dynamics of receptor clusters (Yi et al., 2012).

Stress-fiber-associated proteins

Actin and myosin are the main components of stress fibers and form a functionally strictly controlled actomyosin structure that is responsible for stress fiber contraction. In addition, several actin-binding proteins (ABPs) and focal-adhesion-associated proteins localize to stress fibers, and regulate their assembly and stability (Table 1). The interactions of ABPs with stress fibers are usually highly dynamic, as seen in fluorescence recovery after photobleaching (FRAP) experiments (Schmidt and Nichols, 2004; Hotulainen and Lappalainen, 2006; Endlich et al., 2009; Tojkander et al., 2011). This suggests that stress fibers are dynamic structures, and the proteins that associate with them display constant dissociation and association.

The different ABPs can be classified according to their biochemical functions and their localizations along the stress fibers. α-Actinin, an actin crosslinking protein, displays a punctuate localization pattern on stress fibers that is complementary to the localization of myosin II (Lazarides and Burridge, 1975) (see Fig. 1A). Its two isoforms α-actinin-1 and -4 are expressed in many non-muscle cells. In cultured cells, α-actinin-1 is enriched in stress fibers, whereas α-actinin-4 is more prominently localized to the lamellipodial actin filament network (Honda et al., 1998). In addition to its actin-bundling activity, α-actinin is associated with kinases and signaling proteins, such as PDZ-LIM-containing proteins, thus acting as a mediator for cytoskeleton-targeted signaling (Vallenius et al., 2000, Vallenius and Mäkelä, 2002). A number of other actin filament crosslinking proteins, including fascin, filamin and palladin, localize to stress fibers, but their exact functions in these actomyosin bundles remain largely unknown (Adams, 1995; Wang et al., 1975; Dixon et al., 2008). Importantly, besides their crosslinking activity, many of these proteins have also additional roles in the regulation of cytoskeletal dynamics. Palladin, for example, interacts with the actin-binding proteins profilin and vasodilator-stimulated phosphoprotein (VASP), and might thus function as a scaffolding protein to promote actin dynamics in stress fibers (Boukhelifa et al., 2004; Boukhelifa et al., 2006).

Another group of multifunctional proteins, which localize to stress fibers in a punctuate pattern similar to that of α-actinin, is the calponin family (Strasser et al., 1993). The most extensively studied isoform is calponin-1 (also known as calponin h1), which is expressed in smooth muscle cells and is involved in the regulation of contractility (Winder et al., 1998). The other calponin isoforms, -2 and -3 (h2 and h3, respectively), are found in many non-muscle and muscle cells (Draeger et al, 1991; Hossain et al., 2003). In addition to regulating muscle contraction, calponins have been suggested to crosslink and stabilize actin-based structures, as well as to regulate cell motility (Leinweber et al., 1999b; Danninger and Gimona, 2000). Calponins also associate with several kinases, such as extracellular-signal-regulated kinase 1/2 (ERK1/2) and protein kinase C (PKC), and might thus also act as scaffolding proteins that regulate cytoskeletal dynamics (Leinweber et al., 1999a; Patil et al., 2004).

In addition to myosin II, there are other proteins that localize to stress fibers in a complementary pattern to α-actinin, such as tropomyosins (TPMs), a large family of actin-binding proteins, and caldesmon (CaD, also known as CALD1), which also participate in the regulation of stress-fiber contraction and reorganization (Weber and Groeschel-Stewart, 1974; Lazarides, 1975; Yamashiro-Matsumura and Matsumura, 1988; Castellino et al., 1995). Non-muscle cells express several myosin II isoforms that have distinct binding partners and localization patterns, with the most thoroughly studied being myosin IIA and IIB (Vicente-Manzanares et al., 2009). Myosin II forms bipolar bundles that are indispensable for the formation and maintenance of stress fibers (Bao et al., 2005; Hotulainen and Lappalainen, 2006). Myosin II is recruited to contractile stress fibers by TPMs (Gunning, 2008; Tojkander et al., 2011). In muscle cells, TPMs cooperate with troponin and control contraction by steric inhibition of the actin myosin interface in a Ca^{2+}-dependent manner (McKillop and Geeves, 1993). Additionally, TPMs control actin dynamics by preventing filament depolymerization at pointed ends and by inhibiting the actin filament disassembly that is mediated by actin depolymerization factor (ADF, or cofilin) (Broschat, 1990; Ono and Ono, 2002). Loss or inactivation of specific TPMs have been linked to abnormal actin stress fiber structures that can result from enhanced filament disassembly and impaired myosin recruitment (Gupton et al., 2005; Tojkander et al., 2011). TPMs also stabilize actin filaments in cooperation with CaD (Ishikawa et al., 1989a; Ishikawa et al., 1989b), which binds actin with its C-terminal region and myosin with its N-terminal region, thereby promoting the crosslinking of myosin bundles to actin filaments (Marston et al., 1992;

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Katayama et al., 1995). The interaction of CaD with actin is regulated by phosphorylation, and during the cell cycle CaD phosphorylation leads to disassembly of actin stress fibers (Kordowska et al., 2006). Owing to the similar localization pattern of CaDs and TPMs on stress fibers, and their tightly linked expression levels, the functions of these two protein families in stress fibers are likely to be closely connected (Yamashiro-Matsumura and Matsumura, 1988; Kashiwada et al., 1997). In addition to the above-mentioned proteins, stress fibers also contain several other proteins that function in stress fiber assembly, contractility or repair (Table 1).

Stress fiber assembly

Signaling pathways that operate upstream of actin-binding proteins control the appropriate assembly of stress fibers in a temporal and spatial manner. The small GTPases RhoA, Rac1 and Cdc42 are the central regulators of actin dynamics in a wide range of eukaryotic organisms (Heasman and Ridley, 2008). Among these, at least RhoA directly promotes stress fiber assembly through its effectors, Rho-associated protein kinase (ROCK) and the formin mDia1 (mammalian Dia1; also known as DIAPH1 and DRF1) (Leung et al., 1996; Watanabe et al., 1997). mDia1 facilitates the polymerization of long parallel actin filaments and is important for the formation of dorsal stress fibers (Tominaga et al., 2000; Hotulainen and Lappalainen, 2006), whereas ROCK inhibits ADF- or cofilin-mediated disassembly of actin filaments through activation of LIM domain kinase 1 (LIMK1) (Maekawa et al., 1999). In addition to its direct effects on the actin cytoskeleton, RhoA signaling regulates the transcription of several genes that encode cytoskeletal proteins through myocardin-related transcription factor (MAL, also known as MKL1 and MRTF-A) and serum response factor (SRF) pathway, and thus also controls the composition of the actin cytoskeleton (Hill et al., 1995; Miralles et al., 2003).

Rac1 and Cdc42 coordinate stress fiber assembly in more indirect ways. Rac1 is involved in lamellipodia and membrane ruffle formation by activating the actin nucleating complex Arp2/3, whereas Cdc42 induces filopodia formation by promoting actin polymerization through the formin mDia2 (also known as DIAPH3 and DRF3) (Pollard, 2007). Both Arp2/3-nucleated filaments and mDia2-nucleated filaments act as building blocks for contractile stress fibers, at least in cultured human osteosarcoma U2OS cells. Arp2/3 induces the formation of α-actinin-crosslinked actin filaments, which assemble endwise with mDia2-induced and tropomyosin-decorated actin filaments to yield transverse arcs near the leading edge of the cell (Hotulainen and Lappalainen, 2006; Tojkander et al., 2011). Additionally, filaments generated in filopodial protrusions can be recycled for construction of stress fiber structures (Nemethova et al, 2008; Anderson et al., 2008). The formins mDia1 and mDia2 can also be activated by the small RhoA-related GTPase RhoF (also known as ARHF and Rif), and might therefore also contribute to the formation of stress fibers, at least in certain specific cell types (Fan et al., 2010; Tojkander et al., 2011).

Recent live-cell microscopy studies have provided valuable information regarding the assembly mechanisms of different types of stress fibers. Dorsal stress fibers, which are linked to focal adhesions at their distal ends, are generated through actin polymerization at focal adhesions. As the cell moves forwards, new focal adhesions appear and the elongation of dorsal stress fibers begins from these adhesion sites (Hotulainen and Lappalainen, 2006). The polymerization of dorsal stress fibers is linked to the formation of a contractile stress fiber network. During protrusion, the plasma membrane at the leading edge undergoes constant cycles of extension and retraction. In the

Table 1. Actin stress fiber components

Stress fiber component	Function	References
α-Actinin	Filament crosslinking, signal transduction	(Lazarides and Burridge, 1975; Sjöblom et al., 2008)
Caldesmon	Regulation of contractility, cell motility and stress fiber stability	(Mayanagi and Sobue, 2011)
Calponin	Regulation of contractility, motility, stability of actin-based structures, signal transduction	(Rozenblum and Gimona, 2008)
Serine/threonine protein kinase 35 (CLIK1 or STK35)	Signal transduction	(Vallenius and Mäkelä, 2002)
Coactosin	F-actin binding	(Hou et al., 2009)
Cysteine-rich protein 1 (CRP1)	Actin filament bundling	(Tran et al., 2005)
Fascin	Actin filament bundling	(Yamashiro-Matsumura and Matsumura, 1988; Hashimoto et al., 2011)
FH1/FH2 domain-containing protein 1 (FHOD1)	Stress fiber stabilization	(Jurmeister et al., 2011)
Filamin	Filament crosslinking, mechanosensing	(Brotschi et al., 1978; Ehrlicher et al., 2011)
Myosin phosphatase Rho-interacting protein (MPRIP)	Control of MLC phosphorylation through MLCP	(Mulder et al., 2003; Koga and Ikebe, 2005)
Myosin II	Stress fiber contraction	(Vicente-Manzanares et al., 2009)
NUAK family SNF1-like kinase 1 and 2 (NUAK1 and NUAK2)	Regulation of MLC phosphorylation	(Vallenius et al., 2011)
Palladin	Actin crosslinking, cytoskeletal scaffold	(Parast and Otey, 2000; Mykkänen et al., 2001; Goicoechea et al., 2008)
PDZ-LIM proteins	Signal transduction	(Vallenius et al., 2000; Krcmery et al., 2010)
Rho-associated protein kinase (ROCK)	MLC phosphorylation	(Kawabata et al., 2004)
Septin2 (SEPT2)	Regulation of myosin II activity	(Joo et al., 2007)
Transgelin	Stabilization of stress fibers, regulation of contraction and cell motility	(Assinder et al., 2009)
Tropomyosin	Stabilization of stress fibers, myosin recruitment, regulation of contraction	(Gunning, 2008; Tojkander et al., 2011)
Vasodilator-stimulated phosphoprotein (VASP)	Stress fiber assembly	(Boukhelifa et al., 2004)
Zyxin	Stress fiber stability and repair	(Smith et al., 2010)

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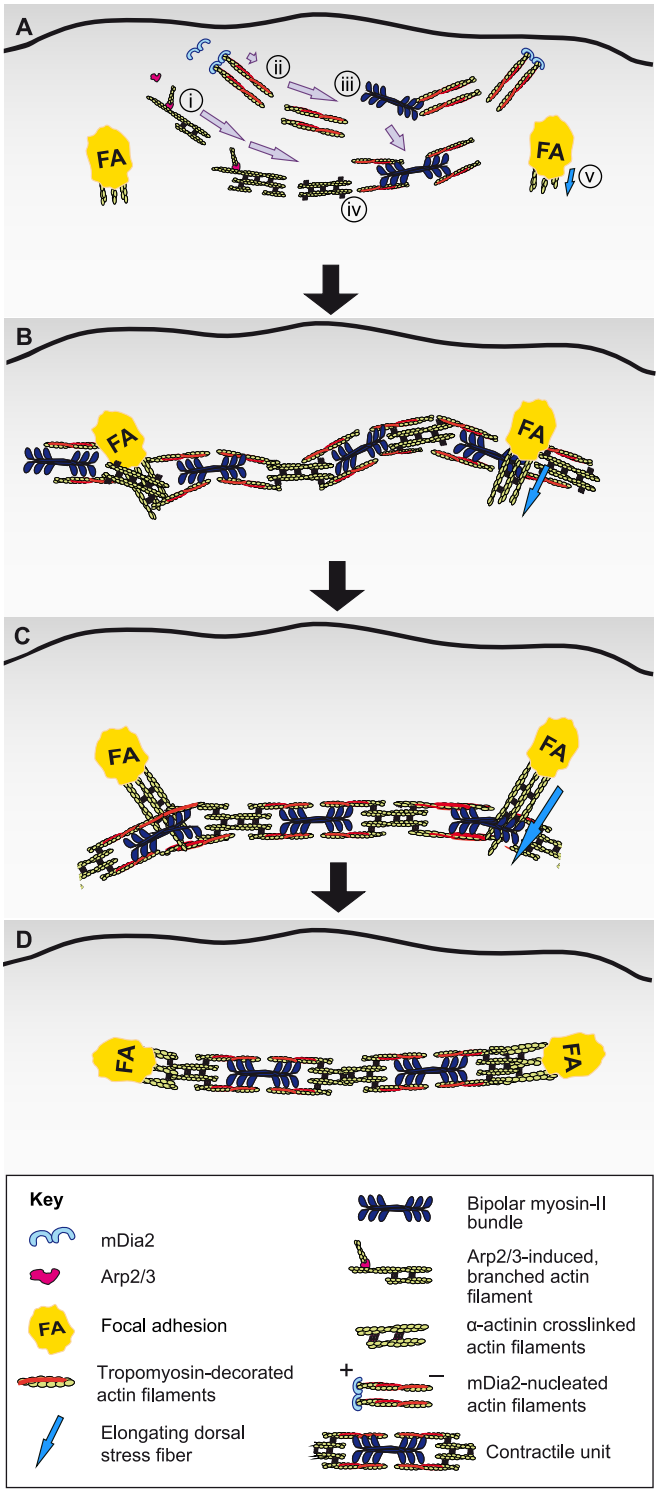
retraction phase, precursors of transverse arcs appear through the condensation of actin and myosin bundles in the lamellipodium (Burnette et al., 2011). More specifically, these structures are generated through the endwise assembly of Arp2/3-nucleated and α-actinin-crosslinked actin filament bundles and of formin-nucleated actin bundles that contain tropomyosin and myosin (Hotulainen and Lappalainen, 2006; Tojkander et al., 2011) (Fig. 3A). These nascent arcs move towards the cell center and collide with immobile focal adhesions (Shemesh et al., 2009). Coupling of actin filaments with adhesion sites probably decreases the velocity of the arc precursors (Hu et al., 2007; Gardel et al., 2008; Burnette, 2011), which then start to condense into mature arcs at the lamellipodium–lamella boundary (Shemesh et al., 2009) (Fig. 3B). These connections between newly formed arcs and elongating dorsal stress fibers occur early in the stress fiber assembly process, and several arcs are able to associate with a single dorsal stress fiber and to flow towards the cell center together with the elongating dorsal stress fiber (Tojkander et al., 2011) (Fig. 3C). In contrast to the assembly of dorsal stress fibers and arcs, ventral stress fibers can be formed from the pre-existing dorsal stress fiber and arc network (Hotulainen and Lappalainen, 2006) (Fig. 3D). In addition, ventral stress fibers can be generated by the fusion of two dorsal stress fibers that are attached to focal adhesions (Small et al., 1998). A schematic model for the mechanisms of stress fiber assembly in cultured U2OS cells is presented in Fig. 3.



Regulation of stress fiber contraction

The contractility of stress fibers is regulated by phosphorylation of the myosin light chain (MLC; these proteins have the symbol MYL in mammals) (Somlyo and Somlyo, 2000). Reversible phosphorylation of MLC on Thr18 and Ser19 increases the assembly of non-muscle myosin II filaments and the actin-activated ATPase activity of the myosin motor domain (Vicente-Manzanares et al., 2009). MLC-phosphorylation-mediated contractility of stress fibers is controlled by at least two distinct pathways, a Ca^{2+}/calmodulin-dependent pathway and a Rho-dependent pathway (Katoh et al., 2001a; Katoh et al., 2001b). The Ca^{2+}/calmodulin pathway works in a similar manner in both smooth muscle and non-muscle cells and leads to the activation of the myosin light chain kinase (MLCK) and subsequent phosphorylation of MLC. The Rho–ROCK pathway results in actomyosin activity either through direct phosphorylation of MLC or by inhibiting the phosphorylation of the myosin light chain phosphatase (MLCP) (Amano et al., 1996; Kimura et al., 1996; Totsukawa et al., 2000). Both phosphorylation pathways generate distinct contractile responses; the Ca^{2+}/calmodulin pathway leads to a local and rapid response, whereas activation of the Rho pathway results in a more sustained response. In addition, the septin SEPT2, a member of a conserved family of

Fig. 3. A hypothelical model for the assembly of stress fibers. (**A**) Formation of arc precursors from two lamellipodial filament populations. Transverse arcs are generated from Arp2/3-nucleated, α-actinin crosslinked lamellipodial actin filaments (i), and formin-nucleated, tropomyosin-decorated lamellipodial actin filaments (ii). The tropomyosin-decorated filaments form a platform for myosin II recruitment and/or myosin II filament assembly (iii). The α-actinin crosslinked and myosin-II-containing actin filament populations assemble endwise with each other to form the precursors of transverse arcs (iv). Dorsal stress fibers elongate through actin polymerization at focal adhesions (v). (**B**) Formation of an intact stress fiber network. Collision of nascent arcs with focal adhesions provokes arc condensation. These mature arcs become connected with the focal-adhesion-attached dorsal stress fibers to generate a stress fiber network. (**C**) Retrograde flow of transverse arcs. The dorsal-attached arcs flow towards the cell center with a similar velocity to that of the dorsal stress fiber elongation. (**D**) Formation of ventral stress fibers. A ventral stress fiber is generated from two focal-adhesion-attached dorsal stress fibers and a transverse arc segment located between the two dorsal stress fibers. Barbed and pointed ends of actin filaments are indicated by '+' and '−', respectively, in the figure key.

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filamentous GTPases, has a role in the regulation of contractile structures through binding and activation of myosin II. Septins are associated with actin stress fibers in interphase cells and with the contractile ring in dividing cells. As inhibition of the interaction between SEPT2 and myosin II in interphase cells results in the loss of stress fibers, the septin-mediated regulation of myosin II activity also appears to be essential for the appropriate assembly of stress fibers and/or their maintenance (Kinoshita et al., 2002; Joo et al., 2007).

Dephosphorylation of MLC and disassembly of stress fibers is mediated by MLCP, which is targeted to actin fibers through interactions of its regulatory subunit MYPT1 with the myosin phosphatase Rho-interacting protein (MPRIP) (Mulder et al., 2003; Surks et al., 2005). Interestingly, the activity of MLCP can be regulated by the tumor suppressor liver kinase B1 (LKB1, also known as STK11) through its interaction with NUAK family kinases, indicating that both phosphorylation and dephosphorylation of MLC are interlinked with multiple signaling pathways (Zagorska et al., 2010; Vallenius et al., 2011).

Are all contractile actomyosin bundles assembled through a common mechanism?

As discussed above, actin stress fibers are composed of a bipolar array of actin filaments and display a periodic localization pattern of α-actinin and myosin (Langanger et al., 1986; Cramer et al., 1997). This resembles considerably the organization of actomyosin arrays in muscle myofibrils. However, the organization of actin filaments in stress fibers is less regular compared with that of mature myofibrils, and actin filament contraction appears to be more constant with occasional or regional relaxation in comparison to the continuous contraction cycles of muscles (Peterson et al., 2004). The protein composition of stress fibers also resembles that of striated and non-striated muscle filaments, but several actin-associated protein families possess muscle-specific isoforms (Ono, 2010). The structural and functional similarities between the distinct contractile systems raise the question of whether actomyosin bundles found in different cell types could be assembled through a common mechanism.

Myofibrils in developing striated muscle cells are generated from premyofibrils, which assemble close to the plasma membrane. Premyofibrils are stress-fiber-like bundles, which display a less regular organization of α-actinin and myosin II compared with that of mature myofibrils. The clear gaps in actin filament bundles, which are typical for mature myofibrils, are not present in premyofibrils. Thus, the organization of actin filaments in premyofibrils resembles that of stress fibers in non-muscle cells (Sanger et al., 2005, Sanger et al., 2009). Although many sarcomeric proteins are present in premyofibrils, they contain non-muscle myosin II instead of the muscle-specific myosin II isoform (Handel et al., 1991; Sanger et al., 2010). Premyofibrils thus display some similarities to the transverse arcs of non-muscle cells and might also utilize similar pathways for their assembly (Sanger et al., 2009; Sparrow and Schöck, 2009). Interestingly, the assembly of the contractile ring during cytokinesis of the fission yeast *Schizosaccharomyces pombe* also involves coalescence of myosin-II-containing nodes to generate a contractile actomyosin structure (Pollard and Wu, 2010). Thus, this process might also be similar to the assembly of contractile transverse arcs at the interface of the lamellipodium and lamellum in animal cells.

The assembly of various contractile actomyosin structures also involves proteins that are shared among them. In stress fibers, formin-nucleated actin filaments become decorated by TPMs to attract myosin II to these structures. Specifically, the Tm4 isoform is involved in recruitment of myosin II to stress fibers in U2OS cells (Tojkander et al., 2011). Interestingly, TPM4 might also contribute to the assembly of premyofibrils (Vlahovich et al., 2008) and to myosin-IIA-rich podosome cores of osteoclasts (McMichael et al., 2006), suggesting that the role of this TPM isoform in the formation of distinct myosin-based structures is conserved. TPMs also regulate actomyosin interactions, including the assembly of contractile ring in fission yeast (Stark et al., 2010; Clayton et al., 2010). Furthermore, common phosphorylation sites in caldesmons are used for the regulation of actomyosin function in both non-muscle and smooth muscle cells (Yamashiro et al., 1995; Yamboliev et al., 2001). It is thus possible that the assembly mechanisms and regulation of contractile machineries are conserved, although tissue-specific protein isoforms might be used by different cell types for the generation of distinct contractile structures. It will therefore be important to study these mechanisms in different cellular contexts and also in a more physiological tissue environment.

Future perspectives

Studies during the past few years have provided valuable new information concerning the mechanisms of stress fiber assembly and the roles stress fibers have in cultured animal cells. However, we still know relatively little regarding the functions of stress fibers in animal development and physiology. Thus, in the future it will be especially important to reveal which of the cell types in the context of the entire animal contain stress fibers and what are the exact functions of contractile actomyosin bundles in these cells. As, in addition to focal adhesions, stress fibers can also be anchored to adherens junctions and the nuclear membrane (Millan et al., 2010; Luxton et al., 2010), it will be also interesting to elucidate the molecular basis of these interactions and to examine the roles of such interactions within a three-dimensional tissue environment.

Although live-cell microscopy studies have provided considerable amount of new information regarding the mechanisms of stress fiber assembly (Hotulainen and Lappalainen, 2006; Nemethova et al, 2008; Anderson et al., 2008; Burnette et al., 2011; Tojkander et al., 2011), many important questions still remain unanswered. For example, we do not know how α-actinin and tropomyosin–myosin-II nodes interact during the formation of transverse arcs, and how dorsal stress fibers and arcs are subsequently connected to each other during assembly of the contractile stress fiber network. It is also important to note that stress fibers contain several tropomyosin isoforms with distinct localization patterns and non-overlapping functions (Tojkander et al., 2011), and we need to investigate whether, for instance, focal-adhesion-anchored dorsal stress fibers comprise several distinct populations of actin filaments. Furthermore, it will important to elucidate the exact functions of the many poorly characterized stress fiber components, such as calponin, palladin and septins, in stress fiber assembly, maintenance and contractility, as well as to decipher the exact mechanisms through which the activities of these and other central stress fiber components are linked to cellular signaling pathways.

Stress fibers also resemble other contractile actomyosin structures, such as the myofibrils of muscle cells, actomyosin bundles in epithelial cells and the contractile ring. Thus, another focus of future research should be on elucidating the similarities and differences in the assembly mechanisms of these distinct actomyosin structures.

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