Actin stress fibres

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Accepted 21 August 2007
Journal of Cell Science 120, 3491-3499 Published by The Company of Biologists 2007
doi:10.1242/jcs.018473

Summary

Animal cell movement is effected through a combination of protrusive and contractile events. Non-muscle cells contain stress fibres – bundles of actomyosin that are the major mediators of cell contraction and that can be compared to the highly organised actomyosin arrays of muscle cells. Recent studies have defined regulatory mechanisms that control stress fibre formation, placing the ROCK protein kinase at the centre of a complex signalling network controlling actomyosin contractility and stress fibre assembly. As we uncover the details of stress fibre construction, it is becoming clear that different categories of stress fibres exist. Some of these structures are less suited for cell motility and more suited to static contraction. In keeping with this, many specialised contractile cell types use stress fibres to remodel tissues and extracellular matrix.

Key words: Stress fibre, Stress fibre, Rho GTPase, Actin, Contractility, Cell migration, Myofibroblast, Myoepithelial

Introduction

We have known for over 50 years that permeabilised non-muscle cells contract in response to ATP (Hoffmann-Berling, 1954). Early studies of non-muscle cells using light (Lewis and Lewis, 1924) and electron microscopy (Abercrombie et al., 1971; Buckley and Porter, 1967) observed long, straight bundles of microfilaments that crossed the cell body and terminated in dense plaques at the base of the cell. The term 'stress fibre' was coined to fit with the idea that these structures arise from the effects of tension on protoplasm (Lewis and Lewis, 1924). Subsequent studies showed, however, that it is the stress fibres themselves that are contractile (Isenberg et al., 1976; Kreis and Birchmeier, 1980).

Stress fibre structure

Stress fibres are composed of bundles of approximately 10-30 actin filaments (Cramer et al., 1997). These bundles are held together by the actin-crosslinking protein α-actinin (Lazarides and Burridge, 1975), although other actin-crosslinking proteins, such as fascin, espin and filamin, have also been detected in these structures (Adams, 1995; Chen et al., 1999; Wang et al., 1975). The staining pattern of α-actinin is periodic along the fibre (Fig. 1A) and alternates with bands containing non-muscle myosin (Weber and Groeschel-Stewart, 1974) and tropomyosin (Lazarides, 1975). These findings led to early models of stress fibre structure and contraction that drew strong parallels with the organisation of muscle sarcomeres (Lazarides, 1975). In such models, actin filaments are held in parallel arrays by α-actinin, which would be analogous to the role of this actin-crosslinking protein at the Z-line of sarcomeres (Fig. 1B). These bands of α-actinin in stress fibres can been seen as electron-dense striations by electron microscopy, with myosin-rich regions appearing as interleaving electron-light regions (Langanger et al., 1984; Langanger et al., 1986). In a sarcomeric model of stress fibre contraction, intercalating arrays of myosin would then slide between the arrays of actin filaments to cause shortening.

Filament orientation

Actin filaments have inherent polarity, each having a plus ('barbed') end and a minus ('pointed') end. Painstaking analysis of the polarity of actin filaments in stress fibres has shown that the sarcomeric model does not describe the complexity of these structures adequately. In a muscle sarcomere, each block of bundled actin filaments must have the opposite polarity to its successive block to allow sliding contraction – the myosin motors move towards the barbed ends of the actin filaments and the gap between each block of actin filaments decreases (Fig. 1B). Analysis of non-muscle cells has shown that the actin filaments in some stress fibres show such alternating polarity, but there is also a range of other possible orientations (Fig. 1B). In some stress fibres, actin filament polarity is uniform; in others, filaments in a bundle show apparently random orientation with respect to each other (Cramer et al., 1997; Svitkina et al., 1997). In motile cells, the majority of stress fibres show overall graded polarity – at each end of the fibre the filament polarity is uniform, barbed ends pointing outwards, but the distribution of filament polarities becomes mixed at the centre of the fibre (Cramer et al., 1997). Establishing the orientation of filaments in stress fibres is important because this defines their contractile properties structures – although not in a way that is necessarily painless or immediately apparent (Fig. 1B).

Stress fibre assembly

Much of our understanding of the polarity of actin filaments in stress fibres has come from studies of how stress fibres assemble. Stress fibres are complex and lengthy structures. Their assembly provokes questions similar to those that can be asked about the assembly of muscle myofibrils (Sanger et al., 2005) – is the periodic alternation of actin and myosin units

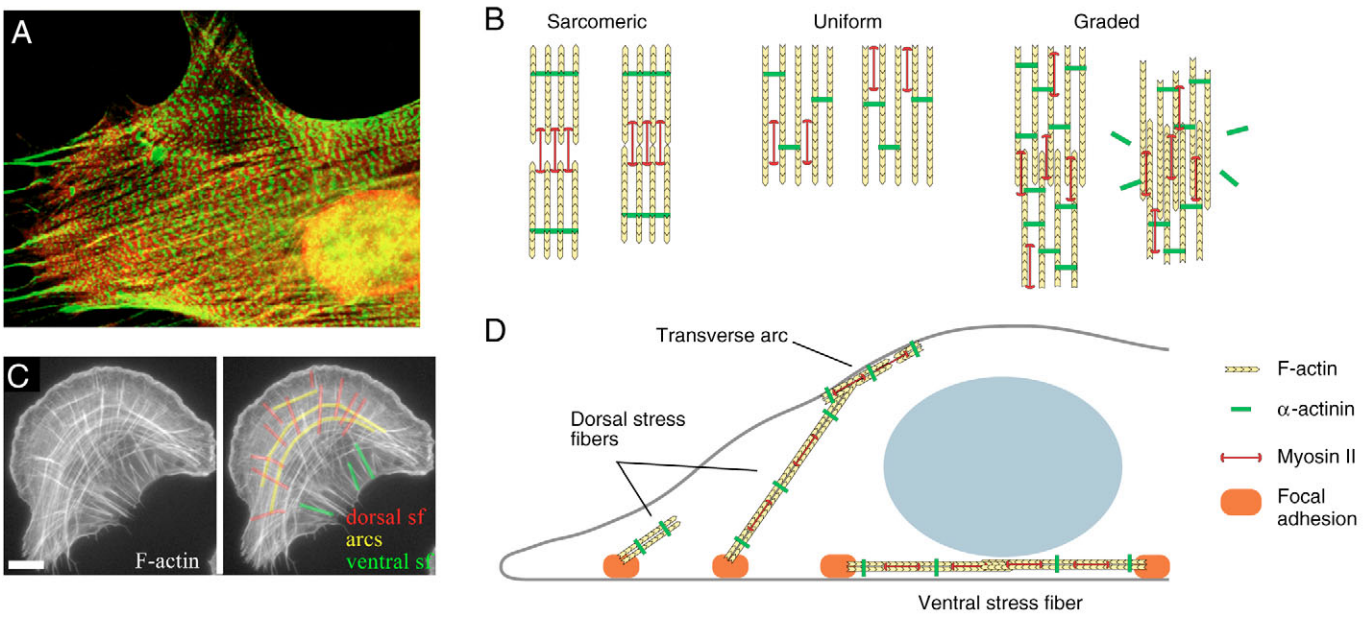

Fig. 1. Actin stress fibre structure. (A) Gerbil fibroma cell stained for non-muscle myosin (red) and α-actinin (green) reveals the periodic banding of these components on actin stress fibres. Reproduced with kind permission from (Peterson et al., 2004). (B) Models of stress fibre structure and contractility. Sarcomeric stress fibres have blocks of actin filaments of alternating polarity and bands of interdigitating non-muscle myosin. The structure is held together by α-actinin and also crosslinked by non-muscle myosin. On contraction (right), myosin slides between the filaments, pulling them towards each other and closing the gap. In stress fibres with uniform polarity, myosin would not be able to cause contraction. In these structures myosin might be instead able to move along the filaments towards the plus (barbed) end. If this myosin was attached to cargo, transport towards the focal contact would occur. Whether the double-headed non-muscle myosin is able to perform this role is unknown, but single-headed myosins are known to be able to transport cargo along actin filaments. Ventral stress fibres show graded polarity and are formed by two fibres of uniform polarity joining at their minus (pointed) ends. Contraction of these structures could occur by myosin driving invasion of filaments in the central region into each bundle. The difference between this form of contraction and the sarcomeric model is that actin filaments become interleaved, which should give rise to mixed polarity of the bundle at the central region. Such contraction would require displacement of α-actinin. (C) U2OS osteosarcoma cells stained for F-actin, displaying the three categories of actin stress fibres (dorsal, red; arcs, yellow; ventral, green). Reproduced with kind permission from (Hotulainen and Lappalainen, 2006). (D) Model of stress fibre formation. Dorsal stress fibres arise from focal contacts at the cell periphery and elongate up through the cell to join transverse arcs at the cell surface. Two dorsal stress fibres may meet at a transverse arc and draw down to the bottom of the cell to form a ventral stress fibre. This model is adapted from (Hotulainen and Lappalainen, 2006).

achieved by the joining of preformed sections, like the building of a bridge, or is making a stress fibre like knitting a scarf, with the correct components being added in as the structure lengthens? The answer is probably both, but different methods are used for different kinds of stress fibre, and first we need to define these differences.

The stress fibres observed in fibroblasts have been divided into three classes on the basis of their subcellular location: ventral stress fibres, dorsal stress fibres and transverse arcs (Small et al., 1998). Ventral stress fibres are the most commonly observed structures (Fig. 1C) and lie along the base of the cell, attached to integrin-rich focal adhesions at each end (Burridge, 1986). Dorsal stress fibres are attached to a focal adhesion at one end only, which tethers them to the base of the cell. The rest of the structures rises towards the dorsal surface, terminating in a loose matrix of actin filaments (Heath and Dunn, 1978). Transverse arcs are bundles of actin that form beneath the dorsal surface of migrating cells, just behind the protrusive lamella. After forming, these structures sweep backwards towards the nucleus, where they disassemble (Heath, 1983).

A beautiful and comprehensive study by Lappalainen and co-workers has shown that each type of stress fibre is assembled by a different mechanism (Hotulainen and Lappalainen, 2006). Imaging of stress fibre formation in living cells shows that dorsal stress fibres elongate from focal adhesions to form short filaments containing α-actinin (Fig. 1). Clusters of myosin are then woven into these structures, displacing α-actinin to give the classic periodic staining. In contrast to this, transverse arcs form by end-to-end joining of short bundles of actin to bundles of myosin – a sectional construction method (Hotulainen and Lappalainen, 2006; Zimerman et al., 2004). It is important to note, however, that other studies support an alternative model for the construction of transverse arcs – where these structures form through the actions of myosin bundles on pre-existing networks of actin filaments. These studies show that aggregates of myosin form at the leading edge of the cell and then travel backwards through the lamella by retrograde flow, becoming associated with the loose network of actin filaments at the base of the protrusion. Detailed ultrastructural analysis of myosin and actin filaments in these cells supports a model in which the effects of myosin contraction on these networks causes their reorganisation into actin bundles into which α-actinin could then become inserted (Verkhovsky et al., 1995; Verkhovsky et al., 1999). Finally, ventral stress fibres, the most commonly

observed structures, form by the end-to-end joining of two dorsal stress fibres to form a structure that is anchored at both ends by a focal adhesion (Hotulainen and Lappalainen, 2006). In some cases the two dorsal stress fibre ends find each other and join directly. In other cases they meet at a transverse arc, which then disassembles as the resulting stress fibre is pulled down to the base of the cell (Fig. 1D).

These three different classes of stress fibre, and their three different modes of formation, provide a potential explanation for the different filament polarities observed in ultrastructural analysis of stress fibres. Elongating dorsal stress fibres should have the uniform polarity seen in stress fibres in the lamellipodia (Cramer et al., 1997; Svitkina et al., 1997). The regular end-to-end joining of actin and myosin bundles seen in transverse arcs should give sarcomeric-like fibres similar to those seen directly below the plasma membrane in ultrastructural studies (Cramer et al., 1997). We propose that the mechanism of formation of ventral stress fibres is consistent with the graded polarity seen in the majority of stress fibres observed in motile fibroblasts (Cramer et al., 1997). The end-to-end joining of two dorsal stress fibres to give a ventral stress fibre should result initially in a structure that has symmetry of filament polarity across the axis of the join. As myosin drives invasion of filaments across this axis, the result should be the mixed/graded polarity observed in vivo (Fig. 1B).

So how might each type of stress fibre contract? Transverse arcs should contract like muscle sarcomeres; however, because these structures are not anchored to the plasma membrane by focal adhesions (Hotulainen and Lappalainen, 2006), it is unclear whether they have an attachment through which this force could be transmitted. The uniform polarity of dorsal stress fibres raises questions about whether these are contractile structures. For myosin to create tension by moving across a set of uniformly polarised filaments, it would have to be linked to a fixed object in the cell that would act as an anchor (Cramer et al., 1997). At present it is unclear whether such anchors exist. One possible use for bundles that display uniform polarity could be myosin-based trafficking – either of vesicle cargoes or proteins – towards focal adhesions at the ventral cell surface (DePina and Langford, 1999). Alternatively, these structures could help to provide rigidity to the cell or simply represent a transient stage in the formation of ventral stress fibres. Finally, ventral stress fibres should be contractile in their central region, where fibres from opposing ends would ratchet over each other, leading to tension across the structure. Because they are tethered at both ends by focal adhesions, this would allow the resultant force to be transmitted to the substratum. In keeping with this, analysis of the contractile forces generated in non-muscle cells shows that the majority of the contractile force that a fibroblast applies to the substrate is aligned with the direction of ventral stress fibres (Burridge, 1981; Harris et al., 1980).

Seemingly opposed to this last model are the results of recent studies by Burridge and co-workers, who measured ventral stress fibre contraction in non-motile cells. By examining the spacing between units of α-actinin in live cells as they contracted, they were able to show that contraction is greatest towards the periphery, and that ventral stress fibres appear to stretch in their central region (Peterson et al., 2004). It is unclear whether this stretching is an active process, or whether it reflects pulling apart of filaments at the weakest point of the structure (Peterson et al., 2004). Harder to understand is the mechanism of contraction in the periphery of the cell. In motile cells, this region shows uniform polarity (Cramer et al., 1997). It is possible that the orientation of filaments is different in non-motile cells. Indeed, the formation of stress fibres in response to RhoA activation in non-motile fibroblasts occurs with very little new actin polymerisation (Machesky and Hall, 1997), suggesting that the predominant mechanism is the bundling of existing actin filaments caused by increased myosin contractility, as originally proposed by Chrzanowska-Wodnicka and Burridge (Chrzanowska-Wodnicka and Burridge, 1996). Clearly, much work remains if we are to find models that fully explain the experimental observations.

In a sarcomeric model of contraction, the shortening of the fibre is allowed because of the intercalation of arrays of actin and myosin. Non-sarcomeric models of stress fibre contraction face a problem – as non-muscle myosin slides actin filaments past each other, α-actinin should act as a roadblock to their movement. A potential solution to this problem comes from observations of the kinetics of the binding of α-actinin to actin filaments, which have been shown to allow cycles of rapid dissociation and reassociation both in vitro (Goldmann and Isenberg, 1993), and live cell imaging (Hotulainen and Lappalainen, 2006). Such dynamic behaviour would allow contracting actin filaments to displace α-actinin, rapid rebinding maintaining the integrity of the structure (Fig. 1B).

Signalling pathways

Actin stress fibres disassemble in response to the *Clostridium botulinum* C3 toxin (Chardin et al., 1989), for which the major cellular targets are three members of the Rho family of small GTPases – RhoA, RhoB and RhoC. Chardin and co-workers first proposed that Rho proteins may be involved in the signalling that controls stress fibre formation. This was subsequently validated by experiments demonstrating that microinjection of activated recombinant RhoA into subconfluent fibroblasts leads to rapid and extensive stress fibre formation (Paterson et al., 1990). The RhoA, RhoB and RhoC small GTPases are very similar (>80% sequence identity), and activation of each stimulates actin stress fibre formation (Giry et al., 1995). The vast majority of work on stress fibre signalling pathways has focused on RhoA, which at this time appears to be the major regulator of stress fibre formation under most physiological conditions. Roles for RhoB and RhoC are highly likely, but possibly in specialised situations or certain cell types.

ROCK

Early breakthroughs in our understanding of the regulation of stress fibres came with the identification of downstream effectors of RhoA, in particular the ROCK/ROK protein kinases (Ishizaki et al., 1996; Leung et al., 1995) and the diaphanous-related formin, mDia1 (Watanabe et al., 1997). ROCK-1 and ROCK-2 are serine/threonine kinases that are activated by RhoA binding and cause prominent stress fibre formation (Leung et al., 1996). ROCK can also be inhibited by overexpression of the small GTPases RhoE (Riento et al., 2003), Gem and Rad (Ward et al., 2002), which block stress

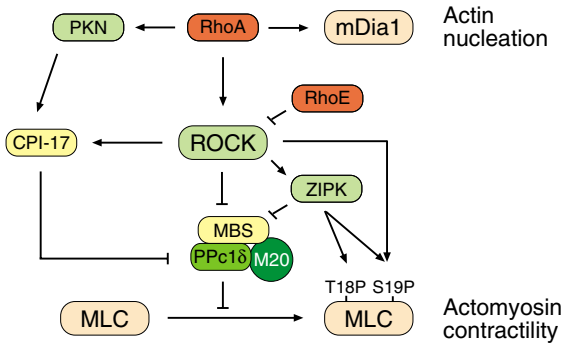

Fig. 2. Signalling pathways controlling stress fibre formation. RhoA-mediated activation of mDia1 at the plasma membrane provides the actin-nucleating activity required for stress fibre formation. RhoA also activates ROCK, which can phosphorylate MLC directly but also inhibits myosin phosphatase through a complex set of pathways. Myosin phosphatase is a trimeric complex comprising the M20 subunit, the PPc1δ catalytic subunit and the regulatory MBS (MYPT) subunit. Inhibition of myosin phosphatase activity downstream of RhoA increases MLC phosphorylation and hence increases actomyosin contractility. ROCK activity is inhibited by the Rho GTPase RhoE and by the small GTPases Rad and Gem.

fibre formation. ROCK is localised in part to stress fibres (Katoh et al., 2001) and phosphorylates at least four targets in the stress fibre pathway (Fig. 2) – all of which lead to increased myosin phosphorylation and increased actomyosin contractility.

ROCK directly phosphorylates myosin light chain 2 (MLC2) at Ser19 (Amano et al., 1996). This is one of the two sites phosphorylated by myosin light chain kinase (MLCK) (Somlyo and Somlyo, 2003), and phosphorylation by ROCK similarly leads to increased stress fibre contractility due to an increase in myosin ATPase activity (Katoh et al., 2001). ROCK also phosphorylates MBS (MYPT) – the regulatory subunit of myosin light chain phosphatase. Phosphorylation of MBS by ROCK inhibits phosphatase activity (Kimura et al., 1996), and so leads to increased MLC2 phosphorylation and increased stress fibre formation. Several ROCK-phosphorylation sites have been identified on MBS (Kawano et al., 1999), of which the two most important appear to be Thr696 and Thr853 [human MYPT1 numbering (Feng et al., 1999; Velasco et al., 2002)]. Thr696 can be phosphorylated by several other protein kinases (Ito et al., 2004) and occupancy of this site appears to be tonic in resting fibroblasts (Ren et al., 2004). Phosphorylation of Thr853 appears to be a clearer marker of acute ROCK activation (Ren et al., 2004; Wilson et al., 2005) and leads to dissociation of the phosphatase complex from myosin (Velasco et al., 2002). ROCK can also phosphorylate and activate CPI-17 in vitro (Koyama et al., 2000) and in smooth muscle cells (Kitazawa et al., 2000). CPI-17 is an inhibitor of myosin phosphatase, and activation of CPI-17 by other protein kinases leads to an increase in actomyosin contractility. Last but not least, ROCK has recently been shown to phosphorylate and activate Zipper-interacting protein kinase [ZIPK (Hagerty et al., 2007)]. Activated ZIPK can in turn inhibit myosin phosphatase activity by phosphorylating the Thr696 site in MBS (MacDonald et al., 2001a) and the Thr38 site in CPI-17 (MacDonald et al., 2001b). ZIPK can also directly phosphorylate MLC2 at both Thr18 and Ser19 (Murata-Hori et al., 1999).

The resulting picture is complex: ROCK lies at the centre of actomyosin contractility, phosphorylating both myosin and multiple myosin regulatory proteins. But which are the predominant pathways in stress fibre formation? CPI-17 is highly expressed in smooth muscle cells in comparison with non-muscle cells (Eto et al., 1997) and it is unclear whether it is a significant target of ROCK outside of smooth muscle (Kolosova et al., 2004; Ren et al., 2004). An elegant study by Matsumura and co-workers targeted MBS and MLC separately to show that ROCK mediated phosphorylation of both proteins is necessary for stress fibre formation in fibroblasts (Totsukawa et al., 2004). Whether these phosphorylations are directly through ROCK, or also indirectly through ROCK-mediated activation of ZIPK, remains to be explored.

mDia1

Activation of ROCK alone does not generate the thick, parallel stress fibres seen after RhoA activation – the activity of mDia1 is also required. ROCK activation produces thick stress fibres, but these typically form a star-like pattern in the centre of the cell (Leung et al., 1996). Overexpression of mDia1 produces parallel actin filaments, but these are not thickly bundled (Watanabe et al., 1997). Crucial work by Narumiya and co-workers showed that both activities are required to recapitulate the effects of active RhoA (Watanabe et al., 1999). The cellular function of mDia1 is to nucleate the polymerisation of actin filaments from cell membranes, nucleation occurring at the barbed end of the nascent filaments (Kovar, 2006). At the site of attachment of stress fibres to focal contacts in the plasma membrane, actin filaments adopt a uniform orientation in which their barbed ends are located at the contact site (Cramer et al., 1997). The simplest model for mDia1 action is that it provides a nucleating activity in the focal contact to form a short actin bundle that can then be elongated into a stress fibre. In keeping with this, isolated focal adhesion complexes nucleate actin polymerisation and localise mDia1 (Butler et al., 2006), and depletion of mDia1 in vivo significantly inhibits the formation of dorsal stress fibres (Hotulainen and Lappalainen, 2006).

Stress fibres and cell migration

Cell motility is generally described as a cyclical process divided into alternating phases of protrusion and contraction. Once the cell has polarised, a lamellipodium protrudes at the front of the cell and attaches to the substrate. Adhesion provides the traction required for generating pulling forces, and translocation ensues by contraction of the cell body and retraction of the tail (Ridley et al., 2003; Small et al., 1998). When the contractile nature of stress fibres was first described, it was speculated that these structures might provide contractile force for cell migration (Kreis and Birchmeier, 1980). At the same time, however, other studies observed that stress fibres are more prominent in stationary cells (Couchman and Rees, 1979), suggesting that stress fibres inhibit cell migration (Burridge, 1981).

Stress fibres and tail retraction

Non-muscle myosin is required for the retraction of the cell tail during migration (Jay et al., 1995; Kolega, 1998) and migrating

fibroblasts show increased MLC phosphorylation in this region (Post et al., 1995). Recent studies have shown that this function is carried out by a specific isoform of non-muscle myosin, myosin IIA (Even-Ram et al., 2007; Vicente-Manzanares et al., 2007). It is also clear that signalling pathways that control stress fibre formation and contractility are important in tail retraction. Migrating cells treated with the ROCK inhibitor Y-26732 leave a long tail behind them (Kolega, 1998), and both RhoA and ROCK are required for tail retraction during migration of leukocytes (Alblas et al., 2001; Worthylake et al., 2001). Further, in migrating fibroblasts and neutrophils, RhoA is concentrated and activated in the tail (Kurokawa and Matsuda, 2005; Wong et al., 2006).

In motile fibroblasts, ventral stress fibres are oriented parallel to the axis of locomotion (Cramer et al., 1997; Oliver et al., 1994), which suggests that force generated by contraction of these structures could drive tail retraction. Tail retraction requires release and/or disassembly of focal adhesions at the rear of the cell, while adhesions at the front are maintained to allow application of traction forces. This disassembly of posterior adhesions is complex and poorly understood, but contractile force is important for this process (Cox and Huttenlocher, 1998; Kirfel et al., 2004). As ventral stress fibres are connected to focal adhesions at both ends, they are well placed to supply the contractile force required for turnover of adhesions at the rear of the cells. One major complication is that stress fibre contractility in non-motile cells causes strengthening of focal adhesions (Chrzanowska-Wodnicka and Burridge, 1996). So, too little contractility would inhibit disassembly of posterior adhesions and tail retraction, whereas too much contractility would either increase adhesion to the point where cells became braced to the substrate, or break adhesions at both ends causing cells to become detached. In keeping with this, excessive RhoA activation inhibits cell migration (Arthur and Burridge, 2001; Cox et al., 2001), and inhibition of stress fibre contractility using the ROCK inhibitor Y-27632 can actually increase migration speed in some situations (Nobes and Hall, 1999; Totsukawa et al., 2004; Wojciak-Stothard and Ridley, 2003). It seems that any contribution to tail retraction made by stress fibre contractility must be carefully balanced and integrated with other pathways controlling cell adhesion at the back and front of migrating cells. Indeed, motile cells have a gradient of adhesion, adhesions being stronger at the front than at the rear (Schmidt et al., 1993).

Finally, it is also important to remember that fibroblasts are not the only motile cells in the village. Fish keratocytes have been the object of detailed study and are an example of cells with higher motility and lower adhesion than fibroblasts. Here it has been shown that the vector of contractile force is perpendicular to the direction of migration, and detailed studies by Small and colleagues have shown how actomyosin contractility contributes to tail retraction in these cells in ways that do not involve stress fibres (Small and Resch, 2005).

Stress fibres and persistence

Kaverina and colleagues have recently studied the interactions between stress fibres and focal adhesions in migrating fibroblasts by live cell imaging. This work shows that, after a ventral stress fibre becomes detached from the disassembling posterior adhesion, it attaches again to a new adhesion in the cell tail. This leads to a reorientation of the stress fibre axis, which becomes aligned with the new direction of migration. The authors propose that stress fibres act a rudders in these migrating cells that define the long axis of the cell and the direction of persistent movement (Rid et al., 2005). Interestingly, the Rho/ROCK pathway that controls stress fibre formation also controls persistence of cell migration by inhibiting Rac and limiting the formation of side protrusions (Ohta et al., 2006; Tsuji et al., 2002; Worthylake and Burridge, 2003). Taken together, it seems that the RhoA/ROCK pathway has a co-ordinating role in this component of cell migration.

Isometric tension and static contraction

By comparison with the uncertainties surrounding the role of actin stress fibres in cell motility, these structures have a straightforward and well-defined role generating contractile forces in tissues. Further, a number of specialised cell types exist to perform these contractile roles.

Myofibroblasts

Fibroblasts in normal connective tissue do not contain stress fibres (Byers et al., 1984; Ehrlich and Hembry, 1984). This fact has mutated on re-telling to create a piece of actin mythology – that stress fibres do not exist outside of a tissue culture dish. There are many situations where cells in the body produce stress fibres; however, these are specific conditions in which cells encounter (and then produce) mechanical force. Early studies of fibroblasts in wounds showed cells that have pronounced stress fibres – these were termed myofibroblasts or granulation tissue fibroblasts (Gabbiani et al., 1971).

Myofibroblasts in dermal wound tissue arise from the differentiation of tissue fibroblasts (Ross et al., 1970) and circulating fibrocyte progenitors (Hinz et al., 2007), both of which migrate into the centre of the wound site. As cells enter the wound they experience tension, which causes them to assemble stress fibres and become proto-myofibroblasts (Hinz et al., 2001a; Tomasek et al., 2002). This effect can be mimicked in vitro by plating fibroblasts on a rigid support (i.e. a plastic tissue culture dish), whereas loss of substrate rigidity causes a rapid loss of stress fibres (Couchman and Rees, 1979; Mochitate et al., 1991). Tension, together with the effects of TGFβ1 and alterations to the composition of the extracellular matrix, then leads to further differentiation of proto-myofibroblasts into myofibroblasts (Tomasek et al., 2002). This differentiation process is accompanied by expression of α-smooth muscle actin (αSMA), which becomes incorporated into myofibroblast stress fibres (Darby et al., 1990). Stress fibres that contain αSMA generate more contractile force than normal stress fibres, which contain only β- and γ-cytoplasmic actin (Hinz et al., 2001b). This increased contractile force strengthens focal adhesions, leading to the formation of the large ‘supermature’ focal adhesions (fibronexi) characteristic of these cells (Hinz, 2006). Interestingly, RhoA has recently been implicated in the induction of αSMA downstream of TGFβ1 during smooth muscle cell differentiation (Chen et al., 2006) – this acute regulator of actin stress fibres might therefore also give rise to long-term changes in stress fibre function by pushing cells to become more smooth-muscle-like.

The majority of myofibroblasts in a slit wound orientate their stress fibres along the long axis of the wound (Fig. 3A), although in the central region of the wound, some show

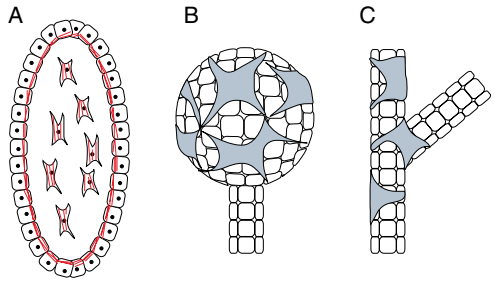

Fig. 3. Contractile non-muscle cells. (A) The arrangement of myofibroblasts in the body of a wound. Actin stress fibres (red) align along the long axis, and the cells exert contractile forces on the extracellular matrix, via integrins spanning the plasma membrane. Epithelial cells at the edge of the wound assemble actomyosin bundles that couple through adherens junctions to form a contractile 'purse-string' structure that helps to close the wound.
(B) Myoepithelial cells (blue) cover the external face of mammary ducts and contract in response to oxytocin to aid expulsion of milk.
(C) Pericytes (blue) similarly cover endothelial cells in mature capillaries and provide contractile force to maintain tone and the passage of fluid.

orientation across the short axis (Petroll et al., 1993). This situation can be presumed to indicate the major vector of tension in such a wound; however, the outcome is at first sight counterintuitive – why doesn't contractile force along the long axis make the wound gape, not close? One plausible explanation is that the force generated by myofibroblasts is largely used to remodel the extracellular matrix in the wound to allow closure. Myofibroblast stress fibres are mechanically linked to the extracellular matrix through their large focal adhesions (Singer et al., 1984) and the contractile forces generated through this coupling cause alignment of collagen fibres in the wound into parallel bundles along the long axis (Gabbiani et al., 1971; Hinz et al., 2001b). This remodelling and contraction of the extracellular matrix would then contribute to the resolution of the wound site (Tomasek et al., 2002). Indeed, myofibroblasts in non-wound sites are found in tissues that require significant matrix remodelling (Sappino et al., 1990) – for example, the developing lung septa (Vaccaro and Brody, 1978; Yamada et al., 2005) and the uterine submucosa (Glasser and Julian, 1986). The role of the contractile forces generated by stress fibres in myofibroblasts is thus not generation of motility but isometric tension and consequent reshaping of the extracellular matrix.

Contractile epithelial cells and myoepithelial cells

Epithelial cells around the border of a wound assemble actomyosin cables at the wound edge that are mechanically linked to those in adjacent cells, forming a contractile 'purse-string' around the edge of the wound (Fig. 3A) (Bement et al., 1993; Martin and Lewis, 1992). Contraction of this structure depends on the activities of RhoA (Brock et al., 1996) and ROCK (Tamada et al., 2007) and provides the forces required to draw the edges of the epithelial sheet together to cover the wound surface. The precise relationship of this actomyosin structure to stress fibres is unclear. Clearly, similar components are involved, although the ends of the purse-string actin cables are connected not to focal contacts but to catenins in adherens junctions (Danjo and Gipson, 1998). This allows mechanical linkage between adjacent cells, and the force to be directed along the wound axis. There are strong parallels between wound healing and the closure of epithelial sheets during the processes of embryogenesis (Martin and Parkhurst, 2004). The same actin-purse-string mechanism is used during dorsal closure, in a process that similarly requires RhoA (Harden et al., 1999; Martin and Parkhurst, 2004). Further, mice lacking ROCK fail to close their eyelids during development or to close their ventral body wall efficiently – both processes have marked similarities to wound healing and dorsal closure (Shimizu et al., 2005; Thumkeo et al., 2005). In all of these events, actomyosin is used not to power cell motility, but to provide a static, asymmetric contraction, which contributes to the orderly closing of a gap in the epithelium. Further work is needed to tell us more about the formation of these purse-string cable structures and their relationship to conventional actin stress fibres.

As fibroblasts can give rise to specialised contractile myofibroblasts, so epithelial cells can give rise to myoepithelial cells (Gudjonsson et al., 2005). These cells form a basket-like network around the epithelial ducts of many exocrine glands. They are best studied in mammary glands, where their static contraction squeezes the duct to expel milk (Gudjonsson et al., 2005; Redman, 1994); however, they are also present in salivary, sweat and lacrimal glands, where they are presumed to play a similar role. Like myofibroblasts, myoepithelial cells contain numerous stress fibres (Haguenau, 1959) and αSMA (Skalli et al., 1986). The contraction of mammary myoepithelial cells is triggered by oxytocin, which causes milk release. Oxytocin stimulation leads to myosin phosphorylation in myoepithelial cells (Olins and Bremel, 1982). The signalling pathways involved are not fully defined – oxytocin mobilises Ca^{2+}, which would lead to MLCK activation and consequent phosphorylation of myosin (Reversi et al., 2005); however, oxytocin can also trigger activation of RhoA and ROCK in other tissues (Tahara et al., 2002). By analogy with smooth muscle, it is possible that acute myoepithelial contraction is regulated largely by Ca^{2+}/MLCK in response to oxytocin, and RhoA/ROCK has a potentiating role, increasing sensitivity and force.

Stress in the vasculature

Cells of the vasculature can experience mechanical stress through hydrostatic pressure and cyclic stretch. The endothelial cells that line blood vessels also experience fluid shear as blood flows across their surface. This stimulates the formation of actin stress fibres (Franke et al., 1984), which become aligned in the direction of flow in vessels experiencing high levels of fluid shear, such as the aorta (Wong et al., 1983), or in response to hypertension (White et al., 1983). Fluid shear leads to RhoA activation in endothelial cells, and the consequent induction of stress fibres requires the action of RhoA and ROCK (Wojciak-Stothard and Ridley, 2003). The generally accepted view is that stress fibre contractility helps endothelial cells to remain flat under flow, and hence resist and reduce fluid shear forces.

Blood vessels are supported by a casing of contractile cells. Larger vessels are surrounded by a layer of vascular smooth muscle cells, which resist hydrostatic pressure and regulate vascular tone. Small capillaries and lymphatic vessels are coated in pericytes, a third subtype of specialised contractile cell (Fig. 3) (Betsholtz et al., 2005). Pericytes are derived from mesenchymal precursors and can further differentiate into

vascular smooth muscle cells. They have thick actin stress fibres and, like myoepithelial cells and myofibroblasts, they express αSMA as well as non-muscle myosin (Herman and D'Amore, 1985). The signals regulating pericyte contractility are unknown at this time.

Conclusions and Perspectives

Perhaps the most productive way to think about stress fibre function is in terms of a spectrum of contractility. At one end of this spectrum are tissue fibroblasts, which have sparse and poorly organised contractile actomyosin bundles. At the other end are smooth muscle cells – highly contractile cells that have highly organised actomyosin arrays. Fibroblasts and epithelial cells can differentiate to become more contractile, and in doing so they take on more of the properties of smooth muscle cells. Highly contractile cells are less likely to be motile but, instead, contractility is used to remodel connective tissue or apply force to tubules and ducts. The role of actin stress fibres in cell motility remains a thorny question. A 'sweet-spot' might exist on the spectrum of stress fibre organisation, where relatively disorganised and dynamic actomyosin arrays could contribute contractile forces to cell movement, without pinning the motile cell to one spot, or contracting it into a ball. In all of these cases, however, actin stress fibres allow cells to respond to imposed mechanical force with a bracing resistance – when you pull on a fibroblast, it is actin stress fibres that let the fibroblast pull back.

The authors thank Lynda Peterson, Keith Burridge and Pekka Lappalainen for permission to reproduce the micrographs of stress fibres in Fig. 1. Stress fibres have been the subject of extensive research for many years. While we have tried to cite primary sources wherever possible, the authors apologise to workers in this field for having omitted many important studies owing to space constraints.

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