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Linking actin dynamics and gene transcription to drive cellular motile functions

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Abstract

Numerous physiological and pathological stimuli promote the rearrangement of the actin cytoskeleton, thereby modulating cellular motile functions. Although it seems intuitively obvious that cell motility requires coordinated protein biosynthesis, until recently the linkage between cytoskeletal actin dynamics and correlated gene activities remained unknown. This knowledge gap was filled in part by the discovery that globular actin polymerization liberates myocardin-related transcription factor (MRTF) cofactors, thereby inducing the nuclear transcription factor serum response factor (SRF) to modulate the expression of genes encoding structural and regulatory effectors of actin dynamics. This insight stimulated research to better understand the actin–MRTF–SRF circuit and to identify alternative mechanisms that link cytoskeletal dynamics and genome activity.

Both during embryonic development and as functional components of mature multicellular organisms, individual cells continuously undergo physical changes in appearance, shape, position and contact with extracellular structures including other cells (BOX 1). These physical changes require cellular motile functions, which are the driving force of many dynamic cell behaviours such as cell migration, guided movement, engulfment, adhesion and contraction. The motile functions of cells are regulated by both physiological and pathological stimuli. The physical basis for cellular motile functions is provided by macromolecular assemblies, including cytoskeletal scaffold structures of actin micro filaments. These scaffolds undergo dynamic changes in both polymerization and their interaction with specific binding proteins. Regarding the actin microfilament, poly merization of monomeric globular actin (G-actin) into a filamentous actin (F-actin) fibre is influenced by local intracellular concentrations of ATP-bound G-actin and by the activity of many actin-binding proteins (ABPs). This determines the rate and direction of

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
UniProtKB: http://www.uniprot.org CRK | DAAM1 | FAK | FHL2 | ILK | JMY | KLF4 | MRTF-A | MRTF-B | NCK1 | SRC | SRF | STARS

FURTHER INFORMATION
Eric N. Olson's homepage: www4.utsouthwestern.edu/olsonlab/index.htm
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polymerization, as well as the shape of the newly generated filament[1]. Such dynamic rearrangements of actin filaments generate the physical force needed for cells to protrude filopodial or lamellipodial membrane extensions and to readjust adhesive contacts, known as focal adhesions, to the cellular environment, as required for motile cell functions.

Although actin microfilament turnover involves the regeneration of depleted ATP–actin levels from ADP–actin pools, enabled by the ABP profilin, cell motility also requires the *de novo* biosynthesis of G-actin. Similarly, activation of protein synthesis is required for the timely generation of other structural components of the actin microfilament network (for example, myosins, actin assembly and disassembly factors, capping proteins and F-actin cross-linking proteins) as well as regulatory components (for example, kinases, phosphatases and myosin light chains (MLCs)). Therefore, cell motility requires the tight temporal coupling of actin dynamics and transcriptional activity.

The requirement for the temporal linkage of actin turnover and transcription necessitates signal transduction mechanisms that communicate the cyto plasmic actin polymerization status to the nuclear genome. How does the transcriptional machinery sense the need for the biosynthesis of components of both the dynamic actin network and the motility apparatus? To approach this question we first review the various cellular receptors that are activated by motility-inducing signals and subsequently lead to activation of the central intracellular regulatory proteins of the Rho family of GTP-binding proteins (FIG. 1). We then focus on relay systems that enable dynamic rearrangements of the cytoskeletal actin microfilament to be communicated to the nucleus by release and nuclear translocation of specific ABPs, thereby eliciting defined changes in the expression of specific gene profiles (FIG. 2).

Box 1 | Motile functions of cells

The living eukaryotic cell must be viewed as a dynamic structure experiencing frequent changes in macromolecular contacts, signal inputs and metabolic flux. As a consequence, cells display continued physical rearrangement and adjustment. Such motility functions require supporting intracellular counter-forces, as generated by dynamic rearrangements of the actin microfilament. Polymerization of monomeric globular actin (G-actin) into filamentous actin (F-actin) polymers is reversible and both reactions are facilitated by numerous actin binding proteins (ABPs).

Cellular motile functions involving dynamic actin microfilament rearrangements include cell migration, spreading, adhesion, contraction and polarization, cell–cell contact and cell–extracellular matrix (ECM) interactions (see the figure). These microfilament rearrangements result in the processes of chemotaxis, mechanotaxis (cell stretching and overload), maintenance of cellular tone, shape changes (branching), engulfment (by phagocytosis), neurite or vascular tip cell extension, axon guidance, morphological rearrangements (such as those required for epithelial–mesenchymal transitions) and intracellular transport (such as RNA localization, protein delivery, membrane trafficking, endocytosis, internalization, secretion and organelle positioning).

Dynamic changes of the actin microfilament contribute to each of these motile functions, either as structural components or as platforms for signal transduction. Microfilament polymerization and depolymerization, accompanied by the association and dissociation of interacting molecular structures, provide the physical scaffold for dynamic changes underlying motility. As discussed in this review, the multitude of cell behaviours involving actin dynamics requires gene expression and *de novo* protein biosynthesis. Selected ABPs function as courier proteins to communicate to the cellular genome the state of actin rearrangements. Such transcriptional relay systems modulate the expression of genes that encode products which contribute to either the execution or regulation of cellular motility functions. Actin structures are indicated in the figure in different shades

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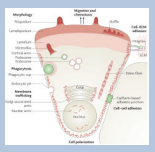


General influences of nuclear actin and nuclear ABPs on overall gene expression are not discussed in detail. Instead, this review emphasizes recent insights into the specific function of G-actin to control both nucleus–cytoplasm shuttling and the nuclear activity of transcriptional cofactors of the myocardin protein family (myocardin itself, plus the myocardin-related transcription factors (MRTFs)) (FIG. 3). These proteins, in turn, control the activity of serum response factor (SRF), a nuclear transcription factor. Thereby, the cytoplasmic concentration of G-actin is reflected by the concentration of MRTF retained in the cytoplasm and is a direct measure of actin dynamics. The release and nuclear translocation of cytoplasmic MRTF on actin polymerization elicits SRF-directed target gene activation. Thereby, the actin–MRTF–SRF circuit allows for the precise modulation of gene expression in concert with cytoskeletal assembly and disassembly. Since SRF target genes encode structural components of the microfilament (for example, actin itself) and regulators of actin dynamics (for example, gelsolin and vinculin), as well as microRnAs (miRnAs) that provide feedback regu lation in these pathways, the MRTF–SRF circuit assumes central importance in directing essential gene activities required for the execution of motile cell functions.

Signal regulation of actin dynamics

The diverse cellular motile functions (BOX 1) are elicited by a host of extracellular stimuli. These stimuli are perceived by cognate receptor proteins that activate different members of the Rho GTPase family through selective Rho guanine nucleotide exchange factors (GeFs) (FIG. 1). The Rho GTPases encompass members of the Rho, Rac and Cdc42 subfamilies[2], which regulate effector proteins that modulate the polymerization equilibrium of G-actin and F-actin in the cytoplasm. G-actin forms complexes with different ABPs, including the nucleating factors profilin, formins and the actin-related protein 2/3 (ARP2/3) complex [1,3]. Activation of Rho GTPases promotes actin polymerization by two downstream signalling modules, one involving the Rho-associated kinase (RoCK)–LiM kinase–cofilin pathway (cofilin is an ABP that can stimulate actin depolymerization and thereby enhance actin poly merization elsewhere), and the other mediated by formin (such as Diaphanous-related formin (DRF)) ABPs. Experimentally, actin dynamics can be effectively modulated using clostridial toxins and other actin-targeting natural compounds[4] (BOX 2).

As outlined in FIG. 1, several types of plasma membrane receptors modulate actin polymerization and cell ular motility by modulating Rho GTPase activity. in the examples discussed below, the link between these receptors and the MRTF–SRF module is emphasized.

Signalling by G protein-coupled receptors

G protein-coupled receptors (GPCRs) are a large family of membrane receptors displaying seven transmembrane helical domains that can couple to heterotrimeric G proteins (guanine nucleotide-binding proteins; consisting of α-, β-, and γ-subunits). Heterotrimeric G proteins with Gα subunits of the types $G\alpha_{12/13}$, $G\alpha_{q/11}$ and $G\alpha_{i/0}$ contribute to actin turnover by engaging Rho GTPases[5] (FIG. 1). GPCRs interact with many ligands, including hormones,

chemokines, bioactive lipids, gastrointestinal peptides, platelet activators and orphan ligands of the developing brain[6]. Activation of the Rho–actin–MRTF–SRF pathway has been clearly demonstrated for the GPCR ligands lysophosphatidic acid (LPA)[7], sphinghosine-1-phosphate (S1P)[7,8] and a neuronal orphan ligand[6].

Receptor Tyr kinase signalling

Numerous Tyr kinase receptors link to Rho GTPases, including those for platelet-derived growth factor (PDGF), insulin, ephrin A (EphA) and ephB proteins, TRKA, TRKB and TRKC, epidermal growth factor (EGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) and MET[9]. Many of these receptors have essential influences on motile cell functions. Activation of the actin–MRTF–SRF pathway has been shown by VEGF receptor signalling in endothelial cells[10], EphA signalling in neurons[11,12] and neurotrophin signalling in dorsal root ganglia (probably through the TRKA, TRKB and TRKC receptors)[13]. The central importance of SRF signalling in these types of signalling events is exemplified by the abnormalities in vasculogenesis[14,15], neuronal axon guidance and synaptic targeting[11,13,16] in *Srf* knock-out mice.

Integrin signalling

Integrin receptors link cells to the extracellular matrix (ECM) and are the main structural organizing components of focal adhesions. Integrin signalling has a big impact on the reorganization of the actin cytoskeleton. Integrin-mediated actin dynamics is pri marily achieved by activating the RHOA and RAC1 GTPases, which involves the regulation of Rho GEFs by the kinases integrin-linked kinase (ILK), focal adhesion kinase (FAK) and SRC[17] (FIG. 1). Integrins also communicate external and internal mechanical stress to the actin cyto skeleton. Such mechano-sensing was shown to activate the actin–MRTF–SRF pathway[18].

TGFβ signalling

The transforming growth factor-β (TGFβ) superfamily of cytokines includes TGFβ-family polypeptides, bone morphogenetic proteins (BMPs) and activins. These ligands act through heterotetrameric complexes of Ser/Thr kinase receptors, which activate different signalling pathways and, in addition, modulate Rho GTPase-mediated actin dynamics[19]. TGFβ-induced alterations in cellular architecture and motility contribute to cellular epithelial–mesenchymal transitions (EMT), which are biologically linked to embryonic tissue movements and to tumour cell invasiveness and metastasis. Activation of the MRTF–SRF module was observed on TGFβ[20–22] and BMP signalling[23].

E-cadherin signalling

Epithelial cell–cell junctions (adherens junctions) are mediated by homophilic inter actions of epithelial cadherins (E-cadherins). Cadherins are functionally linked to the actomyosin cytoskeletal scaffold through catenins[1]. Assembly and disassembly of cell–cell contacts both requires and elicits extensive actin dynamics and activates the MRTF–SRF pathway through the GTPase RAC1 (REFS [20, 24, 25]). The dis assembly of epithelial cell contacts is an initiating event in EMT. The release of catenins (α-catenin, β-catenin and p120-catenin) activates actin dynamics and can directly modulate nuclear protein function[26].

Non-canonical Wnt signalling

The non-canonical, β-catenin-independent Wnt–planar cell polarity (PCP) signalling pathway uses the Wnt signalling component Dishevelled to activate regulators of actin dynamics, including the Rho, Rac and Cdc42 GTPases, ROCK[27] and the formin-related protein Dishevelled-associated activator of morphogenesis 1 (DAAM1)[28]. This pathway was

originally identified in *Drosophila melanogaster* in the establishment of spatial tissue organization, specifically the planar cell polarity of pupal fly wing cells[29]. In vertebrates, Wnt signalling modulates actin dynamics for polarized cell movements during gastrulation and organogenesis[30]. The link of Wnt–PCP signalling to the MRTF–SRF relay is still hypothetical.

Microfilament-to-genome relay systems

In this section, we review the range of currently characterized mechanisms by which cells communicate the dynamic status of their actin microfilaments to the genome.

Cytoplasmic actin dynamics cause changes in both G-actin concentration and F-actin structure; the latter can, in turn, lead to an altered structure or composition of F-actin complexes (F-ACs), such as focal adhesion complexes. Actin is recognized by ABPs, which, for clarity, we suggest are classified here as G-actin binding proteins (G-ABPs), F-actin binding proteins (F-ABPs) and F-actin complex-associated proteins (F-ACAPs) (FIG. 2).

Conceptually, changes in actin dynamics may be communicated to the genome in three ways. First, changes in cytoplasmic monomeric G-actin concentrations can elicit corresponding changes in nuclear G-actin levels. Second, changes in cytoplasmic monomeric G-actin levels can be sensed by G-ABPs, whereby polymerization of G-actin into F-actin can result in the release of G-ABPs, their nuclear translocation and the subsequent modulation of nuclear transcription factors (FIG. 2). This mechanism is best characterized by the MRTF–SRF circuit (FIG. 1; FIG. 3). Last, the status of cytoplasmic actin dynamics can be scored directly by cellular sensors of F-actin filament structure and density. Thus, architectural rearrangements of cytoplasmic F-actin fibres can lead to the release of F-ABPs or F-ACAPs, followed by their nuclear translocation (FIG. 2). In the following section, we provide a brief overview of different relay systems linking actin dynamics to transcriptional genome activity, according to the mechanisms conceptualized above.

Nuclear actin and ABPs

Nuclear actin, in both monomeric G-actin and polymeric F-actin forms, profoundly affects transcriptional responses in general terms[31,32]. it influences gene expression by at least three mechanisms: actin is a component of all three types of RNA polymerases (classes I, II, and III), it contributes to ATP-dependent chromatin remodelling and it binds different ribonucleoprotein (RNP) complexes in eukaryotic cell nuclei, including small nuclear RNAs (snRNAs) and heterogeneous nuclear RNAs (hnRNAs). In these activities, actin functions jointly with both G-ABPs and F-ABPs. G-actin can enter and exit nuclei by diffusion; however, use of its nuclear export sequence, partly in conjunction with members of the exportin protein family or with profilin, regulates its export. This controls the nuclear exit of other G-ABPs, such as MRTFs, which are exported bound to G-actin (see below).

JNK signalling

Signals that elicit dynamic actin rearrangements on activation of Rho GTPases, like the above-mentioned non-canonical Wnt–PCP pathway, were shown to also result in the activation of the Jun N-terminal kinases (JNKs)[33–35], which regulate gene transcription. In different experimental settings, GTPase-mediated JNK activation involved mediation by the kinases mixed lineage kinase 3 (MLK3; also known as MAP3K11)[36] and ROCKs[37], or the adaptor protein CRK[38]. It remains to be shown unequivocally that such mechanisms of JNK activation strictly correlate with the stimulation of actin dynamics.

NF-κB signalling

Cytoplasm-to-nucleus translocation of the transcription factor nuclear factor-κB (NF-κB) can be elicited by regulators of actin dynamics. Expression in endothelial cells of intercellular adhesion molecule 1 (ICAM1), on stimulation by thrombin, involves RHOA–ROCK-mediated activation of nuclear NF-κB[39]. Also, disruption of epithelial cell–cell contacts and associated actin reorganization leads to RHOA–ROCK-mediated activation of protein kinase D (PKD1), resulting in the activation of NF-κB[40]. This scenario may reflect loss of cell–cell contacts during pathological stimulation of endothelial cell motility.

Nuclear hormone receptors

Nuclear hormone receptors, specifically the androgen receptor (AR), are modulated in their transcriptional activity by actin and ABPs, in addition to being under the control of their cognate hormone ligand. Regulation of AR by F-ABPs and G- ABPs can be exerted at the level of cytoplasm-to-nuclear translocation, or by influencing the transcriptional activity of a promoter-bound receptor. ABPs shown to interact with the AR include gelsolin, α-actinin 2, supervilllin, filamin and transgelin[32]. G-actin is part of the AR transcription complex.

The DNA damage response, Nck and JMY

Genotoxic insults affect actin dynamics and elicit transcriptional responses[41,42]. DNA damaging treatment with ultraviolet (UV) light causes two cytoplasmic ARP2/3 effector proteins, junction-mediating and regulatory protein (JMY) and the NCK1–suppressor of cytokine signalling 7 (SOCS7) complex, to translocate to the nucleus. JMY is a G-ABP that can stimulate cell motility. On nuclear translocation, JMY acts as a cofactor of the p53–p300 complex to stimulate the transcription of pro-apoptotic genes[41]. Nuclear translocation of NCK1–SOCS7 follows UV-induced separation from septin proteins[43], which act as F-ABPs. Activation of the DNA damage check-point response requires an as yet undefined nuclear function of NCK1. This highlights new facets of actin microfilamant-to-genome communication that specifically contribute to the cellular genotoxic stress response.

LIM domain-containing proteins

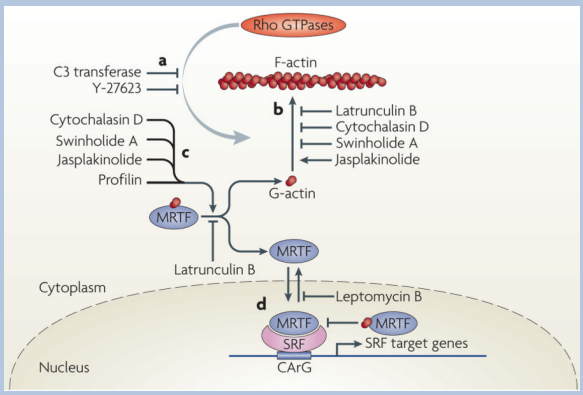
Integrin-mediated engagement of cell–ECM contacts at focal adhesions causes the nuclear translocation of cytoskeletal F-ACAPs[44,45]. The best studied examples are LIM domain-containing proteins and include zyxin, lipoma-preferred partner (LPP), thyroid receptor-interacting protein 6 (TRIP6), paxilin, Hic-5 (also known as TGFB1I1), Cys-rich protein 1 (CRP1), CRP2, antileukoproteinase (ALP), four and a half LIM domains protein 2 (FHL2), LIM and SH3 domain protein 1 (LASP1) and particularly interesting new Cys–His protein 1 (PINCH; also known as LIMS1). These proteins engage in the formation of protein–protein assemblies at focal adhesion-associated actin fibres and, alternatively, can act as nuclear cofactors of transcription or as regulators of nuclear mRNA export. Interestingly, some of these LIM proteins are either cofactors of the transcription factor SRF (for example FHL and CRP proteins)[46,47] or are encoded by SRF-regulated genes (for example zyxin and FHL2)[47,48]. The precise mechanisms of liberation from the focal adhesion are not clear for each of these F-ACBPs, nor are the affected target genes. It seems that LiM protein release from focal adhesions is a mechanism by which changes in the structure and/or density of the F-actin filament are relayed to the nucleus by certain F-ACAP courier proteins for the purpose of modulating gene expression.

Box 2 | *In vitro* modulators of actin dynamics and MRTF–SRF activity

Elucidation of the myocardin-related transcription factor (MRTF)-mediated communication between actin dynamics and transcriptional activation has been helped greatly by the use of compounds that modulate actin activity[51,52,75,76]. The effect of Rho

GTPases on actin polymerization can be inhibited by clostridial toxins such as the RHOA inhibitor C3 transferase (ADP-ribosytransferase) or by the Rho-associated kinase (ROCK)-inhibiting substance Y-27,623 (see the figure, part **a**). Actin-targeting natural compounds impair actin polymerization (latrunculin B, cytochalasin D and swinholide A) or stabilize the filamentous actin (F-actin) polymer (jasplakinolide)[4] (see the figure, part **b**). Interestingly, these compounds also affect the cytoplasmic MRTF–actin complex (see the figure, part **c**). Cytochalasin D, swinholide A and jasplakinolide disrupt the MRTF–globular actin (G-actin) complex, thereby liberating MRTF, whereas latrunculin B inhibits the dissociation of MRTF from G-actin[52,75,76]. Nuclear G-actin also complexes with MRTF, thereby inhibiting the activation potential of serum response factor (SRF) and stimulating nuclear export of MRTF (see the figure, part **d**). Nuclear MRTF–G-actin complexes[76] are affected by actin-targeting compounds in a smilar manner to cytoplasmic complexes. Importantly, these studies reveal that many compounds that were previously thought to primarily modulate actin polymerization equilibrium, also influence the stabilities of actin–actin binding protein (ABP) complexes.

Mutant variants of actin have also been useful in studying the MRTF-dependent regulation of SRF. Actin variants Glu13Arg and Arg62Asp are impaired in polymerization and therefore inhibit MRTF activity, whereas Val159Asn and Ser14Cys stimulate F-actin formation and activate MRTF function[50]. Glu15Ser also stabilizes F-actin while also apparently interacting more tightly with MRTF and promoting its nuclear entry[75]. MRTF variants defective in interacting with actin display constitutive nuclear localization[74]. Structural information on MRTF–G-actin was provided recently by co-crystallization of MRTF-derived RPEL peptides and G-actin in the presence of latrunculin B[77].



The MRTF–SRF circuit

The appreciation that the transcription factor SRF is subject to regulation by Rho GTPases[49] and actin dynamics[50,51] provided the first clue to a hitherto undetected link between the actin cyto-architecture and gene activity. Subsequent identification of G-actin-regulated nucleus–cytoplasm shuttling of the SRF cofactors, MRTFs, revealed for the first time a direct mechanism enabling cytoplasmic G-actin to control nuclear transcriptional activity[52]. MRTFs are G-ABPs, and this regulatory system seems to be of prime importance for the fuelling of actin-based cellular motile function. Thus, the remainder of this Review concentrates on the MRTF–SRF circuit.

Molecular and biological functions of SRF

SRF is a versatile transcription factor, encoded by a single gene that is abundantly expressed in many cell types[53,54]. SRF homologues are found in all species investigated, ranging from

yeast to humans. Homodimeric SRF binds with high affinity and specificity to the palindromic $CC(A/T)_6GG$ DnA sequence, called the CArG-box[55,56], and directs the transcription of numerous target genes on stimulation by various signalling cascades[47,57–61]. SRF associates with target gene promoters to build a regulatory platform for the recruitment of various cofactors[58,62]. Differential cofactor recruitment is best exemplified by the ternary complex factor (TCF) subclass of Ets-type cofactors[63–65] and the myocardin family of co-activators (myocardin and MRTFs)[66–69]. These two types of SRF cofactors respond to distinct signalling inputs (including mitogen-activated protein kinase (MAPK) signalling and actin signalling) and, accordingly, enable SRF to direct the expression of different sets of target genes[70]. MRTFs and TCFs display mutually exclusive interactions with SRF[71,72].

SRF target genes fulfil essential functions (see below), explaining, in part, the dramatic phenotypes seen on *Srf* deletion in mice[73]. A broad range of biological processes is supported by SRF, including gastrulation, development and function of the heart and the cardiovascular system, functioning of all three types of muscle cells, endothelial cell biology and vascularization, development and regeneration of the liver, T cell and B cell activities of the immune system and neuronal functions of the developing and adult brain. At the cellular level, many of these phenotypes can be attributed at least in part to impaired functioning of the actin microfilament[48]. Given the importance of SRF in regulating actin cytoskeletal dynamics, there are nevertheless functions of SRF that go beyond the transcriptional control of cytoskeletal target genes, including contributions to cell survival and apoptosis.

Molecular and biological functions of MRTF proteins

Myocardin, the founding member of the MRTF family (FIG. 3), is expressed specifically in the cardiovascular system[66–68], whereas other MRTF family members display more widespread expression patterns. Myocardin shares homology with MRTF-A (also known as MAL, MKL1 and BSAC) and MRTF-B (also known as MKL2 and MAL16) in a series of conserved domains; to harmonize the nomenclature we suggest using the MRTF designation, which is based on homology criteria of the gene family[69]. These potent transcriptional co-activators associate with SRF through a basic region and an adjacent Glu-rich domain (FIG. 3).

The amino-termini of MRTFs contain three RPEL domains, which form a stable complex with monomeric G-actin[52,74–77], resulting in the sequestration of MRTFs in the cytoplasm. Myocardin contains divergent RPeL domains that do not bind actin efficiently; consequently, homodimeric myocardin is constitutively nuclear and insensitive to cellular stimuli that modulate actin polymerization[68,78]. However, heterodimerization between myocardin and other MRTFs has been proposed to facilitate cooperativity between CArG boxes in SRF-regulated muscle genes[79]. Thereby, myocardin might be subjected indirectly to the regulatory mechanisms operating on MRTFs, specifically G-actin binding.

Knockout studies of myocardin (*Myocd*) and MRTF genes in mice have shown that these SRF co-activators are uniquely required *in vivo* in different cell types and at different developmental stages. Nearly all of the *Myocd* mutant phenotypes reflect abnormalities in aspects of motile cell functions and regulation of SRF-dependent contractile and cytoskeletal genes[80–82]. Mice lacking MRTF-A are viable, but females are unable to nurse their offspring owing to impairments in mammary myoepithelial cells[83,84]. The global deletion of MRTF-B results in embryonic lethality owing to cardiovascular defects, including abnormal patterning of the branchial arch arteries, a double-outlet right ventricle, ventricular septal defects and a thin-walled myocardium[85].

Actin-mediated regulation of MRTF nuclear shuttling

At low actin polymerization states, MRTFs are held in an inactive state in the cytoplasm by reversible complex formation with G-actin[50,52,75]. Thus, MRTFs are *bona fide* G-ABPs. Stimulation of Rho GTPases feeds G-actin into the F-actin filament, thereby liberating MRTFs from G-actin and allowing the nuclear import of MRTF and subsequent activation of SRF-dependent transcription (FIG. 1).

Nuclear G-actin also modulates MRTF functions in multiple ways[76]. First, nuclear export of MRTFs is facilitated by nuclear G-actin. Second, nuclear G-actin prevents nuclear MRTF from activating SRF target genes, so that liberation of the nuclear actin–MRTF complex is required to stimulate SRF. Thus, cellular G-actin regulates MRTFs at three levels: nuclear import, nuclear export and nuclear activation or inactivation of MRTF-SRF-dependent transcription[76] (FIG. 1b). It has also been suggested that MAPK phosphorylation of MRTF stimulates its nuclear export by enhancing the inter action of MRTF with nuclear actin[86]. Therefore, in light of the actin genes themselves being under MRTF-SRF control, a G-actin-dependent, kinetically tuned auto-regulatory feedback loop emerges for Rho-directed control of transcription mediated by MRTF–SRF. Rho activates the initial synthesis of G-actin and ABPs to stimulate F-actin polymerization in the cytoplasm as stimulus-elicited cell biological requirements dictate. With ongoing actin synthesis, G-actin levels rise in both the cytoplasm and the nucleus, thereby downregulating the SRF response by simultaneously impairing the nuclear function and facilitating the export of nuclear MRTF, and by en suring efficient retention of cytoplasmic MRTF (FIG. 1). Accordingly, nucleus–cytoplasm shuttling of MRTF is an essential component of this actin-directed feedback circuit.

The above regulatory mechanisms have been described most thoroughly in muscle cells and cultured fibroblasts. By contrast, in primary neurons a constitutively high nuclear level of MRTF is seen in the absence of stimuli. It will be interesting to identify modulators influencing nuclear MRTF activity in neurons and to explore the potential contributions of nuclear actin[12,76] and nuclear MRTF phosphorylation[86,87] to the neuronal functions of MRTFs.

Positive and negative regulators of MRTF–SRF activity

Numerous cofactors in addition to TCFs and MRTFs exert positive and negative effects on SRF activity. In the cytoplasm of muscle cells, striated muscle activator of Rho-dependent signalling (STARS; also known as ABRA) is a G-ABP that is upregulated during cardiomyopathy[88]. STARS is localized to the sarcomere and, by interacting with monomeric G-actin, promotes nuclear translocation of MRTF-A. STARS thereby syner gizes with MRTF-A to stimulate SRF-mediated transcription. Positive nuclear cofactors of SRF include the Nkx2–5 family of homeodomain proteins[89], members of the GATA family of zinc-finger proteins[90,91] and the CRP family of Cys-rich LIM-only proteins[46]. Negative co factors include the LIM-only protein FHL2 (REFS 47›92), histone deacetylase 4 (HDAC4)[93], the homeo domain protein MSX1 (REF. 94) and the zinc-finger protein krueppel-like factor 4 (KLF4)[95]. Homeodomain-only protein (HOP) dampens SRF activity in cardiac muscle cells by competing with it for myocardin and MRTF-A interaction, and also by recruiting HDAC2 to SRF target genes[96–98]. Members of the myocardin family also engage various partners in addition to or in conjunction with SRF. These include the histone acetyltransferase p300 (REFS 99›100), class II HDACs[99], SMAD4 (REF. 101) (a transcription factor regulated by TGFβ signalling), forkhead box protein O4 (FOXO4)[95] and GATA transcription factors[102]. Such combinatorial associations of SRF with positive and negative partners expand the regulatory potential of SRF by providing cell type specificity, temporal control and signal responsiveness to SRF target genes.

MRTF–SRF target gene functions

SRF target genes, containing functional CArG boxes for SRF binding, have been identified by candidate gene characterization, genome-wide expression profiling of SRF-deficient cells and *in silico* genome queries[47,48,59,60] (for review see REF. 57). Based on these studies, we estimate the number of SRF target genes to be around 300. Chromatin immunoprecipitation (ChIP) with anti-SRF antisera, which provides stringent support for direct gene regulation by SRF, has identified more than 200 SRF target genes[103]. Selective cofactor recruitment (TCF versus myocardin and MRTFs) directs signal-specifi c regulation of different classes of SRF target genes. Class I target genes, which primarily encode proteins with 'immediate–early' cellular functions, including the rapid transcriptional activation of genes encoding proteins involved in the G0–G1 transition, are aided by TCF cofactors[62,63]. Class ii target genes, which are regulated by SRF in conjunction with myocardin or other MRTF cofactors[57], encode at least three types of proteins, namely those involved in muscle-specific and contractile functions, actin microfilament dynamics and cell motility, and miRNA activities. In the following, we restrict our discussion to class II SRF target genes to emphasize their regulation by the myocardin-family transcriptional co-activators.

Muscle-specific and contractile genes

Nearly all smooth muscle-specific genes and many cardiac and skeletal muscle genes are controlled by CArG boxes. Myocardin is a particularly potent activator of SRF-dependent smooth muscle gene expression[79,84,104–106]. Forced overexpression of MRTFs also activates smooth muscle genes in transfected cells, which raises the question of why the endogenous levels of MRTF in many non-muscle cells are not sufficient to drive the expression of muscle genes[59,69,107,108]. It is likely that endogenous MRTF levels are regulated by actin signalling, whereas overexpressed MRTFs bypass this regulatory mechanism to directly activate downstream muscle genes. MRTFs are also required for skeletal muscle differentiation, as shown by the inhibition of skeletal muscle gene expression on expression of dominant-negative MRTF mutants *in vivo* and *in vitro*[59,108]. Many of the contractile functions of muscle cells are enabled by myocardin–SRF- and MRTF–SRF-regulated genes[57].

Genes affecting actin dynamics and cell motility

SRF target genes, which are regulated on recruitment of MRTF cofactors, encode proteins related to actin cytoskeletal activities. SRF-regulated gene products can be grouped into three categories according to their contributions to actin microfilament function: structural (for example, actin), effectors of actin turnover (for example, cofilin 1) and regulators of actin dynamics (for example, talin 1). TABLE 1 lists some well-characterized examples of cytoskeletal SRF target genes. In biological terms, the encoded proteins contribute to development (mesodermal patterning), functioning of the contractile apparatus (muscle cell contraction, vascular tone) and ECM-mediated adhesion and tissue remodelling.

Expression profiling using modulators of MRTF activity (dominant-negative MRTF and treatment with cytocalasin D in the absence or presence of latrunculin B; see BOX 2) identified a subset of ~ 30 MRTF-dependent SRF target genes[7,59,109]. We expect this number to grow with ongoing investigations[110]. ChIP-based evidence for the association of MRTF with SRF at CArG boxes is available but is limited[103]. Despite this limitation, identified MRTF-dependent SRF target genes contribute to cell–ECM adhesion and actomyosin activity. Many structural components of focal adhesions are encoded by SRF–MRTF-regulated genes, including α1, α5, α9 and β1 integrins, as well as talin 1, vinculin, and the syndecan proteins 2 and 4 (REF. 57), such that SRF deficiency leads to impaired formation of focal adhesions[48].

miRNAs

Recent studies have revealed central roles for miRNAs in the cellular circuitry through which SRF and MRTFs control muscle gene expression, cytoskeletal dynamics and stress responsiveness[111]. SRF directly activates the expression of two bicistronic miRNA genes that encode pairs of homologous miRNAs (miR-1-1 and miR133a-2, and miR-1–2 and miR133a-1)[112] (FIG. 4a). These miRNAs, in turn, regulate a large collection of mRNAs, many of which encode proteins that impinge on the SRF–MRTF signalling pathway. miR-133, for example, represses the expression of SRF, thereby establishing a negative feedback loop to precisely titrate SRF expression in muscle cells[113]. A particularly intriguing finding is that miR-1 can substitute for SRF in the induction of mesoderm from mouse embryonic stem cells[114].

A screen for miRNAs regulated by MRTF-A in cardiac myocytes identified several miRNAs with key roles in muscle differentiation, proliferation and phenotypic switching[115]. Many of the same miRNAs were down-regulated in SRF-null compared to wild-type mouse embryonic stem cells. Bioinformatics also showed a disproportionate number of predicted SRF-binding sites associated with miRNA genes[116].

miR-143 and miR-145 are encoded by a bicistronic miRNA gene, controlled by a distal upstream CArG box (FIG. 4b). These two miRNAs are expressed in early cardiac progenitors and throughout the embryonic heart before becoming restricted to all vascular and visceral smooth muscle cells[115,117]. miR-145 knockdown in cultured cells was reported to block smooth muscle gene activation by myocardin, pointing to this miRNA as an essential cofactor of myocardin[117,118]. By contrast, knockout mice lacking miR-143, miR-145 or both are viable and express smooth muscle SRF target genes normally[115]. However, these mice are resistant to vessel remodelling in response to vascular injury. The insensitivity of these mutant mice to vascular injury seems to reflect abnormal regulation of a collection of proteins involved in the modulation of actin cytoskeletal dynamics and stress fibre formation, rendering the mutant mice insensitive to deformation of the vessel wall. For example, these miRNAs target adducin 3, which caps barbed ends of actin filaments and acts as a bridge between the membrane and actin cytoskeleton[115]. Slingshot 2, another target of miR-143 and miR-145, promotes cell motility and enhances F-actin reorganization by dephosphorylating and activating cofilin, an actin severing factor. miR-145 targets KLF4 and KLF5, which repress SRF activity, as well as Slit-Robo GTPase-activating proteins, which modulate cell migration by inactivating the small GTPase Cdc42 and inhibiting actin polymerization[115]. Finally, these miRNAs target MRTF-B, the upstream regulator of their expression, providing a negative feedback loop to precisely titrate miR-143 and miR-145 expression and actin dynamics[115] (FIG. 4b).

Mice lacking miR-143 and miR-145 display a reduction in blood pressure owing to a decreased vascular tone. These findings point to a role for miR-145, and to a lesser extent miR-143, in the control of a feedback loop that modulates a cytoskeletal–transcriptional circuit regulated by SRF.

Another miRNA regulated by SRF together with MRTF is miR-486, a cardiac and skeletal muscle-enriched miRNA expressed from an alternative promoter in an intron of ankyrin 1 (REF. 119). miR-486 acts as a downstream effector for the SRF–MRTF pathway and promotes phosphoinositide 3-kinase (PI3K)–AKT signalling by inhibiting the expression of phosphatase and tensin homologue (PTEN) and FOXO1A, which act as negative regulators of PI3K signalling.

By regulating the expression of miRNAs that affect the signalling system which controls MRTF–SRF activity, the MRTF–SRF partnership generates a balanced regulatory network

to govern cytoskeletal function and signal responsiveness. It is curious that SRF, more so than other transcription factors, is so tightly intertwined with miRNA regulatory circuits. Perhaps this reflects the importance of maintaining tight control over SRF and its downstream targets.

The actin–MRTF–SRF circuit in pathology

MRTF-mediated communication between the actin cytoskeleton and the genome has broad implications for many motile functions of cells. Cell type-specific mouse knock-out studies of *Srf* and MRTF genes suggest that the MRTF–SRF circuit contributes to human pathology. Available evidence supporting this notion is discussed below in the context of two major threats to human health: cardiovascular diseases and cancer.

Cardiovascular diseases

Given the plethora of genes encoding contractile proteins that are regulated by SRF and members of the myocardin family, it is not surprising that this regulatory pathway has a central role in force- and mechano-sensing of muscle cells, such that dysregulation of SRF-dependent gene expression contributes to numerous disease models of the cardiovascular system. During pathological remodel ling of the heart in the settings of hypertrophy and heart failure, SRF-dependent genes are dysregulated. STARS and myocardin, two positive modulators of SRF function, are upregulated during cardiomyopathy and hypertrophy, respectively[88,120,121]. Caspase 3 cleavage of SRF in a failing heart generates a dominant-negative form of SRF that may suppress expression of sarcomeric genes[122]. Thus, SRF is not only required for the appropriate expression of cardiac contractile proteins, but excessive SRF is pathogenic, indicating the requirement of MRTF–SRF activity for cardiac homeostasis. This may explain why miRnAs, which provide robustness and stability to gene regulatory programmes, are so intimately integrated into the MRTF–SRF signalling pathway.

Recently, SRF and myocardin were shown to be overexpressed in small cerebral arteries during Alzheimer's disease, and to have a key role in the progression of the disease by enhancing arterial hypercontractility through the activation of SRF-dependent smooth muscle contractile genes, thereby diminishing cerebral blood flow[123]. in addition, SRF and myocardin control amyloid-β peptide clearance from cerebral vascular smooth muscle cells[124].

SRF also functions as a central regulator of smooth muscle cell phenotypes in response to injury and contributes to pathological smooth muscle cell proliferation in the vessel wall. Signals that diminish myocardin and MRTF activity promote excessive proliferation of smooth muscle cells, as occurs during atherosclerosis and restenosis[125]. The forkhead transcription factor FOXO4 and the zinc-finger protein KLF4 are upregulated during de differentiation of vascular smooth muscle cells *in vivo* [95], resulting in suppression of SRF-dependent transcription. Thus, the MRTF–SRF circuit functions as a nodal sensor of mechanical stress and growth factor signalling to modulate phenotypic switching of smooth muscle cells.

Cancer

Associations of the actin–MRTF–SRF circuit with human cancer biology have been identified, suggesting an MRTF–SRF involvement in the neoplastic process. However, definitive evidence for causative links to clinically relevant carcinogenesis is still lacking.

Infant acute megakaryoblastic leukaemia (AMKL), which correlates with poor prognosis, is associated with the balanced chromosomal translocation t(1;22) (p13;q13), in which the

MRTF-A gene (*MKL1*; also known as *MAL*) is fused to the genomic fusion partner OTT (also known as RBM15)[126–128] (FIG. 3). OTT–MAL is a constitutively nuclear, deregulated activator of SRF, which is not subject to control by G-actin[129–131]. OTT–MAL activates the transcription factor Jκ recombination signal-binding protein (RBP-Jκ), which is normally controlled by canonical notch signalling and is overridden by OTT–MAL to cause abnormal megakaryopoiesis[132].

Myocardin was argued to be a differentiation-inducing tumour suppressor protein that is downregulated in mesenchyme-derived sarcomas[133]. By contrast, leiomyosarcoma (LMS) smooth muscle tumours display overexpression of myocardin[134]. In human prostate cancer, androgen receptor activity was suggested to regulate SRF-dependent expression of FHL2 (REF. 135), which itself is a regulator of SRF activity[47,92] and integrin signalling; the expression of either SRF or FHL2 correlates with poor prognosis.

The general association of MRTF–SRF activity with actin-regulated cellular motile functions links actin–MRTF activity to cancer metastasis. Accordingly, depletion of either MRTF or SRF caused reduced adhesion, spreading, motility, invasion and colonization of metastatic tumour cells in an experimental *in vivo* metastasis assay[109]. In support of MRTF–SRF activity contributing to tumour metastasis, suppressor of cancer cell invasion (SCAI) was found to repress MRTF activity. SCAI was postulated to downregulate MRTF–SRF-mediated expression of β1 integrin, thereby acting as a suppressor of tumour cell invasion[136].

Conclusions and future directions

Although previously unappreciated, it now seems obvious that actin-driven cell motility requires genomic support. Several independent mechanisms can now be outlined that communicate the dynamic status of the actin cytoskeleton to the genome. Microfilament-to-genome communication is mediated by different courier proteins, representing G-ABPs, F-ABPs or F-ACAPs, the cytoplasmic release of which for nuclear translocation is dependent on changes in G-actin levels or F-actin structure and composition. Such courier proteins can function inside nuclei as transcriptional cofactors, as clearly shown for the CRP, FHL2, JMy and MRTF proteins. The control of SRF activity by multiple cofactor interactions and signalling inputs serves as a paradigm for understanding the logic of connecting genome activity with actin cytoskeletal dynamics. The intimate functional link between actin dynamics, MRTF–SRF-regulated gene expression and cellular motile behaviour has been confirmed by genetic means in several cell and organ systems, including embryonic stem cells[48], the developing mouse embryo[73,116,137], muscle tissues[82,138] and the brain[11,139,140]. However, numerous molecular details of the regulatory interactions involved remain to be worked out in the MRTF–SRF circuit and in all the other above-mentioned systems.

We anticipate that new actin-dependent courier proteins will be identified. For each relay system it will be important to characterize comprehensively the associated differential gene expression profiles. The example of MRTF–SRF-directed expression of miRnA-encoding genes indicates that surprising mechanistic insight is to be derived from future studies in this area of cell biology.

Microbial pathogens, both bacteria (for example, *Listeria* Spp., *Samonella* Spp. and enteropathogenic *Escherichia coli*) and viruses (for example, vaccinia virus), subvert actin cytoskeletal functions of the infected host cell by modulating its actin dynamics (for review see REF. 141). it is likely that these influences on micro filament remodelling are accompanied by changes in gene activity and we postulate that this involves the MRTF–SRF circuit. It is equally possible that other, hitherto undetected, G-ABPs might be involved in

such events of host cell infection. In more general terms, actin dynamics might communicate to the genome and thereby combat other cellular insults, as already seen in the context of UV-induced DNA damage.

Vesicle trafficking such as endocytosis, exocytosis and Golgi-to-endoplasmic reticulum transport, which are dependent on dynamic actin rearrangements, might also elicit and even require changes in gene expression. This poorly studied aspect of membrane trafficking warrants closer investigation and may offer surprising new insights.

Embryonic development requires cell movement and motile functions at many essential steps. The precise contributions of microfilament-to-genome communication to these activities are still inadequately understood. Although participation of SRF in mouse gastrulation was noticed early on[73], the MRTF–SRF circuit is expected to contribute to development in many additional ways, including enabling functions during EMT[22] and stem cell programming and reprogramming[117]. Deeper insight into these issues will prove rewarding.

The remarkable progress in identifying actin-dependent SRF cofactors and determining their mechanisms of action has provided new insights into the myriad aspects of cell physiology, development and disease. Given the likely involvement of the actin–MRTF–SRF pathway in tissue remodelling during disease, it will be important to determine how this and similar pathways of cytoskeleton-to-nucleus communication can be therapeutically modulated.

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Glossary

F-actin	A flexible, helical polymer of G-actin monomers that is 5–9 nm in diameter. An F-actin polymer is polar, displaying a plus (+) and a minus (−) end
Focal adhesion	A cellular structure that links the ECM on the outside of the cell to the actin cytoskeleton inside the cell through integrin receptors
Guanine nucleotide exchange factor	A protein that activates a specific small GTPase by catalysing the exchange of bound GDP for GTP
Heterotrimeric G protein	One of a family of heterotrimeric cytoplasmic signal mediators composed of an α-subunit containing a GTP-binding site and intrinsic GTPase activity together with a hydrophobic and an often acylated β- and γ-protein subcomplex. The β- and γ-protein subcomplex may be activated by the dissociation of α–GTP, which is initiated by GTP exchange on the α-subunit
Epithelial–mesenchymal transition	The transformation of an epithelial cell into a mesenchymal cell with migratory and invasive properties
Adherens junction	A cell–cell adhesion complex that contains classical cadherins and catenins that are attached to cytoplasmic actin filaments

SAP domain	(SAFA or SAFB, acinus and PIAS domain). A peptide motif found in several proteins known to contact DNA
Leu zipper	A Leu-rich domain in a protein that binds to other proteins with a similar domain
LIM domain	(Lin11, Isl1 and Mec3 domain) A zinc-binding protein domain that ligates two zinc ions. LIM domains mediate interactions with other proteins and have diverse functions as regulators of gene expression, cell adhesion and motility, and signal transduction
RPEL domain	A protein domain containing the amino acid sequence Arg-Pro-X-X-X-Glu-Leu that is found triplicated in the myocardin family of transcriptional coactivators. RPEL domains form stable complexes with G-actin
Barbed end	The plus (+) end of the polar F-actin polymer that, in contrast to the minus (−) end, is more active with regard to incorporation of G-actin into the polymer (polymer elongation)
Leiomyosarcoma	A rare type of cancer (a soft tissue sarcoma) that is a malignant neoplasm of smooth muscle origin.

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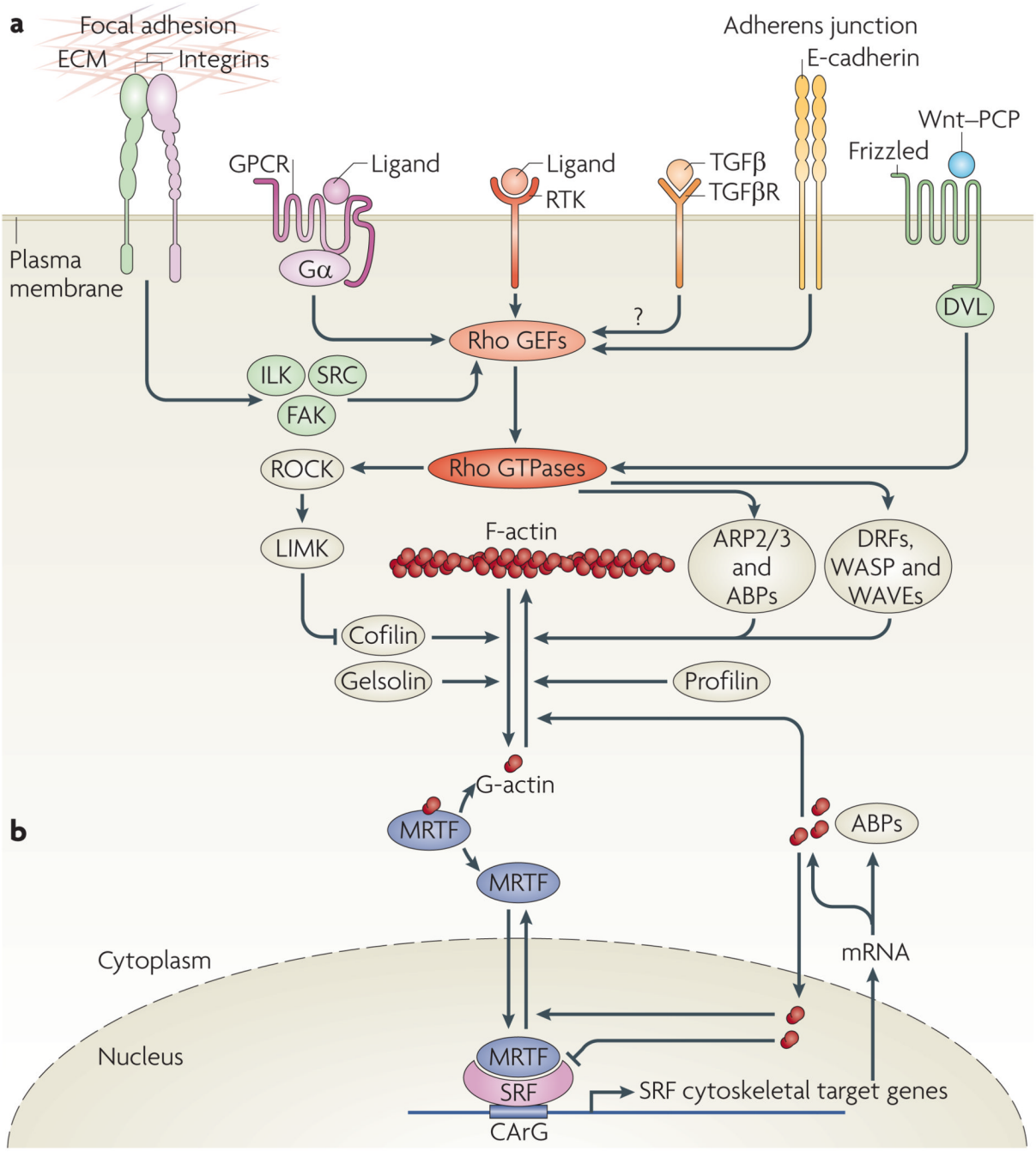

Figure 1. receptors affecting actin dynamics and MrTF-mediated regulation of SrF target genes

a | Cytoskeletal actin microfilament dynamics are affected by the activation of six classes of plasma membrane receptor: receptor Tyr kinases (RTKs), G protein-coupled receptors (GPCRs; with α-subunits of $G\alpha_{12/13}$, $G\alpha_{q/11}$ or $G\alpha_{i/0}$), integrins as structural mediators of focal adhesions, transforming growth factor-β receptors (TGFβRs), E-cadherins at adherens junctions and Frizzled, which mediates the non-canonical Wnt–planar cell polarity (PCP) pathway involving Dishevelled (DVL). These receptors modulate the activity of Rho GTPases[2] through Rho guanine nucleotide exchange factors (GEFs). Effectors of Rho GTPases, including Rho-associated kinases (ROCKs), formins (such as Diaphanous-related formins (DRFs)), Wiskott–Aldrich syndrome protein (WASP), WASP-family verprolin

homologues (WAVEs) and the actin-related protein 2/3 (ARP2/3) complex and other actin-binding proteins (ABPs), orchestrate actin polymerization by incorporating globular actin (G-actin) into the filamentous actin (F-actin) polymer[3]. High levels of cytoplasmic G-actin retain serum response factor (SRF) cofactor proteins, myocardin-related transcription factors (MRTFs), in the cytoplasm. Incorporation of G-actin into the F-actin filament liberates MRTFs to enter the nucleus and interact with the transcription factor SRF[58]. This triggers expression of a subset of SRF target genes, namely cytoskeletal genes. **b** | Activation of SRF class II target genes. Nuclear MRTF can be complexed by nuclear G-actin, which inhibits MRTF-mediated stimulation of SRF-dependent transcription and facilitates MRTF nuclear export[76]. SRF class II target genes that are transcribed as a result of MRTF–SRF activation include actin itself and many genes that modulate actin dynamics, such as gelsolin and vinculin. These newly made proteins, with increasing time and concentration, might stimulate cytoplasmic actin polymerization, complex cytoplasmic MRTF or elevate levels of nuclear G-actin to downregulate MRTF-mediated transcription and stimulate nuclear export of MRTF. FAK, focal adhesion kinase; ILK, integrin-linked protein kinase; LIMK, LIM domain kinase.

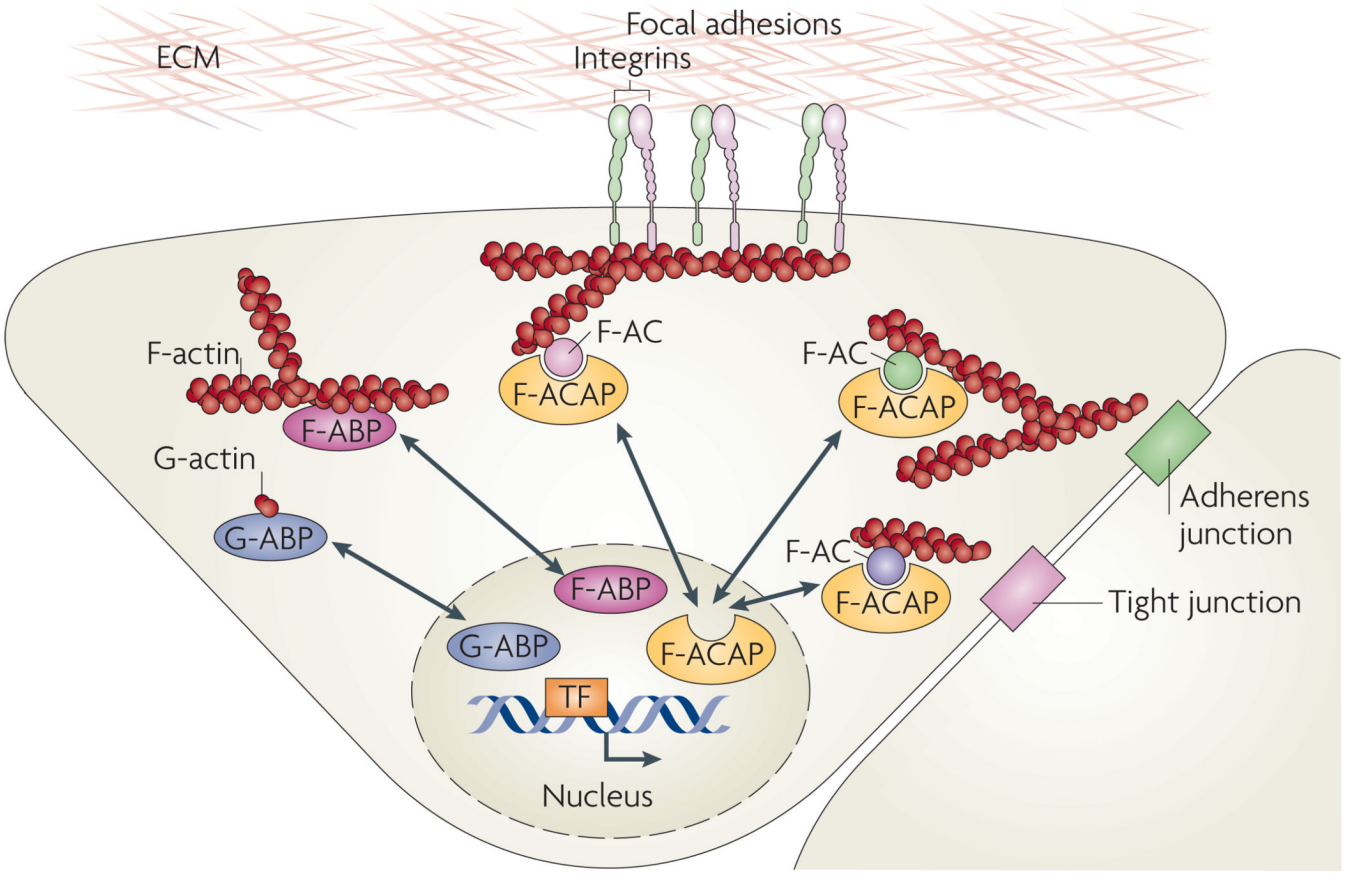

Figure 2. Actin-binding proteins as microfilament messengers

A model summarizing the nucleus–cytoplasm shuttling of three different types of actin-binding proteins (ABPs): globular actin (G-actin)-binding proteins (G-ABPs), filamentous actin (F-actin) binding proteins (F-ABPs) and F-actin complex-associated proteins (F-ACAPs). Examples of G-ABPs include myocardin-related transcription factors (MRTFs), striated muscle activator of Rho-dependent signalling (STARS, also known as ABRA), junction-mediating and regulatory protein (JMY), β-thymosin, profilin, neural Wiskott–Aldrich syndrome protein (N-WASP), the actin-related protein 2/3 (ARP2/3) complex and spire[31]. Examples of F-ABPs include the actin-binding LIM proteins (ABLIMs) cofilin, gelsolin, filamin, α-actinin, supervillin and LIM and SH3 domain protein 1 (LASP1)[31,32], and examples of F-ACAPs include ABL1, integrin cytoplasmic domain-associated protein 1 (ICAP1α), LIM domain proteins and p120-catenin[44]. The shuttling LIM domain proteins zyxin, lipoma-preferred partner (LPP), Cys-rich proteins (CRPs), Hic-5 (also known as TGFB1I1), antileukoproteinase (ALP), paxilin and LIM and SH3 domain protein 1 (LASP1) were shown to directly bind F-actin, whereas LIM kinase, particularly interesting new Cys-His protein 1 (PINCH; also known as LIMS1) and four and a half LIM domains protein 2 (FHL2) are probably indirectly linked to F-ACs[44,45]. F-ACs assemble at the cytoplasmic sides of focal adhesions, cadherin-mediated cell–cell adherens junctions and cell–cell tight junctions. TF, transcription factor.

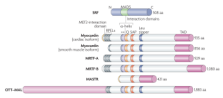

Figure 3. Structure of myocardin family members

Functional domains of homology among the myocardin family proteins are shown and the numbers of amino acids are indicated. Myocardin-related transcription factors (MRTFs) are potent transcriptional coactivators that associate with serum response factor (SRF) through a basic region (++) and an adjacent Glu-rich domain (Q). Between these domains is a short α-helical region with similar secondary structure to a domain in the ternary complex factor protein ELK1, known as a B box, which mediates their interaction with SRF[52,68,71]. Myocard in family proteins contain Arg-Pro-X-X-X-Glu-Leu (RPEL) motifs, which mediate their interact ion with globular actin (G-actin). Members of the myocardin family have a homologous SAP domain, named after SAFA or SAFB, acinus and PIAS, which participates in different kinds of chromosomal DNA metabolism. Deletion of this region disrupts the ability of myocardin to activate a subset of SRF-dependent genes[68]. Myocardin and MRTFs contain powerful transcriptional activation domains (TADs) required for the stimulation of SRF activity. A dimerization motif resembling a Leu zipper mediates homo- and heterodimerization of myocardin and MRTFs. Alternative usage of 5′ exons in the myocardin gene gives rise to proteins with different amino termini. A cardiac-specific splice variant of myocard in contains a unique amino-terminal sequence that confers the ability to interact with the myocyte-specific enhancer factor 2 (MEF2) transcription factor, a MADS-boxtranscription factor related to SRF. This MEF2-interaction domain is also contained in a divergent member of the myocard in family called MEF2-activating SAP transcriptional regulator (MASTR). MASTR lacks the SRF-interaction domain.The OTT (also known as RBM1B)-MAL (also known as MRTF-A) fusion protein of AMKL leukaemia cells contains MRTF-A sequences.

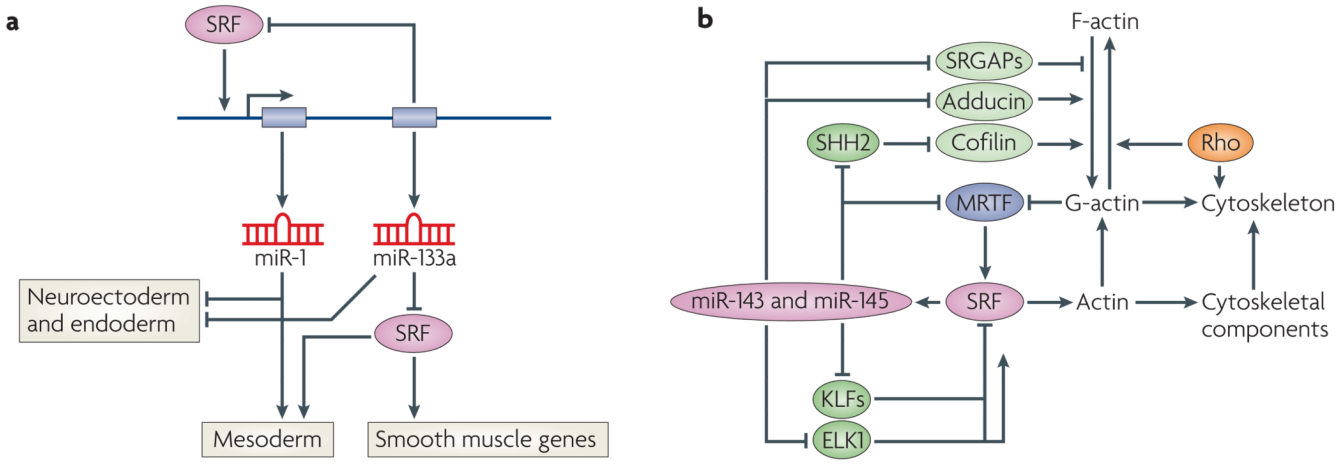

Figure 4. SRF-mediated regulation of miRNAs

a | Regulation of microRNA-1 (miR-1) and miR-133 by sereum response factor (SRF). SRF activates transcription of a bicistronic miRNA cluster encoding miR-1 and miR-133 (REFS [112,113]). miR-133a inhibits SRF expression, establishing a precisely titrated feedback loop to modulate SRF activity. There are two clusters of miR-1 and miR-133a genes in mammalian genomes, which are expressed specifically in cardiac and skeletal muscle cells. A third homologous pair of miRNAs, miR-206 and miR-133b, is expressed specifically in skeletal muscle independently of SRF. Genetic deletion of miR-1 results in phentoypes that suggest it has a role in mesoderm formation. Genetic deletion of the two miR-133a genes results in perinatal lethality owing to cardiac defects. miR-1 and miR-133a also repress neuroectoderm and endoderm genes and promote mesoderm gene expression. miR-1 can substitute for SRF to regulate downstream genes involved in mesoderm development by an undefined mechanism. **b** | Regulation of miR-143 and miR-145 by SRF. SRF activates transcription of a bicistronic miRNA cluster encoding miR-143 and miR-145, which are expressed specifically in cardiac and smooth muscle cells[115,117]. These miRNAs, regulate the expression of numerous mRNAs encoding regulators of actin signalling and myocardin-related transcription factor (MRTF)–SRF activity, thereby establishing an elaborate series of feedback loops to modulate the actin–MRTF–SRF signalling pathway. Targets of miR-143 or miR-145 include the zinc-finger proteins krueppel-like factor 4 (KLF4) and KLF5 (which inhibit SRF), MRTF-B, slingshot 2 phosphatase (SHH2; which controls actin polymerization by cofilin phosphorylation), adducin 3 (which promotes actin polymerization) and Slit-Robo Rho GTPase-activating protein 1 (SRGAP1) and SRGAP2 (which inhibit Rho signalling)[115]. This pathway has been shown to be essential for vascular remodelling in response to injury. In the absence of miR-143 and miR-145, actin stress fibre formation is disrupted, rendering smooth muscle cells insensitive to mechanical stimuli that typically cause vascular stenosis.

Table 1

SRF–MRTF-regulated genes encode actin microfilament effectors

Gene symbol*	Gene name	Function
Structural components of microfilament function		
ACTA1	Actin, alpha 1	Microfilament building
ACTA2	Actin, alpha 2	Microfilament building
ACTB	Actin, beta	Microfilament building
ACTG1	Actin, gamma 1	Microfilament building
ACTG2	Actin, gamma 2	Microfilament building
CALD1	Caldesmon 1	Muscle cell contraction
DMD	Dystrophin	Membrane anchorage
ITGB1	Integrin, beta 1	Focal adhesion component
MYH11	Myosin heavy chain 11	Actomyosin structure
MYH9	Myosin heavy chain 9	Actomyosin structure
MYL9	Myosin light chain 9	Actomyosin structure
TAGLN (SM22A)	Transgelin	Muscle cell contraction
SMTN	Smoothelin	Muscle cell contraction
TPM1	Tropomyosin 1	Muscle cell contraction
VCL	Vinculin	Linking integrin to microfilaments
Effectors of actin turnover		
ACTR3 (ARP3)	Actin-related protein 3	Microfilament turnover and branching
CFL1	Cofilin 1	Microfilament turnover
GSN	Gelsolin	Microfilament turnover
VIL1	Villin 1	Microfilament reorganization
SVIL	Supervillin	Microfilament reorganization
Regulators of actin dynamics		
CTGF	Connective tissue growth factor	An ECM protein that is an integrin ligand and modulates cellular migratory activity
CYR61 (*CCN1*)	Cys-rich protein 61	An ECM protein that is an integrin ligand and modulates cellular migratory activity
FHL1 and *FHL2*	Four-and-half LIM domains 1 and 2	Adherens junction signalling and transcriptional regulation (through SRF binding)
FLNA	Filamin A	Integrin signalling
MMP9	Matrix metalloprotease 9	ECM remodelling and cell migration
MYLK	Myosin light chain kinase	Actomyosin regulation
SRF	Serum response factor	Transcriptional regulation
TLN1	Talin 1	Integrin signalling

ECM, extracellular matrix.

* All the genes listed have promoters with functional serum response factor (SRF)-binding sites, as confirmed by chromatin immunoprecipitation (ChIP). Myocardin-related transcription factor (MRTF) ChIP is currently available only for a subset of these genes, see also REF. [57]. Alternative gene symbols are provided in brackets.