Minireview

Nucleoskeletal regulation of transcription: Actin on MRTF

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Impact statement

Regulation of gene expression is a fundamental cellular process that ensures the appropriate response of a cell to its surroundings. Alongside biochemical signals, mechanical cues, such as substrate rigidity, have been recognized as key regulators of gene expression. Nucleoskeletal components play an important role in mechanoresponsive transcription, particularly in controlling the activity of MRTF-A/SRF transcription factors. This ensures that the cell can balance the internal and external mechanical forces by fine-tuning the expression of cytoskeletal genes.

Abstract

Myocardin-related transcription factor A (MRTF-A) and serum response factor (SRF) form an essential transcriptional complex that regulates the expression of many cytoskeletal genes in response to dynamic changes in the actin cytoskeleton. The nucleoskeleton, a "dynamic network of networks," consists of numerous proteins that contribute to nuclear shape and to its various functions, including gene expression. In this review, we will discuss recent work that has identified many nucleoskeletal proteins, such as nuclear lamina and lamina-associated proteins, nuclear actin, and the linker of the cytoskeleton and nucleoskeleton complex as important regulators of MRTF-A/SRF transcriptional activity, especially in the context of mechanical control of transcription.

Keywords: Actin, nucleoskeleton, myocardin-related transcription factor, serum response factor nuclear lamina, transcription

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Introduction

The main function of the eukaryotic cell nucleus is to provide the environment and appropriate control for storing the genomic material and for gene transcription. One important feature of this regulation is non-random spatial and temporal distribution of chromatin and its regulatory elements in the interphase nuclei. Existence of particular chromosome territories, clustering of modified chromatin, and the long-range contacts that form between promoters and control regions are all relevant features of nuclear organization.[1] Therefore, identifying the mechanisms and molecular players that regulate non-random nuclear organization is of utmost importance. An interesting, but very controversial concept is the nucleoskeleton, which has been proposed to support and regulate nuclear processes as does the cytoskeleton in the cytoplasm.[2] The nucleoskeleton consists of heterogeneous nuclear components, such as lamins and lamina-binding proteins, nuclear pore-linked filaments, nuclear mitotic apparatus, spectrins, titin, nuclear actin, nuclear myosins, and kinesins, which altogether contribute to the specific shape, mechanical properties and functionality of the nucleus and genome.[3,4] Since a rigid structural framework is not supported by current experimental data and the dynamic properties of the cell nucleus, the nucleoskeleton is nowadays mainly considered as a "dynamic network of networks,"[4] and we will be using this definition in our review as well. To date, there is a lot of evidence showing that almost every important aspect of nuclear function is influenced by nucleoskeletal components. Chromatin packaging status, its epigenetic modifications, intranuclear translocation and transcription activation—these and many other essential processes are, directly or indirectly, regulated by nucleoskeletal components. In addition to regulation at the biochemical level, nucleoskeletal proteins, particularly proteins of the nuclear lamina, are also key players in mechanoadaptation and mechanoresponsive functions of the nucleus, which transduce mechanical stimuli emerging from both inside and outside the nucleus.[5] In this review, we will discuss how nucleoskeletal components, in particular nuclear actin, nuclear lamina and lamina-binding proteins, regulate gene expression by utilizing the MRTF-A/SRF transcription complex as an example.

Actin regulates MRTF/SRF pathway activity

Serum response factor (SRF) is a ubiquitously expressed and conserved transcription factor that mediates both the signal-stimulated transcriptional induction of immediate early genes (IEGs), and the activation of cell type-specific genes. It works as a key regulator of cell growth, migration, cytoskeletal organization, differentiation and muscle-specific or neuronal gene expression.[6,7] Expression of SRF target genes is promoted by two families of signal-regulated co-activators, which interact competitively with the DNA-binding domain of SRF, but also contact DNA flanking the SRF-binding site.[8,9] One family is composed of three ternary complex factors (TCFs), Elk-1, Net, and SAP-1 that contain a conserved DNA binding domain ETS (E26 transformation-specific or E-26), and act in the Ras – extracellular signal regulated kinase (ERK) signaling pathway.[10–12] The other SRF co-factor family, the myocardin family, is directly regulated by actin.[8] Actin is a major constituent of the cytoskeleton, and coordinated polymerization of actin monomers (G-actin) to actin filaments (F-actin) plays a key role in processes such as cell shape changes and motility.[13] SRF activation can be regulated by actin polymerization, which can be induced, for example downstream of the small GTPase RhoA.[14] There are three homologous myocardin-related transcription factor (MRTF) proteins: myocardin, MRTF-A (also known as MKL1, MAL), and MRTF-B (MKL2). Myocardin, the founding member of the family and very strong activator of SRF, is expressed in cardiac (longer isoform of myocardin) and smooth muscle cells (shorter isoform of myocardin).[15] MRTF-A and MRTF-B are broadly expressed in a wide variety of tissues and play a critical role in skeletal muscle differentiation.[16] In addition, MRTF-B is also required for normal vascular development and smooth muscle gene expression.[17] MRTF-A is the most ubiquitously expressed mammalian MRTF. It was originally characterized as a fusion with RNA-binding protein 15 (RBM15) in acute megakaryoblastic leukemia.[18] MRTF-A acts as a cofactor for SRF-mediated gene activation in muscle differentiation, but it also regulates expression of a number of cytoskeletal genes in non-muscle cells.[19] To clarify the interplay between SRF and its cofactors and analyze their genomic targets, transcriptional response of fibroblasts to serum stimulation was investigated.[20] ChIP-seq analysis showed that recruitment of SRF cofactors is gene-specific, consistent with predictions from earlier functional studies of model genes,[21] and majority of serum-responsive SRF-linked genes are controlled through MRTF signaling. Furthermore, SRF seems to be the primary targeting agent for the MRTFs. The main role of SRF/MRTF complex was defined as facilitating nucleosome displacement. MRTF itself is required for both RNA Pol II recruitment and for the post-recruitment step in transcriptional activation.[20]

Actin plays a key role in regulating MRTF localization and activity. The conserved N-terminus of MRTFs contains an RPEL (arginine-proline-glutamine-leucine consensus sequence containing) domain that includes three actin-binding motifs (RPEL1, RPEL2, RPEL3), overlapping with an extended bipartite nuclear localization signal (NLS).[22,23] The distinctive feature of myocardin is its exclusively nuclear localization due to divergent RPEL motifs that have a lower affinity for actin compared to the other MRTFs.[24] On the other hand, MRTF-A (and likely MRTF-B) constantly shuttle in and out of the nucleus,[25] and the subcellular localization of both is tightly regulated by actin dynamics. In resting conditions MRTF-A localizes in the cytoplasm due to formation of a pentavalent RPEL-actin complex, where three RPEL motifs and two intervening spacers bind five actin molecules.[23] Structural analysis showed that in this complex, actin-binding sterically occludes the NLS, preventing its recognition by the importin-α/β (Ipoα/β) heterodimer and thus blocking nuclear import of MRTF-A. Mitogenic or mechanical stimuli lead to RhoA-mediated actin polymerization and reduction of the actin monomer (G-actin) pool in the cell. Consequently, G-actin dissociates from MRTF-A, allowing the import factors access to the NLS to promote MRTF nuclear translocation, where it binds to SRF and activates expression of target genes.[22,25] An additional layer of MRTF nuclear import regulation, which is not dependent on actin, is mediated by Ddx19, an RNA helicase required for mRNA export. Mechanistically, Ddx19 regulates the conformation of MRTF-A, facilitating the acquisition of an open conformation, which can then efficiently bind with Importin-β for nuclear import.[26] Taken together, the transcriptional activity of MRTF-A/SRF is tightly regulated by actin dynamics, indicating that this transcription complex integrates signaling inputs from numerous pathways that control the actin cytoskeleton.

Nuclear actin has a key role in regulating MRTF-A/SRF activity

In addition to cytoplasmic actin dynamics, nuclear actin has also been shown to play an important role in controlling MRTF-A/SRF activity by influencing both the subcellular localization and nuclear activity of MRTF-A. Actin-binding is required for Crm1-mediated nuclear export of MRTF-A.[25] Several Crm1-dependent nuclear export sequence (NES) elements have been reported in MRTF-A, and some of them have been shown to be controlled by phosphorylation (see also below),[27,28] but the exact mechanism by which actin promotes nuclear export of MRTF-A is still not clear. Besides regulating MRTF-A subcellular localization, actin also controls intranuclear activity of MRTF-A. Confinement of MRTF-A to the nucleus without disrupting the actin-binding, for example, via blocking MRTF-A nuclear export or fusion to an extra NLS, does not activate SRF-dependent transcription,[25] indicating that actin monomer-binding inhibits MRTF-A transcriptional activity within the nucleus. This view is also supported by experiments with nuclear targeted actin mutants that promote actin polymerization (actin-G15S) and stimulate SRF activity.[29] In addition, ectopic expression of NLS-actin also inhibits expression of MRTF-A target genes leading to decreased cell motility.[30] These data thus point to a repressive role for nuclear monomeric actin in MRTF-A/SRF regulation, although the underlying mechanism is unclear.

The polymerization status of nuclear actin was for a long time under debate and it was suggested that nuclear actin would exist predominantly in its monomeric, or globular, form (G-actin) or form short oligomers.[31] However, development of novel probes has demonstrated that nuclear actin can indeed polymerize in response to specific signals. Baarlink *et al*. showed, for the first time, actin filament assembly in the nucleus in response to serum stimulation. To visualize nuclear actin filaments, they used LifeAct fused with an NLS. The rapid assembly of the nuclear actin filaments is promoted by diaphanous-related formins (mDia1/2) that are well-known actin nucleation and elongation factors, and required for efficient MRTF-A/SRF activation.[32] Also, cell spreading induces nuclear actin polymerization and transcriptional activation of MRTF-A/SRF. Cell spreading is a mechanosensing process that involves the formation of integrin-based adhesion to the substrate. Although the nuclear actin filaments in spreading cells have different shape than those observed after serum stimulation, their nucleation is also driven by mDia1/2 formins. However, nuclear actin polymerization in spreading cells also requires mechanical coupling between the cytoplasm and the nucleus via the linker of nucleoskeleton and cytoskeleton complex (LINC)[33] (Figure 1(a)). Nuclear actin polymerization likely regulates MRTF-A/SRF pathway by releasing MRTF-A from the inhibitory actin monomer.[25] Moreover, the filamentous actin-binding protein filamin A (FLNA) plays a role here. FLNA is necessary for nuclear actin polymerization, but it also interacts with MRTF-A, and is required for expression of MRTF-A/SRF target genes (Figure 1(a)). In the suggested model, MRTF-A-FLNA interaction impairs MRTF-A phosphorylation (see also below), which is a prerequisite for G-actin binding, thereby switching of MRTF-A from its repressive G-actin bound state to an MRTF-A/FLNA complex that transduces actin polymerization into SRF activation.[34] Also, other proteins that regulate nuclear actin polymerization have been shown to influence MRTF-A-SRF activity. One such protein is molecule interacting with CasL (MICAL)-2, which can catalyze the disassembly of nuclear actin filaments in a redox-dependent manner. MICAL2 activity leads to a reduction in nuclear actin levels,[35] likely because the export competent actin monomer pool largely governs nucleo-cytoplasmic shuttling of actin.[36] Reduction of nuclear monomeric actin subsequently promotes MRTF-A/SRF activity.[35]

The transcriptional response of the SRF/MRTF complex to alterations in actin dynamics may be induced by not only biochemical, but also mechanical cues. In particular, actin polymerization pathways regulated by small GTPases have been shown to be activated in response to mechanical stimuli.[37,38] Substrate stiffness can influence the ratio between monomeric and filamentous actin in fibroblasts and epidermal cells, thereby promoting the nuclear localization of MRTF-A and transcription of SRF target genes.[39] Indeed, MRTF-A nuclear translocation and activation are significantly increased in cells on stiff substrates, compared to cells plated on more compliant substrates.[40,41] In fibroblasts, it was found that static tensile forces applied through collagen-coated microbeads activate RhoA-dependent actin assembly, promote nuclear translocation of MRTF-A and transcriptional activation of its target genes.[37] Interestingly, in the cells maintained at tensional homeostasis (with a balance between the external and internal forces), nuclear accumulation of MRTF-A stimulated by serum, drugs that target actin polymerization or mechanical stress, is blocked.[40] These data demonstrate that MRTF-A can act as a mechanical sensor that links actin dynamics to SRF-mediated gene expression.

As mentioned above, MRTF-A is also regulated by phosphorylation. It is subject to extensive Rho-dependent phosphorylation,[8,42,43] and serum-stimulation induces phosphorylation of at least 26 Ser/Thr sites within MRTF-A.[28] Elimination of all 26 phosphorylation sites lead to impaired activation of SRF-dependent genes, suggesting that phosphorylation, which is also inhibited by G-actin binding, contributes positively to transcriptional activation of the SRF/MRTF-A complex. In particular, phosphorylation of Ser98 within the RPEL domain inhibits the formation of the MRTF-A–actin complex, hence, promoting nuclear accumulation of MRTF-A. By contrast, phosphorylation of Ser33 of MRTF-A facilitates its Crm1-dependent nuclear export.[28] Phosphorylation therefore influences MRTF-A activity both positively and negatively.

Actin therefore plays a key role in regulating MRTF-A/SRF activity both in the cytoplasm and in the nucleus. It is likely that cell-type specific differences exist in terms of which pool of actin contributes most to the regulation. In addition, it is important to remember that actin itself constantly shuttles in and out of the nucleus,[44] adding another potential regulatory layer also for the MRTF-A/SRF pathway.

Nuclear lamina and lamina-associated proteins in MRTF-A/SRF complex regulation

During the past few years, structural components of the nuclear lamina, including lamina-binding proteins, have emerged as potential regulators of the MRTF-A/SRF transcription complex. The nuclear lamina is a structural protein framework that underlies the inner membrane of the nuclear envelope and provides mechanical support for the nucleus.[45] The lamina is composed of A- and B-type lamins that form distinct networks in mammalian cells.[46] The A- and B- type lamins have fundamentally different properties and expression patterns: B-type lamins are widely expressed in embryonic and adult cells, but A-type lamins are expressed mainly in differentiated cells.[47] Lamin filaments typically associate with the inner nuclear membrane (INM) and interact with a plethora (more than 150) of transmembrane (TM) proteins,[48] but A-type lamins are also found as a highly dynamic pool in the nucleoplasm, except for the nucleolus.[49–51] In addition to its structural role, the nuclear lamina is implicated in signaling and gene expression. Large portion of the genome is associated with the lamina as lamina-associated domains (LADs). Genes in these domains are transcriptionally silent or express at low levels,[3,52] and enriched in the repressive histone marks.[52,53] However, nuclear lamina may also positively regulate gene expression. Recent studies have

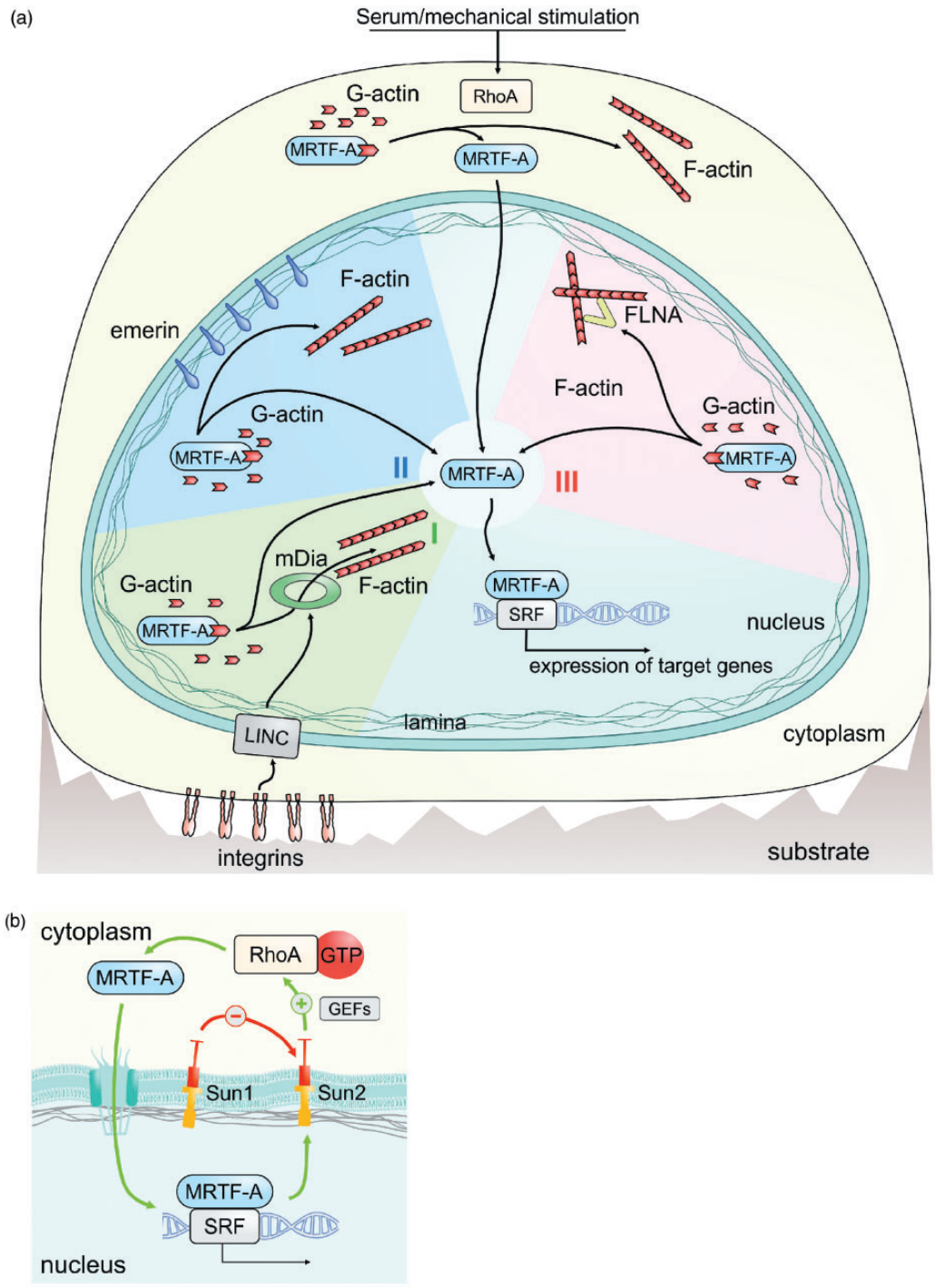

Figure 1. Schematic model of MRTF-A/SRF complex regulation by nucleoskeletal components. (a) Regulation via nuclear actin polymerization and stabilization of filaments. (I) LINC complex mediates cell spreading-induced signaling from integrins to nuclear formins mDia that promote nuclear actin polymerization. (II) Inner nuclear membrane protein emerin facilitates polymerization of nuclear actin via an unknown mechanism. (III) Filamin A (FLNA) facilitates nuclear actin polymerization and links the filaments to MRTF-A/SRF complex. (b) Regulation of RhoA and MRTF-A/SRF activity by LINC complex. SUN2 LINC complexes signal from the nuclear envelope through the cytoplasm to increase the pool of active RhoA and consequently promote MRTF-A/SRF complex activation. SUN1 complexes antagonize this network. (A color version of this figure is available in the online journal.)

revealed that many lamina-binding proteins interact with signaling effectors such as β-catenin,[54] rSMADs,[55] pRB protein,[56] and GLI1 transcription factor.[57] Laminopathies are a group of genetic disorders caused by mutations in genes encoding for nuclear lamina proteins, and various signaling pathways are perturbed in model organisms and samples obtained from laminopathy patients, further highlighting the essential role of the lamina in controlling gene expression. This has led to the hypothesis that, in addition to its barrier function, the nuclear envelope serves as an integration "hub" for important signals in developing and mature tissues. These "signaling" functions are likely to be diverse, ranging from the regulation of "classical" signal transduction pathways, to integration

of cellular mechanical events and to the control of the nucleocytoplasmic transport of specialized cargoes (reviewed in Choi and Worman[58]and Gerace and Tapia[59]).

Nuclear lamina and lamina-binding proteins have attracted interest as potential MRTF-A/SRF regulators for several reasons. First, some nuclear lamina components, such as emerin as well as A- and B-type lamins bind actin,[60,61] which is an established regulator of MRTF-A as discussed above. Second, the nuclear lamina influences gene expression especially in response to mechanical ques by various mechanisms.[62–64] Given the fact that mutations in lamin A affect cardial and skeletal muscles where MRTFs play an indispensable role, lamin A/C has been considered as a potential regulator of the MRTF-A/SRF-dependent transcription. The Lammerding laboratory has made seminal findings on this front, and they have found that fibroblasts derived from lamin A/C-deficient mouse embryos have impaired biomechanical signaling through NF-κB pathway.[62] Based on these data, they proposed that activity of other mechanosensitive transcription factors might also be altered in cells with altered nuclear lamina. Indeed, impaired nuclear translocation of MRTF-A, followed by decreased expression of SRF target genes, is observed in *Lmna*$^{-/-}$ mouse embryonic fibroblasts (MEFs), in lamin A/C downregulated HeLa cells and also in *Lmna* $^{N195K/N195K}$ mouse model with a lamin mutation associated with cardiomyopathy, pointing towards a general effect of lamin A/C knock-out or mutation on the MRTF-A-SRF pathway activity. To explore the molecular background of this effect, FRAP experiments revealed that both nuclear and cytoplasmic actin are more mobile in lamin A/C null or mutant cells. In these cells, emerin, a transmembrane LEM-domain containing protein, is redistributed from the inner nuclear membrane (INM) to the peripheral endoplasmic reticulum. Emerin is multifunctional INM-anchored protein, which has multiple binding partners in the nucleus.[65] It requires lamin A/C for proper localization[66] and has been characterized as an actin pointed-end capping protein that promotes actin polymerization *in vitro*.[60] Indeed, emerin-deficient fibroblasts also display impaired nuclear translocation of MRTF-A, which could be rescued by expression of exogenous emerin, but not by an emerin mutant that did not bind actin.

In the suggested model, emerin regulates nuclear actin polymerization, which controls MRTF-A nuclear export and transcriptional activity. In the lamin A/C null or mutant cells, mislocalization of emerin leads to aberrant nuclear actin polymerization, and consequently to decreased nuclear localization of MRTF-A and decreased expression of MRTF-A-SRF target genes[67] (Figure 1(a)). Although the mechanism by which emerin regulates polymerization of nuclear actin is still somewhat unclear, its functional role in this process is also supported by the findings that both emerin and lamin A/C are required for cell-spreading-induced polymerization of nuclear actin, and consequently for MRTF-A-SRF activation during this process.[33] Interestingly, emerin has also been linked to actin polymerization at the outer nuclear membrane (ONM), forming a mechanosensitive complex with non-muscle myosin, which seems to regulate nuclear actin levels by controlling nuclear import competent actin monomers. Reduced nuclear actin leads to transcription attenuation and Polycomb-dependent gene silencing, which is required for lineage commitment in epidermal stem cells.[68] Emerin may thus regulate nuclear actin by acting both at the inner and at the outer nuclear membranes.

Emerin seems to also play a critical role in the mechanical regulation of MRTF-A activity. The maximum level of MRTF-A nuclear accumulation after stimulation is lower in cells grown on soft substrates, although the kinetics of MRTF-A nuclear accumulation in general is not significantly affected by substrate stiffness. However, experiments with emerin-knock-out (Emd^{KO}) fibroblasts revealed that emerin is required for maximal MRTF-A nuclear accumulation on the stiffest hydrogel substrate and glass coverslips. Similar pattern of MRTF-A nuclear translocation is also observed in case of lamin A/C depletion. Interestingly, emerin seems to be required for steady state expression of MRTF-A/SRF target genes, but not for their transcription activation. Expression of a constitutively active form of MRTF-A bypasses the requirement for emerin in MRTF-A/SRF-dependent gene expression and reverses the focal adhesion defects evident in Emd^{KO} fibroblasts. Nuclear lamina, and especially emerin, seems therefore to be specifically required for coupling ECM mechanics to MRTF-A localization and activity in cells on stiff substrates.[69] However, the mechanism by which emerin operates here needs to be clarified, and it would be important to study, if the ability of emerin to polymerize actin, either at the INM or the ONM plays a role here.

The LINC complex and MRTF-A/SRF complex regulation

The linker of nucleoskeleton and cytoskeleton (LINC) complex has also been implicated in MRTF-A regulation. LINC complex is essential for mechanotransduction to the nucleus, because it provides a physical link between the cytoskeleton and the nuclear envelope.[70,71] This is achieved through the interaction between ONM proteins nesprins, or KASH (named after Klarsicht, ANC-1 and SYNE1 homology) -domain proteins, which connect the nuclear envelope to cytoskeletal elements, and INM proteins SUN1/2 (Sad1 and UNC-84), which bind with lamins and other components of the nuclear lamina.[72] The LINC complex structurally supports the nucleus and translates mechanical cues and alterations in the extracellular matrix into biochemical signals.[73–76] Like lamin A/C and emerin, the LINC complex has also been reported to be required for nuclear actin polymerization upon cell spreading, and consequently for MRTF-A-SRF activation during this process.[33] However, the LINC complex may also impinge on MRTF-A activity also by controlling the upstream signaling pathways (Figure 1(b)). In HeLa cells, LINC complexes containing the Sun2 protein activate the small GTPase RhoA, which is an established regulator of actin cytoskeleton dynamics, thus affecting MRTF-A/SRF complex activity.[77] It is noteworthy that LINC proteins SUN 1 and SUN2 that possess similar domain organization and identical topology at the INM[78] have been found to

have opposing roles in regulating RhoA activity. SUN2 signaling increases the pool of active RhoA. RhoA, in turn, promotes focal adhesion assembly and initiates a MRTF-A/SRF-dependent positive feedback loop. Sun1 antagonizes this network, probably by limiting the activity of SUN2,[77] although precise mechanisms need to be clarified. Biochemically, SUN1 co-precipitates more avidly with nesprins, and consequently incorporates more efficiently into LINC complexes than SUN2 in HeLa cells under normal growing conditions. Ectopic expression of the constitutively active form of MRTF-A was found to increase SUN2 expression and to flip cells into a state favoring SUN2 LINC complex function. Interestingly, overexpression of a SUN1 mutant that is unable to form LINC complexes is sufficient to reduce MRTF-A/SRF target gene expression. This indicates that SUN1 operates also independently of LINC complexes in repressing MRTF-A/SRF. This may take place by regulating Lamin A/C and/or emerin,[79] but the mechanisms need to be clarified.

As a conclusion, many components of the nuclear lamina have been implicated in MRTF-A/SRF regulation, and this process seems to be especially important in response to mechanical stimulation. At least in some cases, the regulation seems to take place at the level of nuclear actin polymerization, but further studies are required to elucidate the mechanisms involved.

Crosstalk between MRTF and YAP/TAZ transcriptional co-activators

In addition to the MRTF-A/SRF pathway, RhoA signaling and mechanical stress also activate the transcriptional response mediated by YAP/TAZ, which are downstream effectors of the Hippo growth control pathway. The core Hippo-pathway consists of a kinase cascade of MST1/2 and Lats1/2, which regulate phosphorylation, and thus subcellular localization and stability of YAP/TAZ transcription coactivators. In the nucleus, YAP/TAZ interact with the TEA domain (TEAD) transcription factor family to regulate expression of a wide variety of genes linked with cell migration, proliferation, and survival (reviewed in Meng *et al.*[80]). Interestingly, mechanical cues from the cytoskeleton have been identified as important regulators of YAP/TAZ activity and these regulatory mechanisms partly recapitulate those that control MRTF family proteins. Like MRTFs, YAP/TAZ localize to the nucleus upon RhoA-induced cytoplasmic actin assembly, although the monomeric/filamentous actin ratio does not seem to play a role in controlling YAP/TAZ activity. Regulation via mechanical cues acts independently of the Hippo cascade.[81,82] In addition, MRTFs and YAP/TAZ may functionally interact to coordinate transcriptional responses to various extracellular stimuli. For example, activation of both MRTF-A and YAP/TAZ pathways is required for transcriptional control of RhoA-regulated genes as well as for cell proliferation.[83] In support of this idea, MRTF is enriched at genomic loci containing TEAD-binding sequences.[20] On the other hand, YAP-TEAD target gene sets include many genes earlier determined as MRTF/SRF targets, and these genes contain binding sites for both MRTF/SRF and YAP/TEAD. In line with this, MRTF and YAP have been shown to function in a mutually dependent manner in cancer-associated fibroblasts (CAFs). MRTF and YAP could each indirectly activate (or inhibit) the other through their ability to affect actin cytoskeleton dynamics.[84] However, YAP has also been suggested to physically interact with MRTF, and this interaction linked to recruitment of the NCOA3 transcriptional coactivator to enhance YAP-TEAD target gene expression, required *in vitro* for LPA-induced cancer cell invasion and *in vivo* for the metastasis of breast cancer cells.[85]

As discussed above, nuclear actin plays a critical role in regulating MRTF-A activity,[25,32] and a recent study suggest that nuclear actin, in its filamentous form, could regulate YAP/TAZ activity as well. The SWI/SNF chromatin remodeling complex has previously been reported to associate with actin filaments *in vitro*.[86] The Piccolo laboratory has demonstrated that at high mechanical stress, nuclear actin filaments interact with AT-rich interactive domain-containing protein 1A (ARID1A) containing SWI/SNF complexes. This antagonizes the interaction between ARID1A-SWI/SNF and YAP/TAZ, allowing YAP/TAZ to bind TEAD to activate transcription.[87] These studies demonstrate that MRTF-A is not the only transcription factor regulated by nuclear actin. Further studies are required to reveal the mechanisms involved, as well as to identify potential other transcription factors that respond to nuclear actin dynamics.

Nuclear actin in gene expression

In addition to regulating the transcriptional activity of the MRTF/SRF and YAP/TAZ-TEAD complexes, actin has also been linked to a number of other processes that regulate gene expression. Actin is a well-established component of a variety of chromatin-modifying and remodeling complexes. It has been linked to ATP-dependent chromatin remodeling complexes such as INO80, SWR1, and SWI/SNF (reviewed in Kapoor and Shen[88] and Klages-Mundt et al.[89]). The interactions with the actin-related proteins (Arps) keep actin monomeric in these complexes.[90,91] Recent structural and functional studies have also started to reveal how actin operates in these complexes, and for example in the Ino80 chromatin remodeling complex, a module containing actin, Arp4 and Arp8 is involved in recognizing the extranucleosomal linker DNA.[92] Actin also associates with all three RNA polymerases: Pol I,[93,94] Pol II,[95] and Pol III,[96] and has been linked to transcription-related processes from pre-initiation complex assembly[97] to transcription elongation.[98–100] Analysis of genome-wide actin-chromatin interactions supports the wide role of actin in transcription, and has revealed that actin binds with essentially all transcribed genes in *Drosophila* ovaries. On most genes, actin is found near the transcription start site (TSS) together with RNA polymerase II, and on highly expressed genes, these proteins are also found on gene bodies. Generally, actin seems to have a positive effect on transcription. Decreasing available nuclear actin levels by inhibiting nuclear import of actin[44] or by polymerizing actin to stable filaments[101] attenuates transcription. As mentioned above, a similar phenomenon is also observed upon activation of the mechanosensitive complex of

non-muscle myosin II and emerin, which leads to decreased nuclear actin by local actin polymerization.[68] Nuclear actin levels are also decreased upon quiescence, and this is linked to reduced global transcription activity.[102] Finally, decreased nuclear actin levels lead to transcriptional defects also *in vivo*. Mutants of the nuclear actin import receptor, RanBP9 (*Drosophila* ortholog of Importin-9), display reduced nuclear actin. These RanBP9 mutant flies lay fewer eggs than control flies, and RNA-seq analysis revealed decreased expression of *chorion* genes in the mutant flies. Mechanistically, the RanBP9 deletion leads to decreased binding of both actin and Pol II to the *chorion* genes, which are required for the eggshell formation.[103] The molecular mechanisms by which nuclear actin affects transcription are still largely unclear. In the future, it will be important to decipher these mechanisms in order to understand how nuclear actin dynamics impinges on both gene specific (for example MRTF/SRF and YAP/TAZ-TEAD target genes) and general transcription.

Conclusion

It has become apparent that the "nucleoskeleton," in addition to providing a structural framework for nuclear functions, also regulates different signaling pathways, modulating the activity of numerous transcription factors, and thereby contributes to key cell fate decisions. Several components of the nucleoskeleton have emerged as important regulators of the MRTF-A/SRF pathway, and this seems to constitute a critical mechanism to sense and maintain the mechanical balance in the cell by appropriate control of cytoskeletal gene expression. Not too surprisingly, this mechanism is impaired in many diseases, such as laminopathies, necessitating deeper understanding of the mechanisms involved. Future studies are needed to reveal the exact mechanisms by which the nucleoskeletal components impinge on MRTF-A/SRF activity. Although several mechanisms may exist, it is also tempting to speculate that many of these may converge at the level of nuclear actin. While the regulation of cytoplasmic actin polymerization has been extensively studied, nuclear actin has only recently been shown to polymerize (reviewed in Grosse and Vartiainen[104]). Consequently, this process is still relatively poorly understood, and for example the mechanisms by which emerin and LINC complex[33] participate in nuclear actin polymerization remain unclear. Beyond MRTF-A/SRF, further studies are also required to reveal how the nucleoskeleton actually operates in the nucleus to control important nuclear processes, such as transcription. If the nucleoskeleton is a "dynamic network of networks,"[4] we must not restrict our analysis only to its individual components, but analyze the system as whole.

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