e-Neuroforum 2011 · 2:1–5
DOI 10.1007/s13295-010-0013-y

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The cytoskeleton and nucleus: the role of actin as a modulator of neuronal gene expression

Introduction

The actin cytoskeleton of cells is primarily associated with the cytoskeletal framework in the cytoplasm built up, e.g by stress fibres. Herein, actin runs through cycles of polymerisation into filamentous F-actin and depolymerisation into monomeric globular G-actin. Thereby, the actin equilibrium is influenced by several actin binding proteins (ABPs). While formin, profilin or Arp2/3 complexes stimulate F-actin binding, the actin-severing proteins gelsolin and cofilin disassemble F-actin, thus providing new nucleation points for polymerisation [6]. Important superior regulators of actin dynamics are the Rho-GTPases RhoA, Rac1 and Cdc42. Via directed polymerisation of the actin cytoskeleton into cellular structures such as stress fibres (RhoA), undulating lamellipodia (Rac1) and finger-shaped filopodia (Cdc42) Rho-GTPases modulate cell motility and adhesion. During the past few years studies have shown that the adjustment of the actin cytoskeleton in the cytoplasm induced by extracellular signalling molecules is not the end of the signal transduction cascade. Instead, the adjustment of the actin polymerisation/depolymerisation balance creates a signal that is relayed to the nucleus, thereby modulating gene expression [17, 22, 25]. This actin-based signalling to the nucleus is virtually unknown for microtubules and intermediate filaments.

Within the nucleus, a gene regulatory complex harbouring the transcription factor serum response factor (SRF) at its center has been identified as the primary acceptor of actin signalling [17, 22, 25]. SRF-regulated genes primarily fall into two classes, the transcription of which is regulated by SRF-recruited transcription factors [13, 22](**Fig. 1**). One mechanism of SRF activation is by MAP kinase-induced phosphorylation of members of the ternary complex factor (TCF) cofactor family, e.g. after serum or growth factor stimulation. In addition to the MAP kinases, SRF also responds to Rho-GTPase-/actin-based signalling in which members of the myocardin-related transcription factor (MRTF) family of SRF cofactors function as sensors. In muscle cells TCF and MRTF cofactors can compete for SRF interaction in which crosslinking of superior signalling pathways (MAP kinases, Rho-GTPases) can be involved [17].

The following gene response is switched on by the interaction of SRF with the named cofactors: (a) With the help of TCFs (e.g. Elk-1) SRF induces the immediate-early gene answer (IEG) which was originally documented in neurons by Morgan et al. [5]. Herein, IEGs like *c-fos*, *Egr1* and *Arc* are induced very rapidly (within minutes) but transiently after stimulus entry (e.g. serum, growth factors). (b) SRF responds not only to Rho-GTPase-/actin signalling, but various genes that encode for actin isoforms (*Actb, Actc, Actg, Acta*) or ABPs (gelsolin, vinculin, tropomyosin, myosin) are under SRF-regulated transcription [13, 17, 22]. Furthermore, SRF abrogates activity of the actin severing protein cofilin [1]. For this purpose SRF can influence the CDK/Lim-Kinase-induced cofilin phosphorylation [14] via *Cdk16* (*Pctaire*) transcription.

The MRTF-SRF complex as a target of actin signalling

The signal transduction generated by actin polymerisation/depolymerisation is carried out by members of the MRTF family such as MRTF-A (=MAL) that function as actin balance sensors. In contrast to other regulatory mechanisms (e.g. phosphorylation of TCFs) the activity of MRTFs is determined to a great extent by their subcellular localisation (cytoplasm vs. nucleus) (**Fig. 1**).

In non-stimulated cells G-actin binds MRTF-A in the cytoplasm, thereby inhibiting the nuclear import of MRTF-A (**Fig. 1**). Additionally, residual nuclear MRTF-A is reduced by actin- and MAP kinase-dependent MRTF-A export [17]. Remarkably, nuclear G-actin can suppress additional activation of MRTF-A and SRF at SRF-regulated promoters. The abovementioned mechanisms (**Fig. 1**) account for a low nuclear MRTF localisation and thus prevent activation of SRF.

Stimulation of cells can induce increased F-actin polymerisation. This is accompanied by a decrease in cytoplasmic (and nuclear?) G-actin levels, thereby releasing MRTF-A. MRTF-A can now enter the nucleus and induce SRF-mediated transcription. In addition to enhanced MRTF-A import, actin-mediated MRTF-A export from the nucleus is reduced [25, 26].

Consequently, G-actin leads to inhibition, while F-actin polymerisation increases SRF-mediated gene activity. As already mentioned above, this mechanism is elaborated for diverse non-neuronal cell types. The importance of this communication of actin signalling with nuclear

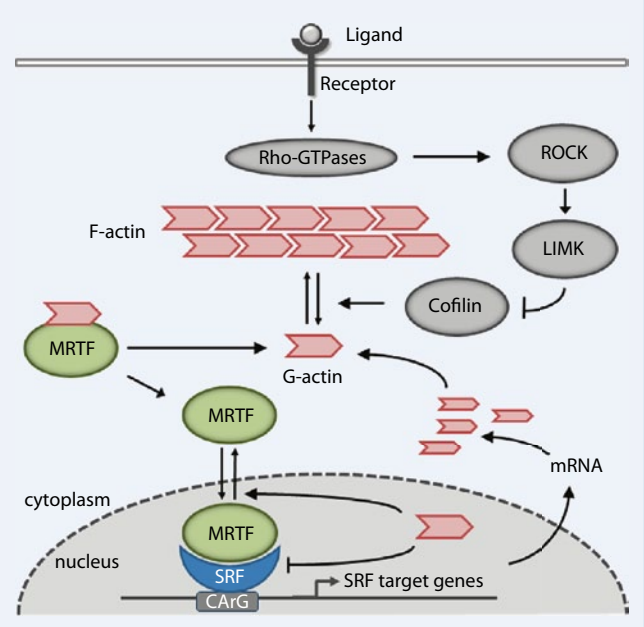

Fig. 1 ▲ Cytoskeletal actin dynamic and myocardin-related transcription factor (*MRTF*)-mediated regulation of serum response factor (*SRF*) target genes. By binding to its receptor signalling molecules trigger the activation of Rho-GTPases. These then activate *ROCK* (Rho Kinase), which phosphorylates *LIMK* (LIM Kinase). Phosphorylated LIMK inhibits cofilin by phosphorylation, so that cofilin-mediated F-actin degradation processes are reduced. In this way the G-/F-actin balance is shifted to F-actin. MRTF that is normally retained in the cytoplasm by association with G-actin can now gain access to the nucleus. Here it promotes the expression of cytoskeletal genes amongst others actin itself as a partner protein of SRF. This increases the cytoplasmic actin level and retains MRTF in the cytoplasm. Additionally, nuclear actin increases MRTF-A export from the nucleus and reduces the MRTF-SRF activity at the promoter of target genes

gene expression in neurons is only at the beginning. However, initial studies show [14, 24] that MRTFs also seem to function as the central sensor of actin signalling in neurons.

The actin-MRTF-SRF triumvirate in neurons

Particular features of neuronal actin dynamics

In non-neuronal cells such as fibroblasts actin stress fibres are rather homogeneously spread throughout the whole cytoplasm. In contrast in neurons F-actin is concentrated to the motile end structures of neurites, so-called growth cones (GC) ([6], **Fig. 2a**). The actin-rich growth cones usually lie far away from the neuronal soma. Hence one could assume that MRTF-A as a possible neuronal sensor of actin signalling needs to be imported into the nucleus over a long distance, e.g. by retrograde transport after the release of G-actin.

Actin-mediated signalling in neurons

Can actin signalling also modulate SRF-mediated gene activity in neurons? The available data indicate that the general mechanism of actin signalling on SRF in neurons is similar to non-neuronal cells [24]. Therefore, different actin mutants with altered polymerisation properties and interaction with ABPs generated by single amino acid changes were used [20, 21]. For instance, the actin mutant G15S increases actin polymerisation. In fact, we observed the incorporation of actin G15S into endogenous F-actin polymers in growth cones [24]. As reported for non-neuronal cells [20, 21] actin G15S increases SRF-mediated gene expression in neurons [24]. Additionally, actin G15S stimulated neurite growth and formation of filopodia in growth cones (**Fig. 2b**).

In contrast to actin G15S, the actin mutant R62D cannot be incorporated in F-actin and thus can contribute to an increase in the G-actin level. Indeed, actin R62D localises primarily outside the growth cones to the neuronal soma [24]. Actin R62D reduced neuronal SRF-mediated gene expression. In addition, actin R62D inhibited neurite growth and altered neuronal morphology in such way that actin R62D-expressing neurons resemble SRF-deficient neurons (**Fig. 2c**). In addition to the cytoplasmic actin R62D, a further ectopic actin R62D relocated to the nucleus was investigated [24]. Therefore, actin R62D was fused to a nuclear localisation signal (NLS) (actin R62D-NLS). Interestingly, actin R62D-NLS could reduce SRF-mediated gene expression and—like cytoplasmic R62D actin—influence neuronal morphology (**Fig. 2d**). Although actin R62D-NLS was separated from the cytoplasmic actin pool neurons looked similar, much like after expression of the cytoplasmic actin R62D variant.

How can actin R62D from the nucleus modulate neuronal morphology to such an extent? One possibility could be that G-actin in the nucleus is bound to MRTF-A in neurons as shown for non-neuronal cells [26]. This nuclear G-actin/MRTF-A complex could then disable SRF-mediated gene expression in neurons. Additionally, MRTF-A export from the nucleus could be increased by G-actin. Such a scenario could explain the phenotypic similarity of actin R62D-expressing and SRF-deficient neurons. The finding that MRTF-A in some but not in all studies was reported to have a constitutive nuclear localisation in neurons is in agreement with such a mechanism [13]. Thus, nuclear G-actin in comparison to the mechanism of the MRTF-A nucleus-cytoplasm translocation would have an outstanding control position for regulation of MRTF-A-SRF activity in neurons. But how could MRTF-A, in the case of a stringent nuclear localisation, adopt its function as a sensor of actin dynamics in neurons? One possibility could be that changes within the growth cone actin dynamics modulate the cytoplasmic G-actin level. Thereby, a guidance

cue, for example, such as ephrin-induced growth cone collapse [11] that leads to a transient F-actin decrease, could increase the G-actin level. This possible increase in the initial cytoplasmic G-actin level could ultimately lead to an increase in nuclear G-actin. Nuclear actin could then bind to MTRF-A and influence SRF-mediated gene activity as mentioned earlier. In contrast, a BDNF-mediated increase in number and length of filopodia [7] that possibly is accompanied by an increased F-actin level could reduce the G-actin level. This would correlate with the already known stimulation of SRF-activity by BDNF [13]. As actin per se harbours no nuclear import signal, the question arises as to how actin reaches the nucleus in neurons? One possible scenario is that actin along ABPs such as profilin, which contain an NLS is imported "piggy back" into the nucleus. In fact, a synaptic activity-induced profilin import that can co-transport actin is reported in neuronal nuclei [3]. Although the exact mechanism modulating SRF gene activity by the actin signalling in neurons, in particular the embedding of MRTF-A in this triumvirate, is not finally clarified; MRTF-A seems to be an important communicator between actin signalling and SRF. This is supported by in vivo data reported in *Mrtf* and *Srf* mouse mutants.

Neuronal phenotypes of *Mrtf* and *Srf* mouse mutants

Forebrain-specific conditional *Srf* mouse mutants show neuronal phenotypes that reflect a dysregulation of both primarily SRF-regulated gene classes (IEGs and cytoskeletal genes) (see Introduction). Thus, various processes that depend on expression of cytoskeletal genes, which contribute to neuronal motility, are impaired in SRF-deficient mice [13]. Defective cell migration of progenitor cells from the subventricular zone to the olfactory bulb was reported in an initial work [1]. Further works then showed that SRF influences neurite growth, neuronal polarisation and axonal control of hippocampal axons in the peripheral nervous system [12, 24, 28]. SRF-deficient neurons display reduced neurite growth, bipolar shape and absent filopodia structures in growth cones ([12, 24], **Fig. 2e**). Several axon guidance cues (ephrin, semaphorin, neurotrophin, reelin) cannot modulate the cytoskeleton within SRF-deficient neurons [24]. This leads to an impaired ephrin-induced growth cone collapse in SRF-deficient neurons and in turn to the formation of novel F-actin and microtubule rings [12]. The latter could be explained by defective activity of the F-actin severing protein cofilin, the activity of which is regulated by SRF [1, 14].

In addition to these phenotypes that primarily reflect the cytoskeletal regulatory function of SRF, processes that are based on normal neuronal activity-induced gene expression, such as IEG induction in SRF-deficient mice [24], are also affected. Thus the IEG response in adult SRF-deficient mice induced either by a new environment (environmental enrichment) or by forced synaptic activity (electroconvulsive shocks) is suppressed [24]. The IEG *Arc* in particular seems to be an important SRF-regulated target gene after induction of synaptic activity [23]. This absent neuronal IEG response correlates with an induced long-term potentiation or long-term depression of hippocampal synapses. This could lead to the disrupted habituation of SRF-deficient mice to a new environment also reported [24]. Recently, the conditional neuronal *Mrtfa/Mrtfb* double mutants were analysed [14] whose phenotype bears a striking resemblance to *Srf* mouse mutants. These phenotypic similarities are so far mainly in terms of neuronal motility. For example, *Mrtfa/Mrtfb* mouse mutants display defective neuronal migration based on the SVZ as well as reduced neurite growth [14]. Furthermore, MRTFs regulate cofilin activity in a comparable manner to SRF (see above) [1, 14]. These results, which prove close cooperation between MRTFs and SRF in neuronal processes in vivo, are supported by further in vitro results. In fact, an MRTF-A-induced change of neuronal morphology requires SRF [12]. Conversely, SRF-mediated gene expression in the presence of a dominant-negative MRTF-A protein is disturbed [27]. In summary, we can confirm that actin-MRTF-SRF build an important functional unit. Basic regulatory mechanisms of this trio seem to be observed between

Abstract

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Abstract
Actin, arranged for example in stress fibres, provides a fundamental cytoskeletal framework function to all cell types. Notably, there is now mounting evidence that, in addition to cytoplasmic cytoskeletal regulation, actin treadmilling provides a signal modulating nuclear gene expression. In altering gene regulation, cytoplasmic and most likely also a nucleus-resident actin provides an additional (gene) regulatory twist to cell motility. So far, the transcription factor serum response factor (SRF) alongside its myocardin-related transcription factor (MRTF) cofactors has emerged as the main target of actin dynamics. In this review, we discuss the impact of actin signalling on nuclear gene expression in the nervous system, where the actin-MRTF-SRF module contributes to various processes including neuronal motility.

Keywords
Actin · Serum response factor · Gene expression · Cytoskeleton · Nucleus

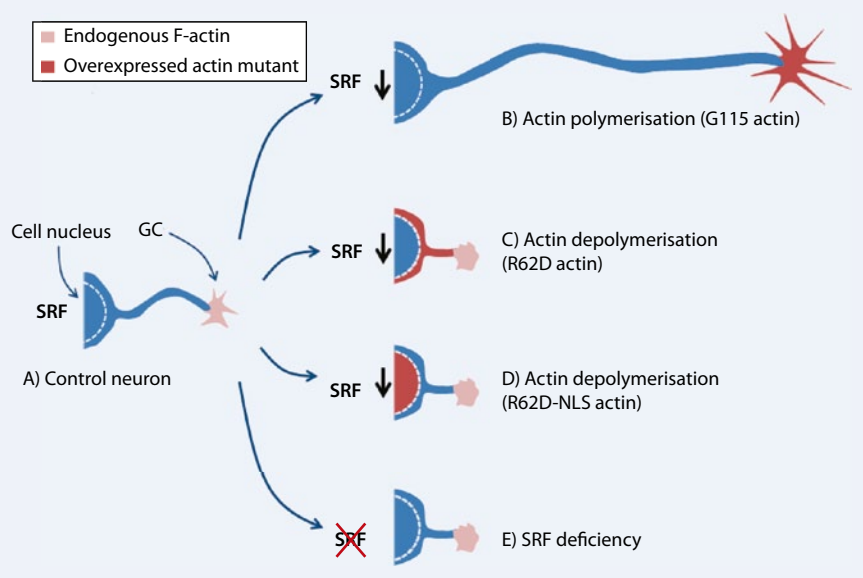

Fig. 2 ▲ Actin signalling changes SRF-mediated gene activity and neuron morphology. *Light red* shows endogenous F-actin, while *dark red* marks the localisation of the overexpressed actin mutant. **A** A control neuron develops several neurites at the end which growth cones (GC) with finger-like filopodia can be found. SRF is localized in the nucleus. **B** Overexpression of the G15S actin mutant shifts the actin balance towards F-actin. The actin mutant is mainly found within the growth cones. G15S actin increases the neurite length and number of filopodia in growth cones. G15S expression increases SRF-mediated gene expression (*upward arrow*). **C** The R62D actin mutant shifts the G/F-actin balance towards monomeric G-actin and localises in complementary regions to endogenous F-actin. Overexpression of R62D actin reduces SRF-mediated gene expression (*downward arrow*) and leads to an altered neuron morphology. Neurites are strikingly short and growth cones are free of filopodia. **D** An R62D mutant fused to a nuclear localisation signal (*NLS*) localises to the nucleus of the neuron. Similar to the cytoplasmic R62D mutant (see **C**), this decreases SRF-mediated gene expression and alters neuron morphology in a similar way. **E** Overexpression of both the cytoplasmic and the nuclear R62D actin mutant (**C** and **D**) result in a phenotype which is also found in SRF-deficient neurons

different cell types, although neuronal-specific features exist that require further investigation.

Excursus: nuclear actin

Studies on actin microfilament have focused mainly on cytoplasmic functions such as cytoskeleton formation. The fact that actin can also be found within the nucleus was long seen as controversial, since nuclear F-actin cannot be made visible cytochemically by phalloidin. In recent years, however, it became clear that nuclear actin can be found in many cell types as well as in neurons [2, 10, 18, 25]. Moreover, a multitude of ABPs such as profilin, cofilin, thymosin [2, 8, 25] and actin related proteins (ARPs) [16] localise to the nucleus, suggesting dynamic regulation of nuclear actin.

The role of nuclear actin in the regulation of MRTF-SRF signalling has already been addressed above. Nuclear actin also plays a role in additional nucleus-specific processes (◘ **Fig. 3**). Beside the regulation of the chromatin structure, actin participates in the control of gene expression. In this context, actin associates with the ATPase subunit of chromatin remodelling complexes of the Brg-associated factor (BAF) family, thereby regulating its activity [16] and binding to chromatin [29]. For the transcription of genetic information, the RNA polymerase is required which, together with other proteins, binds to the DNA, whereby actin participates in the building of the protein complex [28], and by recruiting the motor protein nuclear myosin 1 (NM1) is able to facilitate the progress of transcription [9]. The resulting RNA is complexed by heterogeneous nuclear ribonucleoproteins (hnRNPs), with actin interacting with a series of hnRNPs and consulting histone acetyltransferases, thus facilitating the progression of transcription [15, 16]. Actin also seems to participate in the subsequent RNA export by binding specific hnRNPs that can enclose the RNA until its export from the nucleus [19].

By this means actin builds a molecular platform for various levels of transcription (◘ **Fig. 3**): from chromatin remodelling, RNA polymerase activity and complexation of freshly synthesized RNA up to nuclear export. In the named cases actin seems to function as a regulatory subunit and signal transmitter. To what extent these processes include cytoskeletal structure formation, as shown for *Xenopus* nuclei [4], requires further investigation.

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Acknowledgements. B.K. is supported by the DFG Emmy Noether Programme and Sachbeihilfe KN543/4–1, as well as by the Schram-, Gottschalk- and Gemeinnützige Hertie-Stiftung. H.B. is funded by a graduate fellowship at the Hertie Institute for Clinical Brain Research, Tübingen.

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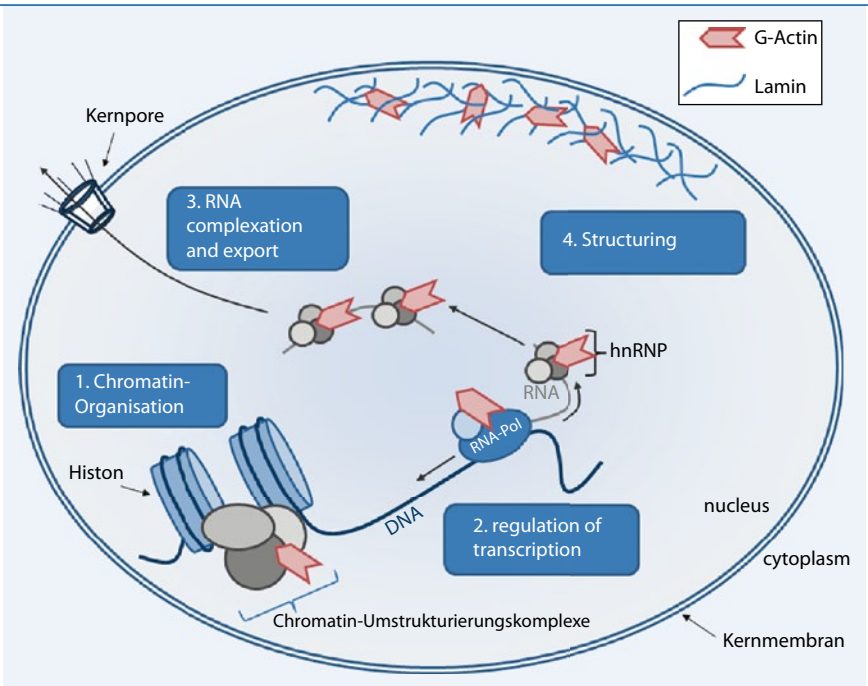

Fig. 3 ▲ Functions of nuclear actin. Actin participates in a multitude of nucleus-specific processes. *1* As a component of chromatin remodelling complexes actin regulates DNA–histone interactions. *2* Actin builds a structural subunit of the RNA polymerase transcription complex. *3* Newly synthesised RNA molecules are enclosed by chaperones (*hnRNPs*). Actin displays a structural component of this complexation and participates in the regulation of the export of RNA transcripts from the nucleus. *4* Actin can also function as a scaffold protein in the nucleus and interact with the nuclear lamina

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