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Mini Review

Extracellular signaling cues for nuclear actin polymerization

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ABSTRACT

Contrary to cytoplasmic actin structures, the biological functions of nuclear actin filaments remain largely enigmatic. Recent progress in the field, however, has determined nuclear actin structures in somatic cells either under steady state conditions or in response to extracellular signaling cues. These actin structures differ in size and shape as well as in their temporal appearance and dynamics. Thus, a picture emerges that suggests that mammalian cells may have different pathways and mechanisms to assemble nuclear actin filaments. Apart from serum- or LPA-triggered nuclear actin polymerization, integrin activation by extracellular matrix interaction was recently implicated in nuclear actin polymerization through the linker of nucleoskeleton and cytoskeleton (LINC) complex. Some of these extracellular cues known so far appear to converge at the level of nuclear formin activity and subsequent regulation of myocardin-related transcription factors. Nevertheless, as the precise signaling events are as yet unknown, the regulation of nuclear actin polymerization may be of significant importance for different cellular functions as well as disease conditions caused by altered nuclear dynamics and architecture.

1. Introduction

Actin is one of the most abundant proteins in the cytoplasm and is an essential player in a plethora of cellular processes such as cellular shape, adhesion, cell motility and cytokinesis (Rottner and Stradal, 2011). Generally, actin can be found in two different states. Monomeric globular actin, termed G-actin, constitutes the G-actin pool from which in turn polymeric filamentous F-actin is assembled (Pollard and Cooper, 2009). Actin filaments display structural polarity because G-actin monomers within a filament are oriented in the same direction. Based on their appearance in electron microscopy, the terminal part is referred to as either barbed or pointed end (Bonder et al., 1983).

Actin nucleation constitutes the initial step in the formation of microfilaments with formins, the Arp2/3 complex and Spire proteins being the three major classes of actin nucleators known to date. Arp2/3 and Spire both bind to pointed ends of actin filaments. While the Arp2/3 complex polymerizes actin filaments into branched arrays, Spire has the ability to assemble linear filaments (Kerkhoff, 2006). Likewise, formins polymerize linear actin filaments through processive association with the barbed end (Faix and Grosse, 2006) via their conserved formin homology (FH) 2 domain. For this, two FH2 domains form a circular head-to-tail homo-dimer thereby stabilizing actin dimers and adding them to the barbed end in a stair-stepping process, while binding of actin by the FH1 domain increases the local G-actin concentration to accelerate actin polymerization (Campellone and Welch, 2010).

Among the different classes of formins, diaphanous-related formins (DRFs) are best characterized (Bogdan et al., 2013). DRFs display a modular domain organization in which the regulatory segment is composed of a GTPase binding domain (GBD) and a diaphanous-inhibitory-domain (DID), both of which are involved in the autoinhibitory regulation of DRFs. The FH2 domain is located at the C-terminus together with the diaphanous-autoregulatory-domain (DAD). In the inactive conformation, DAD binds DID to achieve autoinhibition, while binding of a Rho GTPase was shown to release autoinhibition (Lammers et al., 2005; Kuhn and Geyer, 2014). In addition, it is now appreciated that the DID–DAD autoinhibitory module is further influenced by other cellular signaling processes such as serine/threonine kinases (Kuhn and Geyer, 2014).

Many proteins such as cofilin, profilin, or capping proteins affect actin dynamics and can therefore influence the rate of actin assembly as well as the formation of secondary structures of F-actin such as actin bundles, networks of branched actin filaments or the actomyosin ring responsible for cytokinesis (Pollard and Cooper, 2009).

2. Nuclear actin and extracellular cues

The structure and function of actin within the mammalian cell nucleus is much less clear and hence under intense investigation

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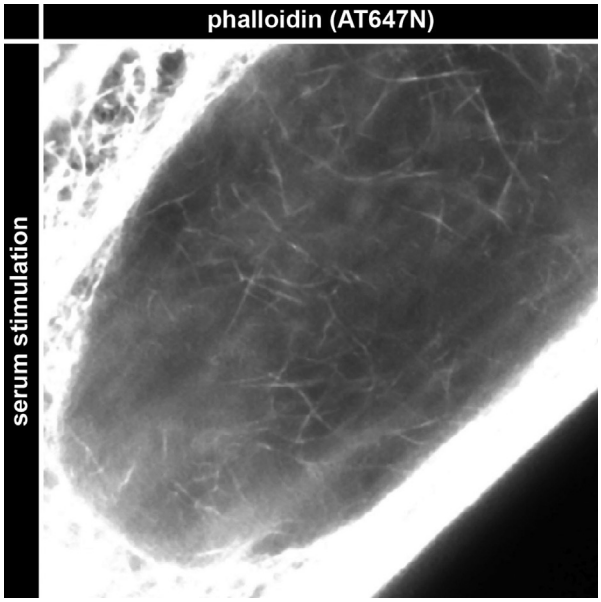

Fig. 1. Visualization of native nuclear actin filaments by STED microscopy. Starved NIH 3T3 fibroblasts were stimulated with fetal calf serum (FCS) for 20 s and immediately fixed with glutaraldehyde. Endogenous F-actin was stained using phalloidin (ATTO 647N-conjugated). The sample was visualized with 633 nm pulsed excitation (confocal) and 775 nm pulsed STED (200 ps pulse width). The image was taken at the MPI for Biophysical Chemistry, Dept. of NanoBiophotonics, Göttingen.

(Belin and Mullins, 2013; Grosse and Vartiainen, 2013). One central issue is to overcome the obstacles in visualizing nuclear actin because its concentration in somatic cell nuclei is very low compared to other models utilized in nuclear actin research, i.e. *Xenopus* oocyte nuclei (Bohnsack et al., 2006; Miyamoto and Gurdon, 2011; Miyamoto et al., 2011). Therefore, it was previously thought that in somatic cells nuclear actin exists mainly or even only in its monomeric form (de Lanerolle and Serebryannyy, 2011; Percipalle, 2013). However, our view on nuclear actin structures is currently changing due to recently described dynamic F-actin foci (Belin and Mullins, 2013) as well as filamentous networks, which can rapidly form upon serum or lysophosphatidic acid (LPA) stimulation (Baarlink et al., 2013) evidently independent of the Arp2/3 complex. In these cases, nuclear actin was visualized by targeting actin probes such as Utrophin or LifeAct to the nucleus, thereby circumventing strong signal interference from cytoplasmic F-actin labeling (Baarlink and Grosse, 2014). Importantly, signal-regulated, rapid nuclear F-actin network assembly was dependent of mDia formins and could be demonstrated without ectopic protein expression using phalloidin labeling, demonstrating the principle existence of native nuclear actin filaments and higher order structures in somatic cell nuclei (Fig. 1) (Baarlink et al., 2013).

3. MRTF-A (MAL, MKL1) is regulated by nuclear actin polymerization

As part of the immediate serum response, nuclear F-actin dynamics are directly linked to myocardin-related transcription factor A (MRTF-A) (also termed megakaryocytic acute leukemia; MAL, or megakaryoblastic leukemia 1; MKL1), which acts as a critical transcriptional coactivator of the serum response factor (SRF) (Olson and Nordheim, 2010). MRTF-A is an actin-binding protein that continuously and rapidly shuttles between the cytoplasmic and nuclear compartment. This shuttling is regulated by compartmentalized actin polymerization (Miralles et al., 2003). G-actin binding to the RPEL domain of MRTF-A is necessary for nuclear export of MRTF-A to the cytoplasm while in turn binding of cytoplasmic actin interferes with access to the nuclear localization signal (NLS) of MRTF-A. Upon release from G-actin, MRTF-A cannot be exported from the nucleus and thus is predestined for SRF-mediated transcription (Treisman, 2013). Therefore, in order to inactivate MRTF-A/SRF activity, MRTF-A requires nuclear actin binding to re-translocate to the cytoplasm (Vartiainen et al., 2007). We recently demonstrated a mechanism in which nuclear formin-dependent polymerization of actin constitutes a critical step efficiently preventing nuclear export of MRTF-A in response to extracellular signals (Baarlink et al., 2013; Baarlink and Grosse, 2014; Esnault et al., 2014).

To study the functional role of endogenous nuclear formins, we generated a genetically encoded, light-switchable tool for DRF activation by fusing the LOV (light, oxygen, or voltage) Jα-domain of *Avena sativa* phototropin-1 (Niopek et al., 2014; Renicke et al., 2013; Wu et al., 2009) to the DAD region of mDia2. Thus, by light-induced uncaging of a DAD domain for binding to endogenous DID-containing DRFs we could spatiotemporally unleash mDia autoinhibition (Fig. 2) resulting in a reversible induction of long and unbranched nuclear actin filaments (Fig. 2). In addition, MRTF-A relocated from the cytoplasm to the nuclear compartment, demonstrating the importance and efficiency of nuclear actin polymerization for MRTF-A regulation (Fig. 3) (Baarlink et al., 2013).

4. Mechanosensing and nuclear actin dynamics

MRTF transcription factors have been recognized to be involved in mechanotransduction or regulation of tension homeostasis by less well-defined mechanisms (Somogyi and Rorth, 2004; McGee et al., 2011; Janmey et al., 2013; Chan et al., 2010; Iyer et al., 2012). Mechanotransduction to the nucleus involves proteins of the nuclear envelope, which communicate with the cytoskeleton. Located in the inner and outer nuclear membrane (INM and ONM) of the nucleus, linker of nucleoskeleton and cytoskeleton (LINC) complexes are recognized for coupling of mechanical signals from the cytoplasm into the nucleus. Force or tension can be transmitted through these cytoskeletal components leading to changes in gene expression (Simon and Wilson, 2011). Fundamental components of LINC complexes are different isoforms of nesprins (nuclear envelope spectrin-repeat proteins) in the ONM and Sun (Sad1 and UNC-84) domain-containing proteins, which are located in the INM. Nesprins contain KASH (Klarsicht, ANC-1 and SYNE homology) domains, essential for binding Sun proteins (especially Sun1 and Sun2). They can also bind the aforementioned cytoskeletal components, either directly or by a variety of linker proteins (Simon and Wilson, 2011). Sun proteins and nesprins represent the core of LINC complexes, as three nesprins bind to a Sun trimer (Sosa et al., 2012).

Inside the nucleus, different proteins are able to interact with LINC complexes. Two of these proteins are emerin and A-type lamins, which are thought to be responsible for mechanotransduction. Mutations in genes coding for lamin A/C or emerin can disrupt nuclear envelope stability causing diseases like laminopathies and Emery–Dreifuss muscular dystrophy.

When considering nuclear actin filaments, emerin has been reported to interact with nuclear myosins (Simon and Wilson, 2011), while A-type lamins and emerin were shown to affect MRTF-A function through actin presumably via modulating the state of nuclear actin polymerization (Ho et al., 2013). Furthermore, matrix elasticity and lamin A/C have been linked to SRF transcriptional activity and the control of actomyosin genes (Buxboim et al., 2014).

Consistent with such a scenario, we could recently show that nuclear actin polymerization is mediated by integrin activation during cell spreading requiring the presence of lamin A/C and emerin (Plessner et al., 2015). Moreover, integrin-triggered or cell