Emerging Properties and Functions of Actin and Actin Filaments Inside the Nucleus

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Recent years have provided considerable insights into the dynamic nature of the cell nucleus, which is constantly reorganizing its genome, controlling its size and shape, as well as spatiotemporally orchestrating chromatin remodeling and transcription. Remarkably, it has become clear that the ancient and highly conserved cytoskeletal protein actin plays a crucial part in these processes. However, the underlying mechanisms, regulations, and properties of actin functions inside the nucleus are still not well understood. Here we summarize the diverse and distinct roles of monomeric and filamentous actin as well as the emerging roles for actin dynamics inside the nuclear compartment for genome organization and nuclear architecture.

Actin is a highly conserved and abundant protein present in two conformations in all eukaryotic cells. Its monomeric form, globular G-actin, reversibly assembles into long helical filaments (F-actin) controlled via the interaction with a myriad of actin-binding proteins (ABPs) (Pollard 2016). As microfilaments, they constitute one of the three major components of the cytoskeleton and play a fundamental role in cell shape and motility, intracellular transport, muscle contraction, and organelle dynamics, among others (Pollard 2016; Titus 2018). It is now known that both actin and actin regulatory factors constantly shuttle between the cytosolic and nuclear compartments (Grosse and Vartiainen 2013; Hyrskyluoto and Vartiainen 2020). Even though a plethora of cytosolic functions of actin have been extensively studied in the past, the diverse and distinct roles for actin dynamics and the actin cytoskeleton within the nuclear compartment are just beginning to emerge (Plessner and Grosse 2019).

In the past decade, the concept of the cell nucleus as a mere repository for genomic information has been replaced by the perception of the nucleus as a highly dynamic organelle, where spatially separated processes strongly affect nuclear and chromosome architecture and organization to guard and control genome functions (Rout and Karpen 2014). However, a potential role for actin structures in these dynamic nuclear events, including transport of molecules, nucle-

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ar shape, and maintenance of nuclear integrity, is surfacing only now. Better microscopy techniques and the use of modified actin-binding probes targeted to the nucleus have allowed for the first direct visualizations of nuclear dynamic actin assembly in live somatic cells and within the interchromatin region (Baarlink et al. 2013, 2017; Belin et al. 2013; Melak et al. 2017). Since then, evidence is accumulating for the involvement of actin dynamics in fundamental nuclear processes like chromatin reorganization (Baarlink et al. 2017), DNA damage repair (DDR) (Caridi et al. 2018; Schrank et al. 2018), transcription regulation and initiation (Wei et al. 2020), or functional control of chromatin remodeling complexes (Jungblut et al. 2020). Here we summarize recent progress in our understanding of the roles and functions of the ancient molecule actin for nuclear dynamics, genome organization, and architecture.

MONOMERIC ACTIN IN THE NUCLEUS

Dynamically Connected Actin Pools in the Cytoplasm and the Nucleus

Because actin is an important protein in both the cytoplasm and the nucleus, there must be mechanisms to ensure appropriate balance and regulation between the cellular actin pools. Indeed, actin uses an active transport mechanism to constantly and rapidly shuttle in and out of the nucleus (Dopie et al. 2012), and this process is subject to regulation at multiple levels to fine-tune the cellular distribution of actin (Fig. 1). First, an actin monomer binds to the small ABP cofilin, and this cargo is imported into the nucleus by the import factor importin-9 (Fig. 1; Dopie et al. 2012). Many proteins that regulate phosphorylation of cofilin, and thus its ability to interact with actin, can influence nuclear import of actin (Dopie et al. 2015). Actin is exported out of the nucleus as a monomer in complex with profilin by RanGTP-bound export factor exportin-6 (Fig. 1; Stuven et al. 2003), which is subject to regulation by several mechanisms that in turn influence nuclear actin levels and play important roles in both development and disease. Suppression of exportin-6 expression leads to massive accumulation of actin in the nucleus of *Xenopus* oocytes (Bohnsack et al. 2006), and the resulting nuclear F-actin meshwork is required to protect the ribonucleoprotein droplets against gravity within these huge nuclei (Feric and Brangwynne 2013). Laminin-111 is an essential component of the basement membrane that regulates cell death and quiescence in many tissues. It enhances exportin-6 activity via attenuation of the PI3K pathway, thereby increasing nuclear export of actin (Fig. 1), which leads to reduction in nuclear actin levels and quiescence in mammary epithelial cells (Spencer et al. 2011; Fiore et al. 2017). Interestingly, this pathway is disrupted in human breast cancer cells resulting in continuous proliferation (Fiore et al. 2017). Moreover, recent results show that the tumor suppressor RASSF1A (Ras association domain family 1 isoform A), which is frequently silenced in many different types of cancers, is required for the efficient interaction between exportin-6 and the RanGTPase (Fig. 1) and consequently for the maintenance of nuclear actin levels (Chatzifrangkeskou et al. 2019).

Because actin is transported across the nuclear envelope as a monomer, and actin monomer levels limit the transport rate of actin in both directions (Dopie et al. 2012), processes that regulate the actin monomer pool, including actin polymerization, influence nuclear actin levels. For example, MICAL-2 is an actin-regulatory protein that is enriched in the nucleus, where it oxidizes actin at methionine 44, leading to depolymerization of nuclear actin and subsequent reduction in nuclear actin levels (Lundquist et al. 2014). On the cytoplasmic side of the nuclear envelope, mechanical strain induces polymerization of actin at the outer nuclear membrane via emerin and nonmuscle myosin. This leads to decreased nuclear actin levels, which plays a role in Polycomb-mediated gene silencing required for lineage commitment of epidermal stem cells (Le et al. 2016). On the other hand, multiple stimuli that induce cyclic AMP (cAMP) regulate nuclear actin levels by inhibiting Rho GTPase-dependent actin polymerization in the cytoplasm. Increased availability of actin monomers for nuclear import thus results in elevated nuclear actin levels (McNeill et al. 2020). Taken

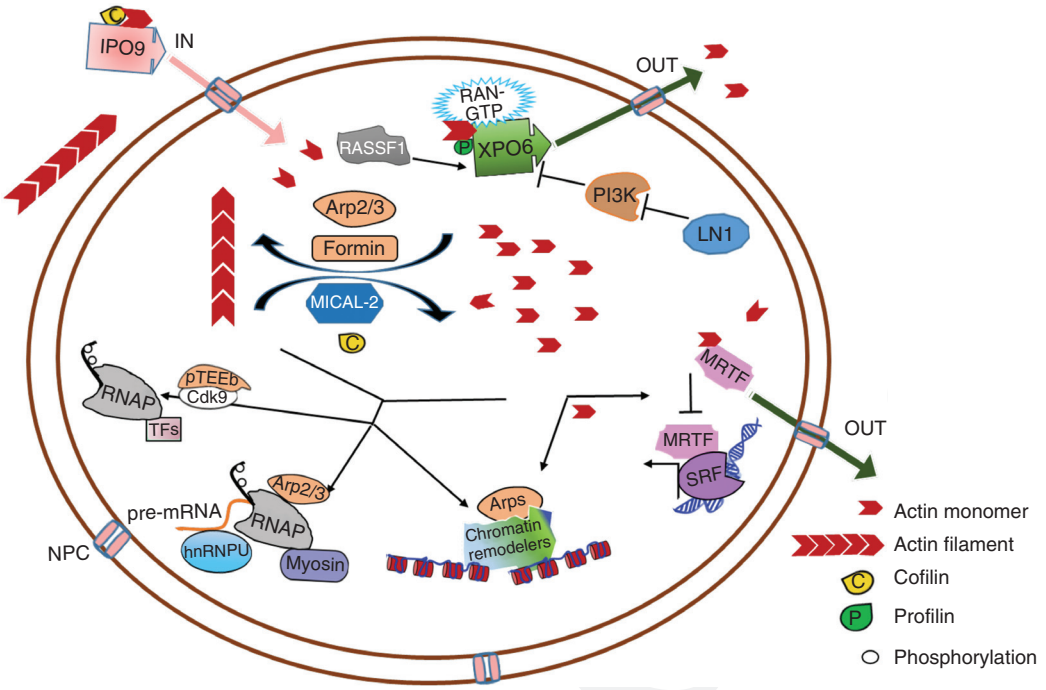

Figure 1. Nucleocytoplasmic shuttling and gene expression–related functions of nuclear actin. In the nucleus, the balance between monomeric and filamentous actin pools is regulated by different actin regulatory proteins, such as Arp2/3 complex and formins, which promote actin polymerization (see also Table 1) and MICAL-2 and cofilin (C), which promote actin filament depolymerization. Actin constantly and rapidly shuttles in and out of the nucleus through nuclear pore complexes (NPCs). Nuclear import is mediated by importin-9 (IPO9) bound to actin monomer and an unphosphorylated cofilin, while monomeric actin is transported out of the nucleus in complex with RanGTP, profilin (P), and exportin-6 (XPO6). RASSF1 enhances the binding of RanGTP to exportin-6, and is thereby required for nuclear export of actin. Laminin-111 (LN1) promotes exportin-6 activity by inhibiting PI3K, thus enhancing nuclear export of actin. Both actin monomers and filaments play functional roles in regulation of gene expression. Actin monomers are integral subunits of several chromatin-remodeling and -modifying complexes together with actin-related proteins (Arps), and actin monomers regulate the subcellular localization and nuclear activity of MRTF-A, the transcription cofactor of SRF. During transcription, actin may influence elongation via positive transcription elongation factor (pTEFb-Cdk9). In addition to actin, actin-binding proteins, such as Arp2/3 complex and nuclear myosins, also interact with the transcription machinery, but the functional form of actin required for RNA polymerase function remains unclear. Polymerized actin may also influence chromatin remodeling.

together, the nuclear pool of actin is in constant and active communication with the cytoplasmic actin networks, and this dynamic process can be used to transmit information from the cytoplasmic cytoskeleton to the nucleus.

Actin as a Signaling Molecule in MRTF/SRF-Mediated Transcription

One of the best-described mediators between the cytoskeleton and nucleus is the myocardin-related transcription factor A (MRTF-A, also known as MKL1 or MAL). MRTF-A is a transcription cofactor of serum response factor (SRF), which regulates many immediate-early, muscle-specific, and cytoskeletal genes (Posern and Treisman 2006). MRTF-A operates as an actin monomer sensor (Vartiainen et al. 2007). In unstimulated conditions, when actin monomer levels are high, MRTF-A is predominantly cytoplasmic, because actin binding occludes the bipartite nuclear localization signal (NLS) em-

Table 1. Signal-regulated F-actin functions in mammalian cell nuclei

Signal input	Actin regulator	Nuclear F-actin properties	Function	References
Serum stimulation	mDia1 and mDia2, MICAL-2	F-actin network	MRTF-A/SRF transcriptional activity	Baarlink et al. 2013; Lundquist et al. 2014
Serum stimulation	N-WASP/Arp2/3	F-actin clusters	Pol II clustering for transcriptional modulation	Wei et al. 2020
Cell spreading	mDia1/2	Thick and short filaments, long-lived (up to 3 h)	MRTF-A/SRF transcriptional activity	Plessner et al. 2015
Mitosis	mDia2	Short dynamic actin filaments	Centromere movement during CENP-A loading	Liu et al. 2018
Mitotic exit	mDia formins	F-actin network	Regulate PCNA loading onto chromatin and initiation of DNA replication	Parisis et al. 2017
Mitotic exit	Cofilin-1, ACTN4	Dynamic and long-lived; bundled short and thick filaments	Actin bundling for nuclear expansion, chromatin decondensation	Baarlink et al. 2017; Krippner et al. 2020
UV/genotoxic agent–induced DNA damage	Spire-1/2, Formin-2	Long, nucleoplasmic filaments; short, nucleolus-associated filaments; dense, nucleoplasmic clusters	DNA double-strand break (DSB) clearance	Belin et al. 2015
Irradiation-induced DNA damage	Arp2/3, WASP	Long filaments spanning the nucleus	Relocalization of heterochromatic DSB to repair sites at nuclear periphery for homology-directed repair	Caridi et al. 2018; Schrank et al. 2018
Replication stress	Not determined	Long filaments spanning the nucleus	Promote replication fork repair	Lamm et al. 2018
GPCR agonists	INF2, mDia formins	Rapidly assembled and short-lived (60–120 sec)	Rapid Ca^{2+} transient, dynamic chromatin organization	Wang et al. 2019
Ca^{2+} elevation	Not determined	Long actin filaments	Long-range movements of myosin VI along F-actin	Grosse-Berkenbusch et al. 2020
T-cell receptor (TCR) activation/immunological synapse formation	Arp2/3	Rapidly assembled and short-lived actin network (60 sec to 8 min)	Ca^{2+} elevation, immune response/effector cytokine expression	Tsopoulidis et al. 2019
Mouse embryo fertilization	Cofilin-1	F-actin network/nucleoskeleton	Regulation of pronuclei formation and function, efficient DNA damage repair	Okuno et al. 2020

bedded within the actin-binding RPEL domain (Vartiainen et al. 2007; Pawłowski et al. 2010; Mouilleron et al. 2011). Moreover, actin binding also promotes MRTF-A nuclear export by Crm1 via an unknown mechanism (Fig. 1; Vartiainen et al. 2007). Upon mechanical or mitogenic signaling that activates RhoA-dependent actin polymerization, actin monomer levels decrease, thus liberating MRTF-A from actin and exposing the NLS for nuclear import and preventing nuclear export. This leads to the accumulation of MRTF-A in the nucleus, and activation of MRTF/SRF target genes (Vartiainen et al. 2007; Pawłowski et al. 2010; Mouilleron et al. 2011). Because actin also regulates MRTF-A in the nucleus, factors affecting nuclear actin levels, such as MICAL-2 (Fig. 1; Lundquist et al. 2014), RASSF1A (Chatzifrangkeskou et al. 2019), and cAMP signaling (McNeill et al. 2020), influence MRTF/SRF-mediated transcription. Finally, signal-induced polymerization of actin in the nucleus (discussed in detail below) has emerged as an important regulator of MRTF/SRF activity (Baarlink et al. 2013; Plessner et al. 2015; Wang et al. 2019). Many cytoskeletal genes, including actin itself, are MRTF/SRF targets (Posern and Treisman 2006), creating a feedback loop where cytoskeletal dynamics regulate the expression of its own constituents.

Monomeric Actin in Allosteric Regulation of Chromatin Remodeling Complexes

In addition to the regulation of MRTF/SRF activity, monomeric actin has a well-established role as an integral component of many chromatin-remodeling (including Ino80 and SWI/SNF families) and -modifying complexes (NuA4 and possibly hATAC) (Fig. 1). These complexes regulate chromatin accessibility for replication, transcription, and repair of DNA damage, and thus play critical roles in most chromatin-related processes. Structural studies by both X-ray crystallography, and more recently by CryoEM, have helped to resolve the role of actin in these multisubunit complexes (Jungblut et al. 2020). Here, actin operates together with actin-related proteins (Arps), and most complexes contain an actin–Arp4 pair bound by the Helicase–SANT-associated (HSA) domain (Szerlong et al. 2008). In addition, the Ino80 HSA domain interacts also with Arp8, while Arp5 forms a distinct module with Ies6. In yeast, SWI/SNF, and RSC complexes, the actin–Arp4 pair is replaced by the Arp7–Arp9 pair (Jungblut et al. 2020). Binding to the HSA domain prevents actin from polymerizing. For instance, in the yeast SWR1, which is the ATPase of a chromatin remodeling complex belonging to the Ino80-family, the barbed end of actin is sequestered by Arp4 and the HSA domain, and the actin molecule adopts a twisted orientation that prevents its contact with another actin molecule and frees it from ATP binding and regulation (Cao et al. 2016). Also, in the Ino80 complex, the HSA domain makes contact along the barbed ends of actin and the Arps and actin is sandwiched between Arp4 and Arp8, which interact with actin using distinct binding modes (Knoll et al. 2018). Functionally, the actin-containing modules play a role in establishing the allosteric control of the motor subunit in these complexes (Jungblut et al. 2020). In the Ino80 complex, the actin–Arp-bound HSA module binds and senses the length of the extranucleosomal/linker DNA to orient the complex, and especially the ATPase, on the nucleosome for remodeling. This is achieved by functional interplay with the Arp5–Ies6 module, which interacts with the acidic patch of the H2A–H2B dimer, and is required for gripping the remodeler on the nucleosome (Brahma et al. 2018; Eustermann et al. 2018; Knoll et al. 2018; Zhang et al. 2019). Also, in the human BAF (SWI/SNF family) complex, the actin–Arp4-containing module bridges the ATPase and the base module, and thus likely couples their motions during chromatin remodeling (He et al. 2020). The functional significance of actin for appropriate SWI/SNF function is further highlighted by studies using β-actin, null-mouse embryonic fibroblasts, which display reduced chromatin association of Brg1, the ATPase subunit of the BAF complex, consequently leading to defects in gene expression (Xie et al. 2018a,b).

Chromatin-modifying complexes controlling histone acetylation have also been reported to interact with actin. NuA4 histone acetyltrans-

ferase (HAT) plays important roles in DNA repair and transcription, and similarly to the chromatin-remodeling complexes, interacts with the actin–Arp4 pair via the HSA domain of Eaf1, which operates as an assembly platform for the complex. CryoEM studies of the yeast NuA4 complex have revealed that the actin–Arp4 pair is located at the peripheral head region of the complex (Wang et al. 2018). Mass spectrometry studies on nuclear actin revealed a potential interaction between actin and the human Ada-Two-A-containing (hATAC) HAT complex, and further experiments demonstrated that actin binds directly, and modulates, the HAT activity of KAT14, which is one of the HAT enzymes in the hATAC complex (Viita et al. 2019). Further structural studies are needed to elucidate the molecular and functional details of this interaction. Interestingly, actin also interacts with histone deacetylases (HDACs) 1 and 2 to modulate its activity. Although this association appears not to be direct (Serebryannyy et al. 2016a), it highlights the multifunctional nature of actin in regulating various proteins and complexes that influence chromatin accessibility for essential nuclear processes.

Although structural data clearly pinpoint a role for monomeric actin in chromatin-remodeling and -modifying complexes, there are some indications that filamentous actin may also play a role. Early studies suggested that Brg1-containing BAF chromatin-remodeling complexes interact with actin filaments in a phosphatidylinositol 4,5-biphosphate (PIP_2)-dependent manner (Zhao et al. 1998; Rando et al. 2002). Similarly, yeast INO80 and SWR1 complexes associate with actin filaments. This interaction depends on the Arp subunits in these complexes and is regulated by the Hsp90 and p23 chaperones (Wang et al. 2020). Moreover, in mammalian cells, ARID1A-containing SWI/SNF complexes associate with nuclear F-actin and this interaction serves as a mechanosensitive switch, which regulates the activity of YAP/TAZ transcriptional coactivators that control cell proliferation (Chang et al. 2018). Binding of ARID1A-containing complexes to actin prevents their association with YAP/TAZ, which are then free to bind the transcription factor TEAD and activate transcription. This pathway may also play a role in tumorigenesis, because ARID1A, and other SWI/SNF subunits, are often mutated in various cancers (Chang et al. 2018). Further biochemical and functional studies are required to elucidate how actin filaments interact with SWI/SNF, and whether the putative roles for both monomeric and filamentous actin on chromatin remodelers are functionally connected.

Actin in RNA Polymerase Functions

Actin has also been linked directly to transcription, mainly through its propensity to copurify with all three RNA polymerases (Fig. 1; Egly et al. 1984; Hofmann et al. 2004; Hu et al. 2004; Philimonenko et al. 2004). Decreased availability of monomeric actin, for example, upon inhibition of its nuclear import (Dopie et al. 2012; Le et al. 2016) by increasing its nuclear export (Spencer et al. 2011) or by persistently forcing nuclear actin into stable filaments (Serebryannyy et al. 2016b), results in impaired transcription. Furthermore, genome-wide binding studies demonstrate that actin is bound to promoter regions of essentially all transcribed genes in *Drosophila* ovaries (Sokolova et al. 2018), suggesting a general role for actin in Pol II–mediated transcription.

The molecular mechanisms by which actin promotes RNA polymerase function still remain somewhat unclear, but roles in several steps of the transcription process have been proposed (Hofmann et al. 2004; Obrdlik et al. 2008). In support, mass spectrometry-based identification of nuclear actin-binding partners revealed association of actin with proteins implicated in preinitiation complex (PIC) assembly, transcription elongation, and pre-mRNA splicing and processing (Viita et al. 2019). Interference with nucleocytoplasmic shuttling of actin results in defects in alternative splicing of reporter gene constructs, potentially implying a role in pre-mRNA splicing, be it direct or indirect. One possible hypothesis is that actin regulates the transcription elongation rate (Viita et al. 2019), which has been shown to affect splice site selection. A role in elongation is also sup-

ported by the observation that on highly transcribed genes, actin binds, together with Pol II, along the gene body (Sokolova et al. 2018). An interesting candidate for connecting actin to the transcription elongation machinery is the positive transcription elongation factor b (P-TEFb), which interacts with actin (Qi et al. 2011), although the biochemical details are still unknown.

To date, it remains unclear whether the functional form of actin, which impinges on RNA polymerase function, is its monomeric or polymeric form. In laminin-111-treated cells that display reduced nuclear actin, the transcription defect can be rescued not only with NLS-tagged wild-type actin, but also with the NLS-tagged actin-R62D mutant, which cannot polymerize (Spencer et al. 2011). This is in contrast to studies done on β-actin knockout MEFs in the context of Pol I–mediated transcription, where actin-R62D does not improve rRNA synthesis unlike wild-type actin (Almuzzaini et al. 2016). Interestingly, actin regulatory proteins, which can control actin polymerization, such as the Arp2/3 complex (Yoo et al. 2007) and its activators N-WASP (Wu et al. 2006), WAVE1 (Miyamoto et al. 2013), and WASH (Xia et al. 2014), as well as motor protein myosins, have also been linked to transcription or transcription-related processes. Myosin-1C and its isoform nuclear myosin 1 (NM1) have been implicated in both Pol I– and Pol II–mediated transcription, possibly via the B-WICH chromatin remodeling complex (Philimonenko et al. 2004; Hofmann et al. 2006; Sarshad et al. 2013; Almuzzaini et al. 2015). Moreover, NM1 is required for activation of *p21* transcription upon DNA damage, which is required for cell-cycle arrest. Here NM1 seems to partner with p53 to control the *p21* promoter through epigenetic mechanisms via the HAT PCAF and histone methyltransferase Set1 (Venit et al. 2020). Curiously, myosin VI, the only myosin traveling toward the minus end of the actin filament, has also been implicated in transcription. Early studies have already indicated the existence of myosin VI in the Pol II transcription complex, as well as the presence of myosin VI on gene promoters and intragenic regions (Vreugde et al. 2006). More recent studies have demonstrated that the transcription coactivator NDP52 regulates myosin VI activity by relieving its autoinhibition and allowing the interaction with DNA through the cargo-binding domain of myosin VI (Fili et al. 2017). The NDP52–myosin VI complex associates with Pol II and is required, for example, for the expression of nuclear receptor target genes (Fili et al. 2017).

Actin, and its binding partners, have therefore a multitude of connections with the RNA polymerase machineries, and could thereby influence the transcription process both as a monomer and as a filament. Whether actin operates here as a cofactor similarly as in chromatin remodeling complexes remains to be elucidated. Further studies are needed to establish the biochemical basis of these interactions, and how nuclear actin dynamics are precisely regulated to facilitate transcription.

FILAMENTOUS ACTIN IN THE NUCLEUS

Multiple Functions of Dynamic Intranuclear Actin Assembly

Although the presence of actin within the nuclear compartment has long been recognized, its dynamic assembly into filamentous structures is still only poorly understood (Percipalle and Vartiainen 2019). Recent methodological and technical advances in imaging have allowed for the first convincing visualization of nuclear F-actin in living mammalian cells; however, its regulation, roles, and functions remain largely unexplored.

Earlier studies exploited *Xenopus laevis* oocytes as a model system to study nuclear F-actin as they are relatively large in size (Dumont 1972) and, as mentioned above, contain high nuclear actin levels because of a lack of expression of exportin-6, thereby drastically increasing the concentration of actin for polymerization (Bohnsack et al. 2006). Wave1 (Wiskott–Aldrich syndrome protein family member 1) belongs to the family of nucleation promoting factors (NPFs) that facilitate Arp2/3-mediated actin polymerization. Several studies revealed an essential role for Wave1-dependent dynamics and

prolonged nuclear F-actin assembly in transcriptional reprogramming by inducing pluripotency genes such as *Oct4* (Miyamoto et al. 2011, 2013) as well as a role in chromatin tethering to the nuclear envelope (Oda et al. 2017).

The high abundance of actin in the cytoplasm makes it challenging to visualize F-actin structures within the nuclear compartment when using traditional actin probes like phalloidin, LifeAct, or utrophin. Progress was made when fluorescently labeled actin-binding domains fused to an NLS were used (Baarlink et al. 2013; Belin et al. 2013; Melak et al. 2017), which helped overcome the predominance of cytosolic over the nuclear actin signal as exemplified in Figure 2. Since then, a multitude of functions have been described for dynamic actin polymerization in the nuclei of living cells in response to several stimuli including serum stimulation (Baarlink et al. 2013; Wei et al. 2020), DNA damage (Belin et al. 2015; Caridi et al. 2018; Schrank et al. 2018), T-cell receptor (TCR) activation (Tsopoulidis et al. 2019), integrin signaling (Plessner et al. 2015), or viral infection (see Table 1; Ohkawa and Welch 2018).

First evidence and visualization of dynamic and transient assembly of F-actin structures in somatic cell nuclei came from a study showing that serum stimulation leads to the rapid formation of an endogenous nuclear actin network (Baarlink et al. 2013). This signal-dependent F-actin polymerization could be spatiotemporally controlled using a light-activatable optogenetic system to release autoinhibition of diaphanous formins (mDia1 and mDia2) localized to the nuclear compartment, thereby demonstrating the dynamic and reversible character of F-actin assembly in somatic cell nuclei.

In another study, nuclear actin filament assembly was observed during cell spreading (Plessner et al. 2015), using a nuclear-targeted nanobody recognizing endogenous actin. Integrin signaling during cell adhesion or spreading or via addition of soluble fibronectin induced the formation of nuclear F-actin depending on

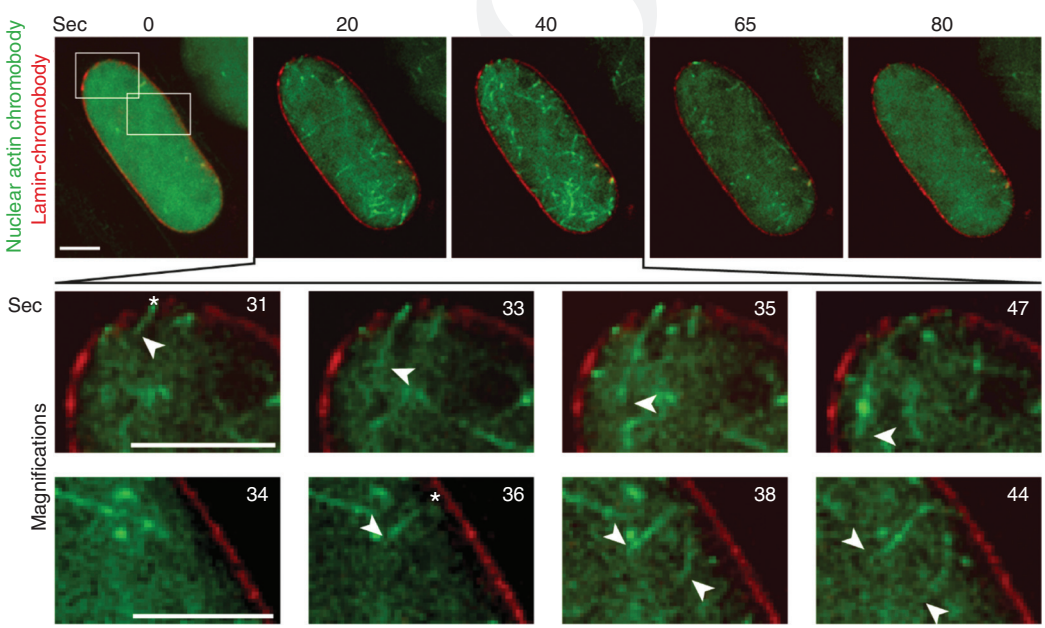

Figure 2. Calcium transiently induces dynamic intranuclear actin polymerization. NIH3T3 cells stably expressing a nuclear actin chromobody (green) were transfected with lamin-chromobody-mCherry (red). Upon stimulation with A23187, actin polymerizes originating from the nuclear envelope (depicted by asterisks). Arrowheads show the tips of growing actin filaments. Scale bar, 5 μm.

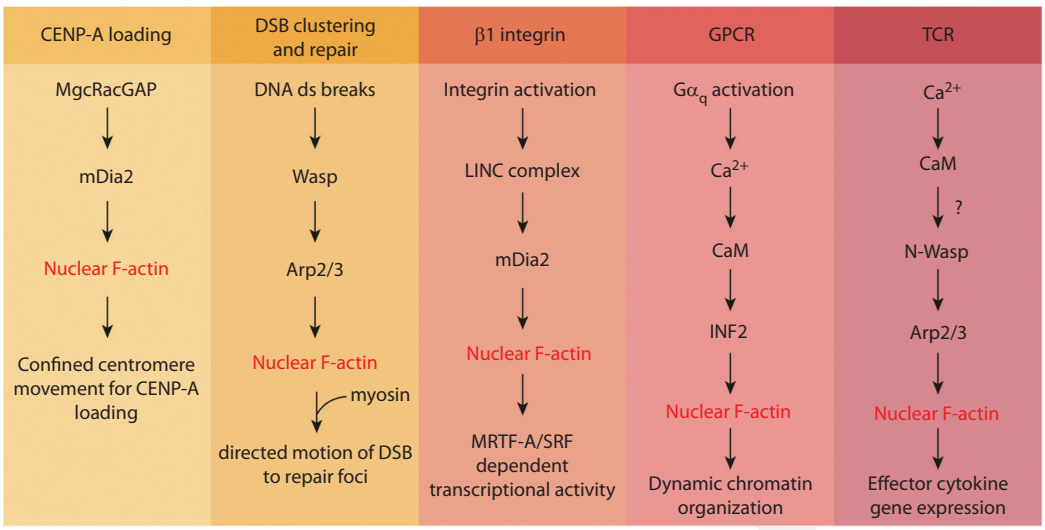

Figure 3. Signaling mechanisms for nuclear F-actin polymerization. Scheme of different signaling events controlling distinct actin assembly factors for nuclear F-actin formation.

the linker of nucleoskeleton and cytoskeleton (LINC) complex and mDia formins (Fig. 3), thereby promoting nuclear retention of MRTF-A (Plessner et al. 2015). Thus, different extracellular stimuli that induce nuclear actin filament formation appear to converge on nuclear formin function (Baarlink et al. 2013; Belin et al. 2015; Plessner et al. 2015). Recently, F-actin assembly upon serum stimulation has also been implicated in transcription regulation by enhancing RNA polymerase II (Pol II) clustering (Wei et al. 2020). Using next-generation transcriptome sequencing and superresolution microscopy, it was demonstrated that the formation of discrete RNA Pol II foci is facilitated through dynamic N-WASP/Arp2/3-dependent de novo polymerization of short actin filaments. Additionally, stimulating cells with IFN-γ, a crucial cytokine in innate and adaptive immunity, led to a similar response where nuclear F-actin promoted enhanced Pol II clustering. Interestingly, these F-actin structures did not have an influence on Pol II clustering and transcription initiation under normal growth conditions, suggesting a general role for dynamic nuclear F-actin assembly and disassembly in providing a dynamic scaffold and thereby regulating Pol II clustering in a signal-dependent manner in response to external stimuli (Wei et al. 2020).

Nuclear Actin Filaments: From Cell Cycle to Chromatin Reorganization

Cellular Signaling

It has become clear in the past few years that dynamic nuclear F-actin polymerization can be triggered and regulated by distinct signaling mechanisms and during different cellular processes. This has revealed not only different temporal characteristics but also differing actin assembly factors and F-actin structures inside the nucleus.

One critical role for nuclear actin assembly has been described after TCR activation, which leads to cytokine expression to drive T-cell proliferation and antibody production (Tsopoulidis et al. 2019). After TCR engagement, a rapid Ca^{2+} increase induces the formation of a dynamic nuclear actin network possibly through nuclear N-Wasp/Arp2/3, and Nck-interacting kinase (NIK) activity (Fig. 3). Importantly, this study links dynamic F-actin structures in the nucleus to critical immune defense mechanisms, allow-

ing for the rapid response of $CD4^+$ T cells to TCR signaling to facilitate T-cell helper functions (Tsopoulidis et al. 2019). However, how these nuclear filaments are mechanistically connected to immediate early transcriptional responses requires further investigation. Another receptor mechanism of how extracellular signals are transmitted from the plasma membrane to the nuclear compartment for functional F-actin assembly has recently been identified (Wang et al. 2019). Stimulation of cells with physiological ligands for G protein–coupled receptors (GPCRs) and downstream activation of $G\alpha_q$ led to transient nuclear Ca^{2+} elevations and Inverted-Formin-2 (INF2)-dependent nuclear F-actin assembly, thereby promoting rapid changes in chromatin dynamics (Fig. 3; Wang et al. 2019). Polymerization of these Ca^{2+}-triggered linear actin filaments appeared to originate from the inner nuclear membrane (INM) (Fig. 2). These findings provide evidence for the INM as a potential signaling hub for intranuclear F-actin assembly (Wang et al. 2019). Even though an interaction between INF2 and the calcium sensor protein calmodulin could be observed in IP experiments (Wang et al. 2019), it remains elusive how precisely INF2 is activated, which receptors of the nuclear envelope might be involved in signal transduction, and what functions the actin filaments might have apart from altering chromatin dynamics.

Long-Range Chromatin Motion

Actin polymerization could also influence transcription by controlling the subnuclear localization of target genes. In budding yeast, the *INO1* locus moves from the center of the nucleus toward the periphery by long-range directed motion upon its transcriptional activation. This movement is dependent on chromatin remodelers INO80 and SWR, as well as on actin-polymerizing proteins, such as the Diaphanous formin homologue Bnr1, which are likely required to create a dynamic pool of short actin filaments in the nucleus (Wang et al. 2020). Interestingly, the movement of the *INO1* locus requires motor activity in the form of myosin 3, which is tethered to the locus via interactions with the transcription factor Put3. The interaction between the chromatin remodelers and actin filaments is thought to further reinforce the connection between the moving locus and the actin filaments, thus fostering long-range transport (Wang et al. 2020). Notably, actin polymerization had previously been implicated in long-range intranuclear movement of chromosomal loci (Chuang et al. 2006; Dundr et al. 2007; Khanna et al. 2014). Although these studies did not directly visualize nuclear actin filaments, long-range chromosomal motion was sensitive to actin depolymerizing drugs such as Latrunculin or could be inhibited by expression of nonpolymerizable actin mutants (Posern et al. 2002).

Only recently, a study performed in mammalian cells presented compelling evidence for ATP-dependent, directed long-range movements of myosin VI along F-actin within the nucleus, possibly enhancing transcription by supporting long-range chromatin rearrangements (Grosse-Berkenbusch et al. 2020). Hence, it is worth speculating whether the long linear actin filaments observed in the nucleus after Ca^{2+} increase also play a role in myosin-mediated transport along F-actin within the nucleus.

Cell Cycle

The histone H3 variant centromere protein A (CENP-A) epigenetically defines centromere regions of the chromosome and needs to be replenished in every cell cycle. Nuclear localization and activity of the formin mDia2 downstream of the MgcRacGAP-dependent GTPase pathway has been identified as a crucial factor for CENP-A loading and maintenance at centrosomes (Liu and Mao 2016, 2017). In a follow-up study, using a utrophin-based probe, mDia2-dependent formation of short dynamic nuclear actin filaments was found to be required for constrained centromere movement during CENP-A loading in G_1 nuclei (Fig. 3; Liu et al. 2018). However, the exact mechanism and potentially spatial regulation of F-actin formation remains to be identified. Interestingly, transient nuclear actin filament formation has been observed during a similar time window in early G_1 phase of the cell cycle in another context (Baarlink et al.

2017). Using PALM and STORM superresolution imaging these filaments were found to consist of both bundled and longer single F-actin structures that assembled dynamically in daughter cell nuclei after mitotic exit to promote nuclear protrusions during nuclear volume expansion and thereby enabling chromatin decondensation. Interfering with the polymerization-competent nuclear actin pool by overexpression of the nonpolymerizable actin-R62D-NLS mutant or the actin exporter exportin-6 resulted in a decreased volume of daughter cell nuclei as well as an increase in chromatin compaction at mitotic exit (Baarlink et al. 2017). Timing and turnover of the observed nuclear actin filaments, which reorganize the postmitotic mammalian nucleus, appear to be tightly controlled by the actin-depolymerizing factor cofilin-1 (Baarlink et al. 2017). Its nuclear activity was observed to be cell-cycle-dependent with an increase of phosphorylated and thus inactivated cofilin-1 during mitotic exit by as-yet-unknown mechanisms. A parallel study implicated a critical role for formin-dependent nuclear F-actin assembly in promoting cyclin-dependent kinase (CDK) and proliferating cell nuclear antigen (PCNA) loading onto chromatin and initiation of DNA replication by as-yet-unknown mechanisms (Parisis et al. 2017). Recently, an involvement of the actin bundling factor α-actinin 4 (ACTN4), in regulating postmitotic nuclear volume expansion and actin assembly has been proposed (Krippner et al. 2020). Performing superresolution STORM imaging, cells expressing an ACTN-4 mutant lacking its actin-binding domain were found to have fewer and thinner actin filaments as opposed to ACTN4-wt expressing cells, that displayed abundant thick F-actin structures (Krippner et al. 2020). Together, these studies emphasize the transient and highly dynamic character of nuclear actin assembly controlled by complex mechanisms as response to various stimuli or cellular processes.

Viral Replication

Well-described functions of F-actin in the cytosol, including scaffolding and transport along the filaments, have long not been observed within the nuclear compartment. Interestingly, several viruses use both cytosolic and nuclear actin-based motility in their life cycle (Wilkie et al. 2016; Ohkawa and Welch 2018). For instance, the baculovirus-type species *Autographa californica* M nucleopolyhedrovirus (AcMNPV) promotes actin polymerization to facilitate its nuclear egress (Ohkawa and Welch 2018). It uses viral Wasp-like proteins to hijack the host system and induce Arp2/3-dependent nuclear actin comet-tails that push the virus against the nuclear envelope (NE) creating nuclear protrusions and finally leading to NE rupture and viral egress into the cytosol where it continues to exploit nuclear-based motility to reach the cell periphery. The human cytomegalovirus (CMV), on the other hand, uses transiently assembled extensive nuclear F-actin networks to control intranuclear polymerization by spatially segregating viral DNA from inactive histones and host DNA to facilitate viral replication (Procter et al. 2020).

Nuclear F-Actin in DNA Damage Repair Mechanisms

Earlier studies had indicated a potential role for nuclear actin in long-range movements and repositioning of chromosomal loci (Chuang et al. 2006; Dundr et al. 2007). More recent work now elucidated a novel regulatory role for actin polymerization in directed chromatin dynamics upon DNA damage and double-strand break (DSB) repair progression (Fig. 3; Andrin et al. 2012; Belin et al. 2015; Caridi et al. 2018; Schrank et al. 2018). As a consequence of DNA damage induced by various endogenous or exogenous triggers, the tightly regulated DDR machinery is activated and recruits and assembles various DDR factors at the site of the lesion (Chatterjee and Walker 2017). Faithfully restoring genome integrity is of particular importance as unresolved DSBs are implicated in a variety of human disorders and cancers (Jackson and Bartek 2009; Chatterjee and Walker 2017). While single-strand breaks are often cleared by different excision repair mechanisms, cells usually employ two main mechanisms to repair DSB: nonhomologous end joining (NHEJ), which is

more error prone and executed throughout the cell cycle, and homologous recombination (HR), which is thought to be restricted to late G_2/S phases, when a sister chromatid is present to function as template for DNA repair (Phillips and McKinnon 2007; Ceccaldi et al. 2016; Chatterjee and Walker 2017). First evidence for a potential involvement of F-actin in DSB repair came from an in vitro study performing pull-down experiments using nuclear cell extracts incubated with purified polymeric actin showing binding of DNA repair proteins including Ku80, Mre11, and Rad51 (Andrin et al. 2012). Later, DNA damage-induced formation of both long actin filaments and dense actin clusters in the nucleoplasm as well as short, nucleolus-associated filaments were identified in living cells using a variety of actin probes (Belin et al. 2015). Simultaneous global knockdown of actin nucleators Spire-1 and Spire-2 or single knockdown of formin-2 completely abolished clearance of chemically induced DNA DSBs in HeLa cells uncovering a new signaling pathway for formin-induced nuclear actin filament formation (Belin et al. 2015). Whether actin nucleation actually takes place inside the nucleus and which function F-actin fibers play in the DNA damage response, however, remains to be elucidated. Only a few years later, using different model organisms, two publications independently uncovered a role for Arp2/3-dependent, actin-based mobility in DSB repair (Caridi et al. 2018; Schrank et al. 2018). It was shown that the actin-NPF WASP was recruited to DSBs independently of the repair mechanism, while Arp2/3 was only enriched and activated at chromatin lesions in G_2 phase, which are cleared by homology-directed repair (Schrank et al. 2018). Arp2/3-mediated actin filaments locally assemble these DSBs and are required for their migration into discrete subnuclear clusters for repair (Schrank et al. 2018). On the other hand, actin filaments observed in *Drosophila melanogaster* cells in response to irradiation-induced DNA damage were longer in size spanning the nucleus (Caridi et al. 2018). Actin filament assembly could be visualized at DSBs in heterochromatic regions through the actin nucleator Arp2/3 that allowed for myosin-dependent, directed movement of repair sites toward the nuclear periphery (Caridi et al. 2018).

CONCLUDING REMARKS

During the last decade, actin has emerged as an essential protein in the nucleus with functional roles in fundamental nuclear processes from gene expression to DNA repair. At the same time, spatially and temporally controlled nuclear organization has emerged as a key concept in nuclear biology. Actin has the inherent capacity to produce force that can be used to drive motility or to create scaffolds for higher-order assemblies, and is therefore an ideal candidate for organizing the dynamic nuclear landscape. With the aid of advanced microscopy and chromosome conformation capture techniques, novel concepts have been established for a dynamic nuclear organization where chromosome loci cluster into large compartments and subcompartments called topologically associating domains (TADs) (Dekker et al. 2013; Schrank and Gautier 2019). Keeping these and the recent findings of F-actin-mediated chromosome repositioning in mind, it is tempting to speculate that future research might uncover a link between spatiotemporally controlled, dynamic nuclear F-actin assembly and the formation of nuclear domains or promoter-enhancer loops, among others. Another interesting avenue for future research are the posttranslational modifications on actin, and how they impinge on nuclear functions of actin (Kumar et al. 2020; Mu et al. 2020). Thus, the actin monomer is not a mere building block for the filament but has itself an important role as a signaling molecule or as a cofactor (e.g., in allosteric control of chromatin-remodeling complexes). How the functions of actin monomers and filaments are connected and how the polymerization process impinges on these activities are important questions for the future.

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Queries

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