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Review

Nuclear actin dynamics in gene expression and genome organization

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ABSTRACT

Although best known from its functions in the cytoplasm, actin also localizes to the cell nucleus, where it has been linked to many essential functions from regulation of gene expression to maintenance of genomic integrity. While majority of cytoplasmic functions of actin depend on controlled actin polymerization, in the nucleus both actin monomers and filaments have their own specific roles. Actin monomers are core components of several chromatin remodeling and modifying complexes and can also regulate the activity of specific transcription factors, while actin filaments have been linked to DNA damage response and cell cycle progression. Consequently the balance between monomeric and filamentous actin must be precise controlled also in the nucleus, since their effects are dynamically coupled. In this review, we discuss the recent data on how actin dynamics is regulated within the nucleus and how this influences the different nuclear processes dependent on actin.

1. Introduction

The key feature of actin is its ability to polymerize into helical filaments, and the well-established functions of actin in the cytoplasm of the cell, such as cell motility, mostly depend on controlled actin filament formation. The monomeric (G-actin) and filamentous (F-actin) actin pools are tightly controlled by numerous actin-binding proteins (ABPs) to allow precise spatial and temporal regulation of cytoplasmic actin dynamics. Interestingly, both forms of actin, as well as numerous ABPs, exist also in the cell nucleus. Actin was detected in the nucleus already over 50 years ago [1–3], but the early studies faced criticism, which consequently hindered the development of the whole field for several decades. However, advances in the field, in particular during the last 15 years, have unequivocally demonstrated not only the presence, but also the functional importance, of actin also in the nuclear compartment, and linked actin to essential nuclear processes from gene expression to DNA damage responses (reviewed in [4]).

Like all proteins synthesized in the cytoplasm, actin must be transported through the nuclear pore complexes (NPC) to the nucleus. Although the size of actin is very close to the passive diffusion limit of the NPC, actin utilizes active transport mechanisms to both enter and exit the nucleus (Fig. 1). Actin is actively imported into the nucleus as a monomer by Importin-9 in a complex with the small ABP cofilin [5] and exported out from the nucleus together with profilin by Exportin-6 [6]. The use of active transport mechanisms for nucleo-cytoplasmic shuttling may indicate the need to actively regulate nuclear actin levels. Indeed, decreased nuclear actin levels have been reported upon cell quiescence [7] and increased nuclear actin levels upon differentiation [8]. Since actin constantly and rapidly shuttles in and out of the nucleus [5], the nuclear and cytoplasmic actin pools are dynamically connected. Actin monomer levels limit the transport rate in both directions, and consequently nuclear actin concentration depends on the cytoplasmic actin pool, import and export activities, as well as the number of interactions sequestering actin in the nucleus [5,9,10]. Further research is required to understand the biochemical basis of nucleo-cytoplasmic of actin, and on how different cellular signals impinge on these processes.

The form of actin in the nucleus remained unclear for a long time, mostly due to the lack of tools to visualize polymeric actin inside the nucleus. Lack of phalloidin staining suggested that nuclear actin could be mostly monomeric, but the development of novel, nuclear targeted probes have proved that nuclear actin can indeed polymerize in response to specific signals (reviewed in [11]). Nowadays it is well established that both actin filaments and monomers have distinct functions in the cell nucleus. This suggests that, like in the cytoplasm, actin dynamics must be very tightly regulated in the nucleus. Indeed, numerous ABPs that have well-established roles in controlling actin polymerization in the cytoplasm, localize also to the cell nucleus (reviewed in [12]). In this review, we discuss the mechanisms that regulate nuclear actin dynamics and the functions of the different forms of actin, monomer and filamentous, in the cell nucleus.

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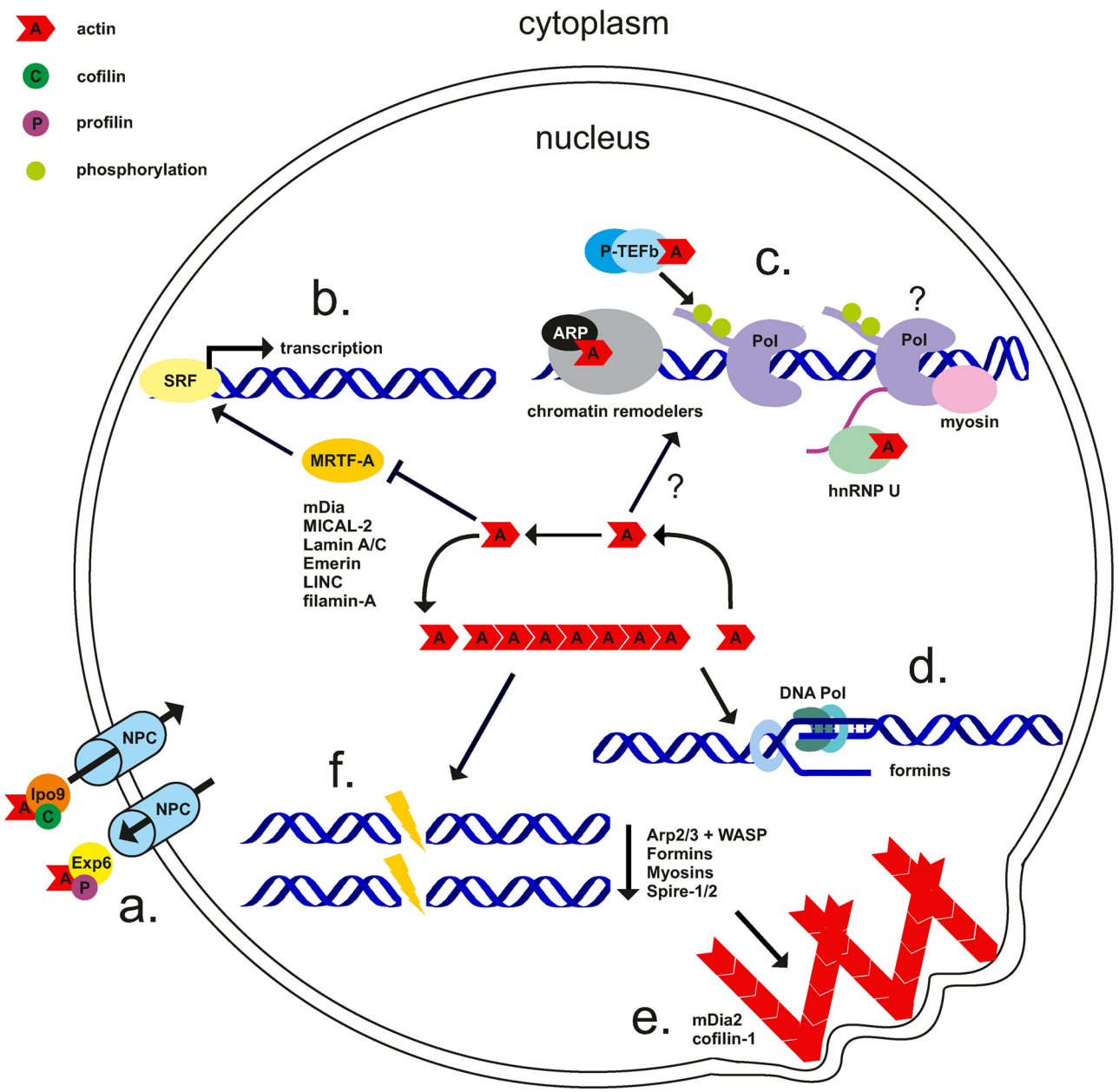

Fig. 1. Functions of monomeric and filamentous actin pools in the nucleus.
(a) Actin is actively transported in and out of the nucleus through the nuclear pore complex (NPC). Nuclear import of actin takes place in a complex with Importin-9 (Ipo9) and cofilin and export complex contains Exportin-6 (Exp6) and profilin.
(b) Actin monomers inhibit the activity of MRTF-A, a transcriptional co-activator of serum response factor (SRF). Formins mDia1/2 are required for nuclear actin polymerization that releases MRTF-A from the actin monomer to activate SRF-mediated transcription upon serum stimulation, and also MICAL-2, lamin A/C, emerin, LINC complex and filamin-A can contribute to the process.
(c) Actin is linked to several steps of transcription. Actin and actin-related proteins (Arps) are subunits of many chromatin modifying complexes. Actin might also associate with RNA polymerases directly, or together with motor protein myosins, and interacts with P-TEFb and hnRNP-U that are required for transcription elongation. The question mark next to the arrow indicates that the functional form of actin during some of these processes has not been established.
(d) Nuclear actin dynamics and formin activity are required for DNA replication.
(e) Actin polymerizes during early G1 phase of the cell cycle and this is linked to nuclear expansion and chromatin decondensation. Polymerization may be mediated by formin mDia2 and cofilin is required for the disassembly of the filaments.
(f) Actin filaments form upon DNA damage in the nucleus, with several actin nucleators implicated in the process. Filaments nucleated by Arp2/3 complex have been linked to double strand break movement within the nucleus.

2. Nuclear actin polymerization upon DNA damage response and during cell cycle

For a long time, the lack of phalloidin-stainable actin filaments, and the relatively low levels of actin in the nucleus compared to the cytoplasm, led to the assumption that there would not be canonical actin filaments in the nucleus [11,13]. However, fluorescence recovery after photobleaching experiments indicated that the nuclear actin pool consists of several kinetically different populations, including a pool corresponding to polymeric actin [5,14]. Subsequent experiments utilizing especially novel, nuclear targeted probes for different forms of actin have unequivocally demonstrated the presence of actin filament also in

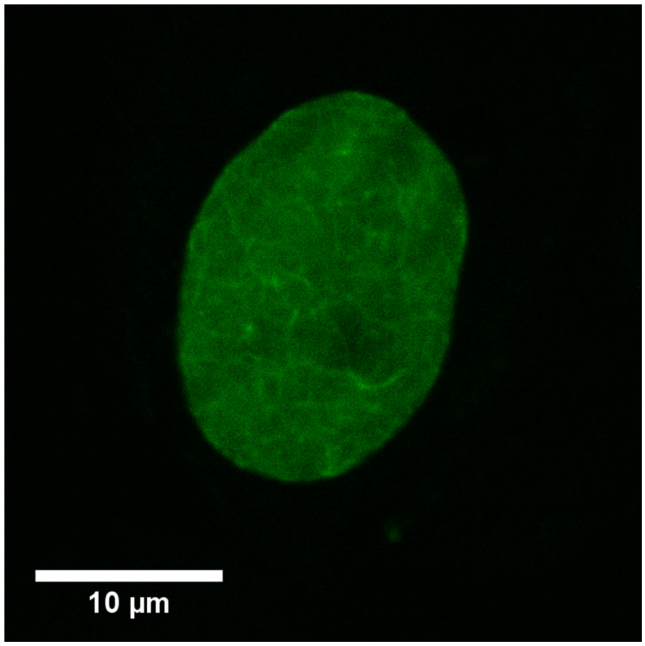

Fig. 2. Nuclear actin filaments.
A spreading NIH 3T3 cell expressing nuclear actin chromobody. Imaging by Henna Moore. Scale bar 10 µm.

the cell nucleus (Fig. 2). Of these probes, UTR230-EN, which is based on the truncated calponin homology domains of utrophin fused with nuclear localization signals (NLS) and GFP, labels submicron length punctate structures, which are excluded from chromatin rich areas [15], implying that these filaments would not be directly involved in processes such as transcription. These puncta are not labeled with phalloidin. It has been postulated that these actin structures could contribute to the viscoelastic nature of the nucleoplasm [15], but their exact function, structural details and regulation have remained unclear. The other widely used probes, nuclear targeted LifeAct [16], which is based on the short peptide from the yeast Abp140 [17] and the nuclear actin chromobody, based on an alpaca nanobody [18] seem to detect filamentous structures only upon specific stimuli or upon certain cellular states as discussed below, and these filaments can also be labeled with phalloidin. It therefore seems that in most situations, there are less polymerized actin in the nucleus than in the cytoplasm. The relationship between the UTR230-EN puncta and the other nuclear actin filaments is unclear, and further studies are required to fully understand the steady state polymerization status of nuclear actin.

Several studies have demonstrated nuclear actin polymerization upon DNA damage (Fig. 1). The genome is constantly challenged by external and internal factors that cause different types of DNA damage. Cells have evolved several different mechanisms, collectively called DNA damage response (DDR) to detect and repair the lesions, while simultaneously controlling other relevant cellular processes, such as cell cycle progression. Utilizing the UTR230-EN probe, the Mullins-lab visualized the formation of nuclear actin filaments with different morphologies upon induction of DNA damage. The formation of these filaments depends on Formin-2 and Spire-1/2 nucleators, and they seem to be needed for double strand break (DSB) clearance and nuclear oxidation upon DDR [19]. Another study utilizing the UTR230-EN probe upon UV-induced DNA damage observed phosphoinositide-mediated nuclear actin polymerization, which was required for the recruitment of Ataxia telangiectasia and Rad3-related protein (ATR), a key DDR protein, to the damage sites. In this case, the formin mDia2 may be involved in promoting nuclear actin polymerization, since it accumulates to the sites of DNA damage before actin [20]. In the cytoplasm, the main function of actin is to provide force for motility, and recent results have demonstrated that actin may perform similar functions during DDR. In fly cells, DSBs in heterochromatin are relocalized from the heterochromatin domain to the nuclear periphery to prevent aberrant recombination [21]. Chiolo-lab showed recently that this relocalization takes place along actin filaments *via* directed motion. The polymerization of actin is mediated by the Arp2/3 complex, which is recruited to the DSBs by Mre11 and HP1a, and activated by Scar and WASH proteins. The heterochromatin repair complex Smc5/6 interacts with the myosin activator Unc45, which activates nuclear myosins to relocalize the DSBs to the nuclear periphery by walking along the actin filaments [22]. At the same time, Gautier-lab showed that in mammalian cells, nuclear actin polymerization by Arp2/3 complex and its activator WASP are required for movement of a subset of DSBs into subnuclear clusters. Interestingly, while WASP seems to interact with all DSBs, it activates Arp2/3-mediated nuclear actin polymerization only at the DSBs undergoing homologous recombination, the other major pathway for DSB repair that is dependent on the homologous DNA template for the repair, and thus restricted to late S and G2 phases of the cell cycle. The movement during DSB clustering seems to be subdiffusive, and enhances DSB end-resection [23]. Taken together, the importance of nuclear actin polymerization for DDR has been clearly demonstrated. However, further experiments are needed to elucidate the mechanisms involved, and *e.g.* to clarify the relative contributions of the different actin filament nucleators in this process, which is crucial for maintaining the genomic integrity.

In addition to movement of DSBs, actin has also been implicated in other intranuclear movement events. For example, repositioning of an interphase chromatin site from the nuclear periphery to the nuclear interior upon targeting of a transcription activator to this site can be prevented by specific actin and nuclear myosin mutants [24]. Similarly, interfering with actin polymerization prevents the translocation of an U2 snRNA array towards Cajal bodies upon transcription activation [25] and the movement of a Hsp70 transgene towards the nuclear speckles upon heat shock [26]. In addition, actin and nuclear myosin have been implicated in the rearrangement of chromosome territories upon serum withdrawal [27] and upon DNA damage [28]. However, in all of these cases the mechanisms have remained obscure and the studies lack concurrent visualization of the putative nuclear actin filaments. Further studies are therefore needed to elucidate, how nuclear actin may contribute to chromatin movement events beyond DNA damage.

Nuclear actin filaments have also been linked to cell cycle control (Fig. 1). Utilizing the nuclear actin chromobody, two studies have reported polymerization of nuclear actin during early G1 phase of the cell cycle [29,30]. Formins [29], and in particular mDia2 [31], have been implied as the actin filament nucleators for this process, and the actin disassembly factor cofilin-1 is critical for the dynamics and turnover of these filaments [30]. The exact role of these filaments is not known, but they have been implicated in nuclear expansion and the concurrent chromatin decondensation after mitosis [30]. Whether localized actin polymerization at the nuclear envelope pushes the membrane similarly as, for example, at the leading edge of a motile cell needs to be firmly proven. On the other hand, actin dynamics and formin activity are essential for DNA replication (Fig. 1), and may contribute to the process through regulation of nuclear transport and loading of replication proteins onto chromatin [29]. Actin may also be involved in epigenetic chromatin maintenance through loading of centromere protein A (CENP-A) to centromeres after replication. The formin mDia2 has been demonstrated to be essential for this process [32] *via* formation of dynamic and short nuclear actin filaments in the G1 phase of the cell cycle that constrain centrosome movement for productive HJURP-mediated CENP-A loading [31].

Taken together, it has become evident that nuclear actin can polymerize into canonical actin filaments upon specific stimuli, and that these filaments have important roles in governing the organization and integrity of the genome. However, we still understand very poorly, how

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nuclear actin filaments perform these tasks at the molecular level. In addition, further studies are needed to elucidate how nuclear actin dynamics are regulated, since most of the regulators characterized to date are actin filament nucleators, and in most cases, it is not clear how the signaling pathways reach the nucleus to influence actin dynamics in this compartment. For example, the mechanisms by which mDia1/2 formins are activated in the nucleus are not known.

3. Actin monomers in chromatin remodeling and modifying complexes

In the cytoplasm, actin monomers are often considered as mere building blocks for the filaments. However, in the nucleus actin monomers have very specific functions. Actin is a stoichiometric subunit of several chromatin remodeling and modifying complexes (Fig. 1), which control chromatin structure and accessibility by regulating nucleosome repositioning and histone modifications, respectively. Actin-containing complexes include BAF [33], INO80 [34], Nua4/TIP60 [35], SRCAP [36], and SWR1 [37,38]. In these complexes, actin is usually found in its monomeric form together with one or more actin-related proteins (Arps) in different combinations. Arps and actin share a similar core structure [39] and ATP-binding motif [40], but insertions and deletion in their surface structures have resulted in unique functional properties for each Arp protein [41]. In eukaryotes, ten Arps have been discovered, out of which Arp1 is most similar with actin and Arp10 the least similar. Arp4-9 localize predominantly to the nucleus and are thus called nuclear Arps [42]. Arp2 and Arp3 are predominantly cytoplasmic, but as mentioned above, are also detected in the nucleus and can regulate actin polymerization in this cellular compartment [22,23,43].

The helicase-SANT-associated (HSA) domain functions as a binding platform for actin-Arp and Arp-Arp pairs in different chromatin remodeling complexes [44]. Yeast genetics and biochemical experiments demonstrated that actin operates as a monomer in the INO80 chromatin remodeling complex [45]. Structural studies have revealed that in the SWR1 complex, the barbed end of actin is sequestered by interactions with Arp4 and the HSA domain, thus keeping actin in the monomeric form. Moreover, in this complex, actin displays a twisted conformation, with its nucleotide-binding pocket occluded, presumably freeing it from regulation by ATP. Interestingly, the structure also implied that actin could interact with the catalytic core of SWR1 [38], and could therefore play a role in regulating the catalytic activity of the complex, or contribute to its chromatin binding. The crystal structure of yeast INO80 revealed binding of the segmented HSA domain to the barbed ends of actin, Arp4 and Arp8 [46]. The Arp4-actin module was similar to the module in SWR1 [38], but Arp8 bound to actin in an unusual side-to-front type of interaction, and curiously the actin was ATP bound in this complex [46]. Arp4 and Arp8 are also able to depolymerize actin filaments *in vitro* [39,45]. Therefore interactions within the chromatin remodeling complexes, and perhaps also interactions with Arps outside these complexes, facilitate the maintenance of the monomeric pool of nuclear actin.

Also the functional relevance of actin in the chromatin remodeling complexes is starting to emerge. Early studies suggested that actin would be needed for the maximal ATPase activity of Brg1 [33]. The actin-Arp4 heterodimer is also crucial for the integrity of the whole Brg1-containing chromatin remodeling complex [47]. In addition, the actin-Arp module plays a critical role in targeting the complexes to chromatin. Indeed, several Arps display considerable affinity towards histones [41,48,49], and analysis of beta-actin null cells have revealed the importance of actin in targeting Brg1-containing BAF complexes to chromatin [50,51]. In the INO80 complex, the module containing the HSA domain, actin, Arp4 and Arp8 is involved in recognition of the extranucleosomal linker DNA, thereby contributing to genome-wide nucleosome positioning [46]. Although the monomeric nature of actin in chromatin remodelers is well-established, interactions with actin filaments might also exist. Brg1-containing complexes have been proposed to interact with pointed ends of actin filaments *via* phosphatidylinositol 4,5-bisphosphate (PIP_2) [52]. Although the functional role of this interaction is not firmly established, it may play a role in controlling the activity of a subset of SWI/SNF complexes. Chang et al. (2018) showed that at high mechanical stress, nuclear actin filaments bind to ARID1A-containing SWI/SNF chromatin remodeling complexes, and thereby prevent the complex from associating with YAP/TAZ, thus favoring the YAP/TAZ activation, and its interaction with the TEAD transcription factor to promote cellular plasticity and tumorigenesis [53]. The interplay between the actin monomer and filament-binding activities of Brg1, and perhaps other components of the chromatin remodeling complexes, needs to be resolved in the future.

Actin can also interact with chromatin modifying complexes without Arps. Recently, our lab utilized two complementary mass spectrometry (MS) techniques, affinity-purification (AP) AP-MS and BioID to identify both stable and dynamic interactions of nuclear actin, and demonstrated that actin is a subunit of the human Ada-Two-A-containing (hATAC) histone modifier complex. Actin binds directly to KAT14, one of the histone acetyl transferases (HATs) in this complex, and can modify its activity both *in vitro* and *in vivo* [54]. Actin may also control histone acetylation *via* its interactions with histone deacetylases HDAC1 and 2. Although the exact binding mechanism is not known, actin appears to be monomeric in the HDAC complexes, and increasing the concentration of actin in cells suppresses HDAC function, while polymerizing nuclear actin to stable filaments increased HDAC activity [55].

Taken together, actin alone, or in combination with Arps, has important roles within several chromatin remodeling and modifying complexes by forming modules needed for interactions with nucleosomes and by regulating catalytic activities of these complexes. Consequently, actin contributes to the key nuclear processes dependent on these chromatin regulators, including transcription, DNA repair and replication [48]. Although interactions with actin filaments may contribute to some of these functions, the established role of the actin monomer necessitates the maintenance of an adequate pool of nuclear monomeric actin. While the interaction with Arps and further sequestration within the chromatin modifying complexes may be sufficient to maintain the monomer pool, also other pathways could exist. Indeed, proteins important for controlling the actin monomer pool in the cytoplasm, such as profilin, thymosin-β4 and cofilin, have all been shown to localize to the nucleus [56,57]. As discussed above, cofilin plays an important role in controlling actin dynamics during G1 phase of the cell cycle [30] and profilin is required for nuclear export of actin [6], but could also play a role in nuclear actin polymerization, since it is required for processive actin assembly by formins [58]. Whether these proteins control the actin monomer pool in the nucleus in other contexts awaits further studies. It seems clear that the balance between nuclear actin monomers and filaments, and hence the balance between nuclear actin polymerization and depolymerization, must be optimized for every specific context.

4. Dynamic nuclear actin in gene expression

Apart from its roles in different chromatin remodeling complex, actin has also been linked to different steps of transcription from gene activation to transcription elongation. Although the exact form of nuclear actin, filamentous or monomeric, during many of these processes is not clear, the importance of the dynamic nature of nuclear actin for transcription is emerging. Regulation of myocardin related transcription factor A (MRTF-A) by actin is the best characterized example of actin-mediated transcriptional control (Fig. 1). MRTF-A is a transcriptional co-activator of serum response factor (SRF), which is an essential transcription factor that regulates the expression of many cytoskeletal and immediate early genes. MRTF-A contains a motif with three RPEL repeats that act as G-actin sensors that passively react to changes in

steady-state concentration of actin monomers, regulating both subcellular localization and nuclear activity of MRTF-A [59]. In unstimulated cells, when actin monomer levels are relatively high, MRTF-A is bound to G-actin, which on one hand masks the nuclear localization signal (NLS) of MRTF-A [60,61] and on the other hand promotes its nuclear export, consequently resulting in cytoplasmic localization of MRTF-A [59]. Serum stimulation, or other treatments that promote actin polymerization, and thus reduce the concentration of actin monomers, cause the dissociation of the actin monomer-MRTF-A complex, promoting the nuclear import and preventing nuclear export of MRTF-A, leading to its nuclear localization [59].

Earlier studies had already implicated the importance of nuclear actin in regulating MRTF-A localization and activity, and demonstrated that MRTF-A can only activate SRF-mediated transcription of target genes, if freed from the actin monomer in the nucleus [59]. Subsequent studies have demonstrated that serum stimulation leads to a rapid and transient polymerization of nuclear actin by mDia1/2 formins, which is required for MRTF-A nuclear accumulation and SRF activation [16]. Also other mechanisms that influence nuclear actin polymerization regulate MRTF-A/SRF activity. For example, mechanical stimuli, such as cell spreading or integrin-engagement, activate signaling pathways that result in nuclear actin polymerization, leading consequently to MRTF-A nuclear localization and SRF-mediated transcription. This pathway again depends on mDia1/2 formins, but also requires an intact nuclear lamina, including lamin A/C and emerin, as well as coupling between the cytoplasmic and nuclear actin networks in the form of LINC complex [18]. In line with this observation, defects in lamin A/C proteins that cause laminopathies disturb nuclear actin polymerization, and thereby impair nuclear translocation of MRTF-A. The key protein here seems to be emerin, which is mislocalized in lamin A/C mutant or deficient cells [62]. Emerin is an integral membrane protein of the nuclear envelope that binds and stabilizes pointed ends of actin filaments, thereby increasing actin polymerization at least *in vitro* [63], and has been implicated in actin polymerization on the cytoplasmic side of the nuclear envelope as well [64]. Of note, also lamin A and lamin B have been suggested to bind actin [65], but whether these activities contribute to nuclear actin polymerization remains to be determined. MICAL-2 regulates nuclear actin through redox modification that leads to depolymerization and lower G-actin levels inside the nucleus. This in turn increases MRTF-A accumulation to the nucleus and MRTF-A/SRF-dependent gene expression [66]. A very recent study demonstrated that Ras association domain family isoform 1A (RASSF1A), which is a tumor suppressor frequently silenced in solid cancer supports the binding of Exportin-6 to Ran GTPase, and thus contributes to nucleo-cytoplasmic shuttling of actin. Interestingly, this pathway is aberrant in cancer cells, which results in increased nuclear actin levels and consequently to decreased expression of MRTF-A-SRF target genes [67]. Still another factor regulating MRTF-A/SRF activity is the F-actin binding protein filamin A, which has been shown to interact with MRTF-A, positively regulating SRF activation and thereby postulated to link nuclear actin polymerization to the MRTF-A/SRF transcription complex [68]. Taken together, while MRTF-A transcriptional activity is regulated by the actin monomers binding to the RPEL domain of MRTF-A [59,60,69], this signaling pathway is totally dependent on the dynamic nature of the actin polymerization and depolymerization cycle taking place either in the cytoplasm or in the nucleus. Notably, as SRF regulates many cytoskeletal genes, including actin itself, actin dynamics throughout the cell regulates the transcriptional homeostasis of the cytoskeleton.

In addition to regulating transcription initiation through transcription factors, actin has also been directly linked to RNA polymerase (Pol) function. Actin co-purifies with all three RNA polymerases [2,70–72] and has been shown to interact with Pol II subunits Rpb5 and Rpb7 [73], as well as Pol III subunits Rpc3, Rpb6 and Rpb8 [72] in co-immunoprecipitation experiments. However, these interactions are poorly understood at the molecular level. Nevertheless, chromatin immunoprecipitation followed be deep-sequencing (ChIP-seq) experiments have demonstrated the genome-wide role for actin in transcription, by showing that actin is found, together with Pol II, on the gene promoters of essentially all transcribed genes and also on gene bodies of highly transcribed genes [74] and on the rDNA transcription unit [75]. Studies both *in vivo* and *in vitro* have demonstrated the requirement for actin in RNA polymerase function. For example, in *in vitro* reconstituted transcription assays actin antibodies or actin depletion inhibit transcription [72,76], while decreased nuclear actin levels impair transcription both in cells [5,64] and in the fruit fly [74]. In line with these observations, treatment of mammary epithelial cells with laminin 111 leads to decreased nuclear actin levels [7] through PI3 kinase-dependent upregulation of Exportin-6 [77], which disturbs transcription by destabilizing Pol II and Pol III binding to transcription sites, eventually leading to cell quiescence [7]. Very interestingly, malignant cells often have defects in this signaling pathway, leading to loss of growth control and tissue homeostasis [77]. Actin thus associates with the RNA polymerase complexes and is required for their function, but, as discussed below, the functional form of actin during transcription, and the molecular mechanisms therein, are still poorly understood.

Binding of actin to both promoters and gene bodies [74] suggests that actin could have a role during several steps of the transcription process. Indeed, actin has been linked both to pre-initiation complex (PIC) formation [78], and to transcription elongation [54,79–81]. During the latter process, established interactions with actin monomers take place. For instance, monomeric actin interacts with Cdk9, the kinase subunit of P-TEFb [79]. Thereby actin could have a role in the pause-release of Pol II, where Pol II pauses in the promoter-proximal region and requires P-TEFb to phosphorylate serine 2 in Pol II C-terminal domain (CTD) in order to continue productive transcription [82]. Indeed, analysis of splice site selection in nuclear actin manipulated cells has indirectly suggested a role for actin in controlling transcription elongation rate [54]. Moreover, actin, likely in its monomeric form, interacts with heterogeneous nuclear ribonucleoprotein U (hnRNP U) and this interaction plays a role in transcription elongation [80]. The actin-hnRNP U interaction might help in establishing transcriptionally active, open chromatin state by recruiting the histone acetyl transferase PCAF to the elongating polymerase [81]. Also our mass spectrometry screen for nuclear actin binding partners revealed several proteins linked to different steps of transcription. Notably, vast majority of these transcription-related interactors were detected only with BioID, and not with the AP-MS technique, indicating dynamic association of actin with the transcription machinery. Moreover, essentially all interactions were also recovered with an actin mutant that cannot polymerize, suggesting that actin does not need its capacity to polymerize in order to interact with these proteins. Besides transcription itself, a large fraction of the nuclear actin interactors obtained from the BioID analysis were related to pre-mRNA processing [54]. Interestingly, actin, in its monomeric form, has been shown localize to nuclear speckles, which also contain many pre-mRNA processing factors [15]. We further investigated the functional implications of actin in pre-mRNA processing by utilizing minigene splicing assays and found that manipulation of nuclear actin levels disturbed alternative splicing [54]. Although these results may suggest a functional role for actin in splicing, the results could also be explained through the involvement of actin in transcription. We had previously shown that similar manipulations of nuclear actin levels impair transcription [5], and we therefore hypothesize that actin could affect Pol II elongation or pausing rate, so that slower elongation by Pol II would leave more time for also the weaker splice sites to commit to splicing [54].

While the above-mentioned data suggest a role for actin monomers in transcription, there is also ample experimental evidence, which seems to imply a role for actin filaments. These include, for example, experiments with actin-binding drugs that prevent actin polymerization and inhibit transcription [14,83]. The involvement of filaments is also supported by the requirement for motor protein myosins during

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transcription. Several members of the myosin superfamily localize to the nucleus [84]. Myosin-1c, particularly the nuclear myosin 1 (NM1) isoform, is the best characterized nuclear myosin, and its nuclear localization depends on phosphoinositide-dependent nuclear import and nuclear retention by its binding partners [85]. Both actin and NM1 have been linked to Pol I-mediated transcription [76], possibly by combining actin polymerization and the motor function of NM1 [83,86–88]. In this context, actin is thought to first associate with Pol I and then with NM1 to regulate chromatin state and cell proliferation [75]. Also Myosin Vb [89] and Myosin II [90] have been linked to Pol I- and Pol II-mediated transcription, respectively. Development of novel probes to study nuclear myosins also in living cells would benefit these studies. In addition to myosins, also actin polymerization regulators, such as Arp2/3 complex and its activator WASp [91] as well as the actin disassembly factor cofilin [92] have been linked to transcription. However, whether they operate together with actin in this context is not known. Nuclear actin polymerization is also required for transcriptional reactivation of the pluripotency gene *Oct4* in a *Xenopus* oocyte transplantation model. The actin signaling protein Toca-1, which is an upstream regulator of N-WASp, enhances Oct4 expression and nuclear actin polymerization in this system [93]. However, also Wave-1 is required for transcriptional reprogramming and associates with active transcription machineries [94], but the interplay between the nuclear actin filaments has not been resolved. Moreover, T cell antigen receptor signaling causes fast and transient nuclear actin polymerization mediated by the Arp2/3 complex in T cells. The signal is mediated by a rise in nuclear calcium ion concentration and regulated by N-WASP and NIK. Importantly, this nuclear actin polymerization is required for the transcription of a specific set of effector cytokines, and thereby needed for the $CD4^+$ T cell effector functions also *in vivo* [43]. However, the mechanisms still remain unclear.

Taken together, actin seems to play several roles during the transcription process. However, apart from the role of the actin monomer in chromatin remodeling complexes and in regulating MRTF-A/SRF transcription factors, the molecular mechanisms by which actin operates *e.g.* together with the RNA polymerases have remained largely unclear. Consequently the functional form of nuclear actin during transcription remains enigmatic. Nevertheless, dynamic nature of the nuclear actin pool seems to be important, since polymerizing nuclear actin into stable filaments impairs the interaction between actin and the RNA polymerase [95].

5. Conclusions

The presence of actin inside the nucleus was first indicated already fifty years ago, but only during the recent decades have we actually gained insights about the functions of nuclear actin and this work is ongoing. The polymerization status of nuclear actin was for a long time the most enigmatic aspect of this whole field. The apparent lack of filamentous actin in the nucleus was puzzling, since the majority of cytoplasmic functions of actin are dependent on spatially and temporally controlled actin polymerization. Nowadays it has become clear that nuclear actin can polymerize in response to specific signals, and that the nuclear actin filaments have important functions in transcriptional regulation, cell cycle and DNA repair. While some of these functions seem to depend on the actin's ability to provide force for movement similarly as in the cytoplasm, it is highly likely that also nuclear specific mechanisms exist. It is therefore important to elucidate, what the actin filaments interact with in the nucleus. In the cytoplasm, hundreds of actin-binding proteins regulate actin dynamics in response to different intra- and extracellular signals. Many of these proteins have been localized to the nucleus [12], but only very few have actually been shown to regulate actin polymerization also in this compartment. This is also an area that would require further attention, and the novel nuclear targeted probes for different forms of actin have made the experiments feasible. Another emerging area is the regulation of actin by different post translational modifications [96], which could be utilized to specifically regulate different aspects of actin functions also in the nucleus. It is also important to remember that in the nucleus, the actin monomers are not just building blocks for the filaments, but have very specific, important functions for example as core components of many chromatin remodeling and modifying complexes. Actin polymerization elicits dynamic changes in the actin monomer and filament pools, and consequently impact processes that depend on either form of actin. Regulation of the transcriptional activity of the MRTF-A/SRF complex is an excellent example of how changes in the different pools of nuclear actin elicit functional outputs [16,59]. However, it is not understood whether nuclear actin polymerization also influences other processes that depend on actin monomers, such as chromatin remodeling. Transient nature of the filaments, as well as the stable association of actin within these complexes [5,54] make this unlikely, but should be nevertheless experimentally verified. Another critical aspect to consider is the fact that actin constantly and rapidly shuttles in and out of the nucleus [5], which means that the cytoplasmic and nuclear actin pools are dynamically connected. In addition to understanding the mechanistic basis of how actin operates within specific subcellular compartments, such as the nucleus, it is therefore critical to start considering the cellular actin networks as a continuum that connects the whole cell, and thereby constitutes a very important mechanism to maintain cellular homeostasis.

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