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Nuclear Actin

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Abstract:

While actin is a highly evolutionarily conserved protein that has been extensively studied for its role in the cytoskeleton, actin also localizes and functions within the nucleus. Tight regulation of both the level and form (monomeric versus filamentous) of nuclear actin is essential for many nuclear processes. Nuclear actin regulates transcription at multiple steps, including initiation, elongation, and mRNA processing. It also regulates chromatin organization, by controlling chromatin-modifiers and mediating chromatin movement during transcription, DNA repair, and DNA replication. Finally, nuclear actin is a critical component of the nucleoskeleton, where it regulates nuclear structure and organization, cellular response to mechanical signaling, and nucleolar structure and function. All of these roles of nuclear actin are critical for maintaining cellular homeostasis, responding to cellular stress, and mediating cell fate decisions.

Key words:

Nuclear actin, transcription, chromatin remodeling, DNA repair, nucleoskeleton, nucleolus, mechanotransduction, cellular stress, cell fate

Key Concepts:

Different forms of nuclear actin regulate distinct aspects of transcription: G-actin promotes the initiation of transcription and mediates the escape from pausing, whereas dynamic actin polymerization is required for transcriptional elongation.

Nuclear G-actin act as a scaffold to regulate the entire RNA biogenesis pathway from initiating transcription to translation.

Both cytoplasmic and nuclear actin dynamics regulate SRF/MAL-dependent transcription.

Nuclear G-actin directly interacts with histone modifiers and chromatin remodeling complexes to regulate chromatin accessibility for transcription, DNA repair, and replication.

Nuclear actin plays critical roles in the repair of DNA double strand breaks, by both regulating chromatin remodeling complex activity, and by F-actin-dependent chromatin movement or stabilization of repair machinery.

Nuclear F-actin is also required for moving chromatin to and maintaining transcription factors, and mediating DNA replication and chromosome segregation.

Mechanotransduction impacts the nucleoskeleton to regulate the level and form of nuclear actin to mediate changes in transcription and chromatin organization that drive the cell specific response to force.

Actin localizes and functions within the nucleolus, and may mediate the nucleolar stress response.

Tight regulation of nuclear actin level and/or polymerization plays critical roles in cell fate transitions.

Introduction:

Actin, a highly conserved and ubiquitously expressed protein, is extensively studied for its cytoskeletal roles. However, actin also localizes and functions within the nucleus. The nuclear localization of actin is highly regulated. In the cytoplasm, actin binds to cofilin, an actin binding protein, and this complex is actively transported into the nucleus by importin 9 (Dopie *et al.*, 2012). Inside the nucleus, actin binds another actin binding protein, profilin, and this complex is exported by exportin 6 (Stuven *et al.*, 2003). This regulated localization of actin to the nucleus strongly suggests it has functions there.

The functions of actin are intimately tied to its form or structure. In the cytoplasm, actin can exist as monomers (G-actin) and filamentous actin (F-actin); F-actin can be organized into networks of filaments giving the cell its shape and allowing the cell to move. Hundreds of actin binding proteins regulate the structure and dynamics of actin in the cytoplasm (Pollard, 2016). Similarly, many actin binding proteins localize to the nucleus (Kristo *et al.*, 2016) and nuclear actin can take on different forms, including G-actin, polymeric actin (non-monomeric and non-filamentous), F-actin, and thick filaments termed actin rods (Kelpsch and Tootle, 2018). Here we review the form-specific roles of nuclear actin in numerous nuclear processes including transcription, chromatin organization, and nuclear structure (Figure 1), and how these functions of nuclear actin contribute to cell homeostasis and fate. See also: DOI: 10.1002/9780470015902.a0001255.pub3.

Nuclear Actin in Transcription

Nuclear actin is widely implicated in regulating transcription (Figure 1). Actin localizes to actively transcribing regions of the chromatin (Percipalle, 2013). Disrupting interactions between nuclear actin and transcriptional machinery, or blocking the nuclear import of actin inhibits global transcription (Percipalle, 2013; Kelpsch and Tootle, 2018). In this section, we discuss the roles of nuclear actin in the different phases of transcription, RNA processing, and regulating specific transcription factors. See also: DOI:10.1002/9780470015902.a0003301.pub3 and DOI: 10.1038/npg.els.0005059

Nuclear actin regulates transcriptional initiation and elongation

Nuclear actin regulates transcription by binding to and regulating all three RNA polymerases (RNAPs). Specifically, in RNAPIII actin binds to the subunits RPABC2 and RPABC3 (Hu *et al.*, 2004). These subunits are common to all three RNAPs, suggesting actin interacts the same way with the basal transcription machinery regardless of which RNAP is involved. Indeed, actin co-immunoprecipitates with both RNAPI and RNAPII (Percipalle, 2013; Kelpsch and Tootle, 2018).

Further, actin localizes to gene promoters (Percipalle, 2013), suggesting actin functions during the pre-initiation and initiation phases of transcription. The pre-initiation complex (PIC) forms at the promoter region prior to RNA synthesis. Actin associates with the PIC (Hofmann *et al.*, 2004; Obrdlik *et al.*, 2008; Viita *et al.*, 2019), and depletion of actin prevents PIC assembly, blocking RNAP recruitment and transcriptional initiation (Percipalle, 2013). While nuclear actin is critical for the initiation of transcription, the form of nuclear actin is debated. Chromatin immunoprecipitation experiments indicate G-actin is bound to gene promoters and is required for RNAPII recruitment (Sokolova *et al.*, 2018) (Figure 2A1). Conversely, actin polymerization and the activity of nuclear myosin I (NMI) is required for RNAPI-dependent transcriptional activation (Percipalle, 2013) (Figure 2A4). Thus, for transcriptional initiation, the polymerization state of nuclear actin may depend on the type of RNAP; alternatively, dynamic polymerization and depolymerization of nuclear actin may be required.

In addition to localizing to promoters, actin is found along the coding sequences of transcribed genes (Percipalle, 2013). This localization is RNA-dependent, and actin is part of both pre- and mature ribonucleoprotein (RNP) complexes (Percipalle *et al.*, 2002; Obrdlik *et al.*, 2008). These data suggest that actin mediates transcriptional elongation. Supporting this idea, RNAP-associated G-actin recruits the positive elongation factor P-TEFb, which is required by RNAPII to escape transcriptional pausing (Qi *et al.*, 2011). Further, G-actin binds heterogenous nuclear RNPs (hnRNPs), which bind nascent transcripts and facilitate transcript elongation (Qi *et al.*, 2011). Following promoter

clearance, the actin-hnRNP U interaction recruits the histone acetyl-transferase PCAF to open chromatin and permit transcript elongation (Obrdlik *et al.*, 2008). Further supporting that G-actin mediates elongation, nuclear actin rod formation blocks RNAPII-dependent transcription and this is rescued by the expression of nuclear targeted, non-polymerizable actin (Percipalle, 2013). Additionally, the actin filament severing protein, cofilin localizes to actively transcribed genes, suggesting cofilin may maintain the monomeric state of actin to promote elongation (Obrdlik and Percipalle, 2011). However, cofilin may act together with profilin, an actin binding protein that promotes polymerization, to mediate actin treadmilling. Indeed, profilin localizes to active genes and is required for on-going transcription (Percipalle, 2013). Additional F-actin binding proteins including Arp2/3, which promotes actin polymerization, and NM1, which moves along actin filaments mediate RNAPII-dependent transcription (Percipalle, 2013). Thus, it is likely that dynamic actin polymerization is required for transcriptional elongation (Figure 2A2).

Nuclear actin and RNA processing

Nuclear actin plays roles in mRNA processing. RNPs mediate mRNA splicing and 3'-end processing, and actin is associated with RNPs during transcription, suggesting it contributes to these processes (Percipalle *et al.*, 2002; Obrdlik *et al.*, 2008; Qi *et al.*, 2011) (Figure 2A3). Indeed, G-actin interacts with spliceosomal small nuclear RNPs, and spliceosomal assembly and activation factors (Viita *et al.*, 2019). Further, using a splicing reporter system, increasing or decreasing nuclear actin impairs splicing (Viita *et al.*, 2019). Actin could regulate splicing directly, or it could have an indirect role due to the co-transcriptional nature of splicing. Viita *et al.* propose that altering actin levels slows down RNAPII transcription, driving the changes in splicing. Actin also contributes to mRNA nuclear export and translation. Actin remains associated with hnRNPs as the transcripts leave the nucleus and localize to polyribosomes for translation. Indeed, actin is found in the 40S pre-mRNP/RNP ribosomal fraction, and is implicated in RNP assembly and disassembly during translation (Percipalle, 2013). Together, these data suggest G-actin acts as a scaffold to regulate the entire RNA biogenesis pathway from initiating transcription to translation.

Nuclear actin and transcription factors

In addition to regulating global transcription, nuclear actin also regulates inducible gene expression and specific transcription factors. For example, nuclear actin regulates serum response factor (SRF) via its coactivator megakaryocytic acute leukemia (MAL, also called MLK1 and MRTF-A). In the cytoplasm, G-actin binds MAL, preventing

its nuclear localization (Hyrskyluoto and Vartiainen, 2020). Similarly, in the nucleus, G-actin inhibits MAL from binding to and activating SRF, and promotes MAL's nuclear export. Both serum stimulated activation of the formin m-Dia and cell spreading induce nuclear actin polymerization, which prevents G-actin from binding MAL, and thereby, promotes MAL/SRF-dependent gene expression (Plessner *et al.*, 2015; Hyrskyluoto and Vartiainen, 2020) (Figure 3C). While mechanisms promoting polymerization drive, depolymerization of nuclear actin inactivates MAL/SRF-dependent gene expression. Indeed, MAL/SRF is inactivated by MICAL-2, which oxidizes a methionine on actin to catalyse the depolymerization of nuclear actin (Hyrskyluoto and Vartiainen, 2020). Nuclear actin polymerization also regulates transcription in response to other stimuli. For example, activation of the T-cell antigen receptor induces actin polymerization by Arp2/3 and N-WASP to regulate cytokine expression (Hyrskyluoto and Vartiainen, 2020). Further, retinoic acid treatment induces nuclear actin polymerization to activate the expression of the Homeobox B gene cluster (Kelpsch and Tootle, 2018). Thus, regulating the level and form of nuclear actin is a critical means of controlling inducible gene expression.

Nuclear Actin in Chromatin Organization

Nuclear actin regulates chromatin organization. Chromatin organization, including packaging by nucleosomes and distribution in the nucleus, is highly regulated and dynamic, and plays critical roles in controlling transcription, DNA replication, and DNA repair (Figure 1). In this section, we review the roles of nuclear actin in chromatin-modifying complexes, DNA repair, and other instances of chromatin movement. See also: DOI: 10.1002/9780470015902.a0005768.pub2 and 0.1002/9780470015902.a0005279.pub3

Nuclear actin and chromatin remodeling

Nuclear G-actin is a key component of chromatin-modifying complexes, including histone modifiers and chromatin remodelers (Klages-Mundt *et al.*, 2018). Histone modifiers alter the post-translational modifications of histones, which make nucleosomes, to alter chromatin accessibility. During transcriptional elongation, G-actin binds hnRNP U to recruit PCAF, which acetylates histones to provide an open chromatin configuration (Obrdlik *et al.*, 2008). Further, G-actin binds and inhibits histone deacetylase (HDAC) 1 and 2, whereas actin polymerization restores their activity (Serebryannyy *et al.*, 2016a). HDACs remove acetyl-moieties from histones, leading to

closed chromatin. Thus, G-actin regulates multiple histone modifiers to activate transcription by controlling chromatin organization.

Nuclear actin also plays pivotal roles in ATP-dependent chromatin remodeling complexes to control nucleosome positioning and composition. G-actin, in complex with actin related proteins (Arps), binds to chromatin remodeling complexes to regulate their structure or function (Figure 2B). A common module in these complexes is actin/Arp4; this module binds the ATPase subunit and recruits additional Arps that are different for each complex (Klages-Mundt *et al.*, 2018). In INO80 (inositol requiring complex), actin/Arp4/Arp8 mediate the interaction with extranucleosomal DNA (Knoll *et al.*, 2018), which is required for processive nucleosome translocation and uniform nucleosome spacing. The actin/Arp4 module also binds to and promotes the ATPase activity of BAF (BRG-1 associated factor; aka BAP and SWI/SNF) (Klages-Mundt *et al.*, 2018). SWR1 (SWI2/SNF2-related, aka SRCAP) chromatin remodeling complex replaces H2A with H2A.Z histone variant in nucleosomes to promote transcription. Actin/Arp4/Arp6 binds to SWR1 and mediates complex assembly, association with nucleosomes, and H2A.Z deposition (Klages-Mundt *et al.*, 2018). The actin/Arp4 module is also a structural component in the histone acetyltransferase remodeling complex NuA4/TIP60 (Nucleosome acetyltransferase of histone 4/Tat-interactive protein 60 kDa). Thus, G-actin, in complex with Arps, is a common and required component of many chromatin remodeling complexes.

Nuclear actin and DNA repair

Multiple forms of nuclear actin mediate the repair of DNA double stranded breaks (DSBs). In order for repair machinery to access DSBs, the surrounding chromatin must be opened by chromatin remodeling complexes (Dion and Gasser, 2013); as discussed above, G-actin is a key regulator of such complexes. Indeed, actin functions within the NuA4/TIP60 complex, which acetylates histone H4 at sites of DNA damage to mark it for repair (Klages-Mundt *et al.*, 2018) (Figure 2C1). Nuclear actin also interacts with and mediates the DNA repair process.

DNA repair occurs by two major mechanisms, homologous recombination (HR), and non-homologous end joining (NHEJ). HR utilizes the homologous chromosome as a template for repair and accurately repairs DSBs. Whereas, NHEJ rejoins the ends of the DSB, which often results in mutations. See also: DOI: 10.1038/npg.els.0005284.

Nuclear F-actin may directly regulate DSB repair. DNA damage induces the nuclear localization of actin and multiple actin binding proteins that promote

polymerization. These proteins co-purify with the DSB and/or DNA repair machinery (Klages-Mundt *et al.*, 2018). Indeed, both HR and NHEJ components bind F-actin *in vitro*. Further, nuclear actin filaments form in response to DNA damaging agents and inhibiting actin polymerization impairs repair (Klages-Mundt *et al.*, 2018). While these data support a role for nuclear F-actin in DNA repair, they did not identify the mechanism.

Recent work provides strong evidence that nuclear F-actin regulates chromatin movement necessary for DSB repair (Figure 2C1). In response to DNA damage, Formin-2 and Spire-1/Spire-2 mediate nuclear actin polymerization, and inhibiting polymerization increases the number of DSBs (Belin *et al.*, 2015); this suggests nuclear F-actin is required for DNA repair. Supporting this, in 2018, two studies simultaneously established nuclear actin polymerization mediates chromatin movement necessary for DNA repair (Caridi *et al.*, 2018; Schrank *et al.*, 2018). Specifically, in *Drosophila* cells, nuclear F-actin is required to repair pericentromeric heterochromatin. Actin, Arp2/3, and multiple myosins are recruited to the DSB site, actin polymerization occurs, and myosins move the DSB along F-actin to the periphery for repair; blocking this relocalization results in aberrant recombination (Caridi *et al.*, 2018). Similarly, in *Xenopus* and mammalian cells, nuclear actin, WASP and Arp2/3 are recruited to DSBs in euchromatin (open chromatin) to cluster repair foci and mediate repair progression (Schrank *et al.*, 2018). Additionally, Arp2/3 mediates the relocalization of damaged rDNA to nucleolar caps for repair (Klages-Mundt *et al.*, 2018). These studies suggest that actin polymerization driven chromatin movement is a common mechanism mediating DNA repair (Figure 2C1).

Nuclear actin and other instances of chromatin movement

Nuclear actin driven chromatin movement has functions beyond DNA repair, including during transcription and DNA replication. Activation of transcription is associated with nuclear actin-dependent movement of the gene locus to regions conducive to transcription. For example, transcriptional activation results in the movement of artificial transgenes away from the periphery; this is delayed by expression of non-polymerizable and accelerated by polymerization prone nuclear actin (Kelpsch and Tootle, 2018). Further, chemically blocking or promoting actin polymerization similarly alters the movement of endogenous gene loci (Kelpsch and Tootle, 2018). These findings suggested nuclear F-actin mediates chromatin movement to promote transcription. Additionally, chromatin remodeling complexes, which require G-actin for their activity (Klages-Mundt *et al.*, 2018), are associated with mediating chromatin

movement (Dion and Gasser, 2013). This may simply be due to their role in opening chromatin, which makes it permissive for movement, or a direct function in movement. Recent work on the movement of chromatin to transcription factories suggests the latter (Wang *et al.*, 2020; Wei *et al.*, 2020).

Transcription factories are comprised of clusters of RNAPII and transcription machinery (Rieder *et al.*, 2012). In yeast, Formin assembles short, dynamic nuclear F-actin, and myosin mediates the translocation of active genes from the center of the nucleus to peripherally located transcription factories (Wang *et al.*, 2020). Myosin processivity depends on chromatin remodelers, which bind to F-actin through their Arps and, thereby, maintain the link to F-actin when the myosin head detaches. In human cells, induction of gene expression by multiple stimuli induces RNAPII clustering and rapid upregulation of transcription; in this context, a dynamic F-actin meshwork produced at the site of transcription by N-WASP and Arp2/3 forms a scaffold to maintain RNAPII clustering (Wei *et al.*, 2020). Together, these studies suggest that nuclear F-actin is required for the movement of chromatin to sites conducive to transcription, and the establishment/maintenance of transcription factories (Figure 2C2).

In addition to mediating chromatin movement during transcription, nuclear F-actin is implicated in DNA replication and chromosome segregation (Figure 1). For example, formin-dependent F-actin mediates centromere maintenance and replication initiation during G1 of the cell cycle (Caridi *et al.*, 2019). Actin also localizes to the spindle during meiosis and mitosis across organisms (Kelpsch and Tootle, 2018). During meiosis, in mammalian oocytes, nuclear F-actin is required for chromosome alignment and segregation (Kelpsch and Tootle, 2018). Given the similarity of replication factories to that of transcription factories, it will be important to determine if F-actin plays similar roles in mediating chromatin movement and scaffolding replication machinery during mitosis and meiosis.

Nuclear Actin and Nuclear Architecture

There are three major nuclear architectural components – chromatin (discussed above), the nucleoskeleton and the nucleolus; all three are carefully coordinated to control nuclear shape and functions (Adam, 2017). Nuclear actin can be detected within each of these structures across organisms (Kelpsch and Tootle, 2018). Perturbing nuclear actin reorganizes chromatin, destabilizes the nuclear membrane, and causes nucleolar coalescence (Kelpsch and Tootle, 2018), suggesting that nuclear actin is an essential regulator in both the nucleoplasmic and nucleolar sub-compartments. In this

section, we discuss the roles of nuclear actin in the nucleoskeleton and nucleolus, and highlight important relationships between the nuclear architectural components. See also: 10.1002/9780470015902.a0026032, 10.1002/9780470015902.a0005975.pub3, and 10.1002/9780470015902.a0001352.pub4

Nuclear Actin and the Nucleoskeleton

The nucleoskeleton is an organized network of lamin intermediate filaments and associated proteins (i.e. emerin) at the nuclear periphery that is critical for nuclear membrane integrity (Adam, 2017). Multiple lines of evidence indicate that nuclear actin is a functional member of and is regulated by components of the nucleoskeleton. For example, actin can be detected in nucleoskeletal preparations (Kelpsch and Tootle, 2018). Lamins can bind and bundle actin *in vitro* (Kelpsch and Tootle, 2018) and, in Drosophila cells, knockdown of lamin reduces nuclear actin filament formation (Dopie *et al.*, 2015). Further, the lamin interacting protein, emerin, promotes actin polymerization *in vitro* and *in vivo* nucleoskeletal components.

One mechanism regulating nucleoskeleton-mediated polymerization of nuclear actin is mechanotransduction (Figure 3). Mechanical forces are sensed by cell surface receptors, and transmitted by cytoskeletal filaments to the Linker of the Nucleoskeleton and Cytoskeleton (LINC) Complex, which spans the nuclear envelope and interacts with the nucleoskeleton (Adam, 2017). In turn, emerin mediates nuclear actin polymerization to promote the transcriptional activity of MAL (Ho *et al.*, 2013; Plessner *et al.*, 2015). Additionally, during mesenchymal stem cell differentiation, mechanical stimuli promotes the nuclear import of actin, ultimately increasing nuclear actin levels and polymerization; these nuclear actin changes are required for the activation of specific transcription factors necessary for determining cell fate (Sen *et al.*, 2015; Sen *et al.*, 2017). Conversely in epidermal stem cells, mechanical signaling decreased nuclear actin levels, which attenuated transcription and silenced chromatin (Le *et al.*, 2016). Thus, mechanotransduction regulates the level and form of nuclear actin to mediate changes in transcription and chromatin organization that drive cell specific responses (Figure 3).

Nuclear actin also plays structural roles within the nucleoskeleton. In Xenopus oocytes, actin is thought to form a gel-like matrix that maintains nuclear integrity (Kelpsch and Tootle, 2018). Additionally, stabilizing nuclear F-actin in the oocytes of multiple organisms blocks nuclear envelope breakdown (Kelpsch and Tootle, 2018). Further, transient nuclear actin filaments are required to expand the nucleus at the end of mitosis (Baarlink *et al.*, 2017). Finally, stable nuclear rods form in response to cellular stress (Kelpsch and Tootle, 2018) and have been observed in diseases where nuclear

architecture is lost (Serebryannyy *et al.*, 2016b). Therefore, nuclear actin structure may broadly alter nuclear organization to regulate essential nuclear functions.

Nuclear Actin and the Nucleolus

Actin also localizes to the nucleolus. The nucleolus is responsible for ribosomal DNA (rDNA) transcription and ribosome biogenesis. It is also an important metabolic sensor and rapidly responds to cellular stress (Boulon *et al.*, 2010; Núñez Villacís *et al.*, 2018) (Figure 4). The nucleolus forms by phase separation and there is an intimate relationship between its structure and function. Disruption of nucleolar structure inhibits its functions and, similarly, disruption of function results in severe structural changes. Indeed, nucleolar formation and structure rely on interactions with chromatin (Tsekrekou *et al.*, 2017). The nucleolus forms around rDNA loci, known as nucleolar organizing regions (NORs). The NORs are heterochromatic, and thus, the nucleolus interacts with and organizes heterochromatin to maintain its silenced state (Tsekrekou *et al.*, 2017) (Figure 2A4). The nucleolus senses and rapidly responds to cellular stress, downregulating rDNA transcription and ribosome biogenesis, which disrupts nucleolar structure (Boulon *et al.*, 2010; Núñez Villacís *et al.*, 2018) (Figure 4D). This nucleolar stress response can allow the cell to survive the stress, and when necessary, drives apoptosis (Núñez Villacís *et al.*, 2018). Thus, nucleolar functions are sensitive to structural changes in both the nucleolus itself and chromatin.

Recent evidence suggests nuclear actin regulates nucleolar structure and function. Indeed, nuclear actin and actin-binding proteins are enriched in the nucleolus (Funaki *et al.*, 1995; Belin *et al.*, 2013; Wineland *et al.*, 2018) (Figure 4). The presence of actin in the nucleolus could sequester it from the nucleoplasm until stimuli prompt its release. Actin also plays an active role in modulating nucleolar functions through well-defined interactions with RNAPI (Percipalle, 2013) and by reorganizing nucleolar structure through regulating chromatin organization (Klages-Mundt *et al.*, 2018). Indeed, in mouse embryonic fibroblasts (MEFs), β-actin knockout results in upregulation of repressive chromatin marks at rDNA loci, blocking rRNA synthesis; this is rescued by polymerization competent actin (Almuzzaini *et al.*, 2016). Further, in *Drosophila*, perturbing nuclear actin level or structure may impact nucleolar structure, and therefore, function (Groen *et al.*, 2015; Kelpsch *et al.*, 2016). These studies lead us to speculate that nuclear actin could be a key regulator of the nucleolus.

Nuclear Actin is a Key Determinant in Cell Homeostasis and Cell Fate Decisions

Nuclear actin regulates numerous processes necessary for maintaining cell function, and mediating tissue development and maintenance. In this section, we discuss the roles of nuclear actin in maintaining cellular homeostasis by participating in the cellular stress response (Figure 4), and mediating cell fate decisions.

Nuclear actin and cell homeostasis

Cells regulate growth, mitigate stress, and when necessary, promote cell death. Cells undergoing stresses – heat shock, DMSO treatment, ATP depletion, and replication stress – accumulate nuclear actin. Once in the nucleus, actin polymerizes into rods, which block transcription (Figure 4C). When the stress is lifted, these rods disassemble restoring baseline nuclear function (Kelpsch and Tootle, 2018). The presence of actin rods is observed in diseases such as intranuclear rod myopathies and neurodegenerative disorders, although the exact mechanisms driving disease progression remain unknown (Serebryannyy *et al.*, 2016b; Kelpsch and Tootle, 2018).

As the nucleolus is a key component of the cellular stress response, it is interesting to consider potential ties between the nucleolar stress response and nuclear actin. The same stresses drive both nuclear actin rod formation and the nucleolar stress response. This response halts canonical nucleolar functions to stop cell proliferation and can activate cell death pathways (Boulon *et al.*, 2010). During stress, nucleolar structure is disrupted, deforming and/or fragmenting the organelle, as well as relocalizing nucleolar components to both nucleolar caps and the nucleoplasm (Boulon *et al.*, 2010) (Figure 4D). Similar nucleolar morphology changes are seen when fascin, an actin bundling protein that localizes to the nucleus and regulates nuclear actin, is lost (Groen *et al.*, 2015; Kelpsch *et al.*, 2016). These data lead us to speculate that disturbances in nuclear actin levels or structure could activate the nucleolar stress response (Duan *et al.*, 2020) (Figure 4).

Nuclear Actin and Cell Fate Decisions

Nuclear actin level and dynamics regulate cell fate. Levels of nuclear actin are actively maintained to support transcription, as quiescent cells have reduced nuclear actin and nuclear actin levels increase during differentiation (Misu *et al.*, 2017). For example, in mammary epithelial cells, nuclear actin levels balance growth and quiescence in response to the basement membrane component laminin-111 (Spencer *et al.*, 2010). Laminin-111 promotes quiescence by upregulating exportin 6 to reduce nuclear actin levels (Fiore *et al.*, 2017). Conversely, increasing nuclear actin levels causes de-differentation and tumorigenesis (Fiore *et al.*, 2017). Additionally, nuclear

actin dynamics drive the differentiation of mesenchymal stem cells into osteogenic and adipogenic fates (Sen *et al.*, 2015; Sen *et al.*, 2017). Osteogenic fate is promoted by branched actin polymerization via Arp2/3, whereas unbranched F-actin results in an adipogenic fate (Sen *et al.*, 2015; Sen *et al.*, 2017). Further, during transcriptional reprogramming of *Xenopus* oocytes, both polymerized nuclear actin and actin-binding proteins are required for expression of pluripotency genes (Misu *et al.*, 2017). Thus, nuclear actin regulates, maintains, and mediates transitions in cellular fate.

Nuclear actin dynamics also regulates chromatin organization to drive changes in cell fate. For example, actin-dependent chromatin organization is necessary for establishing the cellular identities of differentiating MEFs. MEFs from β-actin knockout mice exhibit increased repressive histone modifications, which blocks neuronal reprogramming. Expression of nuclear targeted β-actin rescues these defects (Xie *et al.*, 2018a; Xie *et al.*, 2018b). Similarly, in epidermal progenitor cells, mechanical signaling reduces nuclear actin levels, which represses transcription and promotes H3K27me3-mediated gene silencing to block lineage commitment (Le *et al.*, 2016). These studies establish nuclear actin as a key means of coordinating the chromatin landscape with transcriptional activities to ensure the desired gene expression program.

Conclusion

Though much remains to be learned about the underlying mechanisms by which the different forms of nuclear actin act, nuclear actin regulates vital nuclear functions required for development and cell maintenance. Nuclear actin plays critical roles throughout transcription: G-actin regulates the chromatin state by controlling histone modifiers and chromatin remodelers; F-actin mediates chromatin movement to and/or stabilization of transcription factories; G-actin mediates the initiation of transcription and F-actin mediates elongation; and G-actin contributes to mRNA processing, trafficking, and translation. Specific transcription factors are also regulated by nuclear actin dynamics. Nuclear F-actin also mediates chromatin movement to mediate DNA repair and replication. Further, nuclear actin dynamics contribute to the structure and integrity of the nucleus, and perhaps the nucleolus. All of these nuclear actin functions are modulated by stimuli, including mechanical signaling and cellular stress, to ultimately control cellular functions, fate, and survival.

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Glossary:

Transcription factory – biomolecular condensate of transcription machinery, including the RNA polymerase and the necessary transcription factors, where transcription occurs.

Actin Treadmilling – The barded end of the actin filament grows in length while the pointed end shrinks, which results in a section of the filament to seemingly move along the filament.

Phase separation – physical process in which supersaturated solution of components spontaneously separates into two phases, a dense phase and a dilute phase, and these phases stably coexist.

LINC Complex – a mechanotransductive protein complex that extends from the cytoplasm, where it interacts with cytoskeletal filaments, through the nuclear envelope,

where it interacts with the nuclear lamina; it is comprised of KASH-domain and SUN-domain proteins.

Nucleolar cap – a specific alteration of nucleolar structure in which some nucleolar proteins and nuclear proteins relocalize to the surface of the nucleolus; this structure is observed when nucleolar function is disrupted, such as during cellular stress.

Nucleoskeleton – a dynamic, complex network of proteins that forms an elastic shell at the nuclear periphery that responds to mechanical signaling to control nuclear stiffness, shape, and integrity. It also serves as a platform for organizing and regulating the genome.

Figures:

Figures do not require permission.

Figure 1: Functions of nuclear actin. Schematic of different functions of nuclear actin. The form of nuclear actin mediating each function is indicated as: G-actin = green dashed lines, F-actin = red lines, and actin rods = red line with filled box.

Figure 1

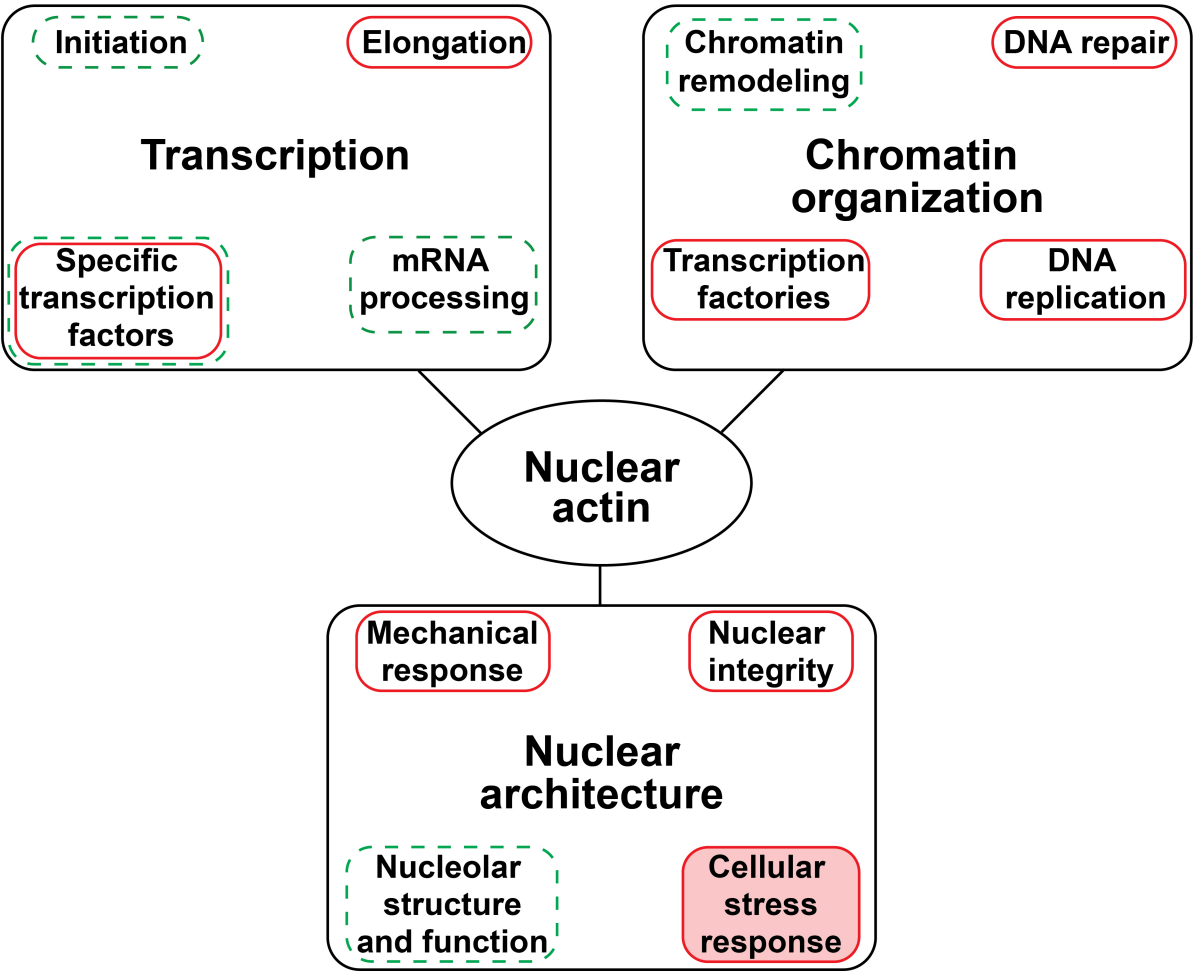

Initiation
Elongation
Transcription
Specific transcription factors
mRNA processing
Chromatin remodeling
DNA repair
Chromatin organization
Transcription factories
DNA replication
Nuclear actin
Mechanical response
Nuclear integrity
Nuclear architecture
Nucleolar structure and function
Cellular stress response

Figure 2: Roles of nuclear actin in transcription and chromatin organization. Nuclear actin has multiple roles during transcription; actin is shown in red. G-actin interacts with the preinitiation complex (PIC) to recruit RNAPII (A1), while actin polymers mediate transcription elongation (A2). G-actin is also implicated mRNA processing, including splicing (A3). Within the nucleolus, polymeric actin is required for RNAPI activity (A4). G-actin also binds to and regulates multiple chromatin remodeling complexes (i.e. INO80) to open the chromatin to allow for transcription (B). Additionally, nuclear actin mediates chromatin movement. During DNA repair (C1), chromatin remodeling complexes which require G-actin (i.e. NuA4) mark the DSB for repair, and F-actin is required for the DSB to be relocalized for repair. F-actin also forms a scaffold that allows for the formation and maintenance of transcription factories (C2).

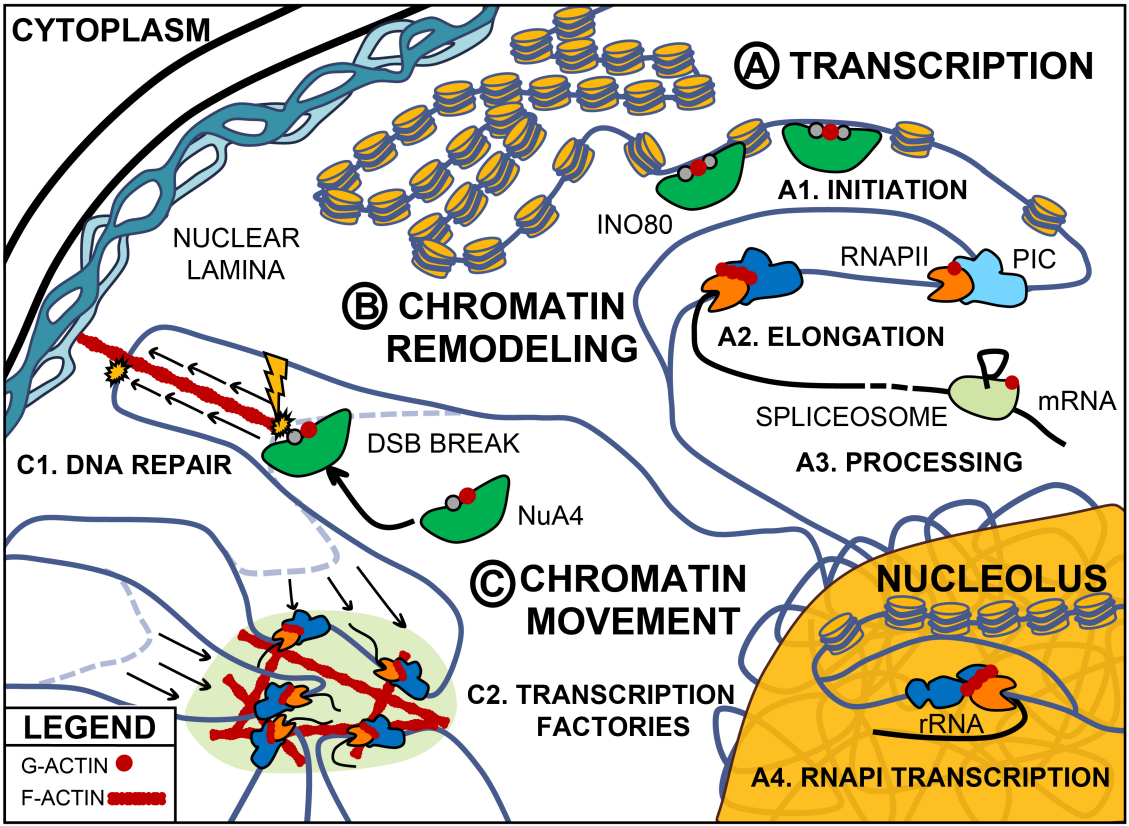

CYTOPLASM
Ⓐ TRANSCRIPTION
A1. INITIATION
INO80
NUCLEAR LAMINA
RNAPII
PIC
Ⓑ CHROMATIN REMODELING
A2. ELONGATION
SPLICEOSOME
mRNA
A3. PROCESSING
DSB BREAK
C1. DNA REPAIR
NuA4
Ⓒ CHROMATIN MOVEMENT
NUCLEOLUS
C2. TRANSCRIPTION FACTORIES
rRNA
A4. RNAPI TRANSCRIPTION
LEGEND
G-ACTIN
F-ACTIN

Figure 3: Roles of nuclear actin in nuclear architecture and mechanical response. Mechanical signaling is transmitted via the cytoskeleton, including cytoplasmic F-actin, to the nucleus via the LINC Complex (A), and induces the nuclear import of actin (B). Both of these events promote the polymerization of nuclear actin (C) to ultimately regulate mechanoresponsive transcription factors, including MAL/SRF (D).

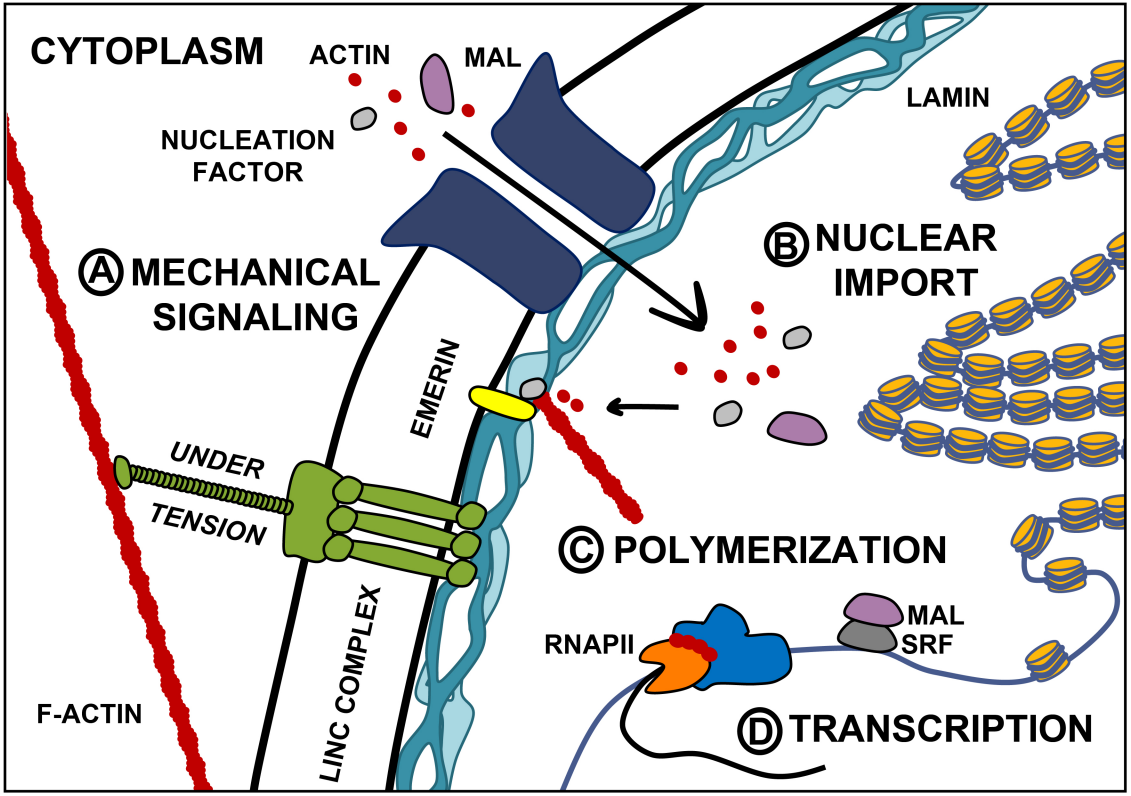
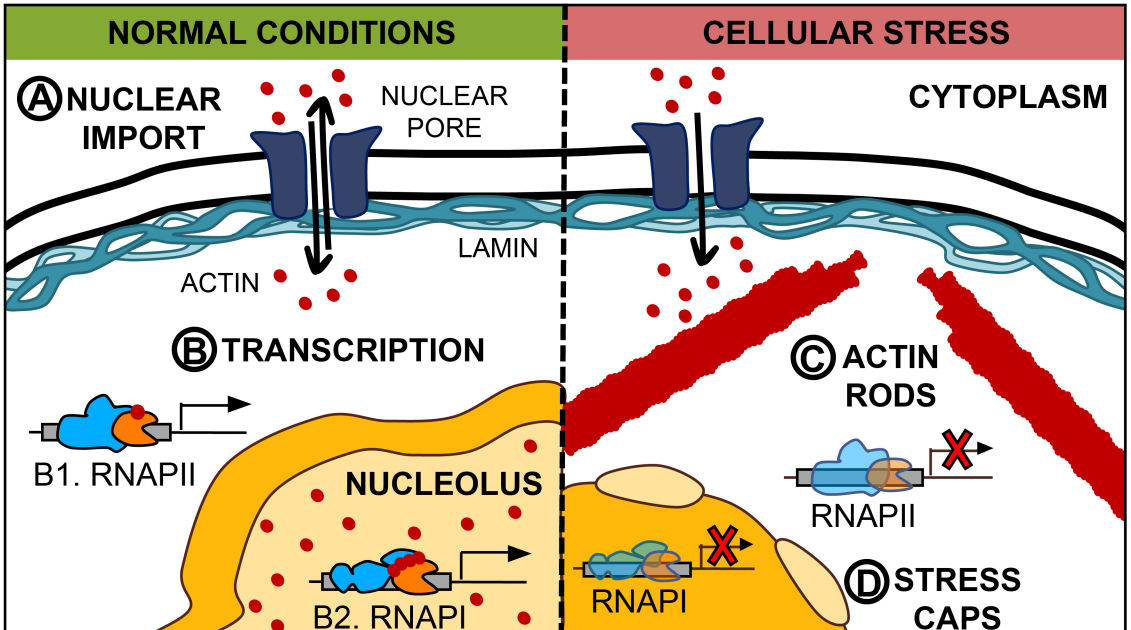

CYTOPLASM
ACTIN
MAL
NUCLEATION FACTOR
LAMIN
Ⓐ MECHANICAL SIGNALING
Ⓑ NUCLEAR IMPORT
EMERIN
UNDER TENSION
Ⓒ POLYMERIZATION
RNAPII
MAL
SRF
F-ACTIN
LINC COMPLEX
Ⓓ TRANSCRIPTION

Figure 4: Roles of nuclear actin in cellular stress. Under normal conditions, cells tightly regulate nuclear actin levels (A) to maintain transcription by both RNAPII (B1) and RNAPI (B2). Cellular stress induces the nuclear import of actin, driving the production of nuclear actin rods (C); this stops RNAPII-dependent transcription. Similarly, the nucleolus responds to cellular stress, stopping RNAPI transcription, which drives the formation of nucleolar caps (i.e. stress caps) (D). Whether nuclear actin dynamics contribute to the nucleolar stress response remains unknown.


NORMAL CONDITIONS
CELLULAR STRESS
A NUCLEAR IMPORT
NUCLEAR PORE
CYTOPLASM
LAMIN
ACTIN
B TRANSCRIPTION
C ACTIN RODS
B1. RNAPII
NUCLEOLUS
RNAPII
B2. RNAPI
RNAPI
D STRESS CAPS