

NUCLEAR ACTIN AND MYOSINS: LIFE WITHOUT FILAMENTS

Primal de Lanerolle and Leonid Serebryanny
Department of Physiology and Biophysics
University of Illinois at Chicago
Chicago, IL 60612

Corresponding author:
Primal de Lanerolle
Department of Physiology and Biophysics
University of Illinois at Chicago
835 South Wolcott
Chicago, IL 60612
primal@uic.edu

ABSTRACT

Actin and myosin are major components of the cell cytoskeleton, with structural and regulatory functions that impact many essential cellular processes. Actin and myosin were traditionally thought to function only in the cytoplasm. However, it is now well accepted that actin and multiple myosins are found in the nucleus. Increasing evidence on their functional roles has highlighted the importance of these proteins in the nuclear compartment.

INTRODUCTION

Myosins are a large family of motor proteins that interact with actin, a major cytoskeletal component, and use the energy released from ATP hydrolysis to power muscle contraction, cell motility and organelle movement^{1,2}. Decades of studying actin and myosin II have led to two major concepts: actin and myosin must form filaments¹ to do work and they are only found in the cytoplasm². The discovery of myosin I by Pollard and Korn³ challenged the former and the discovery of an isoform of myosin I in the nucleus^{4,5} challenged the latter. However, nuclear myosin I (NMI) does not form filaments and nuclear actin filaments are only observed under specialized conditions. Thus, the question of how actin and myosin perform physiologically relevant functions in the nucleus persists. This review will present evidence in support of the physiological functions of nuclear actin (Figure 1) and myosins (Figure 2 and Table 1) and describe some of the important questions regarding molecular motors in the nucleus.

NUCLEAR ACTIN

Nuclear actin structure

Although the existence of nuclear actin is now undisputed, the absence of nuclear actin filaments⁶ is perplexing. Filamentous actin in the cytoplasm is routinely visualized by staining with phalloidin. Despite exceeding the critical concentration for polymerization⁷, phalloidin-stained nuclear actin filaments are rarely seen. Filamentous structures have been observed in the nucleus only with antibodies that recognize unique actin conformations, and then only in specific cell types and under specific conditions^{8,9}. This extends to the multiple types of nuclear actin in plants¹⁰. While nuclear actin is predominantly monomeric, ~20% of actin in the nucleus has

characteristics of a very dynamic polymeric population, which might represent oligomers¹¹, and has led to the use of the term "polymeric actin" in reference to nuclear actin.

Interestingly, Latrunculin A, an actin polymerization inhibitor, causes the formation of actin rods that stain with phalloidin⁹ by increasing monomeric actin in the cytoplasm and the nuclear translocation of actin/cofilin complexes. Similarly, several stress related conditions, including DMSO treatment¹², heat shock¹³ and ATP depletion¹⁴, also generate nuclear actin rods. Physiologically, mutations in the α -skeletal muscle actin gene lead to intranuclear rod myopathy, a disease characterized by the formation of nuclear rods containing actin and α -actinin¹⁵. Actin rod formation decreases the mitotic index and may contribute to the muscle weakness seen in this myopathy³⁵. Thus, the formation of intranuclear actin rods may represent a stress response that includes a decrease in transcription.

Nuclear actin filaments appear to play a crucial role in viral infection¹⁶. Baculovirus infection results in the translocation of the host ARP2/3 complex to the nucleus, where it interacts with a viral WASp-like protein to stimulate actin polymerization¹⁷. Pseudorabies virus and herpes virus infections also result in the formation of nuclear actin filaments^{16,17}. Although the mechanism by which viruses induce the formation of actin filaments is not known, a viral kinase may be involved¹⁶ and the physical association of nuclear actin filaments with Herpes viral capsids appears to be necessary for the correct formation of viral assembly sites and viral transcription¹⁸. Viral capsid proteins co-localize with myosin V, suggesting that viral capsids travel along nuclear actin filaments using myosin V as a motor¹⁸. Herpes simplex virus replication involves the formation of replication compartments that grow, move and merge with each other in a nuclear actin and NMI dependent manner¹⁹. Collectively, these data suggest that viruses may use actin and different myosins to accomplish specific objectives.

Nuclear actin regulation and functions

The absence of canonical nuclear actin filaments under physiological conditions could be due to post-translational modifications or regulation by actin binding proteins (ABPs) and actin related proteins (ARPs) that bestow actin with nucleus-specific properties. However, although SUMOylation has been implicated in the nuclear retention of actin²⁰ and despite undergoing many other post-translational modifications in the cytoplasm²¹⁻²⁶, whether actin is similarly modified in the nucleus and how such modifications affect the structure, function or trafficking of nuclear actin is unknown.

The nuclear roles of ABPs and ARPs and their influences on actin have been studied more intensely. Two actin-binding proteins, cofilin and profilin, have been implicated in the import/depolymerization and the export/polymerization of actin, respectively²⁷. Actin does not contain a nuclear localization signal and its nuclear import appears to require an interaction with cofilin²⁸. In contrast, nuclear export involves exportin 6, a channel dedicated to the translocation of actin and actin-profilin complexes²⁹. The actin regulatory protein CapG was shown to localize to the nucleus and nucleolus and may therefore affect nucleolar actin dynamics³⁰.

The roles of ABPs and ARPs in the nucleus also hint at the functions of actin. Actin partners such as supervillin²⁷, gelsolin^{27,31}, α -actinin^{27,32} and nuclear myosins³¹ interact with liganded steroid receptors and act as transcriptional coactivators²⁷. The actin nucleating ARP2/3 complex has also been implicated in transcription by RNA polymerase II *in vitro* and *in vivo* in association with a neuronal Wiscott-Aldrich syndrome protein (N-WASp) complex^{33,34}. Actin and ARPs are important in recruiting chromatin remodeling complexes³⁵⁻³⁹ and many chromatin remodeling complexes associate with at least one ARP and actin^{35,39}, further supporting a transcriptional role for actin.

Indeed, actin is functionally involved in virtually all the steps of transcription. Actin binds to all three polymerases⁴⁰⁻⁴² and may be needed to form the active forms of RNA polymerase I and II. Exogenous actin stimulates the rate of *in vitro* transcription by RNA polymerase II more than eight-fold, technically making it a transcription factor⁴². Actin is also involved in forming high order structures required for export and conformational changes of mRNA through its association with pre-mRNP (messenger ribonucleoprotein) or hnRNP (heterogeneous RNP) particles in *C. tentans* and rat liver nuclei^{43,44}. The interaction of actin with hnRNPs regulates RNA polymerase II elongation and the recruitment of histone acetyltransferases (HATs) in a transcription dependent manner^{45,46}. Specifically, a complex of hnRNP U, actin and other proteins binds to the Ser2/5- and Ser2-phosphorylated C-terminal tail domain of RNA polymerase II (CTD), along with PCAF HAT, to couple the elongation phase of transcription with the processing of transcripts^{36,37}. Furthermore, the interaction between Cdk9, which phosphorylates Ser2 of the RNA polymerase II CTD, and actin stimulates RNA polymerase II transcription by promoting chromatin remodeling³⁸.

Actin connecting the cytoplasm and nucleus

Ultimately, the nucleus has to respond to environmental changes that are initially detected by cell surface receptors and communicated via the cytoskeleton⁴⁷. One mechanism for connecting the nucleus to its external environment involves the binding of proteins that have nuclear functions to the actin cytoskeleton. PREP2⁴⁸, a transcriptional activator, and YY1⁴⁹, a transcriptional repressor, bind to actin filaments in the cytoplasm and translocate to the nucleus when actin is depolymerized. In contrast, the transcriptional co-activator MAL interacts with cytoplasmic unpolymerised actin in the absence of serum and translocates to the nucleus when actin polymerization increases⁵⁰. Mechanical cues can also be relayed from the cell exterior to

the nucleus by the concerted action of the cytoplasmic actin cytoskeleton and force sensors, as was recently observed with the transcriptional regulators YAP and TAZ⁵¹. Thus, changes in the physical characteristics of the cytoskeleton, resulting from changes in the environment, can directly regulate nuclear functions by sequestering or releasing specific proteins.

The connection between the cytoplasm and the nucleus can be achieved more directly through the LINC (Linkers of the Nucleoskeleton to the Cytoskeleton) complex, which includes nesprin and the SUN proteins⁵². This complex physically connects the nuclear lamins on the inside of the nuclear envelope to the actin cytoskeleton, microtubules⁵³ and intermediate filaments⁴⁷ in the cytoplasm. Lamins form a meshwork under the inner nuclear membrane that binds to chromatin, providing a relatively direct pathway for transducing cytoskeletal changes to the nuclear interior. Indeed, cells lacking lamins A and C have decreased nuclear stiffness^{47,54} and lamin A mutants result in progeria, muscular dystrophies and other diseases⁴⁷. Nuclear lamins also bind the spectrin repeat protein, emerin⁵⁴. Emerin associates with proteins involved in signaling, RNA processing, chromatin remodeling and DNA replication⁵⁵ and also stably interacts with spectrin, NMI and actin-ADP⁵⁵. Thus, emerin, like other spectrin repeat proteins, has the capacity to link actin and the nucleoskeleton⁵⁴. These results highlight the complex mechanisms that cells employ to communicate extracellular events to the nucleus via the cytoskeleton.

NUCLEAR MYOSINS

Nuclear Myosin I

NMI is an isoform of myosin IC that has a 16 amino acid N-terminal sequence that is essential for its nuclear localization⁵. Class I myosins are the largest group of unconventional

myosins⁵⁶ and are characterized by three domains: an actin-binding head that hydrolyses ATP to move along actin filaments, a linker neck domain that is regulated by calmodulin binding and a tail that, unlike myosin II, is too short to self-aggregate into filaments⁵⁶. The myosin IC tail does, however, contain regions, such as a positively charged domain and a putative pleckstrin homology domain, that can bind various molecules, including DNA⁵⁷ and phospholipids⁵⁸.

Although both NMI and actin have been implicated in transcription by RNA polymerase I⁵⁹⁻⁶¹ and II^{6,62} (Figure 3), subtle differences have been noted in their functions. NMI is necessary for forming the first phosphodiester bond during transcription initiation and purified NMI stimulates transcription by RNA polymerase II⁶², but not as much as actin⁴². Like actin, NMI interacts with RNA polymerase I⁵⁹⁻⁶² and ribonucleoproteins to aid in elongation and rRNA maturation^{59,63}. Immunoelectron microscopy has shown that NMI is found in the dense fibrillar component of the nucleolus, the site of active rDNA transcription^{4,64,65}. In contrast, actin is found largely in the nucleolar fibrillar center, the site of inactive RNA polymerase I⁶⁶, even when transcription is inhibited^{64,65}. Actin binds RNA polymerase I directly, whereas the interaction of NMI is indirect and mediated by TIF-1A, a transcription factor phosphorylated by growth associated kinases⁶¹. The NMI-TIF-1A interaction suggests that NMI plays a role in the growth dependent regulation of rDNA transcription^{60,61}.

The motor function of nuclear actin and myosin I is crucial for the functional compartmentalization of the nucleus. The nuclear centre includes early replicating chromosomes while the periphery contains late replicating heterochromatic chromosomal regions⁶⁷. How this compartmentalization is established and how genes on different chromosomes are coordinately expressed have been open questions in cell biology. Chuang *et al.* revealed that the movement of an engineered chromosome locus is an active process powered by actin and NMI by showing

that actin and NMI mutants inhibit the repositioning of the gene locus⁶⁸. Other studies have corroborated these results by demonstrating that chromosome repositioning requires an NMI-containing motor complex⁶⁹⁻⁷¹.

ATP hydrolysis by actin and NMI has also been implicated in regulating the association of the transcription machinery and the rate of transcription. Ye *et al.* showed that NMI motor activity and stabilized polymeric actin are necessary for efficient rDNA transcription⁶⁰. They also reported that a mutant NMI lacking the tail domain, reduced the level of RNA polymerase I found at both promoter and transcribed regions, but had no effect during elongation. These studies, along with data from multiple CHIP assays using different antibodies, suggest that NMI adopts different conformations when associating with the promoter or transcribed regions^{60,61,72}. These findings have revealed small parts of what could be an elegant motor function coupled to transcription or a dynamic platform for structural regulation.

NMI also interacts with topoisomerase II⁷³ and with the chromatin remodeling complex WSTF/SNF2h⁷⁴, indicating additional roles for NMI in DNA damage repair and chromatin regulation and highlighting the multifunctionality of this motor.

Other nuclear myosins

Embryonic myosin II heavy chain is found in the nuclei of proliferating myoblasts and is down-regulated during differentiation⁷⁵. Myosin Va and b have also been observed in the nucleus. Myosin Va is phosphorylated by calcium-calmodulin dependent protein kinase II and phospho-myosin Va localizes in nuclear speckles, the sites of RNA processing in transcriptionally active cells⁷⁶, raising the possibility that it is involved in splicing. Myosin Vb, in contrast, interacts with β -actin and RNA polymerase I in the dense fibrillar component of nucleoli⁷⁷. This association can be altered by blocking RNA polymerase I, suggesting a role for

myosin Vb in rRNA transcription. These observations, along with the role of myosin Va in viral infection¹⁶⁻¹⁸, reinforce the idea that myosins have separate functions in the nucleus.

Myosin VI has also been implicated in transcription by RNA polymerase II⁷⁸. ChIP assays demonstrated its recruitment to the promoter and intergenic regions of active genes. Moreover, myosin VI depletion down-regulates gene expression and antibodies against myosin VI inhibit transcription in a runoff assay⁷⁸. The presence of both NMI and myosin VI in RNA polymerase II complexes is intriguing. In contrast to NMI, myosin VI has a large and highly variable step size and can act as an anchor at high mechanical loads^{79,80}. Myosin VI is also a backward motor⁷⁹, raising the possibility that these two myosins are involved in separate aspects of transcription.

Myosin XVI, another nuclear myosin, contains an ankyrin repeat domain in the N-terminal head region that mediates an interaction with protein phosphatase 1⁸¹. Expression of different domains of this myosin demonstrated that the C terminus is necessary for nuclear localization. The N terminal ankyrin repeat domain targeted the protein to the nucleolus and the tail region of myosin XVI associated with stress induced nuclear actin rods and nuclear profilin, indicating that different regions may direct the protein to function in distinct nuclear compartments. Indeed, myosin XVIb localizes in a nuclear compartment that contains proliferating cell nuclear antigen (PCNA) and cyclin A, and over-expressing the full length protein or the tail region decreases BrUTP incorporation and delays S-phase progression. Together, these data suggest that myosin XVI may affect the cell cycle and proliferation⁸¹.

Myosin XVIIIb is principally found in cardiac and skeletal muscle⁸². Although it is cytoplasmic in myoblasts, a fraction localizes in the nucleus upon differentiation. Myosin XVIIIb is necessary for myofibrillar development⁸³ and a candidate tumor suppressor gene⁸⁴

because mutations in this protein have been found in a variety of cancers⁸⁵. This myosin interacts and co-localizes with the HOMER2 protein in membrane protrusions and stress fibers and the expression of HOMER2 enhances the ability of myosin XVIIIb to suppress anchorage independent growth⁸⁴. Because its nuclear functions remain undefined, it is unclear how these data relate to the nuclear form of myosin XVIIIb.

DISCUSSION

Outstanding questions

The available data raise a number of important questions. The first of these relates to the form of actin in the nucleus. Elucidating whether nuclear actin is primarily monomeric or polymeric, how it is affected by post-translational modifications, ARPs and ABPs, how it relates to different myosins and where it is located in pre-initiating and elongating transcription complexes will greatly increase our understanding of how motors work in the nucleus. The absence of a reasonable picture of how actin and myosins associate with each other and with other nuclear components is one of the factors retarding progress in this field. Fortunately, the technology related to super resolution microscopy is evolving rapidly and 3D images of the nucleus could clarify these interactions.

A related question is *why* there are no canonical actin filaments in the nucleus. It is possible that technical limitations have prevented their visualization to date. Another possibility is that canonical actin filaments in the nucleus confer a developmental disadvantage. Thin filaments found in sarcomeres of skeletal and cardiac muscle are the most stable^{1,2} actin filaments in any cell type. They are an excellent example of form following function because the structure of the sarcomere enhances both reliability and speed of muscle contraction. Cell

motility is much slower in comparison and requires a dynamic cytoskeleton. Thus, bacteria and archaea have unusually dynamic filaments⁸⁶ and motile cells sacrifice speed and dependability for flexibility and plasticity. Nuclear actin can be visualized as part of a structural and evolutionary continuum that stretches from thin filaments in muscle cells to filopodia and other dynamic actin structures in motile cells to actin polymers in the nucleus. In this continuum, thin filaments are the most highly organized, least plastic and evolutionarily most recent adaptation. In contrast, the organization of actin in the nucleus would be the most plastic and evolutionarily oldest. The nature of this organization, how it is regulated and how it serves the nuclear functions of actin are prime issues to be addressed in future work.

The absence of nuclear actin and myosin filaments is also consistent with the evolution of these proteins. Evolution depends on adapting new functions for existing proteins. Actin⁸⁷ and myosin^{88,89} developed separately and almost certainly had separate functions, and because actin and myosin have to work together to perform as a motor, their initial, individual functions were probably structural in nature. Moreover, two of the three earliest myosins⁸⁸ are found in the nucleus (myosin I and myosin V) and neither forms filaments. It is conceivable that, at some point, actin and myosin co-operated to perform a function that provided an advantage to a cell. This event is likely to have triggered the evolution of multiple myosin families, diverging in their ability to form filaments. In this context, having non-mutually exclusive structural and motor functions in the nucleus seems reasonable.

The lack of filamentous actin and myosin questions how force is generated in the nucleus. The sliding filament model of muscle contraction is central to our understanding of force production and filaments clearly enhance the efficiency and speed of force generation¹. However, force could also be generated if myosin anchored to a nuclear component pulled on an

actin molecule bound to something else. For instance, actin binds to all three polymerases, indicating that it is “anchored” to them and NMI can bind to DNA through its tail domain⁵⁷, suggesting a potential binding partner during transcription. The specificity and stability of these interactions could be increased by DNA binding proteins, ABPs or transcription factors, but whether the strength of these interactions and the amount of force they generate is sufficient to support transcription remains unclear.

Future directions

Understanding how nuclear actin and myosin are regulated is equally important. The actin-myosin II interaction in muscle contraction is regulated by calcium. Likewise, calcium transients have been demonstrated in the nucleus⁹⁰ and may contribute to nuclear dynamics⁹¹. Nuclear myosin II is reportedly regulated by calcium-dependent phosphorylation⁹² and phosphorylated myosin Va is found in the nucleus⁷⁶. PIP₂ and PIP kinases associate with nuclear speckles and nucleoli⁹³⁻⁹⁵ suggesting that proteins with lipid binding domains, such as myosin or ABPs, can localize to speckles to mediate the aggregation of actin by altering its organization, capping, or polymerization. Nuclear PIP₂ also regulates chromatin remodeling activity³⁵. Based on its regulation of the cytoskeleton, PIP₂ could control interactions of nuclear actin with myosins, gelsolin, cofilin, profilin, and WASp⁷, which have all been identified in the nucleus²⁷.

Finally, two of the greatest challenges in the future will involve describing all the nuclear myosin isoforms and obtaining an integrated picture of how they interact with each other and with nuclear actin. Sequence analysis has shown that myosins VII, IX, X and XV in mammals have putative classical nuclear localization signals (Wilma A. Hofmann and Primal de Lanerolle, unpublished) and future work on their subcellular localization and activities will provide a complete picture of their physiological functions. An important consideration in these studies

will be to determine how the characteristics of individual myosins, such as step size, number of heads, processivity and cargo binding domains, fit with the requirements of individual nuclear processes. The available literature supports the notion that specific myosins perform distinct nuclear functions and paints a picture of nuclear actin and myosins regulating multiple processes that are crucial for key cellular functions. Learning more about the molecular details of the nuclear roles of actin and myosin will undoubtedly increase our understanding of both the nucleus and molecular motors, and will perhaps change how we think about them.

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REFERENCES

- 1 Sellers, J. R. Fifty years of contractility research post sliding filament hypothesis. *J Muscle Res Cell Motil* **25**, 475-482 (2004).
- 2 Schmidt, A. & Hall, M. N. Signaling to the actin cytoskeleton. *Annu Rev Cell Dev Biol* **14**, 305-338 (1998).
- 3 Pollard, T. D. & Korn, E. D. Acanthamoeba myosin. I. Isolation from *Acanthamoeba castellanii* of an enzyme similar to muscle myosin. *J Biol Chem* **248**, 4682-4690 (1973).
- 4 Nowak, G. *et al.* Evidence for the presence of myosin I in the nucleus. *J Biol Chem* **272**, 17176-17181 (1997).
- 5 Pestic-Dragovich, L. *et al.* A myosin I isoform in the nucleus. *Science* **290**, 337-341 (2000).
- 6 Pederson T. As functional nuclear actin comes into view, is it globular, filamentous, or both? *J Cell Biol.*:1061-1064. (2008)
- 7 Pollard, T. D., Blanchoin, L. & Mullins, R. D. Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. *Annu Rev Biophys Biomol Struct* **29**, 545-576 (2000).
- 8 Gonsior, S. M. *et al.* Conformational difference between nuclear and cytoplasmic actin as detected by a monoclonal antibody. *J Cell Sci* **112 (Pt 6)**, 797-809 (1999).
- 9 Schoenenberger, C. A. *et al.* Conformation-specific antibodies reveal distinct actin structures in the nucleus and the cytoplasm. *J Struct Biol* **152**, 157-168 (2005).
- 10 Kandasamy, M. K., McKinney, E. C. & Meagher, R. B. Differential sublocalization of actin variants within the nucleus. *Cytoskeleton (Hoboken)* **67**, 729-743 (2010).
- 11 McDonald, D., Carrero, G., Andrin, C., de Vries, G. & Hendzel, M. J. Nucleoplasmic beta-actin exists in a dynamic equilibrium between low-mobility polymeric species and rapidly diffusing populations. *J Cell Biol* **172**, 541-552 (2006).
12. Fukui Y, Katsumaru H. Dynamics of nuclear actin bundle induction by dimethyl sulfoxide and factors affecting its development. *J Cell Biol.* (1980) 84: 131-40.
- 13 Nishida, E. *et al.* Cofilin is a component of intranuclear and cytoplasmic actin rods induced in cultured cells. *Proc Natl Acad Sci U S A* **84**, 5262-5266 (1987).
- 14 Pendleton, A., Pope, B., Weeds, A. & Koffer, A. Latrunculin B or ATP depletion induces cofilin-dependent translocation of actin into nuclei of mast cells. *J Biol Chem* **278**, 14394-14400 (2003).
- 15 Domazetovska, A. *et al.* Intranuclear rod myopathy: molecular pathogenesis and mechanisms of weakness. *Ann Neurol* **62**, 597-608 (2007).
- 16 Taylor, M. P., Koyuncu, O. O. & Enquist, L. W. Subversion of the actin cytoskeleton during viral infection. *Nat Rev Microbiol* (2011).
- 17 Goley, E. D. *et al.* Dynamic nuclear actin assembly by Arp2/3 complex and a baculovirus WASP-like protein. *Science* **314**, 464-467 (2006).
- 18 Feierbach, B., Piccinotti, S., Bisher, M., Denk, W. & Enquist, L. W. Alpha-herpesvirus infection induces the formation of nuclear actin filaments. *PLoS Pathog* **2**, e85 (2006).

- 19 Chang, L. *et al.* PNAS Plus: Herpesviral replication compartments move and coalesce at nuclear speckles to enhance export of viral late mRNA. *Proc Natl Acad Sci U S A* **108**, E136-144 (2011).
- 20 Hofmann, W. A. *et al.* SUMOylation of nuclear actin. *J Cell Biol* **186**, 193-200 (2009).
- 21 Abe, A., Saeki, K., Yasunaga, T. & Wakabayashi, T. Acetylation at the N-terminus of actin strengthens weak interaction between actin and myosin. *Biochem Biophys Res Commun* **268**, 14-19 (2000).
- 22 Karakozova, M. *et al.* Arginylation of beta-actin regulates actin cytoskeleton and cell motility. *Science* **313**, 192-196 (2006).
- 23 Wang, J. *et al.* Stable and controllable RNA interference: Investigating the physiological function of glutathionylated actin. *Proc Natl Acad Sci U S A* **100**, 5103-5106 (2003).
- 24 Pekiner, C. *et al.* Glycation of brain actin in experimental diabetes. *J Neurochem* **61**, 436-442 (1993).
- 25 Aslan, M. *et al.* Nitric oxide-dependent generation of reactive species in sickle cell disease. Actin tyrosine induces defective cytoskeletal polymerization. *J Biol Chem* **278**, 4194-4204 (2003).
- 26 Thom, S. R., Bhopale, V. M., Mancini, D. J. & Milovanova, T. N. Actin S-nitrosylation inhibits neutrophil beta2 integrin function. *J Biol Chem* **283**, 10822-10834 (2008).
- 27 Gettemans, J. *et al.* Nuclear actin-binding proteins as modulators of gene transcription. *Traffic* **6**, 847-857 (2005).
- 28 Iida, K., Matsumoto, S. & Yahara, I. The KKRKK sequence is involved in heat shock-induced nuclear translocation of the 18-kDa actin-binding protein, cofilin. *Cell Struct Funct* **17**, 39-46 (1992).
- 29 Stuken, T., Hartmann, E. & Gorlich, D. Exportin 6: a novel nuclear export receptor that is specific for profilin.actin complexes. *EMBO J* **22**, 5928-5940 (2003).
- 30 Hubert, T., Van Impe, K., Vandekerckhove, J. & Gettemans, J. The F-actin filament capping protein CapG is a bona fide nucleolar protein. *Biochem Biophys Res Commun* **377**, 699-704 (2008).
- 31 Ambrosino, C. *et al.* Identification of a hormone-regulated dynamic nuclear actin network associated with estrogen receptor alpha in human breast cancer cell nuclei. *Mol Cell Proteomics* **9**, 1352-1367 (2010).
- 32 Huang, S. M., Huang, C. J., Wang, W. M., Kang, J. C. & Hsu, W. C. The enhancement of nuclear receptor transcriptional activation by a mouse actin-binding protein, alpha actinin 2. *J Mol Endocrinol* **32**, 481-496 (2004).
- 33 Yoo, Y., Wu, X. & Guan, J. L. A novel role of the actin-nucleating Arp2/3 complex in the regulation of RNA polymerase II-dependent transcription. *J Biol Chem* **282**, 7616-7623 (2007).
- 34 Wu, X. *et al.* Regulation of RNA-polymerase-II-dependent transcription by N-WASP and its nuclear-binding partners. *Nat Cell Biol* **8**, 756-763 (2006).
- 35 Zhao, K. *et al.* Rapid and phosphoinositol-dependent binding of the SWI/SNF-like BAF complex to chromatin after T lymphocyte receptor signaling. *Cell* **95**, 625-636 (1998).
- 36 Kukalev, A., Nord, Y., Palmberg, C., Bergman, T. & Percipalle, P. Actin and hnRNP U cooperate for productive transcription by RNA polymerase II. *Nat Struct Mol Biol* **12**, 238-244 (2005).

- 37 Obrdlik, A. *et al.* The histone acetyltransferase PCAF associates with actin and hnRNP U for RNA polymerase II transcription. *Mol Cell Biol* **28**, 6342-6357 (2008).
- 38 Qi, T. *et al.* G-actin Participates in RNA Polymerase II-dependent Transcription Elongation by Recruiting Positive Transcription Elongation Factor b (P-TEFb). *J Biol Chem* **286**, 15171-15181 (2011).
- 39 Blessing, C. A., Ugrinova, G. T. & Goodson, H. V. Actin and ARPs: action in the nucleus. *Trends Cell Biol* **14**, 435-442 (2004).
- 40 Grummt, I. Actin and myosin as transcription factors. *Curr Opin Genet Dev* **16**, 191-196 (2006).
- 41 Hu, P., Wu, S. & Hernandez, N. A role for beta-actin in RNA polymerase III transcription. *Genes Dev* **18**, 3010-3015 (2004).
- 42 Hofmann, W. A. *et al.* Actin is part of pre-initiation complexes and is necessary for transcription by RNA polymerase II. *Nat Cell Biol* **6**, 1094-1101 (2004).
- 43 Percipalle, P. *et al.* Actin bound to the heterogeneous nuclear ribonucleoprotein hrp36 is associated with Balbiani ring mRNA from the gene to polysomes. *J Cell Biol* **153**, 229-236 (2001).
- 44 Percipalle, P. *et al.* Nuclear actin is associated with a specific subset of hnRNP A/B-type proteins. *Nucleic Acids Res* **30**, 1725-1734 (2002).
- 45 Percipalle, P. *et al.* An actin-ribonucleoprotein interaction is involved in transcription by RNA polymerase II. *Proc Natl Acad Sci U S A* **100**, 6475-6480 (2003).
- 46 Sjolinder, M. *et al.* The growing pre-mRNA recruits actin and chromatin-modifying factors to transcriptionally active genes. *Genes Dev* **19**, 1871-1884 (2005).
- 47 Jaalouk, D. E. & Lammerding, J. Mechanotransduction gone awry. *Nat Rev Mol Cell Biol* **10**, 63-73 (2009).
- 48 Haller, K., Rambaldi, I., Daniels, E. & Featherstone, M. Subcellular localization of multiple PREP2 isoforms is regulated by actin, tubulin, and nuclear export. *J Biol Chem* **279**, 49384-49394 (2004).
- 49 Favot, L., Hall, S. M., Haworth, S. G. & Kemp, P. R. Cytoplasmic YY1 is associated with increased smooth muscle-specific gene expression: implications for neonatal pulmonary hypertension. *Am J Pathol* **167**, 1497-1509 (2005).
- 50 Vartiainen, M. K., Guettler, S., Larijani, B. & Treisman, R. Nuclear actin regulates dynamic subcellular localization and activity of the SRF cofactor MAL. *Science* **316**, 1749-1752 (2007).
- 51 Dupont, S. *et al.* Role of YAP/TAZ in mechanotransduction. *Nature* **474**, 179-183 (2011).
- 52 Luxton, G. W., Gomes, E. R., Folker, E. S., Vintinner, E. & Gundersen, G. G. Linear arrays of nuclear envelope proteins harness retrograde actin flow for nuclear movement. *Science* **329**, 956-959 (2010).
- 53 Razafsky, D. & Hodzic, D. Bringing KASH under the SUN: the many faces of nucleocytoskeletal connections. *J Cell Biol* **186**, 461-472 (2009).
- 54 Shumaker, D. K., Kuczmarski, E. R. & Goldman, R. D. The nucleoskeleton: lamins and actin are major players in essential nuclear functions. *Curr Opin Cell Biol* **15**, 358-366 (2003).
- 55 Holaska, J. M. & Wilson, K. L. An emerin "proteome": purification of distinct emerin-containing complexes from HeLa cells suggests molecular basis for diverse roles

- including gene regulation, mRNA splicing, signaling, mechanosensing, and nuclear architecture. *Biochemistry* **46**, 8897-8908 (2007).
- 56 Coluccio, L. M. in *Myosins Vol. 7 Proteins And Cell Regulation* 95-124 (Springer Netherlands, 2008).
- 57 Hofmann, W. A., Johnson, T., Klapeczynski, M., Fan, J. L. & de Lanerolle, P. From transcription to transport: emerging roles for nuclear myosin I. *Biochem Cell Biol* **84**, 418-426 (2006).
- 58 Hokanson, D. E. & Ostap, E. M. Myo1c binds tightly and specifically to phosphatidylinositol 4,5-bisphosphate and inositol 1,4,5-trisphosphate. *Proc Natl Acad Sci U S A* **103**, 3118-3123 (2006).
- 59 Fomproix, N. & Percipalle, P. An actin-myosin complex on actively transcribing genes. *Exp Cell Res* **294**, 140-148 (2004).
- 60 Ye, J., Zhao, J., Hoffmann-Rohrer, U. & Grummt, I. Nuclear myosin I acts in concert with polymeric actin to drive RNA polymerase I transcription. *Genes Dev* **22**, 322-330 (2008).
- 61 Philimonenko, V. V. *et al.* Nuclear actin and myosin I are required for RNA polymerase I transcription. *Nat Cell Biol* **6**, 1165-1172 (2004).
- 62 Hofmann, W. A. *et al.* Nuclear myosin I is necessary for the formation of the first phosphodiester bond during transcription initiation by RNA polymerase II. *J Cell Biochem* **99**, 1001-1009 (2006).
- 63 Obrdlik, A. *et al.* Nuclear myosin 1 is in complex with mature rRNA transcripts and associates with the nuclear pore basket. *FASEB J* **24**, 146-157 (2010).
- 64 Kysela, K. *et al.* Nuclear distribution of actin and myosin I depends on transcriptional activity of the cell. *Histochem Cell Biol* **124**, 347-358 (2005).
- 65 Philimonenko, V. V., Janacek, J., Harata, M. & Hozak, P. Transcription-dependent rearrangements of actin and nuclear myosin I in the nucleolus. *Histochem Cell Biol* **134**, 243-249 (2010).
- 66 Raska, I., Shaw, P. J. & Cmarko, D. Structure and function of the nucleolus in the spotlight. *Curr Opin Cell Biol* **18**, 325-334 (2006).
- 67 Spector, D. L. The dynamics of chromosome organization and gene regulation. *Annu Rev Biochem* **72**, 573-608 (2003).
- 68 Chuang, C. H. *et al.* Long-range directional movement of an interphase chromosome site. *Curr Biol* **16**, 825-831 (2006).
- 69 Hu, Q. *et al.* Enhancing nuclear receptor-induced transcription requires nuclear motor and LSD1-dependent gene networking in interchromatin granules. *Proc Natl Acad Sci U S A* **105**, 19199-19204 (2008).
- 70 Dundr, M. *et al.* Actin-dependent intranuclear repositioning of an active gene locus in vivo. *J Cell Biol* **179**, 1095-1103 (2007).
- 71 Mehta, I. S., Amira, M., Harvey, A. J. & Bridger, J. M. Rapid chromosome territory relocation by nuclear motor activity in response to serum removal in primary human fibroblasts. *Genome Biol* **11**, R5 (2010).
- 72 Percipalle, P. *et al.* The chromatin remodelling complex WSTF-SNF2h interacts with nuclear myosin 1 and has a role in RNA polymerase I transcription. *EMBO Rep* **7**, 525-530 (2006).

- 73 Smukste, I., Bhalala, O., Persico, M. & Stockwell, B. R. Using small molecules to overcome drug resistance induced by a viral oncogene. *Cancer Cell* **9**, 133-146 (2006).
- 74 Percipalle, P. & Farrants, A. K. Chromatin remodelling and transcription: be-WICHed by nuclear myosin I. *Curr Opin Cell Biol* **18**, 267-274 (2006).
- 75 Rodgers, B. D. Insulin-like growth factor-I downregulates embryonic myosin heavy chain (eMyHC) in myoblast nuclei. *Growth Horm IGF Res* **15**, 377-383 (2005).
- 76 Pranchevicius, M. C. *et al.* Myosin Va phosphorylated on Ser1650 is found in nuclear speckles and redistributes to nucleoli upon inhibition of transcription. *Cell Motil Cytoskeleton* **65**, 441-456 (2008).
- 77 Lindsay, A. J. & McCaffrey, M. W. Myosin Vb localises to nucleoli and associates with the RNA polymerase I transcription complex. *Cell Motil Cytoskeleton* **66**, 1057-1072 (2009).
- 78 Vreugde, S. *et al.* Nuclear myosin VI enhances RNA polymerase II-dependent transcription. *Mol Cell* **23**, 749-755 (2006).
- 79 Buss, F., Spudich, G. & Kendrick-Jones, J. Myosin VI: cellular functions and motor properties. *Annu Rev Cell Dev Biol* **20**, 649-676 (2004).
- 80 Altman, D., Sweeney, H. L. & Spudich, J. A. The mechanism of myosin VI translocation and its load-induced anchoring. *Cell* **116**, 737-749 (2004).
- 81 Cameron, R. S. *et al.* Myosin16b: The COOH-tail region directs localization to the nucleus and overexpression delays S-phase progression. *Cell Motil Cytoskeleton* **64**, 19-48 (2007).
- 82 Salamon, M. *et al.* Human MYO18B, a novel unconventional myosin heavy chain expressed in striated muscles moves into the myonuclei upon differentiation. *J Mol Biol* **326**, 137-149 (2003).
- 83 Ajima, R. *et al.* Deficiency of Myo18B in mice results in embryonic lethality with cardiac myofibrillar aberrations. *Genes Cells* **13**, 987-999 (2008).
- 84 Ajima, R. *et al.* HOMER2 binds MYO18B and enhances its activity to suppress anchorage independent growth. *Biochem Biophys Res Commun* **356**, 851-856 (2007).
- 85 Bleeker, F. E. *et al.* Mutational profiling of cancer candidate genes in glioblastoma, melanoma and pancreatic carcinoma reveals a snapshot of their genomic landscapes. *Hum Mutat* **30**, E451-459 (2009).
- 86 Skarp, K. P. & Vartiainen, M. K. Actin on DNA-an ancient and dynamic relationship. *Cytoskeleton (Hoboken)* **67**, 487-495 (2010).
- 87 Goodson, H. V. & Hawse, W. F. Molecular evolution of the actin family. *J Cell Sci* **115**, 2619-2622 (2002).
- 88 Richards, T. A. & Cavalier-Smith, T. Myosin domain evolution and the primary divergence of eukaryotes. *Nature* **436**, 1113-1118 (2005).
- 89 Hofmann, W. A., Richards, T. A. & de Lanerolle, P. Ancient animal ancestry for nuclear myosin. *J Cell Sci* **122**, 636-643 (2009).
- 90 Rodrigues, M. A., Gomes, D. A., Andrade, V. A., Leite, M. F. & Nathanson, M. H. Insulin induces calcium signals in the nucleus of rat hepatocytes. *Hepatology* **48**, 1621-1631 (2008).
- 91 Bootman, M. D., Fearnley, C., Smyrnias, I., MacDonald, F. & Roderick, H. L. An update on nuclear calcium signalling. *J Cell Sci* **122**, 2337-2350 (2009).

- 92 Li, Q. & Sarna, S. K. Nuclear myosin II regulates the assembly of preinitiation complex for ICAM-1 gene transcription. *Gastroenterology* **137**, 1051-1060, 1060 e1051-1053 (2009).
- 93 Mortier, E. *et al.* Nuclear speckles and nucleoli targeting by PIP2-PDZ domain interactions. *EMBO J* **24**, 2556-2565 (2005).
- 94 Boronenkov, I. V., Loijens, J. C., Umeda, M. & Anderson, R. A. Phosphoinositide signaling pathways in nuclei are associated with nuclear speckles containing pre-mRNA processing factors. *Mol Biol Cell* **9**, 3547-3560 (1998).
- 95 Osborne, S. L., Thomas, C. L., Gschmeissner, S. & Schiavo, G. Nuclear PtdIns(4,5)P₂ assembles in a mitotically regulated particle involved in pre-mRNA splicing. *J Cell Sci* **114**, 2501-2511 (2001).
- 96 Jung E. J., Liu, G., Zhou, W., Chen, X. Myosin VI is a mediator of the p53-dependent cell survival pathway. *Mol Cell Biol.* **26**, 2175-86 (2006).

FIGURE LEGENDS

FIGURE 1. THE ROLES OF ACTIN IN THE NUCLEUS: Monomeric, G-actin can polymerize to form canonical actin filaments (F-actin) in the cytoplasm. Actin in complex with cofilin can enter the nucleus via nuclear pore complexes (NPC), while profilin-actin complexes are exported via exportin 6. Nuclear actin appears to be highly dynamic. It can interact with all three polymerases and engage in transcription, it can undergo post-translational modifications, like SUMOylation, or it can form actin polymers (shown as groups of three or fewer monomers to differentiate from F-actin in the cytoplasm). Helical rod bundles consisting of actin and cofilin have been detected in the nucleus under various stressful conditions and in the disease intranuclear rod myopathy. The formation of actin rods or polymers and the recruitment of nuclear myosin I or myosin Va appear to be important in viral replication. LINC complexes, which bind to lamins inside the nucleus and to actin filaments in the cytoplasm, appear to be important in outside-in nuclear signaling. The cytoplasmic actin cytoskeleton can also influence nuclear activity. The transcription factors PREP2, YY1 and MAL bind to cytoplasmic actin and changes in actin polymerization can sequester, or release these factors so that they can enter the nucleus to regulate transcription. Steroid receptors that translocate to the nucleus following binding of their activating ligands also interact with actin, actin binding proteins and myosins and act as transcriptional co-activators of genes regulated by steroid hormones.

FIGURE 2. MYOSIN IN THE NUCLEUS: Myosins are involved in many nuclear functions. NMI and actin are important for transcription by RNA polymerase II and I, through structural roles, motor functions or a combination of the two. Myosin VI is also implicated in transcription

by RNA polymerase II (see also Figure 3A). The motor activity of NMI and actin is also critical for positioning and organizing chromatin and the expression of estrogen receptor-activated genes. In the nucleolus, NMI and Myosin Vb are localized in a transcription dependent manner to the dense fibrillar component, while actin is in fibrillar centers. Myosins are also part of nuclear scaffolding complexes containing lamins and emerin. The latter associates with actin, NMI, myosin II heavy chain, spectrin and others, thus providing a link to the nucleoskeleton and chromatin. Myosin XVIb may regulate the cell cycle by interacting with cyclin A and proliferating cell nuclear antigen (PCNA), whereas binding to protein phosphatase 1 (PP1) may control its activity and its nuclear transport through the nuclear pore complex (NPC). Adult and embryonic myosin II have also been described in the nucleus, with functions in regulation of gene expression and differentiation, respectively. Myosin XVIIIb has also been implicated in the transcription of genes required for differentiation. Myosin Va is found in speckles and is thought to be involved in RNA processing.

FIGURE 3. ACTIN AND MYOSINS IN TRANSCRIPTION:

Panel A: Transcription by RNA Polymerase II: Actin interacts with RNA polymerase II and is necessary to form the pre-initiation complex. The actin binding protein WASp has been discovered in the nucleus and modulates transcription independently or with the ARP2/3 complex. Actin is needed to enhance the activity of several chromatin remodeling proteins and the elongation machinery, including BAF, PCAF, Cdk9, P300, and P-TEFB, following phosphorylation (P) of the C-terminal domain of the polymerase. Actin also induces the activity of hnRNP U, a post-transcriptional modifier of mRNA. Nuclear myosin I (NMI) is needed to create the first phosphodiester bond in transcription initiation. Myosin VI (MVI), a backwards

motor, has also been implicated in transcription, posing the intriguing possibility that NMI and MVI have counteracting motor functions (represented by white arrows). Myosin Va has been discovered in nuclear speckles, suggesting a role in splicing.

Panel B: Transcription by RNA Polymerase I: Actin also binds to polymerase I and a polymerized form of actin is suggested to be necessary for rRNA transcription. NMI is recruited by phosphorylated (P) TIF-1A and the binding of the phospho-TIF-1A-NMI complex results in an initiation competent form of polymerase I. NMI also has a role in chromatin remodeling by the WSTF/SNF2h complex and rRNA maturation. Actin is proposed to act in concert with NMI, either as a structural complex or a supplemental motor in transcription. Myosin Vb has also been implicated as a regulator of polymerase I transcription.

TABLE 1 MYOSINS IN THE NUCLEUS

Myosin Type	Abbreviation	Potential Nuclear Functions
Nuclear Myosin I	NMI	Transcription by RNA pol I ^{4,59-61} and II ^{5,62} ; DNA damage repair ⁷³ ; Chromosome translocations ⁶⁷⁻⁷⁰ ; Virus replication ¹⁹
Myosin II	MII	Embryonic MII in myoblast differentiation ⁷⁵ ; Adult MII in pre-initiation ⁹²
Myosin Va & Vb	MVa and MVb	MVa: Splicing ⁷⁶ ; Virus replication ¹⁸ ; MVb: Transcription by RNA pol I ⁷⁷
Myosin VI	MVI	Transcription by RNA pol II ⁷⁸ ; DNA damage repair ⁹⁶
Myosin XVI	MXVI	Cell cycle and proliferation ⁸¹
Myosin XVIII		Myofibrillar development ^{82,83} ; Putative tumor suppressor gene ^{84,85}

Figure 1: Actin in the Nucleus

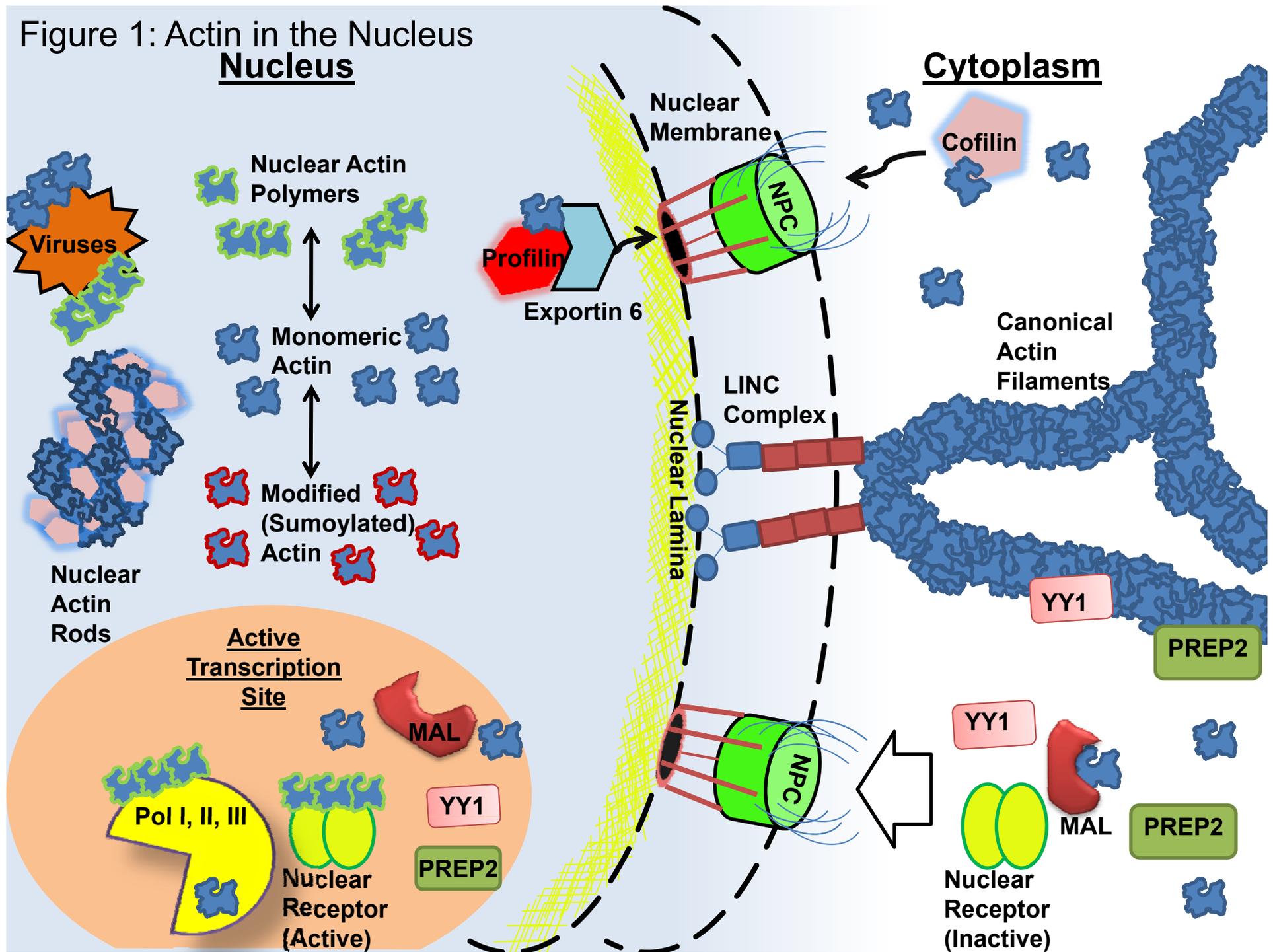


Figure 2: Myosins in the Nucleus

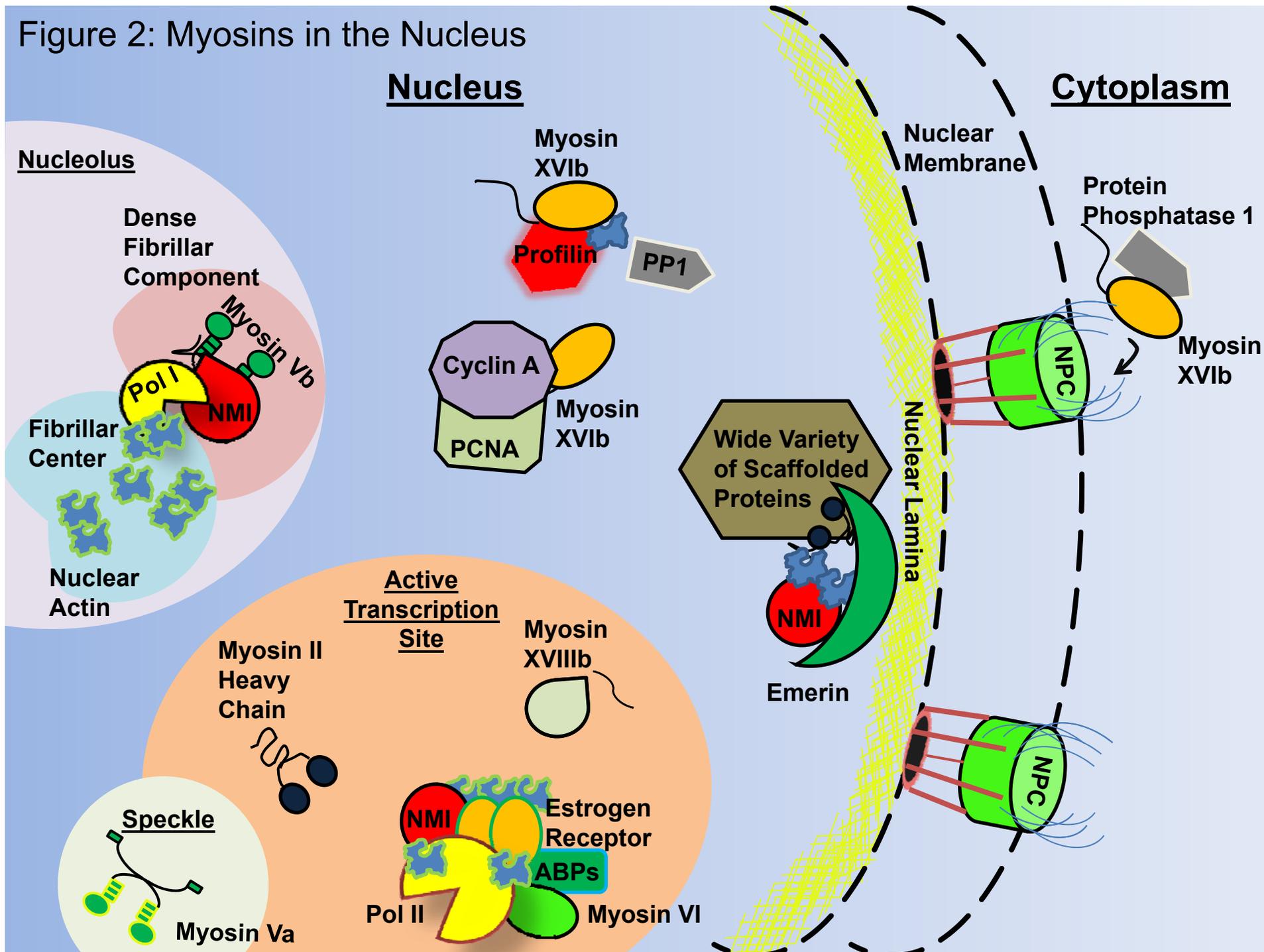


Figure 3a: Actin, Myosins and Interacting Proteins
In Transcription by RNA Polymerase II

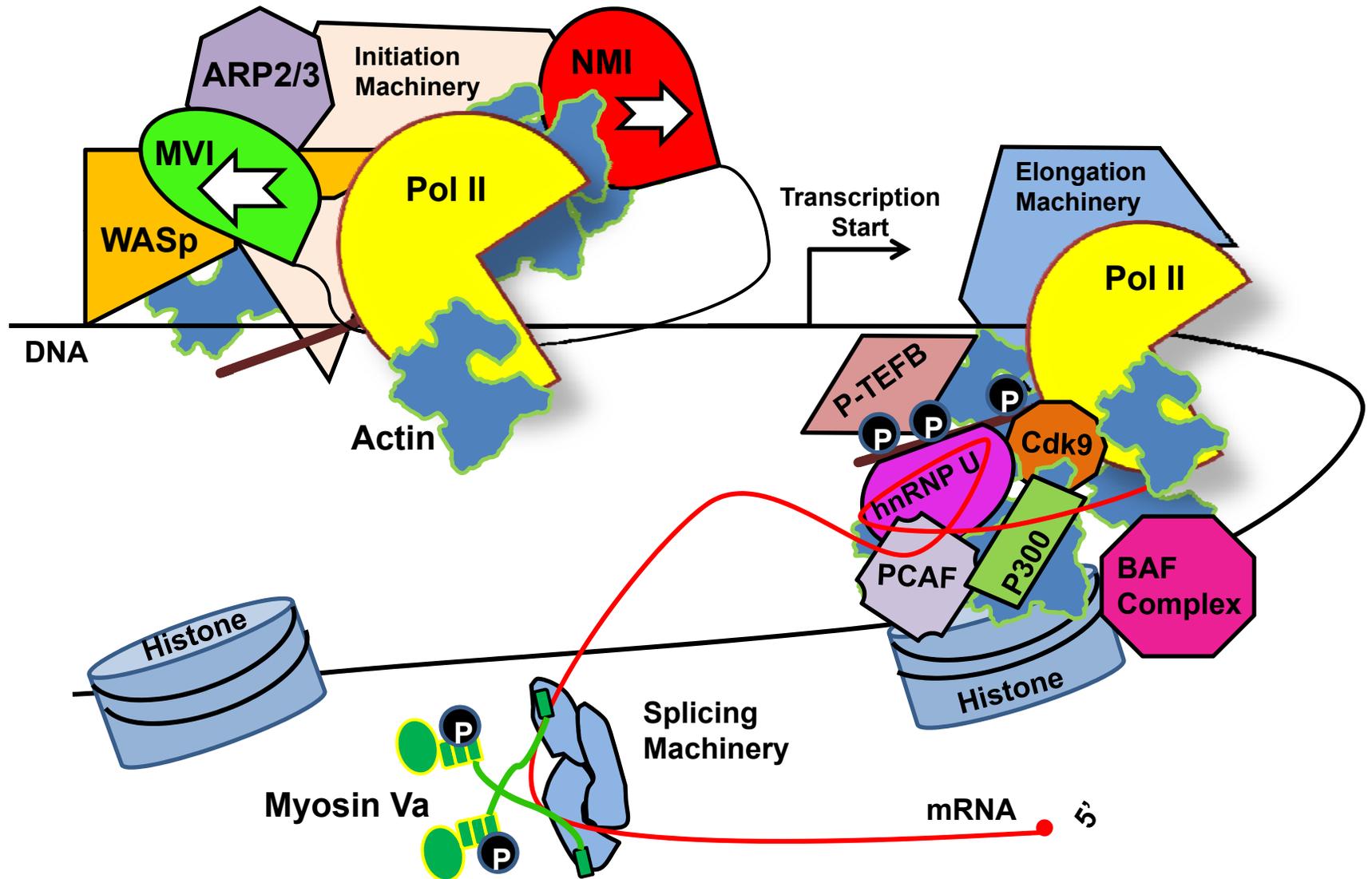


Figure 3b: Actin, Myosins and Interacting Proteins
In Transcription by RNA Polymerase I

