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The nucleoskeleton: lamins and actin are major players in essential nuclear functions

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The nucleoskeleton is composed of many interacting structural proteins that provide the framework for DNA replication, transcription and a variety of other nuclear functions. For example, the type-V intermediate filament proteins, the lamins, and their associated proteins (e.g. Lap2α) play important roles in DNA replication and transcription. Furthermore, actin, actin-related proteins and other actin-associated proteins likewise appear to be important in nuclear functions because they are components of chromatin-remodeling complexes and are involved in mRNA synthesis, processing and transport. Newly described nuclear proteins that contain both actin- and lamin-binding domains could be involved in regulating molecular crosstalk between these two types of nucleoskeletal proteins. This range of activities might help to explain why genetic defects in some of the nucleoskeletal proteins contribute to an ever-expanding list of human diseases.

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Abbreviations

AR-CMT2	autosomal recessive Charcot–Marie–Tooth disorder type 2
Arp	actin-related protein
BAF	barrier to autointegration factor
BAF complex	Brg/hBrm-associated factor
DCM	dilated cardiomyopathy
EDMD	Emery–Dreifuss muscular dystrophy
FPLD	familial partial lipodystrophy
IF	intermediate filament
LA/B/C	lamin A/B/C
LAP	lamin-associated protein
LGMD-1B	limb girdle muscular dystrophy 1B
MAD	mandibuloacral dysplasia
PCNA	proliferating cell nuclear antigen
pol	RNA polymerase
RFC	replication factor C
RNP	ribonucleoprotein
SR	spectrin repeat
XLB3	*Xenopus* lamin B3

Introduction

Over the past few years, there has been an explosion of interest in structural proteins within the nucleus and their roles in regulating essential activities ranging from nuclear assembly and shape to DNA replication and transcription [1–3]. Intriguingly, many of these proteins are members of the same families of cytoskeletal proteins that have captured the interest of the cell biological community for many years. In light of this, we use the term 'nucleoskeleton' as a general descriptor for this growing group of proteins. To date, the most extensively studied nucleoskeletal proteins are the nuclear lamins, which are members of the type-V intermediate filament (IF) protein family, and lamin-associated proteins (LAPs). In addition, nuclear forms of actin, actin-associated proteins, and actin-related proteins (Arps) have recently received a significant amount of attention.

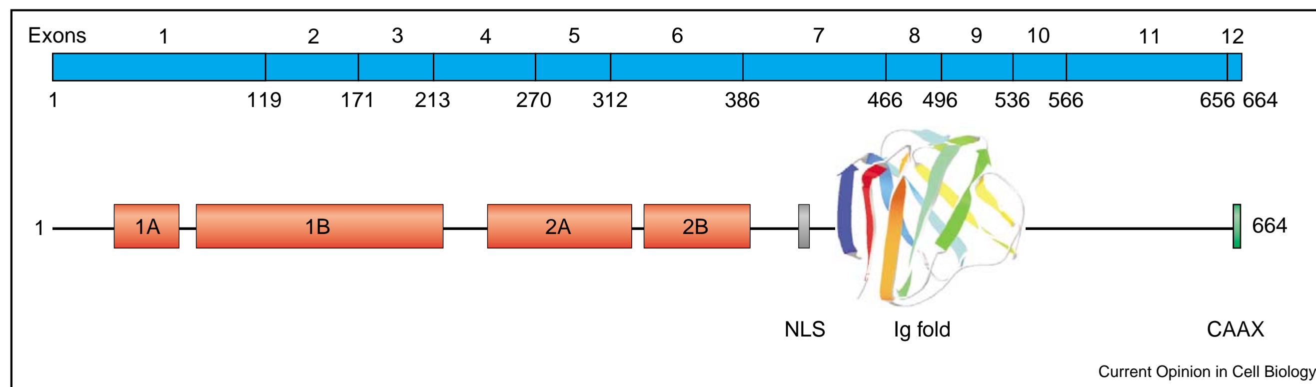
In this review, we first consider the structure and function of the nuclear lamins, then nuclear actin, and in conclusion we speculate about the possible molecular crosstalk between these two nucleoskeletal systems.

The nuclear lamins

The lamins are the major proteins comprising the nuclear lamina, which forms a molecular interface between the inner nuclear envelope membrane and chromatin [4]. In lesser amounts, lamins are also located throughout the nucleoplasm [5]. In humans, there are three genes encoding the lamins, *LMNA*, *LMNB1* and *LMNB2*. There are two major A-type lamin proteins (lamin A [LA] and C [LC]) and two major B-type lamins (LB1 and LB2). All vertebrate cells express at least one B-type lamin, whereas the A-types are developmentally regulated and expressed in differentiated cells [1,6]. Similar to cytoskeletal IF, the lamins consist of a central α-helical rod domain flanked by non-α-helical amino- and carboxy-terminal domains. The carboxyl terminus contains both a nuclear localization sequence and, in the majority of lamins, a highly conserved immunoglobulin fold [7,8••] (Figure 1).

Cytoskeletal IF proteins assemble into around 10 nm filaments both *in vitro* and *in situ*. Similar filaments are rarely seen in electron microscopic studies of the lamina, except in *Xenopus* germinal vesicles where a lattice of 10 nm IF has been reported [9]. In most nuclei, the ultrastructure of the lamina appears amorphous and varies in thickness [10,11] (Figure 2). In one study, using anti-lamin immunogold electron microscopy, a few clusters of

Figure 1



Primary structural elements in lamin A. The 12 exons are indicated along with their corresponding amino acid residues. The central rod domain is identified by the subdomains 1A, 1B, 2A and 2B (red; see [13••]). In addition, the nuclear localization signal (NLS) is shown as a grey box (residues 417–422) and a CAAX sequence is shown at the carboxyl terminus (green [1]). The structure of the immunoglobulin (Ig) fold is shown (residues 436–544 [8••]).

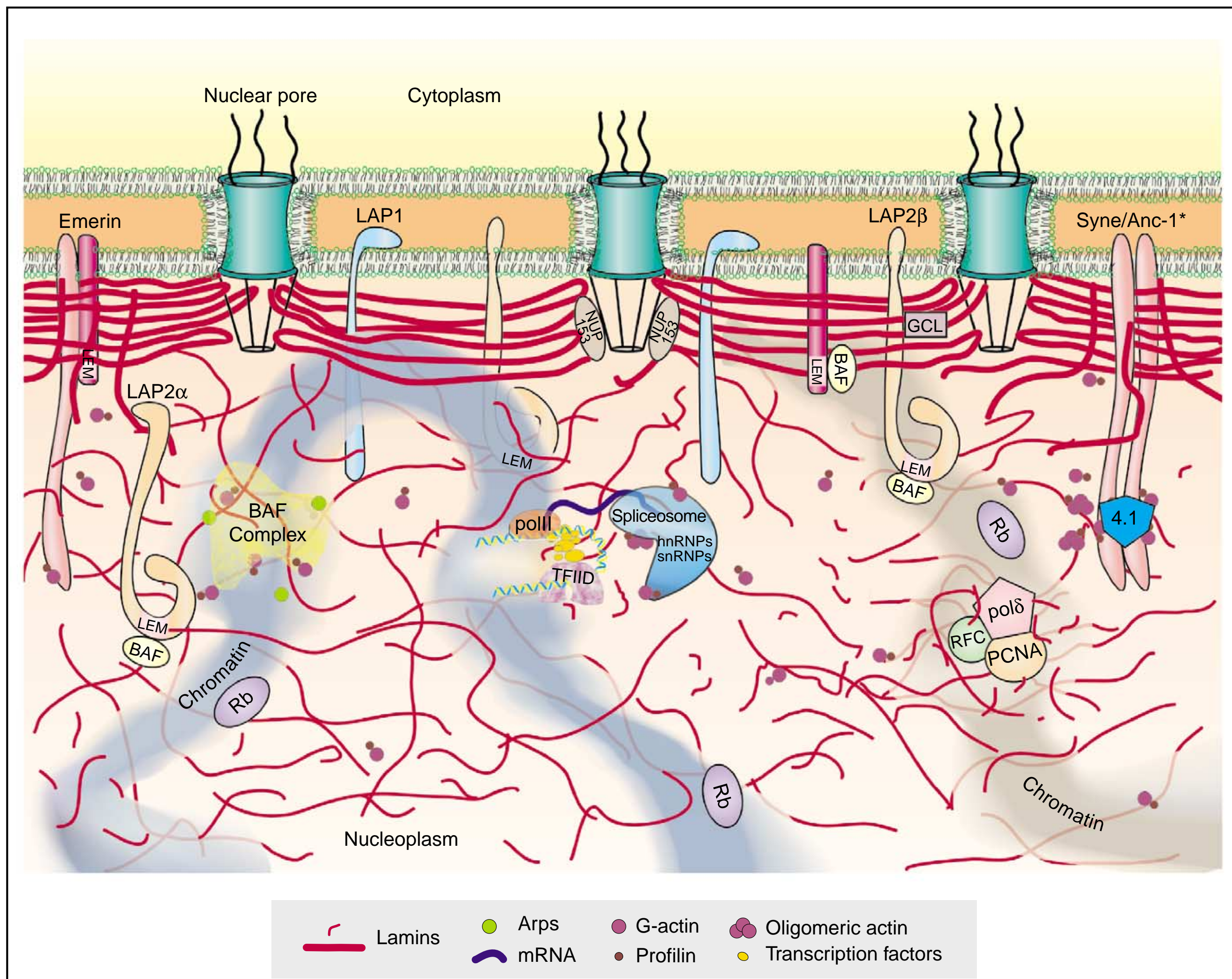
gold particles were seen. Some of these clusters appeared to be associated with filamentous structures of unknown composition [12]. *In vitro* studies have revealed that the basic building block of lamin polymers is a dimer. These dimers interact in a head-to-tail fashion to form protofilaments which aggregate laterally, frequently forming paracrystalline arrays [13••,14]. The inability of most lamins to assemble into 10 nm diameter IFs might be related to the fact that they contain six additional heptads in their central rod domain compared with cytoskeletal IF proteins. Interestingly, it has been shown that the *Caenorhabditis elegans* lamin, which contains only four additional heptads, can form 10 nm filaments *in vitro* [14]. These studies suggest that lamins can assemble into a wide range of structures, and this property might reflect the different functions that have been attributed to the lamins *in vivo*. In addition, there is also evidence that lamins form nucleoplasmic structures. Immunofluorescence and GFP–lamin imaging show that these appear as either tight foci or as relatively general nucleoplasmic fluorescence [5,15]. The nucleoplasmic lamins are assembled into stable structures, as shown by a slow rate of fluorescence recovery after photobleaching (FRAP) in interphase nuclei [5]. FRAP analyses also demonstrate that the lamins in the lamina are even more stable. These findings suggest that the assembly states and functions of the nucleoplasmic and peripheral lamins may be different [5]. Therefore, determining the protein–protein interactions responsible for the polymerization and depolymerization of lamin structures is basic to understanding the central roles that lamins play in many processes. For example, during cell division in most eukaryotic cells, lamin depolymerization is required for nuclear disassembly, and lamin polymerization is required for nuclear reassembly in daughter cells (for reviews, see [1,16]).

Although the lamins are the major structural components of the lamina, there are a growing number of other components present in this region of the nucleus, some of which are known to interact with lamins. These include proteins associated with the inner nuclear envelope, such as the LAP1, LAP2β [17], emerin [18] and Syne1 (also termed 'Myne1' and 'Nesprin') [1,19]. There is evidence that two nucleoplasmic proteins bind lamins, including LAP2α [20] and the retinoblastoma transcription factor pRb [21]. There is also evidence that proteins in the lamina or in the nucleoplasm can link the lamins to chromatin. These include emerin, Lap2α and -β, which share an homologous 40-amino-acid LEM (LAP2, emerin, MAN1) domain that binds the barrier to autointegration factor (BAF). BAF, in turn, can bind directly to DNA, thus linking lamins and chromatin (Figure 2; [1]).

Lamins and DNA replication

There is a significant amount of evidence that lamins play a role in DNA replication. This was first suggested by assembling nuclei *in vitro*, using interphase extracts of *Xenopus* eggs following a reduction in the endogenous lamin B3 (XLB3) by immunodepletion. Under these conditions, DNA synthesis was significantly inhibited [22,23]. Lamins have also been implicated in DNA replication at the cellular level by the finding that DNA replication sites or replication foci co-localize with nuclear lamins. In NIH 3T3 cells, it has been shown that LB co-localizes with replication foci, as visualized by BrdUTP (bromodeoxyuridine triphosphate) incorporation and proliferating cell nuclear antigen (PCNA) staining during mid to late S phase [24]. Interestingly, it has recently been shown that in the early stages of DNA synthesis of human WI38 cells, replication foci are associated with LA [25] (Figure 2). However, in other cells, such as HeLa, some

Figure 2



A representation of the nucleus showing the location of lamins and actin and some of their proposed interactions. Lamins are represented by thick red lines near the nuclear envelope, indicating a concentration in the lamina, and as thin red lines throughout the nucleoplasm. Some inner nuclear membrane lamin-binding proteins are shown (LAP1, LAP2β, emerin and Syne/Anc-1*). An example of a lamin interaction with nuclear pores is shown for NUP153. Proposed functional roles for lamins in large complexes are indicated in the two chromosome domains (indicated in blue and light brown). Lamins are associated with both the DNA replication complex containing DNA polymerase δ, RFC and PCNA; the transcription complex containing pol II and TFIID; and spliceosomes. The interaction of actin with the spliceosome through heterogeneous ribonucleoproteins (hnRNPs) and small nuclear ribonucleoproteins (snRNPs) is also shown. The proposed functions of actin and Arps in chromatin remodeling is indicted by its interaction with the BAF complex (large yellow area). LAP2 and emerin share a ~40-residue homology domain called LEM (pink box) that binds to BAF, the barrier to autointegration factor. Through BAF, lamins can interact with chromatin. Protein 4.1 is shown binding to dimerized Syne/Anc-1* and actin. Syne/Anc-1* is also shown to form a complex with emerin, indicating another potential link between the lamina and actin. (*) Syne/Anc-1 has many names in the published literature, including Myne, Nesprin and NUANCE, and is related to a *Drosophila* protein MSP300. (See text for full details.) This figure is adapted from [1], and reproduced with permission from Cold Spring Harbor Laboratory Press, copyright © 2002.

investigators report that there is no precise co-localization between replication foci and lamins, although they do lie in close proximity to each other [26]. Perhaps these different patterns of lamins and replication foci reflect differences in the cell types used for these studies.

In light of the difficulties using fixed and stained cells for light microscopy, other approaches have been developed for studying the role of lamins in DNA replication. For example, the addition of dominant-negative mutant lamins to *Xenopus* nuclei assembled *in vitro* has provided an opportunity to correlate the role of lamins in DNA synthesis using structural and biochemical techniques. The mutant lamins used consisted of amino-terminal deletions of XLB3 or human LA. When added during assembly or after nuclei have assembled, lamin organization is

disrupted as demonstrated by a loss of a prominent lamina and the induction of lamin aggregates within the nucleoplasm. Under these conditions, DNA synthesis is inhibited >95% and PCNA and replication factor C (RFC), two known replication factors, co-localize with the lamin aggregates [27,28] (Figure 2). This disruption does not affect the distribution of the replication initiation factors, DNA polymerase α, ORC2 or MCM3 [28]. Taken together, these results suggest that lamins play a role in organizing or assembling a nucleoplasmic scaffold upon which the active elongation process of DNA replication takes place.

In contrast, some investigators have suggested that lamins may not be required for DNA replication. For example, a highly concentrated nucleoplasmic extract of *Xenopus* nuclei, devoid of nuclear envelopes and a lamina, was capable of supporting DNA replication [29]. However, in intact nuclei, there is little doubt that lamins and their normal assembly states are required for DNA replication, as demonstrated by the experiments described above. The lamins might therefore play a critical role in providing a platform to regulate the location and timing of events that occur during S phase in mammalian cells [30].

Lamins and transcription

The change in lamin expression that is concurrent with the onset of cell differentiation provided the first clue that lamins might be involved in transcription. For example, in *Xenopus*, XLB3 is the only lamin found in developing embryos until the mid-blastula transition, when XLB1 is translated from maternal-stored mRNA. This is coincident with the initiation of RNA polymerase II (pol II)-dependent transcriptional activity. Furthermore, the expression of XLB2 increases dramatically during gastrulation [31,32]. In mammalian systems, such as the mouse, A-type lamins are not expressed until cells and tissues begin to differentiate [33], while B-type lamins are expressed throughout development [34]. In light of these findings, it is surprising that the $LMNA^{-/-}$ mouse appears to develop normally for two to three weeks after birth before exhibiting a wide range of deleterious phenotypes resulting in death by around week eight [35] (see below).

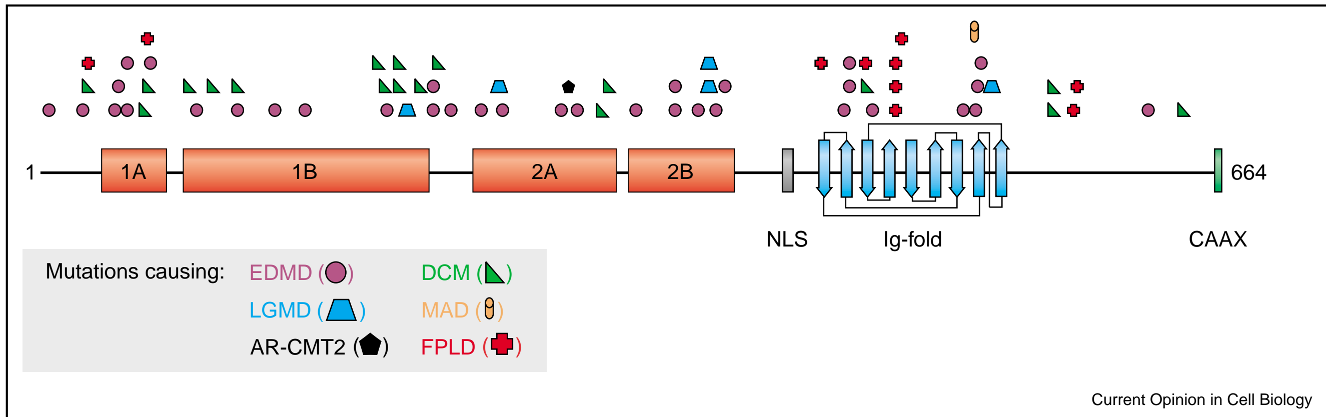
More direct evidence in support of a role for LA and LC in transcription is through their binding to the transcription factor pRb, as demonstrated by blot overlay and immunoprecipitation experiments [21,36]. There is evidence that pRb also binds to LAP2α [37], suggesting that *in vivo*, a complex containing a LAP, LA and pRb is involved in regulating transcription. In addition, the LB1 binding partner [17], LAP2β, has been shown to interact with the vertebrate transcription factor mGCL (mammalian germ-cell-less) [38] (Figure 2). Other evidence supporting a role for lamins in transcription comes from studies using dominant-negative mutants to disrupt normal lamin assembly and organization in *Xenopus* and mammalian nuclei. Under these conditions, there is a significant inhibition of pol II activity, but not pol I or pol III activity. Within these lamin-disrupted nuclei, there is also an alteration in the organization of the splicing factors B″ and Y12, but not the gene-specific transcription factor Sp1. Interestingly, TATA binding protein (TBP) co-localizes with the lamin aggregates that form in lamin-disrupted nuclei [39••]. This result is further supported by findings with an unusual LA/C monoclonal antibody (LA-2H10), which exclusively co-localizes with speckles or splicing-factor compartments, and does not stain the lamina by immunofluorescence [40,41••] (Figure 2). The reactivity of this unusual antibody suggests that it recognizes a structural epitope of lamins that is only available in the region of splicing-factor compartments, but not in the lamina or elsewhere in the nucleoplasm. Taken together, the available data suggest that lamins and their binding partners play important roles in gene regulation.

Linking lamins to human diseases

Many interesting possibilities for lamin functions are being uncovered through patients with mutations in *LMNA*. To date, there are around 124 mutations (G Bonne, personal communication) in *LMNA* that cause laminopathies [1] corresponding to six disease categories: Emery–Dreifuss muscular dystrophy (EDMD); dilated cardiomyopathy (DCM); familial partial lipodystrophy (FPLD); mandibuloacral dysplasia (MAD); autosomal recessive Charcot–Marie–Tooth disorder type 2 (AR-CMT2); and limb girdle muscular dystrophy 1B (LGMD-1B) (Figure 3). The age of onset and the symptoms of these diseases varies over a wide range, but there are overlapping similarities, including muscle wasting, locomotory problems, fat redistribution, and cardiovascular problems. The $LMNA^{-/-}$ mice (see above) also exhibit signs of muscular dystrophy, including an abnormal gait, weak forelimbs and a loss of skeletal muscle mass soon after birth. In addition, cultured $LMNA^{-/-}$ fibroblasts have abnormal nuclear shapes. These appear as blebs in the region of the nucleus characterized by a reduction in LB, LAP2β and the nuclear pore protein NUP153 [35]. Virtually identical alterations are seen in the nuclei of fibroblasts grown from biopsies of FPLD patients [42]. Similar nuclear blebs or herniations have also been related to the presence of HIV-1 protein Vpr, which causes cells to arrest in the G2 phase of the cell cycle. These blebs are devoid of nuclear lamins and pore complexes. Occasionally, the blebs rupture, permitting cell cycle regulatory factors to exchange between the nucleus and the cytoplasm, and in this fashion Vpr may cause G2 arrest [43••].

Analyses of the effects of the lamin mutations in normal cells have been carried out using transient transfection with LA cDNAs carrying mutations found in various laminopathies. For example, human LA mutations mimicking EDMD in the α-helical central rod domain frequently cause a dramatic reorganization of the lamins into nucleoplasmic aggregates, whereas mutations in the

Figure 3



Locations of the published LA mutations that cause human diseases. There are six known categories of diseases: EDMD (circles), DCM (triangles), LGMD (trapezoid), MAD (cylinder), AR-CMT2 (pentagon) and FPLD (cross). The distribution of mutations across the entire protein chain illustrates that there is no obvious correlation between the site of a mutation and the clinical diagnosis. In fact, it has been reported that when residue 527 is mutated to phenylalanine, it causes EDMD, whereas a mutation to histidine causes MAD. Most of the mutations are missense, but there are a few nonsense mutations (Q6X, E111X, R225X and Y259X), frameshifts (321 FS, 386 FS, 466 FS, 536 FS and 571 FS) and deletions (ΔE112, R196S + ΔLQT 197–199, ΔK208 and ΔK261).

non-α-helical domain do not appear to significantly alter the organization of the endogenous lamin network [44,45]. The wide range of phenotypes that accompany LA mutations leaves little doubt that lamins play a central role in many cellular functions, most likely through interactions with other proteins, such as LAPs, to modify chromatin organization and gene expression.

Nuclear actin and actin-binding proteins in the nucleus

Reports of nuclear actin were initially viewed with great scepticism, as many cell biologists believed that it was simply a cytoplasmic contaminant [2]. Today, a variety of structural and functional roles have been attributed to nuclear forms of actin. Under normal conditions, nuclear actin does not appear to form filaments, indicating that it is probably in its monomeric, or G-actin, form. This is supported by a lack of staining for F-actin with fluorescent phalloidin [46] and the finding that a monoclonal antibody (2G2), which recognizes only G-actin, yields a punctate nuclear-staining pattern [47] (Figure 2). Electron microscopy shows G-actin associated with the nucleoplasmic filaments located adjacent to nuclear pore complexes.

Antibody staining further indicates that actin is associated with nascent Balbiani ring mRNA transcripts on the polytene chromosomes of the dipteran *Chironomus tentans*. Nuclear actin remains associated with the mature mRNA particle (the Balbiani ring granule) as it moves through the nuclear pore to become associated with polysomes [48••]. In rat liver nuclei, actin has also been identified in pre-messenger ribonucleoprotein (RNP) complexes containing 40s heterogeneous RNP (hnRNP) particles [49], and actin antibodies can inhibit nuclear export of RNA [50]. Nuclear actin also is an integral component of complexes involved in modifying or remodeling chromatin [3]. For example, the chromatin remodeling BAF complex (Brg- or hBrm-associated factor; not to be confused with the other nuclear BAF, barrier to autointegration factor [1]) contains tightly bound β-actin, the actin-related protein BAF53, and numerous other proteins [51] (Figure 2). These results suggest that nuclear actin might play a critical role in mRNA processing and transport.

Nuclear forms of actin-related proteins and chromatin remodeling

Arps were first discovered in the cytoplasm where they play an important role in regulating the nucleation and branching of actin filaments [52]. There is now evidence that Arps are also components of nuclear complexes. For example, the BAF53 subunit of the BAF complex (see above) is homologous to Arp3; the yeast SWI/SNF chromatin-remodeling complex contains Arps 7 and 9; the yeast Act3p/Arp4 protein binds to histones; the *Drosophila* HP1 heterochromatin regulatory factor co-localizes with Arp 4 [46]; and isolated human nucleoli appear to contain Arps 2 and 3 [53]. Chromatin-remodeling complexes use the energy of ATP hydrolysis to render nucleosomal DNA more accessible to a variety of factors. It is conceivable that actin and Arps function to regulate the remodeling process [54].

Other actin-binding proteins

A novel form of myosin I that localizes to the nucleus has a unique 16-residue amino-terminal extension that differentiates it from the cytoplasmic forms of myosin. An

antibody directed against this myosin blocks RNA synthesis *in vitro* and appears to co-localize with pol II in HeLa cells [55]. However, there is no evidence that nuclear actin and myosin interact in a functional complex.

Profilin, originally described as a cytoplasmic actin-binding protein, has recently been identified in the nucleus. Purified chicken antibodies directed against human profilin I label nuclei in a speckle-like pattern [56••]. These speckles show extensive co-localization with the small nuclear RNP (snRNP)-associated Sm proteins and with Cajal bodies. Following treatment of cells with actinomycin D, profilin and snRNPs reorganize together into larger aggregates but profilin no longer co-localizes with Cajal bodies. These results suggest that profilin might play a role in pre-mRNA processing. Furthermore, *in vitro* this same profilin antibody interferes with RNA splicing, suggesting that actin–profilin complexes are functional components of the spliceosome [56••].

Cytoplasmic profilin binds tightly to G-actin and facilitates nucleotide exchange. Upon binding a specific inositol polyphosphate, phosphatidylinositol 4,5-bisphosphate PtdIns(4,5)P_2, the ATP-containing G-actin is released to membrane-associated sites of actin polymerization [57]. There is increasing evidence that inositol polyphosphates may also regulate nuclear functions. For example, PtdIns(4,5)P_2 has been localized to nuclear speckles [58], which resemble interchromatin granule clusters [59], and this molecule appears to be necessary for pre-mRNA splicing *in vitro* [59]. Furthermore, two recent papers provide evidence that nuclear inositol polyphosphates could also regulate chromatin-remodeling complexes [60,61]. The presence of profilin, actin and inositol polyphosphate in the nucleus raises the interesting possibility that lipid-derived signaling molecules might regulate gene expression by modulating the interactions of profilin with actin and/or proline-rich binding partners.

There have been numerous recent reports describing isoforms of protein 4.1 and novel nuclear proteins characterized by spectrin repeats (SRs). These proteins were originally described as components of the red blood cell membrane-associated cytoskeleton. In that system, protein 4.1 links small actin oligomers with spectrin. When *Xenopus* interphase extracts have been depleted of the nuclear isoform of protein 4.1, normal nuclear assembly is inhibited [62••]. The nuclear SR-containing proteins, Syne-1 and -2, were originally identified in skeletal muscle cells as components of the nuclear envelope [63]. Subsequently, these proteins and various splice variants were found by other groups. These were named Myne-1 [19], Nesprin-1 and -2 [64,65••], and NUANCE [66]. Similar proteins have been identified in *C. elegans* (ANC-1) [67] and in *Drosophila* (MSP-300) [68]. Recently, it has been suggested that this family of proteins be called the Syne/ANC-1 proteins [69]. The largest forms of these proteins are enormous and could contain up to 12,000 amino acids. It is likely that even more proteins will be identified — each of these genes has a very large number of potential splicing sites [65••,66]. These proteins also contain a carboxy-terminal transmembrane domain that presumably anchors the protein to the nuclear envelope [65••,66]. In further support of the nuclear envelope association, numerous antibodies directed against these SR proteins generate intense nuclear staining patterns. Since these staining patterns are similar to LA immunofluorescence patterns, they appear to be inner nuclear membrane components. It has also been shown that these proteins co-immunoprecipitate with LA, and there is evidence that the carboxy-terminal half of Nesprin-1α directly binds to LA [65••,70] (Figure 2). Interestingly, some of these proteins contain a calponin homology domain, which is known to bind to actin [65••,66].

Conclusions: Perhaps lamin and actin interact in the nucleus

The finding that Syne/Anc-1* (or whatever name(s) the workers in this field finally decide upon) contain lamin- and actin-binding domains raises the interesting possibility that these proteins serve to link the inner nuclear membrane, the lamins comprising the lamina and nuclear actin. These SR proteins might bind to monomeric or short oligomeric forms of actin, and this binding could be stabilized by nuclear protein 4.1, similar to the situation in the red blood cell membrane cytoskeletal system. Despite the evidence suggesting that the major form of nuclear actin is monomeric, it is possible that very short actin filaments are present in the nucleus but do not stain with phalloidin because they are bound to other proteins. For example, cofilin can change the twist of F-actin, thereby preventing the binding of phalloidin [71].

Many of the components originally defined as cytoskeletal have counterparts in the nucleoskeleton. It is also known that there are bridging elements that connect IFs with actin. For example, the IF-associated protein plectin can form bridges between vimentin and actin filaments, thereby providing a potential mechanism for coordinating the activities of these two cytoskeletal systems. There is also evidence that suggests that a form of plectin is located at the nuclear surface and that it binds to LB [72]. The recent finding that members of the Syne/Anc-1*/Nesprin/NUANCE family bind to LA and contain an actin-binding domain lends further support to the existence of bridging molecules [19,65••,70]. It is worth mentioning that the nuclear form of myosin could also interact with both actin and lamins, as it has recently been found that myosin Va is a neural IF-associated protein [73]. To continue this speculation, it is possible that the type-V IF proteins, the lamins, and the nuclear forms of actin could be structurally and functionally linked by a variety of different molecular mechanisms. Such molecular crosstalk could provide a

dynamic structural framework involved in facilitating essential nuclear functions, including DNA replication and transcription, most likely through lamin-binding proteins and Arps. Therefore, it is not surprising that mutations in the nucleoskeletal proteins, especially the lamins, can cause a variety of human diseases. It is therefore essential to learn more about the structure and function of the individual nucleoskeletal systems and the molecular crosstalk that governs their activities.

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- •• of outstanding interest

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