Lamina-Independent Lamins in the Nuclear Interior Serve Important Functions

T. DECHAT, K. GESSON, AND R. FOISNER

Max F. Perutz Laboratories, Department of Medical Biochemistry, Medical University of Vienna, Vienna 1030, Austria

Correspondence: roland.foisner@meduniwien.ac.at

Nuclear lamins were originally described as the main constituents of the nuclear lamina, a filamentous meshwork closely associated with the inner nuclear membrane. However, within recent years, it has become increasingly evident that a fraction of lamins also resides throughout the nuclear interior. As intermediate-filament-type proteins, lamins have been suggested to fulfill mainly structural functions such as providing shape and mechanical stability to the nucleus. But recent findings show that both peripheral and nucleoplasmic lamins also have important roles in essential cellular processes such as transcription, DNA replication, cell cycle progression, and chromatin organization. Furthermore, more than 300 mutations in the gene encoding A-type lamins have been associated with several human diseases now generally termed laminopathies and comprising muscular dystrophies, lipodystrophies, cardiomyopathies, and premature aging diseases. This review focuses on the lamina-independent pool of lamins in the nuclear interior, which surprisingly has not been studied in much detail so far. We discuss the properties and regulation of nucleoplasmic lamins during the cell cycle, their interaction partners, and their potential involvement in cellular processes and the development of laminopathies.

The nucleus of an eukaryotic cell can be roughly divided into two major compartments: the nuclear envelope (NE), which is composed of the outer and inner nuclear membrane (ONM and INM, respectively), nuclear pore complexes (NPCs) and nuclear lamina, and the nucleoplasm, which contains the chromatin, various structural subcompartments, and soluble nuclear components (for review, see Pederson 2010). In contrast to the well-characterized cytoskeletal framework in the cytoplasm, consisting of actin filaments, intermediate filaments (IFs), and the microtubules, still little is known about the structural scaffold within the nucleus, often termed the nucleoskeleton. The best-studied component of the nucleoskeleton is a filamentous protein meshwork at the nuclear periphery, the nuclear lamina (for review, see Gruenbaum et al. 2005), but the structural organization of the nuclear interior is poorly understood. The main components of the nuclear lamina and probably also of the internal nucleoskeletal structures are type-V IF proteins, the nuclear lamins (Gerace and Blobel 1980; Aebi et al. 1986; Hozak et al. 1995; Barboro et al. 2002).

Nuclear lamins are classified into A and B types, based on sequence homologies, expression patterns, and structural, biochemical, and dynamic properties (for reviews, see Broers et al. 2006; Verstraeten et al. 2007; Dechat et al. 2010). All A-type lamins in mammals, of which the major isoforms are lamin A and C, are derived from a single gene, *LMNA*, by alternative splicing. In contrast, the two major B-type lamin isoforms in mammals, lamin B1 and B2, are encoded by different genes, *LMNB1* and *LMNB2*, respectively. Whereas at least one B-type lamin is expressed in all metazoan cells, A-type lamins are found only in vertebrates (with the exception of *Drosophila melanogaster*, which also

expresses an A-type lamin), and their expression is developmentally regulated (for reviews, see Melcer et al. 2007; Dechat et al. 2008). Regarding the latter, A-type lamins, for example, are not expressed in human and mouse embryonic stem cells (Constantinescu et al. 2006; Butler et al. 2009) and during mouse embryogenesis until day 10–12 (Stewart and Burke 1987; Rober et al. 1989). In particular, A-type lamins have been linked to various diseases that are collectively called laminopathies and include skeletal muscle diseases (e.g., Emery-Dreifuss muscular dystrophy [EDMD]), cardiomyopathies, lipodystrophies, and premature aging syndromes (e.g., Hutchinson-Gilford progeria syndrome [HGPS]) (for review, see Worman et al. 2010).

On the molecular level, lamins display the typical tripartite structure common to IFs, consisting of an α -helical rod domain, a globular amino-terminal head, and a carboxy-terminal tail domain (Fig. 1) (for review, see Herrmann et al. 2009). Within their amino terminus, lamins contain a nuclear localization signal (NLS) located right after the rod domain (Loewinger and McKeon 1988) and a structural motif similar to an S-type immunoglobulin (Ig) fold (Dhe-Paganon et al. 2002; Krimm et al. 2002). In addition, lamin A and B-type lamins possess a CAAX box at their carboxy-terminal end that leads to their excessive posttranslational processing (for review, see Rusinol and Sinensky 2006). This processing involves the farnesylation and carboxymethylation of the cysteine residue and removal of the -AAX tripeptide and appears to be important for the close association of lamins with the INM (Krohne et al. 1989; Rusinol and Sinensky 2006; Dechat et al. 2007). Although B-type lamins remain farnesylated and carboxymethylated and are thus tightly membrane associated, lamin A is further processed by the

Disease-causing lamin A/C mutations

Posttranslational modifications of lamin A/C

Identified binding domains within lamin A/C

Figure 1. Molecular domain organization of prelamin A and lamin C. (NLS) Nuclear localization signal. The CAAX motif is post-translationally modified. (Arrow) Site of the last proteolytic cleavage by Zmpste24/FACE1. (White) Lamin A- and (black) lamin C-specific regions at the carboxyl terminus. Shown are the position of disease-causing mutations (*top*) and posttranslational modifications (*bottom*) as well as reported interaction domains for lamin A/C-binding partners (*bottom*) within the lamin polypeptide. Mutation sites are derived from the UMD-LMNA database (http://www.umd.be/LMNA/W_LMNA/gene.shtml). Shown are only missense mutations, in-frame deletions and insertions, and splice mutations leading to HGPS. Data for modifications were obtained from the UniProt database (<http://www.uniprot.org/>). (*Bottom*) Interaction partners were grouped into proteins involved in (continuous frames) gene expression, signaling, and cell cycle control; (dashed frames) chromatin organization; and (dotted frames) other processes. See text for details and references.

zinc metalloprotease Zmpste24/FACE1 (Corrigan et al. 2005). In this last step, an additional 15 amino acids are cleaved off of the carboxyl terminus of lamin A, including the farnesylated/carboxymethylated cysteine residue. Therefore, lamin A can also be found in a membrane-independent nucleoplasmic pool (see below).

On the structural level, lamins form parallel and in-register coiled-coil dimers that further associate in a head-to-tail fashion to form polar polymers (Heitlinger et al. 1991; Stuurman et al. 1998). In vitro, these polymers assemble into higher-order structures including ~10-nm filaments and paracrystalline structures (Heitlinger et al. 1991; Stuurman et al. 1996; Karabinos et al. 2003; Foeger et al. 2006; Ben-Harush et al. 2009). However, little is known about the assembly, composition, and structure of lamin polymers in cells, both at the NE and in the nuclear interior. Within past years, increasing evidence has accumu-

lated that nucleoplasmic lamins are not only assembly intermediates before their integration into the peripheral nuclear lamina but they also represent a distinct pool of lamins that is tightly regulated and serves important functions in many cellular processes. In this review, we focus on this newly emerging concept and discuss the structure, regulation, dynamics, and functions of the lamina-independent pool of lamins. We also discuss the role of lamin-binding proteins in the regulation of the structure and function of nucleoplasmic lamins.

STRUCTURE AND DYNAMICS OF NUCLEOPLASMIC LAMINS

Unlike cytoplasmic IFs, which are well characterized by various light and electron microscopy methods (Svitkina et al. 1995; Eriksson et al. 2009), lamin structures in the nu-

cleus have only been analyzed and microscopically visualized at higher resolution in a few studies. At the nuclear periphery, the reported lamina structures range from a regular meshwork of ~10- to 15-nm filaments in *Xenopus* oocyte germinal vesicles and in vitro assembled sperm pronuclei (Aebi et al. 1986; Zhang et al. 1996; Goldberg et al. 2008) to more irregular meshworks described in mammalian cells (Belmont et al. 1993; Schermelleh et al. 2008; Shimi et al. 2008). These meshworks are most likely formed by separate A- and B-type lamin networks that overlap and interact with one another (for review, see Dechat et al. 2010). The structural properties, assembly state, and composition of intranuclear lamins, however, remain an enigma. Although a few ultrastructural studies suggest lamin filaments or a lamin-containing lattice throughout the nuclear interior (Hozak et al. 1995; Barboro et al. 2002, 2010), indirect immunofluorescence microscopy and analyses of green fluorescent protein (GFP)-tagged lamins in cells reveal either a uniformly nucleoplasmic lamin "veil" or intranuclear lamin foci (Bridger et al. 1993; Moir et al. 1994, 2000; Broers et al. 1999; Kennedy et al. 2000; Muralikrishna et al. 2004; Naetar and Foisner 2009). Regarding the latter, such foci may not be mistaken with invaginations of the nuclear membrane described in cultured cells (Fricker et al. 1997; Broers et al. 1999).

Fluorescence recovery after photobleaching (FRAP) and fluorescence correlation spectroscopy (FCS) analyses of ectopically expressed GFP-tagged lamins in cells revealed a much higher mobility of nucleoplasmic versus peripheral A-type lamins, which are more or less immobile, indicating that A-type lamins within the nuclear interior reside in a lower assembly state compared to those integrated in the nuclear lamina (Broers et al. 1999; Moir et al. 2000; Shimi et al. 2008). In addition, FCS data demonstrate that within the nucleoplasm, B-type lamins are less mobile, whereas the majority of A-type lamins reside in two mobile fractions, one fast and one slow moving (Shimi et al. 2008). The estimated molecular masses of these mobile fractions range from ~1.3 MDa (fast) to ~0.8–2.9 GDa (slow). Interestingly, the slow-moving lamin A fraction appears to be dependent on the presence of lamin B1, because down-regulation of lamin B1 leads to a twofold increase in the mobility of this fraction. However, it is not clear if these mobile fractions represent polymers exclusively composed of lamins or lamin-containing multiprotein complexes. Further support for the association of nucleoplasmic A-type lamins with more dynamic structures that reside in lower assembly states comes from their extractability following detergent treatment of cells (Moir et al. 2000; Muralikrishna et al. 2004; Naetar et al. 2008).

The most dramatic changes in the dynamic behavior of lamins occur during mitosis. To facilitate NE breakdown (NEBD) and subsequent disassembly of the nucleus in metazoan cells, the lamin meshworks are depolymerized (Heald and McKeon 1990; Collas 1998; Panorchan et al. 2004). This is achieved by sequential phosphorylation of lamins at specific sites by various mitotic kinases including cyclin-dependent kinase (Cdk) 1 and protein kinase C (PKC) (Heald and McKeon 1990; Peter et al. 1990; Ward and Kirschner 1990; Fields and Thompson 1995; Collas 1999; Kuga et al. 2010). During this process, A-type lamins

become solubilized and first accumulate within the nuclear interior and later, following NEBD, disperse throughout the cytoplasm (Georgatos et al. 1997; Dechat et al. 2007). In contrast, B-type lamins remain associated with the NE until NEBD and subsequently become distributed throughout the cytoplasm, partly soluble and partly membrane associated (Meier and Georgatos 1994; Georgatos et al. 1997; Beaudouin et al. 2002; Dechat et al. 2007). A- and B-type lamins also behave differently during nuclear reassembly at the end of mitosis. Whereas B-type lamins relocalize exclusively to the newly forming NE, A-type lamins initially relocalize to a spatially defined structure at the surface of the segregating sister chromatids, called "core" region, and subsequently accumulate within the nuclear interior (Broers et al. 1999; Moir et al. 2000; Dechat et al. 2004, 2007; Haraguchi et al. 2008). During G₁ phase, A-type lamins are detected as mobile, unpolymerized components within the nucleoplasm and are gradually integrated into a stable polymer at the nuclear periphery as cells progress through G₁ (Moir et al. 2000). Thus, A-type lamins, in particular, are found in the nuclear interior in G₁ phase of cycling cells (Bridger et al. 1993; Dechat et al. 2000b). In contrast, in S-phase cells and during in vitro differentiation of myoblasts into myotubes, the nucleoplasmic pool of A-type lamins is greatly reduced (Muralikrishna et al. 2001; Markiewicz et al. 2005; Naetar and Foisner 2009). Importantly, the nucleoplasmic localization of A-type lamins has also been detected within whole tissues (Naetar et al. 2008), showing that nucleoplasmic lamins are not just an artifact of in vitro cell cultures. Although proliferating progenitor cells in the mouse epidermis contain nucleoplasmic A-type lamins, nonproliferating, differentiated cells in the suprabasal layers of the epidermis are depleted of the nucleoplasmic lamin pool.

In addition to A-type lamins, B-type lamins have also been reported to localize in the nuclear interior either in nucleoplasmic foci, where they colocalize with sites of DNA replication (Moir et al. 1994), or are diffusely distributed (Moir et al. 2000). In contrast to the mobile nucleoplasmic A-type lamins, B-type lamins residing within the nuclear interior are immobile, similar to those integrated in the nuclear lamina, and therefore suggested to be associated with more stable structures (Moir et al. 2000; Shimi et al. 2008).

REGULATION OF NUCLEOPLASMIC LAMINS

The fact that A-type lamins accumulate in the nuclear interior at the end of mitosis and during G₁ phase and that nucleoplasmic lamin A was detected in tissues only in proliferating progenitor cells leads to the hypothesis that the nucleoplasmic pool of lamin A represents intermediates of lamin A complexes during their assembly into the lamina after mitosis (Fig. 2). In this scenario, the lamina-independent pool of lamins in G₁ phase is generated in the preceding mitosis, but there are also other possibilities. For example, newly synthesized prelamin A initially localizes in the nuclear interior after its import into the nucleus (Goldman et al. 1992; Lutz et al. 1992; Dyer et al. 1999) and is posttranslationally modified at its carboxyl terminus with a hydrophobic farnesyl residue. The latter

Figure 2. Potential regulation of nucleoplasmic lamins. Mitotically solubilized, mature lamin and newly synthesized prelamins A are imported into the nucleus through NPCs. Prelamin A is processed and finally integrated into the lamina. Mature lamin either polymerizes into the lamina or is retained in the nucleoplasm with the help of LAP2 α and, likely, specific modifications. Nucleoplasmic LAP2 α -lamin complexes serve important functions in gene expression and chromatin organization. (ONM) Outer nuclear membrane, (INM) inner nuclear membrane.

leads to its translocation to the INM, where the last 15 amino acids containing the farnesyl group are cleaved off by Zmpste24/FACE1, a protease residing in the ER and INM (Lehner et al. 1986; Lutz et al. 1992; Sinensky et al. 1994; Barrowman et al. 2008). Fully processed, mature lamin A is then assembled into the lamina (Fig. 2). Therefore, newly synthesized lamin A within the nuclear interior most likely comprises prelamins A and farnesylated prelamins A, but not mature lamin A. The half-life of these two prelamins A forms in the nucleoplasm may range from less than 30 min to 3 h (Lehner et al. 1986; Goldman et al. 1992; Lutz et al. 1992), and it is unclear whether their nucleoplasmic localization is only related to posttranslational processing or also specific cellular functions.

The third possibility is a dynamic exchange of lamin subunits between the filamentous lamina and nucleoplasmic pool (Fig. 2). This mechanism has been previously demonstrated for cytoplasmic keratin and vimentin IFs (for review, see Eriksson et al. 2009), but has not yet been proven experimentally for lamins.

Independent of which of these pathways is relevant for lamins in the nucleoplasm, the lamina-independent pool has to be regulated in some way. What keeps a certain fraction of A-type lamins in the nucleoplasm in G₁ phase and prevents it from rapid polymerization and incorporation into the lamina? First insights into the potential regulation of nucleoplasmic lamins come from a recent study on a lamin-binding protein termed lamina-associated polypeptide (LAP)2 α (Naetar et al. 2008).

LAP2 α is one of six splice variants in mammals encoded by the *LAP2* gene (Harris et al. 1995). Although most LAP2 isoforms are transmembrane proteins of the INM interacting with B-type lamins, LAP2 α localizes throughout the nuclear interior, because it misses a transmembrane domain, and interacts specifically with A-type lamins in vitro and in situ (see Dechat et al. 2000b; for review, see Dechat et al. 2000a). Genetic ablation of *Lap2 α* in mice reveals that the nucleoplasmic pool of A-type lamins is lost in proliferating primary fibroblasts derived from LAP2 α -deficient mice (Fig. 3) as well as in proliferating progenitor cells in the epidermis of LAP2 α -deficient mice (Naetar et al. 2008). Importantly, expression of LAP2 α , but not of a LAP2 α mutant deficient in lamin A/C binding, can rescue the nucleoplasmic pool of A-type lamins in the LAP2 α -deficient fibroblasts. In addition, the siRNA-mediated knockdown of LAP2 α in primary human dermal fibroblasts also led to a decrease in nucleoplasmic lamins (Pekovic et al. 2007). These results led to the hypothesis that LAP2 α is essential and sufficient for targeting or stabilizing lamins A/C within the nuclear interior (Fig. 2). In line with this hypothesis is the finding that following myoblast differentiation, the loss of nucleoplasmic lamins goes hand in hand with a down-regulation of LAP2 α (Markiewicz et al. 2005).

It is still unclear how LAP2 α prevents lamin A/C assembly and integration into the lamina (Fig. 2). A clue for a potential mechanism comes from a recent study on importin- α /*Xenopus* lamin B3 (XLB3) complexes (Adam et

Figure 3. Indirect immunofluorescence microscopy of mouse primary fibroblasts derived from wild-type (*Lap2α*^{+/+}) or LAP2α-deficient (*Lap2α*^{-/-}) mice depicting LAP2α (left) and lamins A/C (right). Bar, 10 μm.

al. 2008). This study shows that the interaction of XLB3 with importin-α inhibits XLB3 polymerization in vitro and prevents the assembly of XLB3 into the lamina in *Xenopus* egg extract nuclear assembly assays. Furthermore, this interaction is dependent on the presence of the NLS in XLB3 and can be inhibited by RanGTP. Similar to the situation regarding the importin-α/XLB3 interaction, the binding of LAP2α to the amino-terminal tail of lamins A/C (Fig. 1) (Dechat et al. 2000b) might counteract lamin polymerization.

Despite the important role of LAP2α in targeting lamins A/C in the nuclear interior, additional cell-cycle-dependent regulatory pathways likely exist based on the following observation. In immunofluorescence microscopy, LAP2α localizes in the nucleoplasm in proliferating cells throughout the cell cycle, yet nucleoplasmic lamins A/C are only detectable in G₁ phase and are lost in late S phase (Naetar and Foisner 2009). These results are consistent with several models: For example, nucleoplasmic lamin A may change its conformation or interaction partners following progression from G₁ to S phase, causing the masking of antibody epitopes, as suggested previously in other cases (Dyer et al. 1997; Tunnah et al. 2005). However, our observations that nucleoplasmic lamins are undetectable in S phase by various antibodies reacting with different lamin domains makes this scenario unlikely (Dechat et al. 2000b; Dechat et al., unpubl.). We therefore speculate that modifications of lamins and/or LAP2α may regulate their interactions and localization (Figs. 1 and 2).

Lamin phosphorylation has long been known to regulate lamin disassembly during mitosis, and dephosphorylation of lamins by protein phosphatase 1 at the end of mitosis is required for their assembly into the nuclear lamina (Thompson et al. 1997; Steen et al. 2000). It is also known that lamins are phosphorylated during interphase (Eggert et al. 1993; Schneider et al. 1999; Otto et al. 2001; Kuga et al. 2010), but besides a potential phosphorylation-dependent regulation of nuclear import (Hennekes et al. 1993; Leukel and Jost 1995), the effects of interphase-specific lamin phosphorylation remain to be elucidated. In addition to phosphorylation and farnesylation/carboxymethylation, lamin A is also modified by ADP ribosylation (Adolph 1987), sumoylation (Zhang and Sarge 2008),

and probably glycosylation (Ferraro et al. 1989) and acetylation (Kim et al. 2006; Choudhary et al. 2009). However, only for sumoylation has the modified residue in lamins A/C (lysine 201) and its functional relevance been reported. Regarding the latter, mutations within the sumoylation consensus sequence in lamin A associated with familial dilated cardiomyopathy lead to a decrease in lamin A sumoylation, an altered nuclear localization of lamin A, and an increase in cell death (Zhang and Sarge 2008). In addition to lamins, LAP2α is also phosphorylated (Gajewski et al. 2004) and potentially acetylated (Choudhary et al. 2009) in interphase. However, it is unknown whether these modifications affect the interaction of LAP2α with lamins A/C.

FUNCTIONS OF LAMINS WITHIN THE NUCLEAR INTERIOR

Although previous studies suggested that the presence of nucleoplasmic A-type lamins is simply a consequence of their specific assembly pathway after mitosis, more recent data also indicate roles of the nucleoplasmic lamin pool in various cellular processes (see below). Nevertheless, there are still numerous open questions: Do internal lamins have specific functions that do not overlap with those of the peripheral lamina? Do nucleoplasmic lamins provide scaffolding platforms for components of signaling pathways, similar to what has been described for the lamina, or do they actively engage in regulatory pathways?

Most of the newly reported functions of lamins, both at the NE and in the nuclear interior, are based on the identification of novel interaction partners of lamins, including cell cycle regulators, transcription factors, and proteins involved in chromatin organization (Fig. 1) (Dorner et al. 2007; Vlcek and Foisner 2007). However, in many cases the physiological significance of these interactions must still be demonstrated. Below, we discuss the best-studied processes, in which nucleoplasmic lamins are likely involved.

- **Nucleoplasmic lamins in gene transcription, cell cycle progression, and differentiation.** Retinoblastoma protein (pRb), the major cell cycle regulator protein and transcriptional repressor, has been shown to bind to both A-type lamins and LAP2α (Ozaki et al. 1994; Markiewicz et al. 2002a, 2005). pRb's major activity in cell cycle control is its interaction with and inhibition of E2F-type transcription factors in G₁ phase, which facilitates cell cycle exit (Giacinti and Giordano 2006). Hyperphosphorylation of pRb by cyclin-dependent kinases causes its release from and subsequent activation of E2F, which in turn drives the expression of E2F target genes necessary for cell cycle progression. Although original studies suggested that nucleoplasmic lamin A/C-LAP2α complexes are important for the anchorage and localization of pRb in the nucleus (Markiewicz et al. 2002a; Pekovic et al. 2007), other studies of lamin A/C knockout cells found lamins A/C to be important for pRb protein stability (Johnson et al. 2004; Nitta et al. 2006). More recent studies show additional roles of lamin A/C-LAP2α in the control of pRb activity (Dorner et al. 2006; Naetar et al. 2008).

LAP2 α and lamins A/C primarily bind hypophosphorylated pRb at E2F promoter sequences and mediate efficient pRb-dependent repression of E2F target genes (Mancini et al. 1994; Markiewicz et al. 2002a; Dorner et al. 2006; Pekovic et al. 2007). Thus, complexes of nucleoplasmic lamins A/C and LAP2 α have been proposed to serve as cell cycle regulators that promote cell cycle exit by enhancing pRb repressor activity. This model is supported by the observation that in LAP2 α -deficient epidermis and cells, which both lack nucleoplasmic lamins A/C, pRb activity is compromised and progenitor cells show hyperproliferation and delayed differentiation (Pekovic et al. 2007; Naetar et al. 2008). Similar effects were detected in lamin A/C-deficient epidermis (Naetar et al. 2008). However, it is not completely clear how LAP2 α -lamin A/C affects pRb activity mechanistically. One possibility is that the complex regulates pRb (de)phosphorylation. This hypothesis is in line with a report showing that TGF β -mediated cell cycle arrest (which requires PP2A-dependent dephosphorylation of pRb) is less efficient in lamin A-deficient cells due to an impaired pRb dephosphorylation (Van Berlo et al. 2005). The latter model is consistent with a scaffolding role of nucleoplasmic lamin A/C complexes allowing efficient recruitment of regulatory enzymes of specific pathways. However, a more direct role of lamin A in transcriptional repression is also possible, as indicated by findings that artificial targeting of lamin A to a promoter of a reporter gene mediates its efficient repression (Lee et al. 2009).

Because both LAP2 α -deficient (Gotic et al. 2010) and lamin A-deficient (Frock et al. 2006) mouse myoblasts (or myoblasts expressing an EDMD-causing lamin A mutant) (Favreau et al. 2004) show impaired differentiation *in vitro*, it is possible that lamin A/C-LAP2 α complexes also regulate muscle-specific pRb activity (Gotic and Foisner 2010), which is required during early muscle differentiation (Huh et al. 2004). This is supported by expression-profiling analyses of muscle biopsies from lamin-linked EDMD patients, which revealed an impairment of the pRb-MyoD pathway in muscle regeneration (Bakay et al. 2006). In addition, lamin A has also been shown to interact directly with cyclin D3 (Mariappan et al. 2007), which was found to reorganize lamins A/C during myoblast to myotube conversion in a pRb-dependent manner (Mariappan and Parnai 2005). Thus, nucleoplasmic lamins may have multiple roles in regulating pRb-linked pathways during myogenic differentiation.

Lamin A also interacts with other differentiation-specific transcription factors, such as the sterol response-element-binding protein (SREBP) 1 (Lloyd et al. 2002) and the zinc-finger transcription factor MOK2 (Dreuillet et al. 2002). Because both transcription factors localize primarily in the nucleoplasm (Kim and Spiegelman 1996; Arranz et al. 1997; Capanni et al. 2005; Dreuillet et al. 2008), it is likely that they mostly interact with lamins A/C in the nuclear interior. However, besides a mislocalization of these transcription factors following the expression of pathogenic lamin A

mutants (Capanni et al. 2005; Hubner et al. 2006; Dreuillet et al. 2008), the role of lamins A/C in regulating these complexes is not clear yet.

Lamins have also been implicated in the control of gene expression by influencing upstream components or transcriptional coregulators of various signaling pathways, such as the Notch and the ERK pathway (Espada et al. 2008; Favreau et al. 2008; Gonzalez et al. 2008; Scaffidi and Misteli 2008; Emerson et al. 2009; Muchir et al. 2009). It has been proposed that scavenging of signaling molecules (ERK) and transcriptional regulators (c-Fos, SKIP) at the lamina may either inhibit the pathway by preventing their binding to respective promoters (Ivorra et al. 2006; Scaffidi and Misteli 2008) or facilitate signaling by mediating activating reactions (e.g., ERK-mediated activation of c-Fos; Gonzalez et al. 2008). In principle, however, all of these interactions of signaling components with lamins could also occur in the nuclear interior. In fact, many of the regulatory functions attributed to peripheral lamins in previous studies may be accomplished at least in part by the nucleoplasmic pool.

- **Nucleoplasmic lamins in chromatin organization.** In vertebrates, the nuclear lamina has been proposed to generate a heterochromatic “gene silencing” environment at the nuclear periphery (Guelen et al. 2008). However, translocation of a reporter gene to the nuclear periphery does not always mediate its repression. Whereas tethering genes to lamin-binding integral INM proteins emerin or LAP2 β led to full or partial transcriptional repression (Finlan et al. 2008; Reddy et al. 2008), tethering by lamin B1 did not affect transcription (Kumaran and Spector 2008). With respect to nucleoplasmic lamins, little is known about their potential roles in chromatin organization. However, because lamins have been reported to bind DNA and histones (for review, see Dechat et al. 2009), the histone-binding proteins RBBP4/7 (Pegoraro et al. 2009) and ING1 (Han et al. 2008), and the DNA cross-linking protein barrier-to-autointegration factor (BAF) (Holaska et al. 2003; Haraguchi et al. 2007), it is conceivable that lamins in the nuclear interior contribute to chromatin organization and epigenetic gene regulation. In line with this hypothesis, ING1 mislocalizes to the cytoplasm in lamin A/C-deficient cells (Han et al. 2008), and nucleoplasmic levels of RBBP4/7 are decreased in fibroblasts expressing a lamin A mutant associated with HGPS (Pegoraro et al. 2009). These findings suggest that nucleoplasmic lamins may provide a platform for chromatin-associated/remodeling proteins and thereby regulate their stability, localization, and/or activity.

In particular, the LAP2 α -lamin A complex may be involved in chromatin organization within the nuclear interior, because LAP2 α itself interacts with chromatin by several means: a carboxy-terminal chromatin targeting domain (Vlcek et al. 1999) and an amino-terminal LAP2, emerin, MAN1 (LEM) and LEM-like domain (Cai et al. 2001). The LEM motif is found in a group of chromatin-associated proteins, mostly localized in the INM (for review, see Wilson and Foisner 2010), and

mediates binding to BAF, whereas the LEM-like motif directly binds DNA (Cai et al. 2001). Thus, a multitude of interactions of the LAP2 α -lamin A complex with DNA and chromatin proteins may contribute to their potential role in chromatin organization and/or gene regulation.

- **Nucleoplasmic lamins in other cellular functions.** Support for a role of lamins in DNA replication comes from the findings that lamin A localizes to intranuclear early-replication foci (Kennedy et al. 2000) and that lamins interact directly with the proliferating cellular nuclear antigen (PCNA), a component of the replication machinery (Shumaker et al. 2008), although the molecular mechanisms are still unclear.

Other proteins present within the nucleoplasm and reported to interact with lamins A/C *in vitro* include PKC (Martelli et al. 2000, 2002), nuclear actin (Sasseville and Langelier 1998; for reviews, see Shumaker et al. 2003; Maraldi et al. 2004; Mehta et al. 2008), and a protein termed Lamin companion (Lco1) (Vlcek et al. 2004). However, the physiological role of these interactions is yet to be determined.

ROLE OF NUCLEOPLASMIC LAMINS IN THE DEVELOPMENT OF LAMINOPATHIES

Within the last 10 years, several human diseases now called laminopathies have been linked to mutations in lamin genes, primarily *LMNA*, and in genes encoding proteins associated with lamins (Worman et al. 2010). Studying the various laminopathies helped tremendously in identifying novel functions of lamins (see above), yet the molecular disease mechanisms are still poorly understood. Disease-causing mutations have been proposed to impair lamin assembly (affecting lamin complex stability) or lamin interactions (interfering with their specific role in signaling) (for reviews, see Parnaiik 2008; Andres and Gonzalez 2009; Prokocimer et al. 2009; Dechat et al. 2010). Interestingly, many laminopathy-causing mutations are single-point mutations in *LMNA*, distributed along the whole lamin A/C coding sequence (Fig. 1) and leading to the expression of a mutated protein with a single amino acid exchange. These mutated lamins are often mislocalized in nucleoplasmic foci/aggregates or are distributed diffusely in the nucleoplasm (for examples, see Östlund et al. 2001; Muchir et al. 2004; Hubner et al. 2006; Wiesel et al. 2008). Furthermore, the most prominent HGPS-linked lamin mutation induces missplicing of the *LMNA* transcript and generates a permanently farnesylated prelamin A variant tightly tethered to the INM (Dechat et al. 2007). Thus, many disease-linked mutations affect the localization of lamin A, either increasing or decreasing the nucleoplasmic pool of lamin A (for examples, see Markiewicz et al. 2002b; Gilchrist et al. 2004; Muchir et al. 2004; Broers et al. 2005; Dahl et al. 2006; Hubner et al. 2006; Wiesel et al. 2008) and it is conceivable that at least some of the pathologies of these diseases are linked to a misregulation of the functions of nucleoplasmic lamins in, e.g., gene expression, cell cycle progression, and chromatin organization (Gotzmann and Foisner 2006). In line with this hy-

pothesis, HGPS fibroblasts show heterochromatin loss and impairment of epigenetic pathways (Goldman et al. 2004; Shumaker et al. 2006) and misregulation of adult stem cell differentiation (Scaffidi and Misteli 2008), and mouse models of progeria show stem cell defects in the epidermis (Espada et al. 2008).

Intriguingly, lamin-mediated pathways, likely also those mediated by nucleoplasmic lamins, appear to have a role in normal aging, because the mutant lamin protein associated with HGPS and/or farnesylated prelamin A are also found enriched in cells of healthy elder individuals (Scaffidi and Misteli 2006; Cao et al. 2007; McClintock et al. 2007; Rodriguez et al. 2009).

CONCLUSIONS

Since the discovery of lamins in the late 1970s, and their subsequent cloning and identification as IF proteins in the 1980s, research on lamins has gone through several periods of excitement but also periods of disinterest. After the initial boom during the analyses of their biochemical properties, in which lamins were recognized as mostly structural components of the nucleus, many scientists interested in development- and disease-relevant cellular processes turned away from the field. With the discovery of an increasing number of mutations in lamins that are linked to human diseases, starting in 1999, and the report of an unexpected muscle phenotype in the first lamin knockout mouse model, a second wave of excitement began. During the last 10 years, numerous novel and often unexpected functions of lamins in chromatin organization, gene expression, and cell signaling have been reported and much still must be done to study the mechanistic details of these functions and their relevance for human diseases. In more recent years, investigators also started to realize that the function of lamins is not only restricted to the nuclear periphery; lamins seem to have important regulatory roles throughout the entire nucleus. Yet, mostly because lamins in the nuclear interior are not abundant and thus difficult to study, reports on their assembly state, regulation, and functions are just beginning to emerge. In the future, we will have to revisit whether some of the functions previously attributed to the lamina, particularly those involved in signaling pathways and gene expression, are also linked to the nucleoplasmic lamin pool.

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T. Dechat, K. Gesson and R. Foisner

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