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When Lamins Go Bad: Nuclear Structure and Disease

Katherine H. Schreiber[1] and **Brian K. Kennedy**[1,2,3]

[1]Buck Institute for Research on Aging, 8001 Redwood Blvd., Novato, CA 94945

[2]Aging Research Institute, Guangdong Medical College, Dongguan 523808, Guangdong, China

Abstract

When mutations in nuclear lamins were first identified in skeletal and cardiac muscle diseases, the molecular events underlying pathogenesis were mere points of speculation. As more and more unrelated diseases were linked to lamins and other nuclear envelope proteins, nuclear structure and disease became an increasingly prominent research focus. Today, the disease mechanisms remain unresolved, but incredible progress has occurred. Nuclear envelope dysfunction is not only associated with altered nuclear activity, but also impaired structural dynamics and aberrant cell signaling. Building on these findings, small molecules are being discovered in animal models that may become effective therapeutic agents.

Introduction

Since their discovery more than 35 years ago as constituents of the nuclear lamina (Gerace et al., 1978), the nuclear lamins have been the subject of intense speculation regarding their possible roles in almost everything that happens in the nucleus. Early studies focused on biochemistry and cell biology with the goal of achieving a basic understanding of the principles governing nuclear organization. The nuclear envelope entered the medical realm in the mid-1990s when mutations in emerin were identified in patients with Emery-Dreifuss muscular dystrophy (EDMD) (Bione et al., 1994). The *LMNA* gene, encoding all A-type nuclear lamins, was linked to EDMD a few years later (Bonne et al., 1999), and since then links between nuclear structure and human disease have been studied extensively in labs throughout the world.

With nearly 15 diseases attributed to *LMNA* mutations, including a range of dystrophic and progeroid syndromes, and mutations in genes encoding associated nuclear envelope proteins causing an overlapping set of diseases, the questions and experimental approaches have evolved. Why do alterations in nuclear envelope proteins confer disease? What are the mechanisms underlying disease pathology? Do A-type lamins have a role in normal aging? Can effective therapies be developed for these debilitating diseases? While a range of exciting discoveries have been made in the last decade, there remain a great many unknowns. Here, we seek to frame the current questions, propose possible paths toward mechanistic understanding and briefly evaluate the therapeutic possibilities that are starting to emerge. Given the amount of interest and momentum in the lamin field, it is feasible that

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[3]To Whom Correspondence should be addressed: bkennedy@buckinstitute.org.

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therapies to rescue the pathogenic consequences of misbehaved nuclear structural components will be developed in the not-too-distant future.

Nuclear lamins

The nuclear envelope is comprised of two membranes, the outer nuclear membrane, which is continuous with the endoplasmic reticulum, and the inner nuclear membrane, which associates with the nuclear lamina. Nuclear pore complexes perforate the nuclear envelope to allow transport between the cytoplasm and nucleus. The nuclear lamina is primarily composed of nuclear lamins, which were originally identified as lamins A, B and C (Gerace et al., 1978). These proteins constitute the only class of intermediate filament proteins in the nucleus and form associated filamentous structures that underlie the nuclear envelope and interact with neighboring proteins (Gerace and Huber, 2012). Lamins A and C, as well as two other variants (C2 and AΔ10), are classed as A-type lamins and are encoded by the *LMNA* gene through alternative splicing. Three different lamin B family members (B-type lamins) are encoded by two genes (lamin B1 by *LMNB1* and lamins B2 and B3 by *LMNB2*).

A- and B-type lamins have fundamentally different properties, perhaps most importantly by virtue of their different isoelectric points, which dictate that B-type lamins stay associated with the nuclear envelope during mitosis while A-type lamins become soluble. Expression patterns differ as well, with B-type lamins expressed in most or all cell types and A-type lamins expressed during cell differentiation in many different developmental lineages (Rober et al., 1989). At the cellular level, both classes of proteins have been ascribed structural roles in the nucleus as well as a range of other activities including coordination of transcription and replication. While specific functions of A-type lamins remain somewhat elusive, a number of recent discoveries point to key interactions between lamins and cell proliferation, differentiation and stress response pathways.

Both A- and B-type lamins undergo post-translational processing based on a C-terminal CaaX motif that dictates a series of modifications (Weber et al., 1989); only lamin C avoids this by virtue of alternative splicing of the *LMNA* transcript that lacks the C-terminus. As a first step, the cysteine residue is farnesylated. Next, proteolytic processing leads to cleavage after the cysteine residue, followed by a carboxymethylation of the new C-terminal residue. Many membrane associated proteins, including Ras undergo this processing event. However, in the case of lamin A, isoprenylation is a transient event since a second proteolytic event mediated by the zinc metalloproteinase, Zmpste24, leads to excision of another 15 amino acids. Due to this cleavage, mature lamin A lacks the modified cysteine. This process is clearly important to pathologic states, since laminopathies are linked to altered processing of lamin A as well as loss of function mutations in *ZMPSTE24*.

The reasons for farnesylation of lamin A remain to be elucidated despite extensive efforts. Until recently, thinking has been that the transient farnesylation event was needed, through association of the hydrophobic farnesyl group with the nuclear envelope, to provide initial recruitment of lamin A to the nuclear periphery (Hennekes and Nigg, 1994). After assembly into filaments, farnesylation of lamin A may no longer be required. Consistent with this hypothesis, the nucleus has been shown to be the site of both lamin A carboxymethylation and proteolytic cleavage by ZMPSTE24. However, several recent studies using mice and/or cells engineered to express mutant forms of lamin A indicate that farnesylation is not required for recruitment (Davies et al., 2011). For instance, when only a non-farnesylated version of lamin A is expressed, normal localization of the lamin A variant to the nuclear periphery was observed (Davies et al., 2010; Lee et al., 2010), although mice generated in this manner develop cardiomyopathy (see below). In addition, mice expressing only lamin C

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(not farnesylated) or only a mature (pre-processed) lamin A are (surprisingly) normal and have apparently correct localization of the respective protein to the nuclear periphery . While these studies do not preclude a more subtle role for lamin A processing in filament assembly or envelope association, they raise serious questions about the importance of these events in the mouse and provide an interesting puzzle to be pieced together by future studies.

Diseases linked to mutations in nuclear structure proteins

The number of different diseases linked to mutations in *LMNA*, at least 15 by now, surpasses that of any other human gene. It is hard to establish absolute numbers, however, since many of the associated syndromes have overlapping pathologies. Nevertheless, the range of tissues and functions that can be adversely affected by mutation in *LMNA* is striking (Table 1). Diseases include the aforementioned Emery-Dreifuss muscular dystrophy (EDMD2/3) (Bonne et al., 1999) and a second muscular dystrophy (Limb-girdle, LGMD1B) (Muchir et al., 2000), which affects different skeletal muscle groups. Patients with both forms of muscular dystrophy also present with dilated cardiomyopathy, which is often the cause of mortality. Other *LMNA* mutations lead to dilated cardiomyopathy (CDM1A) without skeletal muscle involvement (Fatkin et al., 1999). Finally a form of congenital muscular dystrophy has more recently been linked to mutations in *LMNA* (Quijano-Roy et al., 2008), as well as Heart-hand syndrome, which couples a range of cardiac defects to brachydactyly (Renou et al., 2008).

Pathology associated with *LMNA* mutations is not restricted to striated muscle tissue, as other diseases confer loss of adipose tissue, including Dunnigan-type familial partial lipodystrophy (FPLD2) (Shackleton et al., 2000), Mandibuloacral dysplasia (MAD) (Novelli et al., 2002), generalized lipoatrophy (Caux et al., 2003), Restrictive dermopathy (RD) (Navarro et al., 2004) and other overlapping disorders. Highlighting the importance of Lamin A processing, mutations resulting in loss of *ZMPSTE24* function, which result in partially processed lamin A, lead to both MAD and RD (Agarwal et al., 2003; Navarro et al., 2005). However, links between lamin A processing and pathology extend beyond mutations in *ZMPSTE24* and connect with another set of disorders termed progeroid, which give rise to the appearance of premature aging. The most noted of these is Hutchinson-Gilford progeria syndrome (HGPS), a severe disorder for which symptoms, including cachexia, alopecia and atherosclerosis become apparent shortly after birth. Death results from heart attack or stroke usually before the patient reaches the age of 20. The most common *LMNA* mutation leading to HGPS, G608G, does not affect coding sequence but instead creates a cryptic splice site leading to removal of 50 amino acids in the C-terminus of lamin A (De Sandre-Giovannoli et al., 2003; Eriksson et al., 2003), resulting in a protein named progerin. A similar splicing mutant has been identified that leads to removal of an extra 40 amino acids (90 total) in a patient diagnosed with RD (Navarro et al., 2004), leading to speculation that RD is a more severe version of HGPS, although the two diseases do not overlap entirely. This splicing event removes the cleavage site for ZMPSTE24, creating a permanently farnesylated protein that likely causes a dominant gain-of-function toxicity. Other mutations in *LMNA* that do not obviously affect C-terminal splicing lead to HGPS as well as other generally less severe progeroid pathologies (Cao and Hegele, 2003; Chen et al., 2003; Verstraeten et al., 2006). Finally, with regard to *LMNA* mutations, homozygous loss of lamin A function leads to Charcot-Marie Tooth Syndrome, characterized by loss of peripheral nerve myelination (De Sandre-Giovannoli et al., 2002).

Before leaving A-type lamins, it is worth noting the interesting connections that have arisen with cancer progression (Butin-Israeli et al., 2012). Most laminopathies are not associated with cancer, but an increasing range of tumors are characterized by down-regulation of A-

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type lamin expression (Broers et al., 1993; Kaufmann, 1992) although results differ in tumor types. Recalling that this family of lamins is expressed in differentiated cells, but not stem cells, speculation has developed that A-type lamins may act as tumor suppressors, perhaps by blocking de-differentiation into a more stem cell-like state. A-type lamins have also been ascribed roles in regulating cell proliferation and the DNA damage response, either of which could be linked to cancer progression (Redwood et al., 2011). Among these activities, A-type lamins are required to stabilize the retinoblastoma tumor suppressor protein (Johnson et al., 2004). This may be relevant because the one tumor described in HGPS patients (the sample size is quite small) is an early onset osteosarcoma (Shalev et al., 2007), one of the most common tumors linked to homozygous mutation of the *Rb* locus (Friend et al., 1986). Although progerin can stabilize pRb levels (Nitta et al., 2006), the HGPS patient with osteosarcoma had a rare T623S *LMNA* mutation that has not been tested with regard to pRb stability (Shalev et al., 2007).

Mutations in genes encoding other nuclear envelope proteins are also associated with disease (described in more detail in (Mendez-Lopez and Worman, 2012)). In addition to *emerin* and *LMNA*, mutations in *SYNE1* and *SYNE2* (encoding nesprin-1 and nesprin-2), *TMEM43* (encoding LUMA) and *TMPO* (encoding LAP2alpha) are all associated with dilated cardiomyopathy and muscular dystrophy (Liang et al., 2011; Taylor et al., 2005; Zhang et al., 2007). These genes encode proteins that all interact as part of the linker of nucleoskeleton and cytoskeleton (LINC) complex, suggesting that altered LINC function may underlie striated muscle pathology (Puckelwartz and McNally, 2011). Unrelated *SYNE1* and *SYNE2* mutations are also linked to autosomal recessive spinocerebellular ataxia type 8 and autosomal recessive arthrogyrposis, respectively (Attali et al., 2009; Gros-Louis et al., 2007). *LEMD3*, encoding MAN1, an LEM domain containing protein, is also associated with disease, with mutations linked to a series of disorders associated with increased bone density (Hellemans et al., 2004). In addition, mutations in BANF1, encoding the nuclear envelope protein BAF that binds DNA and is involved in chromatin organization and nuclear envelope assembly, are associated with Atypical progeria (Puente et al., 2011).

Not to be left out, *LMNB1* and *LMNB2* mutations are both linked to rare diseases. Autosomal dominant mutations in *LMNB1* lead to adult-onset leukodystrophy, which is characterized by central nervous system demyelination (Padiath et al., 2006). In the case of *LMNB2*, individuals with heterozygous mutations are susceptible to acquired partial lipodystrophy, likely triggered by one of several autoimmune diseases (Hegele et al., 2006). Finally, the lamin B receptor (LBR), which interacts with B-type lamins and may serve to help link them to the nuclear envelope and chromatin, is also a target for mutation in two syndromes: homozygous mutations in LBR cause Greenberg skeletal dysplasia (Waterham et al., 2003), whereas heterozygous mutations are associated with Pelger-Huet anomaly, a benign condition characterized by altered chromatin organization in granulocytes (Best et al., 2003; Hoffmann et al., 2002). Given the rate of new discoveries of disease association with nuclear structural factors, it is fair to speculate that new diseases will continue to emerge.

Disease mechanisms: Mouse models lead the way

How could altered function of nuclear structural components lead to such a wide range of diseases? In the beginning, there were few connections between lamins and known disease mechanisms; however, lamins were known to be important for a wide range of nuclear functions, including replication and transcription. Many of the initial ideas were based on changes observed at the level of cell biology. For instance, the shape of the nucleus was found to be disrupted in fibroblasts lacking A-type lamins, with enhanced nuclear deformation and sensitivity to mechanical stress (Lammerding et al., 2004). Emerin-deficit

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cells have similar properties and reduced mechanical stress could explain part of the pathology associated with diseases such as dilated cardiomyopathy and muscular dystrophy, where affected tissues are under regular strain (Lammerding et al., 2005). However, cells isolated from human and mouse tissue from the various laminopathies, all display abnormal nuclear structure. These phenotypes range from abnormal nuclear shape to nuclear blebbing and even dispersal of DNA into the cytoplasm. While these observations may relate to disease, they do not clearly differentiate one laminopathy from another and researchers have turned to more detailed assessments of cellular function to generate more recent hypotheses.

Theories to explain the pathology associated with nuclear structure defects have emerged largely from two areas: (1) an extensive set of mouse models and, more recently, (2) studies of stem cells expressing a range of mutant forms of A-type lamins. An informative starting point for the former was the generation of mice lacking A-type lamins (Sullivan et al., 1999). In addition to being cachexic, these mice present with a subset of the pathologies associated with *LMNA* mutation, including muscular dystrophy, dilated cardiomyopathy and Charcot-Marie Tooth Syndrome and succumb to the cardiac phenotype at about six weeks of age. *Lmna*$^{+/-}$ heterozygous mice also develop the cardiac pathology, although at a slower rate, and mice expressing two different *LMNA* alleles associated with striated muscle disease recapitulate at least some of the human phenotypes (Arimura et al., 2005; Mounkes et al., 2005). One assertion arising from these findings is that the muscle and peripheral myelination diseases result from reduced A-type lamin function. This is not surprising for Charcot-Marie Tooth Syndrome, which is a recessive disorder in humans (De Sandre-Giovannoli et al., 2002). However, both dominant and recessive mutations have been identified in the muscle pathologies and one possibility is that autosomal dominant alleles have a dominant negative effect, interfering with intermediate filament assembly or some other property of A-type lamins. Haploinsufficiency also likely explains the onset of disease in many cases.

It should be noted that the *Lmna*$^{-/-}$ mouse described originally may in fact not be a null allele of the gene (Sullivan et al., 1999). Recent evidence suggests that this mouse expresses a still incompletely characterized, truncated 54kD protein derived from a splicing event, that bypasses the removed exons (Jahn et al., 2012). While the dust has not settled from this finding, most data suggest that the lamin A variant expressed in this mouse is hypomorphic. Interestingly, another *Lmna*$^{-/-}$ model has been derived through disruption with a reporter gene and this mouse presents with defective development of heart liver and somites leading to death before weaning (Kubben et al., 2011). This latter mouse is more consistent with a homozygous *LMNA* nonsense mutation that resulted in the complete absence of A-type lamins and was associated with the death of a newborn patient (van Engelen et al., 2005). Clearly, these findings call for some re-evaluation of studies performed in the *Lmna*$^{-/-}$ mouse despite its past and continued value to the field.

One recent, highly informative mouse model was engineered to homozygously express a non-farnesylated version of lamin A in the absence of lamin C (Davies et al., 2010). These mice were expected to resemble the phenotype of mice lacking *ZMPSTE24* (see below), but instead present with cardiomyopathy. The investigators sought to determine whether the cardiac pathology was attributable to gain-of-function toxicity or a hypomorphic, reduced function of the lamin A variant. To distinguish, they generated a mouse expressing a non-farnesylated allele over a null, finding that this mouse has a more severe phenotype, consistent with further reduced lamin A function. If the pathology were a result of toxicity, the heterozygous mouse would have had a less severe cardiac phenotype. These findings are consistent with the data that cardiomyopathy derives from reduced lamin A function.

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While striated muscle pathology represents one cluster of mouse *LMNA* models, progeria characterizes the other. In this case, the data is generally supportive of a model whereby lamin A variants with processing defects show dominant onset of a subset of features associated with Hutchinson-Gilford progeria syndrome. These models are covered in detail in a recent review (Zhang et al., 2012). Recall that the primary human lesion associated with Hutchinson-Gilford progeria syndrome is a heterozygous G608G mutation that creates a splicing defect and leads to permanently farnesylated lamin A. Of the many different HGPS models, much debate centers around which ones are the best to develop mechanistic explanations and therapies for human patients. Most of the models, including *Lmna* mutants and *Zmpste24*$^{-/-}$, present with a subset of phenotypes characteristic of progeroid mice, including cachexia, reduced bone density and rib fractures, loss of subcutaneous fat, kyphosis, alopecia and premature death. However, a model generated to express human progerin from a BAC clone does not exhibit these phenotypes, instead displaying arterial smooth muscle defects (Varga et al., 2006). While the differences are unknown, both types of models may have advantages. For instance, the BAC progerin model mimics atherosclerosis, which by leading to heart attacks and strokes results in mortality in most patients. Therefore, studies in this mouse explore effects on what may be the most important pathology in children with disease. However, the rapid presentation and wider array of phenotypes in the other mice offer clear advantages as well. Of note, some of the progeria model mice display cardiac defects more consistent with dilated cardiomyopathy (Davies et al., 2010; Yang et al., 2011). One point worth considering is that a *LMNA* mutation could lead to gain-of-function toxicity for some phenotypes and loss-of-function for others.

In the next two sections, we focus in on the two classes of *LMNA*-associated disease about which we understand the most: striated muscle disease and progeroid disorders. The exciting progress in these two areas has led to possible therapeutic approaches.

Disease mechanisms and possible therapies for *LMNA*-associated striated muscle diseases

Interesting findings have emerged on several fronts with respect to *LMNA*-associated dilated cardiomyopathy with conduction defects and muscular dystrophies. While these findings do not yet come together in a neat package, continued studies may begin to generate such a composite understanding. The fact that *LMNA* mutants leading to EDMD2/3 so closely resemble X-linked EDMD, caused by Emerin mutations, must be considered for any mechanistic disease model. Unlike A-type lamins, emerins reside in the inner and outer nuclear membranes, interacting with lamins in the former case and with microtubules in the latter. Lamin A/C binding to emerin is required for its localization to the nuclear envelope (Vaughan et al., 2001). This raises the possibility that emerins might be a conduit by which the nuclear lamina communicates with the cytoskeleton. However, no clear understanding has emerged as to how and why the lamin A/C-emerin interaction is important in skeletal and cardiac muscle.

The linker of nucleoskeleton and cytoskeleton (LINC) protein complex, consisting on SUN1 and 2 as well as Nesprin 1 and 2, also connect A-type lamins to the cytoskeleton with Sun proteins directly interacting with lamin A/C at the inner nuclear membrane and Nesprins in the lumen (Mejat and Misteli, 2010). Nesprins cross the outer nuclear membranes and connect to the cytoskeleton in the cytoplasm. In addition to linking the nucleo- and cytoskeleton, LINC complexes have a wide range of cellular functions, including in cell division, centrosome-nucleus association, nuclear migration and positioning. Disruption of any of these activities could contribute to disease progression. An interesting recent study has implicated SUN1 in disease progression, albeit through an unexpected mechanism. In *Lmna*$^{-/-}$ mice, SUN1 is dramatically overexpressed and directed to the Golgi, presumably

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after nuclear occupancy sites are saturated (Chen et al., 2012a). RNAi-mediated knockdown of SUN1 rescued nuclear defects in cell culture and knockout of SUN1 significantly extended the survival of *Lmna*$^{-/-}$ mice. While many questions remain unresolved, this report suggests that one significant problem associated with reduced A-type lamin function is SUN1-mediated toxicity in the Golgi.

Another intermediate filament factor, desmin, serves as a linking factor between lamins and many cytoplasmic structures in striated muscle cells. Desmin mutations can result in desmin-related myopathies (DRM), which are characterized by cardiac and skeletal muscle weakness with a highly variability of presentation. Inherent in DRM at the cellular level is disruption of desmin filaments and accumulation of desmin-containing protein aggregates. Interestingly, cardiomyoctes from *Lmna*$^{-/-}$ mice display disrupted desmin networks and elevated protein levels (Nikolova et al., 2004). This may not be the case for skeletal muscle as electron micrographs of muscle biopsies from human patients failed to detect abnormal desmin localization (Frock et al., 2012; Piercy et al., 2007). The authors of this study also looked at murine embryonic stem cells transfected with a human EDMD mutation and differentiated into cardiomyocytes, finding no defects in desmin localization. These latter findings appear to differ from the in vivo studies described above and may suggest that knockout of A-type lamins, as opposed to expression of an EDMD missense mutation, is required to induce abnormal desmin localization. Alternatively, the cell culture model may not recapitulate events regarding desmin.

Myoblasts generated from *Lmna*$^{-/-}$ mice are reported to have differentiation defects, suggesting that reduced regenerative potential of adult stem cells may combine with increased damage to myofibers from mechanical stress sensitivity to explain the rapid onset of dystrophic pathology (Frock et al., 2006). Interestingly, a small percentage of *Lmna*$^{-/-}$ myoblasts respond normally to differentiation signals whereas a majority fail to induce the differentiation program. The majority of proliferating *Lmna*$^{-/-}$ myoblasts also display reduced levels of both MyoD and desmin. Stable transfection of desmin rescued the differentiation defects of these cells, implying that reduced desmin levels during the proliferation phase may in part be responsible for the inability of cells to respond to differentiation cues. Stable expression of MyoD also rescued differentiation defects. With respect to *EDMD* mutations, MyoD transformed human patient fibroblasts were reported to differentiate normally (Piercy et al., 2007). Again, the differences may be attributable to the relative severity of the *LMNA* mutation or they may have been suppressed in the latter case due to artificially high MyoD levels (Frock et al., 2006; Piercy et al., 2007).

In recent years, it has become apparent that *Lmna* mutations can lead to altered activation of major signal transduction pathways in the cell (Figure 1). While the mechanisms connecting the nuclear envelope to cell signaling have not been fully elucidated, the findings are important since (1) altered signaling can be linked to pathological progression and (2) in some cases small molecules are available as therapeutic options to correct signaling defects. In cardiac tissue, three different branches of the MAP kinase signaling pathways have been found to be aberrantly activated in a mouse model homozygously expressing the human *LMNA* H222P mutant associated with dilated cardiomyopathy (Muchir et al., 2007b; Muchir et al., 2012). One of these, the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway was also upregulated in *Emerin*-deficient mice, while the Jun N-terminal kinase (JNK) pathway was not elevated and the p38α pathway remains to be tested (Muchir et al., 2007a). Elevated ERK1/2 phosphorylation has also been detected in human cancer cells lines where A-type lamin or Emerin expression was inhibited by an siRNA approach and in cardiac tissue from *Lmna*$^{-/-}$ mice (Frock et al., 2012; Muchir et al., 2009b). In *Lmna*$^{-/-}$ hearts, aberrant phosphorylation could be corrected by restoration of lamin A expression specifically in cardiomyocytes, indicating that the defects are cell autonomous (Frock et al.,

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2012). Finally, at least elevated p38α phosphorylation has been detected in heart tissue from human dilated cardiomyopathy patients (Muchir et al., 2012).

A variety of MAP kinase inhibitors have been generated and many have been tested in the clinic for other disease indications. Worman and colleagues have tested several of these in *Lmna*$^{H222p/H222p}$ mice, finding that inhibition of each branch of the Map kinase pathway either delays onset or progression of cardiac symptoms (Muchir et al., 2009a; Muchir et al., 2012; Wu et al., 2010). Given that some of these inhibitors appear to be relatively well tolerated in humans, these findings lead to a potential therapeutic route for dilated cardiomyopathies associated with *LMNA* mutation. Potential benefits for muscular dystrophy have not been assessed.

How does *LMNA* mutation lead to activation of the MAP kinase pathways? While the answer to this question remains to be determined, ideas have emerged. For instance, MAP kinases are known to be activated by mechanical stress and reduced A-type lamin function is associated with impaired activation of mechanosensitive genes in cardiomyocytes. A second, more direct model has potentially emerged that involves direct interaction between ERK1/2 and A-type lamins in the nucleus. ERK1/2 is reported to interact with lamin A and the retinoblastoma protein pRb at the nuclear periphery. Stabilization of pRb by A-type lamins is important to maintain normal cell cycle control (Nitta et al., 2006). Upon serum stimulation of quiescent cells, ERK1/2 phosphorylates c-Fos releasing it to stimulate Ap-1 activation and also dislodges pRb from A-type lamins, leading to pRb phosphorylation and E2F activation (Gonzalez et al., 2008; Ivorra et al., 2006; Rodriguez et al., 2010). It is unclear presently how disruption of the ERK1/2-A-type lamin interaction by *LMNA* mutation affects ERK1/2 activation but this question needs to be investigated.

Equally unclear are the pathways downstream of MAP kinases that mediate cardiac pathology. Two possibilities have emerged. The first involves an observation that connexins are mis-localized in mice expressing a different mutant associated with DCM (N195K). Here, connexin 43 was found to be mis-localized and not associated with gap junctions, a finding that could explain conduction defects associated with altered A-type lamin function (Mounkes et al., 2005). Expression of another DCM mutant (E82K) was found to lead to downregulation and mis-localization of connexin 43 in neonatal myocytes (Sun et al., 2010). Finally, a recent study has demonstrated mis-localization of connexin 43 in heart cardiomyocytes of *Lmna*$^{-/-}$ mice (Frock et al., 2012). Re-expression of lamin A rescued aberrant ERK1/2 phosphorylation and restored connexin 43 localization. Given that connexins are known substrates of ERK1/2, the possibility exists that aberrant activity of this pathway disrupts normal connexin43 localization and interferes with cardiac conduction (Chen et al., 2012b).

Two recent studies point to the involvement of another major signal transduction pathway in *LMNA*-related cardiac and skeletal muscle disease. In *Lmna*$^{-/-}$ mice, the mTORC1 pathway was found to be upregulated in cardiac and skeletal muscle, leading at least in the heart to impaired autophagy (Ramos et al., 2012). Reduced mTORC1 signaling by the specific kinase inhibitor rapamycin, led to enhanced cardiac function and survival with indications of improved skeletal muscle function, although the latter possibility needs to be more fully explored. A similar study conducted in the *Lmna*$^{H222P/H222P}$ mouse led to highly overlapping findings, suggesting that aberrant mTORC1 signaling may be a common feature of this class of laminopathies (Choi et al., 2012). Among several upstream activators of mTORC1 are ERK1/2 MAP kinases and one possibility is that increased mTORC1 signaling occurs by this mechanism. However, there are numerous upstream activators of mTORC1 that need to be more fully explored. The possibility of testing rapamycin as a treatment for LMNA-associated DCM is intriguing since the drug has been tested in a wide range of

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clinical trials and is approved for multiple disease indications. However, there are side effects such as dyslipidemia and impaired insulin signaling that, while generally manageable, must be considered for treatment of cardiac disease. Elevated mTORC1 signaling, which is classically associated with increased protein translation and cell growth, is already linked to forms of cardiac hypertrophy. However, general levels of translation do not appear to be elevated in the *Lmna*$^{-/-}$ heart (Ramos et al., 2012), suggesting that other pathways are offsetting the translational effects of mTORC1 in this scenario. Interestingly, rapamycin has been reported to improve autophagic flux and suppress nuclear blebbing in fibroblasts expressing progerin, indicating that suppression of the mTOR pathway may be efficacious in *LMNA*-associated progeria models as well (Cao et al., 2011).

Given the remarkable progress in this cluster of *LMNA*-associated diseases, it has been possible to move from identification of *LMNA* mutations in EDMD and DCM to possible therapeutic approaches in under two decades (Figure 2). Whether the current drugs will prove efficacious in humans remains to be seen. Even if this is not the case, new candidate therapeutic approaches will surely continue to emerge.

Disease mechanisms and possible therapies for *LMNA*-associated progerias

Although very rare, progeria syndromes have long been of great interest based in part on the hypothesis that by learning the mechanisms underlying their pathology, insights will be made into the normal aging process. This assumption is yet to be validated and researchers in the aging field have a wide range of viewpoints. One thing is clear. The studies into *LMNA*-associated progerias have yielded major biological insights and provided hope that therapeutic approaches can be developed to slow the impact of these very severe syndromes. In this section, the latest findings in progeria and lamin A processing will be discussed.

A large body of work suggests that HGPS mutants in *LMNA* at least in part confer toxicity by virtue of being permanently farnesylated. Several deformations of the nucleus were found in cells expressing progerin or other non-farnesylated versions of lamin A, and several studies indicated that these phenotypes could be rescued by a class of drugs that inhibit farnesyltransferases (Young et al., 2006). These drugs were initially generated based on their ability to block Ras farnesylation and the promise that that would inhibit tumor progression. While cancer studies continue, their development has been fortuitous to the study HGPS. Not only do they rescue cellular defects but they have beneficial properties when delivered to HGPS mouse models, extending survival and improving other physiological readouts including bone and cardiovascular defects (Capell et al., 2008; Yang et al., 2008b). These findings, together with the fact that FTIs have good safety profiles in the clinic, were cause for great optimism, leading to the first clinical trial in human patients with HGPS. Initial findings were recently reported, showing variable rates of improvement in vascular function, enhanced bone rigidity and improved sensorineural hearing in 25 patients treated with Ionafarnib for at least two years (Gordon et al., 2012).

One reason FTIs may have limited potency is that lamin a variants can become geranylgeranylated, especially when farnesyltransferase activity is blocked (Varela et al., 2008). This has led to the assumption that blocking the HMG-CoA reductase pathway upstream, in a manner that inhibits both lamin A modifications, might have enhanced efficacy. Consistently, combined treatment of *Zmpste24*$^{-/-}$ mice with two such agents, statins and aminobisphosphonates, enhances survival and improves several pathologies. Another potential approach has emerged in a mouse that is genetically engineered to have the exact G608G mutation (G609G in mice) (Osorio et al., 2011). As in the human case, alternative splicing leads to progerin production and progeroid phenotypes. Interestingly,

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treatment of the mice with a morpholino-based therapy therapy that prevents pathogenic splicing, delays pathology and extends survival suggesting an alternative therapeutic approach.

Genetic studies support the toxicity of farnesylated lamin A in progerias. For instance, mice lacking *Zmpste24* develop progeroid features linked to the toxicity of an unprocessed lamin A, since deletion of one copy of *LMNA* in this background improves the range of phenotypes (Fong et al., 2004). Extensive studies by Young and colleagues have further elucidated the role of farnesylation in vivo. Mice engineered to express a non-farnesylated version of progerin still develop progeroid features, albeit at a slower rate (Yang et al., 2008a). However, mice expressing a non-farnesylated version of prelamin A do not develop progeroid features as described earlier (Davies et al., 2010). One possible interpretation of these studies in that farnesylation may be required for toxicity in the case of prelamin A but that the 50 amino acid deletion in progerin also contributes to disease progression.

Several lines of evidence implicate enhanced DNA damage and/or an impaired DNA damage response pathway in the etiology of HGPS. HGPS cells have higher levels of reactive oxygen species and greater rates of basal DNA damage (Viteri et al., 2010). These findings are likely connected since a reduction in ROS by exposure to n-acetylcysteine reduces double strand break formation. These alterations lead in part to enhanced activation of DNA response pathways, including enhanced ATM and RAD3-associated foci, that may adversely affect cell cycle proliferation. An interesting and unusual feature of HGPS cells is persistent basal levels of phosphorylated γH2AX foci marking double strand breaks that also stain positive for Xeroderma pigmentosum group A protein (XPA) (Liu et al., 2008), a component of nucleotide excision repair. No other related factors are upregulated, suggesting that the foci have an abnormal set of repair proteins and the type of DNA damage in HGPS cells may have unique features.

Cells from mice lacking *Zmpste24* also exhibit a significant delay in recruitment of 53BP1 to sites of DNA repair after induction of double strand breaks (Liu et al., 2005). p53 targets such as GADD45, p21 and ATF3 were also elevated and deletion of p53 was sufficient to rescue some of the progeroid phenotypes of the *Zmpste24*$^{-/-}$ mouse (Varela et al., 2005). While p53 targets were not elevated in HGPS fibroblasts, inactivation of the transcription factor was sufficient to suppress premature senescence (Kudlow et al., 2008). More recent data indicates that ATM and NEMO pathways become activated and promote NF-κB dependent inflammation in both *Zmpste24*$^{-/-}$ and *Lmna*$^{G609G/G609G}$ mice (Osorio et al., 2012). Genetic and pharmacological interventions of these pathways slow progeroid pathology and enhance survival. These findings are particularly interesting since (1) they suggest that NF-κB inhibitors may be effective therapeutic agents and (2) enhanced inflammation may be a major driving of normal aging processes. Furthermore, the tissues affected by altered lamin A processing have remained unresolved. Progeria involves systemic pathology and one possibility is that defects in every tissue cause cell autonomous phenotypes. More likely, defects in a smaller set of tissues lead to systemic responses that impact the whole organism. Enhanced NF-κB signaling could mediate such a systemic effect.

A more straightforward approach to understanding the role of A-type lamins in DNA damage responses may involve loss-of-function studies. In contrast to progeroid models, loss of A-type lamins leads to 53BP1 degradation by the proteasome (Gonzalez-Suarez et al., 2009). In its absence, repair of double strand breaks proceeds more slowly, hindering effective non-homologous end joining (Redwood et al., 2011). Homologous recombination is also compromised through a transcriptional mechanism by which enhanced proteasome dependent degradation of pRb and p107 leads to repression of RAD51 and BRCA1

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(Redwood et al., 2011). It remains unclear why enhanced protein turnover of pRb and 53BP1 occur in the absence of A-type lamins, but the hypothesis has been put forward that A-type lamins may have a general role in promoting the stability of several nuclear regulatory factors through keeping proteasome-dependent degradation in check (Parnaik et al., 2011). It should also be noted that many of these properties may explain why loss of A-type lamin expression could have tumor promoting properties.

In addition to impaired DNA damage response pathways, telomere dysregulation may also contribute to progeroid pathology. HGPS fibroblasts in culture experience faster telomere shortening and progerin expression in normal fibroblasts recapitulates this phenotype, as well as enhancing formation of signal free ends (Decker et al., 2009). Enhanced telomere attrition may contribute to proliferation defects and early senescence, since telomerase expression restores both properties in fibroblasts (Benson et al., 2010; Kudlow et al., 2008). One role of telomerase may be to enhance resolution of DNA damage foci, which were found to localize in regions near telomeres (Benson et al., 2010). The mechanisms by which this might occur and the extent to which altered telomere dynamics promotes progeroid pathology remains to be determined.

Given that HGPS (and other laminopathies) primarily affect tissues of mesenchymal origin, altered mesenchymal stem cell function may be a major site of progerin-induced dysfunction. Gene expression profiling in fibroblasts expressing progerin provide support for this assertion as Notch signaling was found to be highly enhanced (Scaffidi and Misteli, 2008). Elevated Notch activity associated with progerin expression was found to promote expression of a range of differentiation markers in human mesenchymal stem cells. As a possible mechanism, progerin was found to disrupt nuclear matrix association of SKIP, a co-activator of Notch genes, leading to its release into the nucleoplasm and activation of targets. Reduced mesenchymal stem cell function could promote a subset of progeroid phenotypes in vivo, but this remains to be tested.

The Wnt/β-catenin pathway is altered in a variety of laminopathies as well. In both *Zmpste24*$^{-/-}$ and HGPS mice, reduced β-catenin levels were detected and cell proliferation defects could be rescued by inhibition of Gsk-3β, leading to β-catenin stabilization (Espada et al., 2008; Hernandez et al., 2010). Notably the Wnt pathway may be disrupted in mice lacking emerin as well (Markiewicz et al., 2006; Tilgner et al., 2009). Given that the Wnt pathway may have critical roles in maintaining adult stem cell function with age, the role of this pathway in laminopathies needs further interrogation.

Adult stem cells may also be impaired in progeroid laminopathies due to impaired SIRT1 function. A recent study has demonstrated that A-type lamins interact with the protein deacetylase and that preprocessed lamin A disrupts this association in *Zmpste24*$^{-/-}$ cells, leading to reduced deacetylase activity and rapid in vivo stem cell depletion (Liu et al., 2012). Treatment of mice with resveratrol restores SIRT1 activity, reduces the pathology and extends survival, indicating that enhancing SIRT1 activity may be another therapeutic approach in progeroid disorders associated with *LMNA* mutation.

Conclusions

Since the identification of diseases caused by mutations in genes encoding for nuclear lamina proteins in 1999, research has been dedicated toward understanding the molecular mechanisms leading to these specific phenotypes. Understanding how the nuclear lamina interacts with structural proteins, chromatin, transcription factors, and other signaling partners will likely give us an understanding of mechanistic links to disease. At this moment, the puzzle is starting to come together, but the overall picture of how lamins

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regulate all of these pathways and how this regulation leads to disease is still underway. Understanding the mechanisms by which mutations in lamins cause these rare diseases will provide molecular insight into other common conditions that laminopathies model (such as muscle diseases, cardiomyopathy, aging?). Additionally, since mutations in the nuclear lamina result in rapid aging-like disease, defining the role of the nuclear lamina in regulating normal human longevity will be of great importance.

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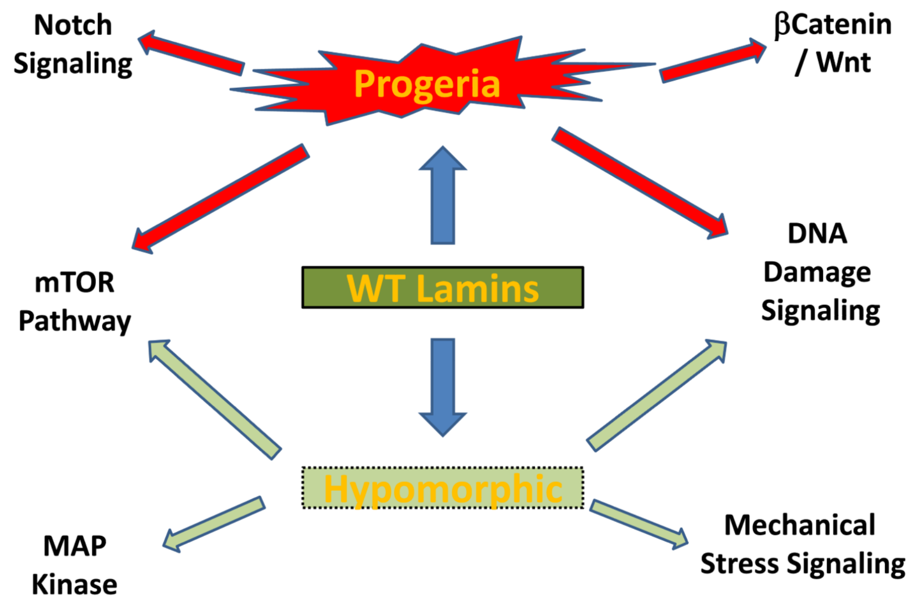

Figure 1. Signaling pathways disrupted by *LMNA* mutations

Recent years have seen several discoveries of signal transduction pathways that are altered in *LMNA* mutant backgrounds associated with gain-of-function toxicity, loss-of-function or both. A list of pathways are provided that are described in detail in the text.

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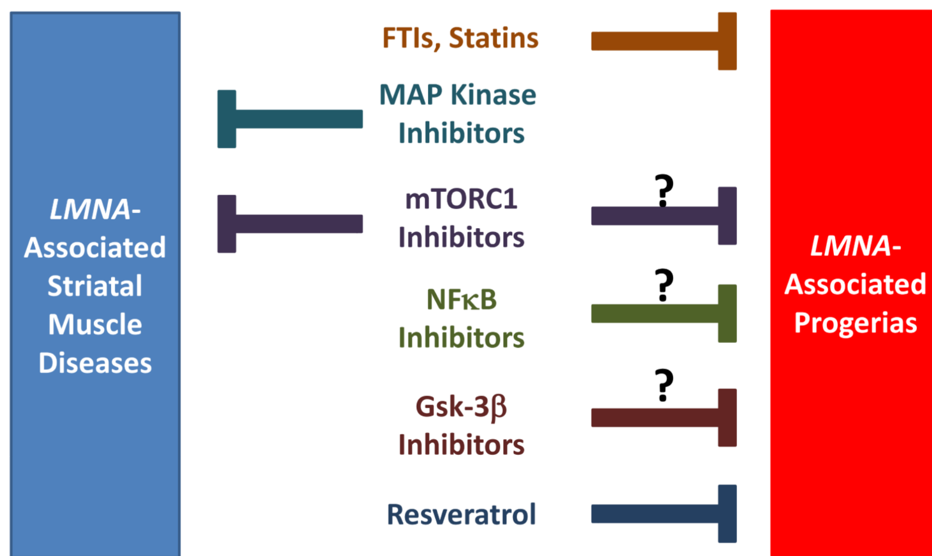

Figure 2. Potential therapeutic approaches to laminopathies

Several small molecules have been proposed as treatments for laminopathies. The major ones are listed with arrows indicating the diseases to which they may have efficacy. Question marks indicate that animal data has yet to be presented. Notably, FTIs have been tested in human children with HGPS with promising initial results (Gordon et al., 2012).

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Table 1

Diseases Caused by Mutations in Genes Encoding Lamins and Lamin-Associated Proteins

Striated Muscle Diseases	**Gene Mutated**
Emery-Dreifuss muscular dystrophy	*LMNA, EDMD, SYNE1, SYNE2, TMEM43, TMPO*
Limb-girdle muscular dystrophy	*LMNA*
Dilated cardiomyopathy	*LMNA, EDMD, SYNE1, SYNE2, TMEM43, TMPO*
Cogenital muscular dystrophy	*LMNA*
Heart-hand syndrome	*LMNA*
Lipodstrophy	
Dunnigan-type familial partial lipodystrophy	*LMNA*
Mandibuloacral dysplasia	*LMNA, ZMPSTE24*
Lipoatrophy	*LMNA*
Partial Lipodystrophy	*LMNB2*
Premature Aging	
Atypicial Werner Syndrome	*LMNA*
Hutchinson-Gilford Progeria Syndrome	*LMNA*
Restrictive Dermopathy	*LMNA, ZMPSTE24*
Atypical Progeria Syndrome	*BANF1*
Peripheral Nerve Disorders	
Charcotte-Marie Tooth Syndrome	*LMNA*
Adult-onset leukodystrophy	*LMNB1*
Spinocerebellular ataxia type 8	*SYNE1*
Bone Diseases	
Buschke-Ollendorff Sydrome	*LEMD3*
Melorheostosis	*LEMD3*
Osteopoikilosis	*LEMD3*
Greenberg skeletal dysplasia	*LBR*
Other	
Pelger-Huet Anomaly	*LBR*
Arthrogryposis	*SYNE2*