

## Laminopathies: Multiple disorders arising from defects in nuclear architecture

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Lamins are the major structural proteins of the nucleus in an animal cell. In addition to being essential for nuclear integrity and assembly, lamins are involved in the organization of nuclear processes such as DNA replication, transcription and repair. Mutations in the human lamin A gene lead to highly debilitating genetic disorders that primarily affect muscle, adipose, bone or neuronal tissues and also cause premature ageing syndromes. Mutant lamins alter nuclear integrity and hinder signalling pathways involved in muscle differentiation and adipocyte differentiation, suggesting tissue-specific roles for lamins. Furthermore, cells expressing mutant lamins are impaired in their response to DNA damaging agents. Recent reports indicate that certain lamin mutations act in a dominant negative manner to cause nuclear defects and cellular toxicity, and suggest a possible role for aberrant lamins in normal ageing processes.

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### 1. Introduction

In eukaryotic cells, the nucleus is segregated from the cytoplasm by a double-layer membrane termed nuclear envelope. The outer nuclear membrane is contiguous with the endoplasmic reticulum, while the inner nuclear membrane is associated with a filamentous network of proteins called the nuclear lamina (Fawcett 1966). The nuclear pore complexes, which mediate import and export of molecules, are embedded in the nuclear envelope at regular intervals. Major components of the lamina are a group of nuclear proteins termed lamins which belong to the intermediate filament superfamily of proteins (reviewed by Goldman *et al* 2002; Gruenbaum *et al* 2005). Lamins have been shown to be involved in a number of nuclear processes, and increasing evidence suggests that lamins can interact directly with proteins that function in diverse cellular pathways.

In studies carried out by several groups in the past decade, >150 mutations in the human lamin A gene (*LMNA*) have been linked to at least 10 highly degenerative, heritable, rare disorders that primarily affect muscle, adipose, bone or neuronal tissues and also result in premature ageing syndromes. These disorders have been collectively termed laminopathies. The human lamin A gene has been mapped to chromosome 1q21.2 – 1q21.3 (Wydner *et al* 1996); mutations observed in laminopathies have been linked to *LMNA* using both positional cloning and candidate gene approaches. It is indeed rare to find different mutations in the same gene giving rise to distinct, possibly unrelated diseases. Though we are far from understanding how lamin mutations result in disease, phenotypes of the various diseases have given valuable clues about the functional roles of lamins, especially in tissue-specific differentiation.

**Keywords.** Adipocyte differentiation; DNA repair; lamins; muscle differentiation; nuclear lamina

Abbreviations used: ATM, Ataxia-telangiectasia-mutated; ATR, ATM-and-Rad3-related; CMT, Charcot-Marie Tooth disorder; DCM, dilated cardiomyopathy; EDMD, Emery-Dreifuss muscular dystrophy; FPLD, familial partial lipodystrophy; HGPS, Hutchinson-Gilford progeria syndrome; MAD, mandibuloacral dysplasia; pol II, polymerase II.

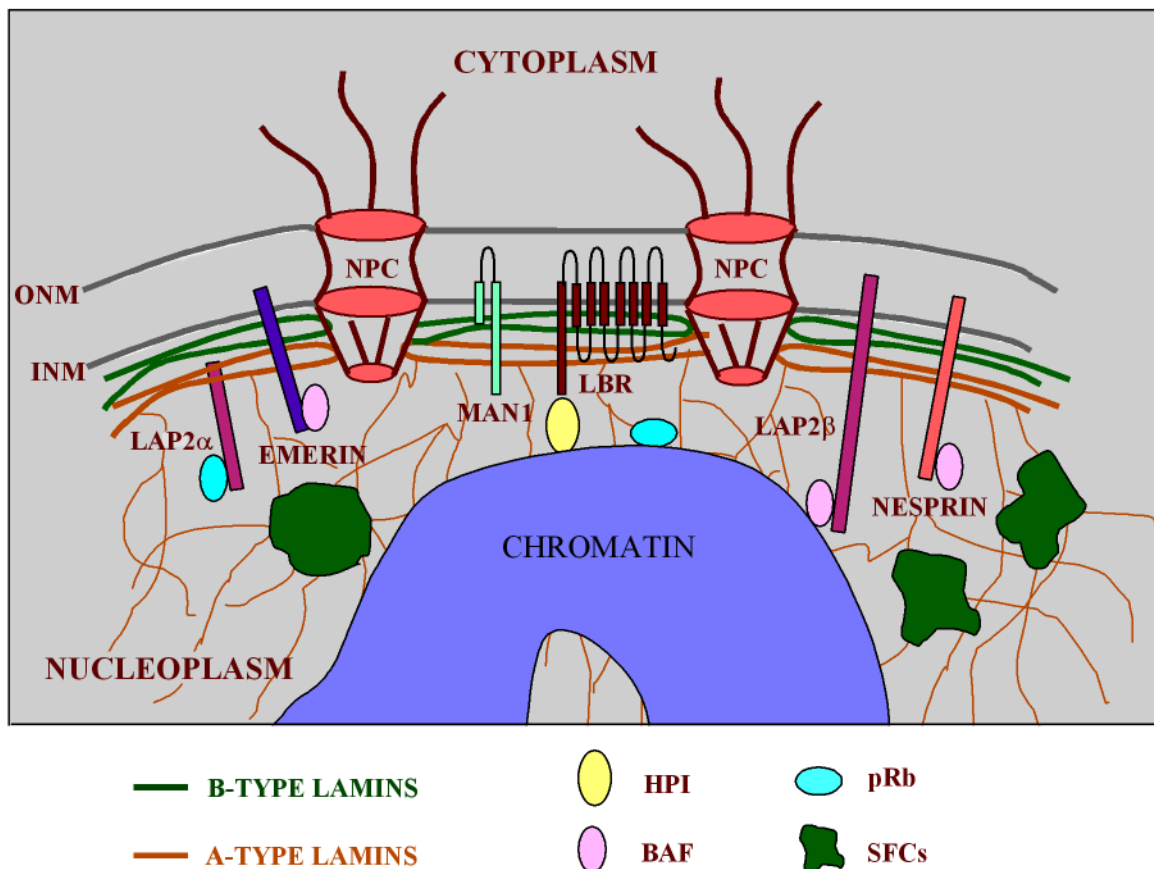
In this review, we will briefly summarize the structure and functions of lamins, and then discuss the laminopathies with reference to the actual disease conditions in humans and studies in model organisms as well as in cell culture, and finally describe possible mechanisms of pathogenesis. Further details of clinical findings can be found in the original references and have been recently reviewed by Jacob and Garg (2006).

## 2. Lamin structure and functions

### 2.1 Structural organization

Lamins typically form a stable polymer situated beneath the inner nuclear membrane, but have also been located in the interior of the nucleus, as shown in figure 1 (Goldman *et al* 1992; Hozak *et al* 1995; Jagatheesan *et al* 1999). The lamina plays an essential role in maintaining the integrity of the

nuclear envelope and provides anchoring sites for chromatin, and is hence considered to be an important determinant of interphase nuclear architecture (reviewed in Stuurman *et al* 1998; Goldman *et al* 2002; Shumaker *et al* 2003; Gruenbaum *et al* 2005). Two major kinds of lamins are present in vertebrate cells: B-type lamins (B1 and B2) that are found in nearly all somatic cells, and A-type lamins (A and C) which have been detected primarily in differentiated cells. The lamin genes are highly conserved across species. Lamins A and C (henceforth called lamin A/C) are alternatively spliced products of the lamin A gene, *LMNA*, whereas lamins B1 and B2 are coded by two separate genes, *LMNB1* and *LMNB2*. Additional splice variants are germ cell-specific lamins C2 and B3 which are encoded by *LMNA* and *LMNB2* respectively, and a minor somatic cell isoform termed lamin A $\Delta$ 10. *Drosophila melanogaster* has two lamin genes, lamin Dm0, which is expressed in most cells and lamin C, whose expression is developmentally regulated. *Caenorhabditis elegans* has only one lamin gene, *lmn-1*, which is expressed



**Figure 1.** Schematic representation of nuclear envelope. The major components of the nuclear envelope and some of the nuclear proteins that interact with the lamins are shown here. The A-type lamins are concentrated at the periphery (thick lines) and also distributed throughout the nucleoplasm (thin lines). ONM, outer nuclear membrane; INM, inner nuclear membrane; NPC, nuclear pore complex; LAP, lamina-associated polypeptide; LBR, lamin B receptor; HPI, heterochromatin protein 1; pRb, retinoblastoma protein; BAF, barrier-to-autointegration factor; SFC, splicing factor compartment.

in all cells except for mature sperm. Genome sequence analysis of yeast and *Arabidopsis* indicates that these species do not have lamins and, though functional homologs are likely to exist, these have not yet been definitively identified. Thus lamins appear to have evolved in animal cells.

Lamins belong to the intermediate filament family of proteins and contain a characteristic central  $\alpha$ -helical rod domain, flanked by relatively flexible N-terminal and C-terminal segments. The C-termini of lamins A, B1 and B2 bear a CaaX motif (C is cysteine, a is an aliphatic amino acid and X is any amino acid) which is post-translationally modified by farnesylation, and, in the case of lamin A, is subjected to a further maturation step by which pre-lamin A is proteolytically cleaved to give mature lamin A. *In vitro* studies have shown that all three domains of the protein are essential for lamin assembly and functions. The rod domains of two lamin monomers associate to form a two-stranded coiled-coil  $\alpha$ -helix, which is the basic dimeric subunit involved in formation of the higher order structure of the lamin filament. Head-to-tail interactions of lamin dimers lead to formation of 2–3 nm diameter protofilaments which have the propensity to associate laterally in different configurations such as parallel, staggered or half-staggered to give rise to the 10 nm lamin filament (Stuurman *et al* 1998). Although formation of the 10 nm filament has not been observed *in vitro* with vertebrate lamins, the assembly of the ~10 nm filaments has been observed with *C. elegans* lamin (Karabinos *et al* 2003). It has been suggested that higher-order lamin assembly might also involve interactions with other molecules *in vivo*.

## 2.2 Assembly and disassembly of lamins

During mitosis, phosphorylation of essential serine residues on either end of the rod domain of lamin by p34/cdc2 kinase results in depolymerisation of the lamina into dimers and tetramers. The lamina is reassembled towards late telophase and in early G1 phase of the cell cycle; lamin B appears to be incorporated when the nuclear membranes and pores are assembling in the nascent nuclear envelope, whereas lamin A/C is imported through the nuclear pores towards the end of cytokinesis (reviewed in Gant and Wilson 1997). An important finding reported recently is that lamin B is essential for formation of a matrix-like network required for assembly of the mitotic spindle (Tsai *et al* 2006). In addition to the typical nuclear rim pattern, lamin A and B tagged with green fluorescent protein (GFP) have been observed to form a veil of fluorescence in the nucleoplasm of interphase cells (Broers *et al* 1999; Moir *et al* 2000b). During apoptosis, lamins are cleaved by caspases, and this helps to disassemble the lamina and allows the nuclear events of apoptosis such as reduction in nuclear size and chromatin condensation to proceed (Cohen *et al* 2001).

## 2.3 Lamin-binding proteins

Majority of the proteins associated with the inner nuclear membrane interact with lamins and/or chromatin. Prominent lamin-binding proteins are emerin, lamin B receptor, and lamin-associated-polypeptides (LAPs) 1 and 2. Emerin, LAP2 and another envelope protein MAN1 possess a 40-residue folded motif called the LEM domain (derived from LAP, emerin, MAN1) that binds directly to barrier-to-autointegration factor (BAF), a conserved DNA binding protein that is involved in higher-order chromatin structure and is important for nuclear assembly (reviewed in Zastrow *et al* 2004). LAP2 $\alpha$  is an important binding partner for lamin A in the interior of the nucleus (Dechat *et al* 2000; Markiewicz *et al* 2002). These findings suggest that interactions between lamins and its binding partners might influence chromatin structure and gene activity. Moreover, many of the mutations that cause laminopathies also result in reduced amounts of emerin at the nuclear envelope (Östlund *et al* 2001; Raharjo *et al* 2001; Vigouroux *et al* 2001).

A newly described class of proteins termed nesprins (also called Syne/ANC-1 proteins) span the outer nuclear membrane, and smaller nesprin isoforms localized in the inner nuclear membrane are able to bind to lamin A as well as emerin. The nesprins are large proteins with several spectrin repeats, and exist in many forms with tissue-specific expression patterns due to alternate splicing (Mislow *et al* 2002; Zhang *et al* 2002). Some of the nesprins contain a calponin homology domain which is known to bind to actin; nesprins have also been localized in multiple cytoplasmic compartments and in association with the actin cytoskeleton (Zhang *et al* 2005). Genetic studies suggest that the nesprins are required for the anchorage of the nucleus to the cytoskeleton (Starr and Han 2003), and may thus be involved in correct nuclear positioning as well as transmission of regulatory signals from the cell surface to the nucleus. A new class of inner nuclear membrane proteins called the SUNs are likely to provide a link between the cytoplasm and nuclear interior as they can bind to nesprins at the outer nuclear membrane (Padmakumar *et al* 2005). A recent study has reported binding of the lamins to nuclear titin, a protein essential for mitotic chromosome condensation, suggesting a possible role for titin in nuclear organization as well (Zastrow *et al* 2006). Loss-of-function mutations in *Drosophila* lamin Dm0 suggest that lamins might be involved in additional functions such as cytoplasmic organisation and cell polarity (Guillemain *et al* 2001).

## 2.4 Lamins and DNA replication

Several studies indicate that a normal lamina organization is required for DNA replication (Newport *et al* 1990; Meier *et al* 1991). Although it is possible to replicate DNA *in vitro*

with purified factors in the absence of lamins (Walter *et al* 1998), an intact lamina is essential for DNA replication in assembled nuclei and cells. When N-terminal deletion mutants of human lamin A (Spann *et al* 1997; Moir *et al* 2000a) or *Xenopus* lamin B1 (Ellis *et al* 1997) were added to assembled nuclei, they disrupted lamin organization and inhibited DNA synthesis. A direct role for lamins in the spatial organization of DNA replication has been suggested by evidence of colocalization of lamin B with replication foci and replication factors such as PCNA in NIH 3T3 cells (Moir *et al* 1994), and colocalization of lamin A with replication factors at specific stages of S phase in primary mammalian cells (Kennedy *et al* 2000).

### 2.5 Lamins and gene regulation

Early studies on the developmental and tissue-specific expression of A-type lamins in mouse and *Drosophila* pointed to a possible involvement of lamins in gene regulation (Röber *et al* 1989; Riemer *et al* 1995). A-type lamins have been shown to associate with specific transcription factors such as the retinoblastoma protein (pRb) (Mancini *et al* 1994; Ozaki *et al* 1994), sterol response element binding protein 1 (SREBP1) (Lloyd *et al* 2002), a Kruppel-like protein, MOK2 (Dreiuillet *et al* 2002) and more recently, c-Fos (Ivorra *et al* 2006). Lamin A/C can bind to protein phosphatase 2A to modulate TGF- $\beta$ 1 signalling in fibroblasts (van Berlo *et al* 2005). There is evidence that localization of specific genes at the nuclear periphery may lead to transcriptional silencing. In *Drosophila*, insertion of the *gypsy* insulator into a gene sequence causes its translocation to the nuclear envelope (Gerasimova and Corces 1998). Association of the POU domain repressor protein Oct-1 with lamin B1 has been correlated with its repressor activity (Imai *et al* 1997). Lamin B together with LAP2 $\beta$  forms functional complexes with the transcription factors germ-cell-less (GCL) and DP to repress E2F (de la Luna *et al* 1999; Nili *et al* 2001). Lamins can also bind directly to histones (Taniura *et al* 1995).

Definitive evidence of a role for lamins in organization of transcription has been provided by the following studies. Goldman and colleagues have shown that expression of an N-terminal deletion mutant of lamin A,  $\Delta$ NLA, in cells leads to disruption of the lamina and inhibition of RNA polymerase II (pol II) transcription, without affecting pol I or pol III transcription (Spann *et al* 2002). Our group has shown that nucleoplasmic lamins termed lamin speckles are relocalized into enlarged domains upon treatment with transcriptional inhibitors, and that disruption of these domains in various mammalian cells disrupts splicing factor compartments and inhibits RNA pol II transcription (Kumaran *et al* 2002). We have shown that lamin speckles colocalize with splicing factor compartments using a monoclonal antibody LA-2H10

that specifically recognizes A-type lamins (Jagatheesan *et al* 1999). The epitope sequence for this antibody has been determined to be NIYSEEL, which corresponds to residues 209-215 in lamin A/C and is present exclusively in A-type lamins (Bh Muralikrishna and S Thanumalayan, unpublished data). This peptide blocks antibody reactivity in immunofluorescence assays whereas sequences with lower homology in lamins B1 or B2 have no effect. Furthermore, RNAi to lamin A/C disrupts lamin speckles in addition to the peripheral lamina, and also disrupts splicing factor compartments, suggesting an important role for lamin speckles in the maintenance of splicing factor compartments (B V Gurudatta, unpublished data). Splicing factor compartments are dynamic domains involved in the storage and recruitment of splicing factors (Spector 2003). Previous studies have indicated presence of the lamins in purified preparations of splicing factor compartments (Mintz *et al* 1999).

## 3. Laminopathies

### 3.1 Muscular dystrophies

The clinical condition termed Emery-Dreifuss muscular dystrophy (EDMD) was first described by Alan Emery and Fritz Dreifuss in the 1960's (Emery and Dreifuss 1966). This is marked by contractures of the elbows, achilles tendons and posterior neck, slow progressive muscle wasting and cardiomyopathy with atrioventricular conduction block. The gene responsible for X-linked EDMD was identified by a positional cloning approach and named 'emerin' (Bione *et al* 1994). Emerin is an inner nuclear membrane protein which has been shown to interact with A-type lamins and, together with another protein, BAF, mediates lamin-chromatin interactions (Zastrow *et al* 2004). Emerin was shown to be deficient in cells of patients suffering from EDMD (Manilal *et al* 1996; Nagano *et al* 1996). Subsequently an autosomal dominant form of EDMD has been attributed to mutations in *LMNA* (Bonne *et al* 1999; Brown *et al* 2001). Most of the mutations are missense mutations and a few are small deletions; mutations are found in all the exons of the gene except exon 12. Both familial and sporadic mutations have been identified. For the same mutation, there is considerable variability in the range of symptoms observed in different families as well as within a family; some patients show the full clinical symptoms of EDMD whereas others show only cardiac symptoms (Bonne *et al* 2000). Hence the occurrence of modifier genes cannot be ruled out. A rare autosomal recessive form of EDMD is caused by the inheritance of two different mutant *LMNA* alleles (Raffaelli Di Barletta *et al* 2000). A case of autosomal recessive EDMD has been documented to be due to homozygosity for a H222Y

mutation (Sanna *et al* 2003); the parents of the individual did not show skeletal or cardiac dysfunction. In addition, missense mutations and splicing defects in *LMNA* have been linked to autosomal dominant limb girdle muscular dystrophy type 1B (LGMD1B) (Muchir *et al* 2000). LGMD1B is a slowly progressing disease characterized by weakness and wasting of shoulder and pelvic muscles due to necrosis, and is accompanied by cardiac conduction defects in several patients. A lethal phenotype for a newborn child from an LGMD1B family has been attributed to homozygosity for a Y259X nonsense mutation (Muchir *et al* 2003; van Engelen *et al* 2005).

### 3.2 Dilated cardiomyopathy

Dilated cardiomyopathy (DCM) may be genetic in origin or brought about by a variety of factors such as viruses, alcohol, toxic chemicals etc. The familial cases of DCM are due to mutations in a number of different genes. Autosomal dominant DCM type 1A is associated with mutations in *LMNA* (Fatkin *et al* 1999). DCM is a progressive disease and is characterized by ventricular dilation and systolic dysfunction. DCM is usually accompanied by conduction defects, as well as skeletal muscle involvement in patients with *LMNA* mutations. Due to the risk of sudden death among such patients, an effective therapeutic intervention is the use of an implantable cardioverter-defibrillator to treat possibly lethal tachyarrhythmias (Meune *et al* 2006).

### 3.3 Lipodystrophies

Dunnigan-type familial partial lipodystrophy (FPLD) is an autosomal dominant disorder characterized by loss of fat tissue from the extremities and excess fat accumulation on the face and neck, beginning at puberty. This is accompanied by insulin-resistant diabetes, hyperlipidemia, and atherosclerotic vascular disease. The gene responsible for FPLD has been identified as *LMNA* by several groups (Cao and Hegele 2000; Shackleton *et al* 2000; Speckman *et al* 2000). Approximately 90% of the mutations are localized to exon 8, with substitutions at arginine at 482 position being found in 75% of cases. Cardiomyopathy and possible symptoms of LGMD have been reported in FPLD patients with mutations in exon 1 (Garg *et al* 2002; van der Kooi *et al* 2002) but not in FPLD patients with mutations in exons 8 or 11.

Generalized lipodystrophy has been associated with *LMNA* mutations (Caux *et al* 2003) and is accompanied by insulin-resistant diabetes and cardiomyopathy. A patient with Seip syndrome and showing generalized lipodystrophy together with features of progeroid syndromes including short stature, scleroderma-like skin, and early graying of

hair has been reported to harbour a T10I *LMNA* mutation (Csoka *et al* 2004a).

### 3.4 Progeria syndromes

The most dramatic effects of mutations in *LMNA* have been observed in the premature ageing disorder Hutchinson-Gilford progeria syndrome (HGPS), and were reported by two groups in 2003 (De Sandre-Giovannoli *et al* 2003; Eriksson *et al* 2003). HGPS is a very rare disorder (about one in a million) that leads to early mortality, usually in the second decade of life. HGPS is an autosomal-dominant condition that is characterized by short stature, early thinning of skin, loss of subcutaneous fat, premature atherosclerosis and cardiac failure leading to death. Majority of the cases are due to a *de novo* missense mutation (GGC to GGT) in exon 11 that does not cause an amino acid change (G608G), but leads to creation of an abnormal splice donor site that results in expression of a truncated pre-lamin A protein (also termed progerin) with loss of 50 amino acids from the C-terminus, but retention of the C-terminal CaaX motif. Other missense mutations such as R471C and R527C have also been described which do not result in expression of truncated pre-lamin A protein (Cao and Hegele 2003). *LMNA* mutations that lead to atypical progeroid syndromes have been reported (Csoka *et al* 2004a). Chen *et al* (2003) have described cases with atypical Werner's syndrome with an early adult onset phenotype and less severe disease severity than HGPS. The patients were found to have missense mutations in *LMNA*. Recently, a case of autosomal recessive HGPS has been reported with a K542N mutation in *LMNA* (Plasilova *et al* 2004); phenotypes in the family of this patient overlapped with those observed in mandibuloacral dysplasia (described below).

### 3.5 Neuropathy

An autosomal recessive mutation at R298C of *LMNA* gives rise to an axonal neuropathy termed Charcot-Marie-Tooth disorder (CMT) type 2B (De Sandre-Giovannoli *et al* 2002). Patients showed sensory impairment with some reduction in motor nerve conduction velocity. The heterozygous carriers did not exhibit clinical signs of neuropathy. Recently, cases have been reported with the dominant *LMNA* mutations E33D and R571C, which result in overlapping symptoms of muscular dystrophy and neuropathy, and with cardiac disease (Goizet *et al* 2004; Benedetti *et al* 2005; Vital *et al* 2005).

### 3.6 Bone and skin disorders

Mandibuloacral dysplasia (MAD) is a rare, autosomal recessive disorder characterized by postnatal growth retardation, skull and facial anomalies, skeletal abnormalities,

mottled skin pigmentation, partial lipodystrophy and signs of premature ageing (Novelli *et al* 2002). Several patients have been reported to have the R527H homozygous mutation in *LMNA*. A patient with a R527C/R471C heterozygous mutation, reported to have atypical progeria, also showed symptoms of MAD (Cao and Hegele 2003). In addition, MAD is caused by mutations in *Zmpste24* protease which is involved in the processing of pre-lamin A to lamin A (Agarwal *et al* 2003). Metabolic studies have revealed hyperinsulinemia in many subjects of MAD.

Mutations in *LMNA* as well as *Zmpste24* are associated with restrictive dermopathy, a rare disorder characterized by intra-uterine growth retardation, tight and rigid skin with erosions, facial malformation, bone mineralization defects and early neonatal mortality (Navarro *et al* 2004, 2005; Shackleton *et al* 2005). Some of the mutations in *LMNA* or *Zmpste24* led to creation of premature termination codons, resulting in truncated proteins. One case has been diagnosed with the G608G mutation identical to that observed in HGPS (Navarro *et al* 2004), with phenotypes in between those of HGPS and restrictive dermopathy. Thus HGPS, MAD and restrictive dermopathy appear to represent a clinical spectrum of related disorders, with the severity depending on the dysfunction of lamin A/C. Recently a case has been reported with a combination of myopathy and progeria, bearing the S143F mutation (Kirschner *et al* 2005).

At this juncture, the role of other factors or modifying genes in laminopathies cannot be ruled out. It is also possible that more genetic disorders may be attributed to mutations in *LMNA* or related genes in future studies. Mutations in the inner nuclear membrane protein, lamin B receptor, which interacts with lamins and chromatin, lead to an autosomal dominant genetic condition termed Pelger-Huët anomaly (Hoffman *et al* 2002), which is characterized at the cellular level by blood granulocytes displaying abnormal nuclei and chromatin, and is associated with varying levels of developmental delay, epilepsy and skeletal abnormalities. A recessive disorder called Greenberg skeletal dysplasia is a lethal chondrodystrophy which has also been attributed to the lamin B receptor gene (Waterham *et al* 2003). Mutations in *MAN1* cause inherited diseases that mainly affect bone and skin tissues (Hellems *et al* 2004). A mutation in the *LAP2α* gene has been linked to DCM (Taylor *et al* 2005). At present, no mutations in the lamin B genes have been linked to human disease, suggesting that loss of one of the B-type genes is either without consequence or results in death in early development, as observed in a mouse knock-out of the lamin B1 gene (Vergnes *et al* 2004).

#### 4. Animal models for laminopathies

Valuable insights into the functions of *LMNA* have been obtained by gene targeting studies in mice. A lamin A/C

gene knock-out in exons 8–11 leads to the development of muscular dystrophy symptoms resembling EDMD and results in mortality by 2 months of age (Sullivan *et al* 1999). The *Lmna*<sup>-/-</sup> animals also show loss of white adipose tissue (but not partial lipodystrophy or insulin resistance), cardiac dysfunction (Nikolova *et al* 2004), non-myelinated axons and reduced axon density (De Sandre-Giovannoli *et al* 2002). Heterozygous *Lmna*<sup>+/-</sup> mice, however, do not show any symptoms that could arise due to haploinsufficiency. This is unlike the case of the human EDMD Q6X mutation, which results in a stop codon at position 6 and effectively encodes only one copy of intact lamin A (Bonne *et al* 1999). A mouse model for progeria has been reported for the L530P mutation by a gene knock-in technique (Mounkes *et al* 2003). Although this mutation is associated with EDMD in humans, the aberrantly spliced forms of lamin A observed in this mouse model might have given rise to the progeria-like symptoms. A mouse model for EDMD has been obtained by gene targeting of a mouse *Lmna* fragment with a H222P mutation by a knock-in approach (Arimura *et al* 2005). This mutation had been earlier identified in a family with autosomal dominant EDMD (Bonne *et al* 2000). Homozygous knock-in mice developed skeletal muscle degeneration and cardiac dysfunction by adulthood but did not show symptoms at earlier stages. Another mouse model with knock-in of the N195K mutation, which leads to DCM in humans, shows features characteristic of this disease. At the cellular level, organization of the cardiomyocytes was disrupted, together with misregulation of factors required for normal cardiac development (Mounkes *et al* 2005). Another useful mouse model is the *Zmpste24*-null mouse which develops DCM and muscular dystrophy, as well as progeria-like features (Bergo *et al* 2002; Pendás *et al* 2002). Unprocessed pre-lamin accumulates in the nuclei of these mice, leading to irregular nuclei with herniations and nuclear blebs. A progerin knock-in model has been reported wherein heterozygous mice exhibit growth retardation and fibroblasts from these mice show aberrant nuclear morphology (Yang *et al* 2005). In a recent report, a mouse transgenic model bearing the human *LMNA* gene with a G608G mutation shows a progressive loss in vascular smooth muscle cells (Varga *et al* 2006), as seen in HGPS patients (McClintock *et al* 2006). It may be noted that most of these mouse models show disease symptoms only in the homozygous state, unlike the autosomal dominant nature of the human diseases, where only one allele is mutated. Thus all features of the human diseases might not be faithfully reproduced by the mouse models.

In *C. elegans*, which has a single lamin gene, loss of lamin causes abnormal heterochromatin organization, unequal separation of chromosomes and abnormal distribution of nuclear pore complexes, leading to embryonic lethality (Liu *et al* 2000). Mutations in *Drosophila* lamin Dm0

(a B-type lamin) display developmental abnormalities in organogenesis, locomotion, tracheal development and nuclear positioning in the oocyte and eye, as well as aberrant nuclear morphology (Lenz-Bohme *et al* 1997; Guillemain *et al* 2001; Patterson *et al* 2004; Osouda *et al* 2005). In a recent study, mutations in *Drosophila* lamin C, an A-type lamin, have been shown to be lethal at the pre-pupal stage, and an R401K mutation (homologous to the R386K mutation that causes EDMD in humans) showed defects in nuclear morphology (Schulze *et al* 2005). A detailed study carried out by us indicates that *Drosophila* lamin C is essential for tissue-specific development and chromatin organization (B V Gurudatta, L S Shashidhara and Veena K Parnaik, manuscript in preparation).

## 5. Mechanisms of pathogenesis

There is currently considerable interest in understanding how mutations in *LMNA* lead to a broad spectrum of diseases. The major question is how do mutations in lamin A, which is expressed in nearly all differentiated tissue types, cause several diseases, some of which are tissue-specific. Two hypotheses have been proposed to explain the clinical observations. The “mechanical stress” hypothesis states that abnormalities in nuclear structure, resulting from mutations in lamin A, weaken the nuclear lamina-envelope network and thus lead to increased susceptibility to cellular damage by physical stress (Sullivan *et al* 1999). The “gene expression” hypothesis proposes that nuclear lamin plays a role in tissue-specific gene expression which can be altered by mutations in lamin A (Wilson 2000). A number of studies have been carried out with cells from patients and mouse models, as well as exogenously expressed mutants, to address this problem (reviewed by Hutchison and Worman 2004; Worman and Courvalin 2004; Smith *et al* 2005; Mattout *et al* 2006). Broadly speaking, it may be concluded that the above hypotheses are not mutually exclusive. Furthermore, effects on gene expression can impair downstream events in diverse ways, as will be evident from the following discussion.

### 5.1 Effects on nuclear integrity

Abnormal nuclear morphology has been observed in cells from patients with various laminopathies. Fibroblasts from patients with EDMD, LGMD, CMD and FPLD show abnormal nuclear phenotypes with nuclear blebbing and aberrant lamin foci in up to 20% of cells (Vigouroux *et al* 2001; Capanni *et al* 2003; Favreau *et al* 2003; Muchir *et al* 2004). Severe nuclear abnormalities have been reported in HGPS cells, including lobulation, blebbing and loss of heterochromatin (Eriksson *et al* 2003; De Sandre-Giovannoli *et al* 2003; Goldman *et al* 2004). It has been proposed that

the accumulation of pre-lamin A leads to pathogenesis, and this is supported by evidence for improvement of nuclear morphology by inhibiting abnormal splicing (Scaffidi and Misteli 2005), blocking farnesyl transferase activity in cells (Capell *et al* 2005; Columbaro *et al* 2005; Glynn and Glover 2005; Mallampalli *et al* 2005; Yang *et al* 2005; Varga *et al* 2006), knocking out the *Zmpste24* gene in a mouse model (Fong *et al* 2006a), or reducing the levels of progerin by RNA interference assays (Huang *et al* 2005). Importantly, a recent report indicates that a farnesyl transferase inhibitor administered to *Zmpste24* deficient mice can decrease progeria-like disease symptoms and improve survival (Fong *et al* 2006a), raising possibility of the beneficial effects of these drugs in humans. It has been recently reported that nuclei from old individuals acquire defects that are similar to those seen in cells from HGPS patients, and this has been attributed to accumulation of progerin (Scaffidi and Misteli 2006). A study with *C. elegans* has shown that there are age-dependent changes in nuclear shape and loss of peripheral heterochromatin in non-neuronal cells, supporting the hypothesis that defects in nuclear architecture might be a cause for normal ageing. The rate of these changes is affected by the insulin/IGF-1-like signalling pathway, which plays an important role in the ageing process (Haithcock *et al* 2005).

The *Lmn*<sup>-/-</sup> mouse model has provided an informative system to study defects associated with lamin A deficiency, in particular those leading to muscular dystrophy and cardiomyopathy. Fibroblasts from *Lmn*<sup>-/-</sup> mice have aberrant nuclear morphology, show herniations of the envelope and mislocalize emerin. In response to mechanical strain, these fibroblasts exhibit increased nuclear deformations and defective mechanotransduction, together with reduced expression of genes activated by NF-κB (Lammerding *et al* 2004). *Lmn*<sup>-/-</sup> mice also develop DCM, and cardiomyocytes from these mice show abnormal nuclear architecture with relocalization of heterochromatin to the nuclear interior, and, interestingly, changes in localization of the cytoplasmic filament protein desmin, thus leading to contractile dysfunction (Nikolova *et al* 2004). These two studies suggest that mutations in lamin A may cause striated muscle disease by impaired nuclear mechanics and secondary changes in gene expression, thus supporting the mechanical stress hypothesis for lamin pathogenesis. A further report on loss of mechanical stiffness in *Lmn*<sup>-/-</sup> fibroblasts is consistent with this hypothesis, and also suggests that a loss of physical interactions between lamins and the cytoskeleton may lead to general cellular weakness (Broers *et al* 2004). Interestingly, a recent report indicates that mice which have been targeted to express only lamin C and not lamin A are healthy and exhibit only minimal nuclear abnormalities (Fong *et al* 2006b). Hence lamin A and pre-lamin A appear to be dispensable in the mouse.

Exogenous expression of several lamin A/C mutants in mouse or human cells causes aberrant nuclear morphology, altered lamina assembly, mislocalization of emerin and disruption of the endogenous nuclear lamina (Östlund *et al* 2001; Raharjo *et al* 2001; Vigouroux *et al* 2001; Bechert *et al* 2003; Favreau *et al* 2003). Aberrant nuclear morphology results in cellular senescence, downregulation of transcription and apoptosis. Most of the mutants in the rod domain which cause EDMD affect lamin assembly and show increased mobility in live cells (Gilchrist *et al* 2005; Broers *et al* 2005). Some of the mutations in the C-terminal domain affect binding to emerin though the mutant proteins correctly assemble into the nuclear rim. The R482W mutation which causes FPLD does not affect filament stability (Gilchrist *et al* 2004), and this is consistent with predictions from the crystal structure of the C-terminal globular domain (Dhe-Paganon *et al* 2002).

### 5.2 Effects on muscle differentiation

Since the majority of mutations in *LMNA* affect muscle tissue, there is considerable interest in understanding the role played by A-type lamins in muscle development and effects of mutations on this process. Based on the mechanical stress hypothesis mentioned above, it is possible that muscle cells under high mechanical stress are unable to survive due to loss in nuclear integrity, leading to loss in cells and a dystrophic phenotype. The second possibility is that lamin A/C plays a specific role in muscle differentiation and mutations in lamin A/C may affect the muscle differentiation programme, leading to aberrant or incomplete differentiation due to misregulation of muscle-specific gene expression, which eventually leads to a dystrophic syndrome.

Favreau and coworkers have analysed the differentiation capacity of a stable clone of C2C12 myoblasts expressing a

common EDMD-causing lamin A mutation at arginine 453, R453W (Favreau *et al* 2004). These cells were deficient in expression of myogenic markers like myogenin, did not exit the cell cycle properly and were eventually targeted for apoptosis. Further, these cells showed persistence of the hyperphosphorylated form of pRb, though pRb is hypophosphorylated during normal myogenesis. Also, the muscle regulator Myf5 was expressed at high levels unlike in normal cells. Cells expressing the R482W FPLD mutation were able to differentiate normally. Subsequent studies have shown that the EDMD mutation W520S also inhibits myogenin expression and blocks myoblast differentiation (Markiewicz *et al* 2005).

The authors have investigated the effects of a range of EDMD missense mutants on C2C12 myoblast differentiation (unpublished results). The mutations studied were G232E, Q294P and R386K which cause autosomal dominant EDMD (Bonne *et al* 2000) and R482L that is prevalent in FPLD patients (Shackleton *et al* 2000). These mutants were expressed as GFP-fusion proteins in C2C12 myoblasts, under conditions wherein levels of ectopically expressed proteins were 20-30% of endogenous lamin A. As seen from table 1, G232E, Q294P and R386K predominantly assembled into intranuclear aggregates and often disrupted nuclear morphology, whereas R482L and wild-type GFP-lamin A were typically incorporated into the nuclear rim in ~90% of transfected cells after 24 h of transient transfection (see figure 2). As the G232E, Q294P and R386K mutations are in the rod domain of the protein (see figure 2A), these are likely to disrupt filament assembly and form aggregates, as observed earlier with FLAG-tagged R386K (Östlund *et al* 2001), though low amounts of exogenous protein may be incorporated into the peripheral lamina. When transfected myoblasts were induced to differentiate to multinucleated myotubes by serum depletion, there was extensive loss of cells expressing lamin aggregates, whereas cells displaying

**Table 1** Properties of C2C12 myoblasts expressing GFP-lamin A.

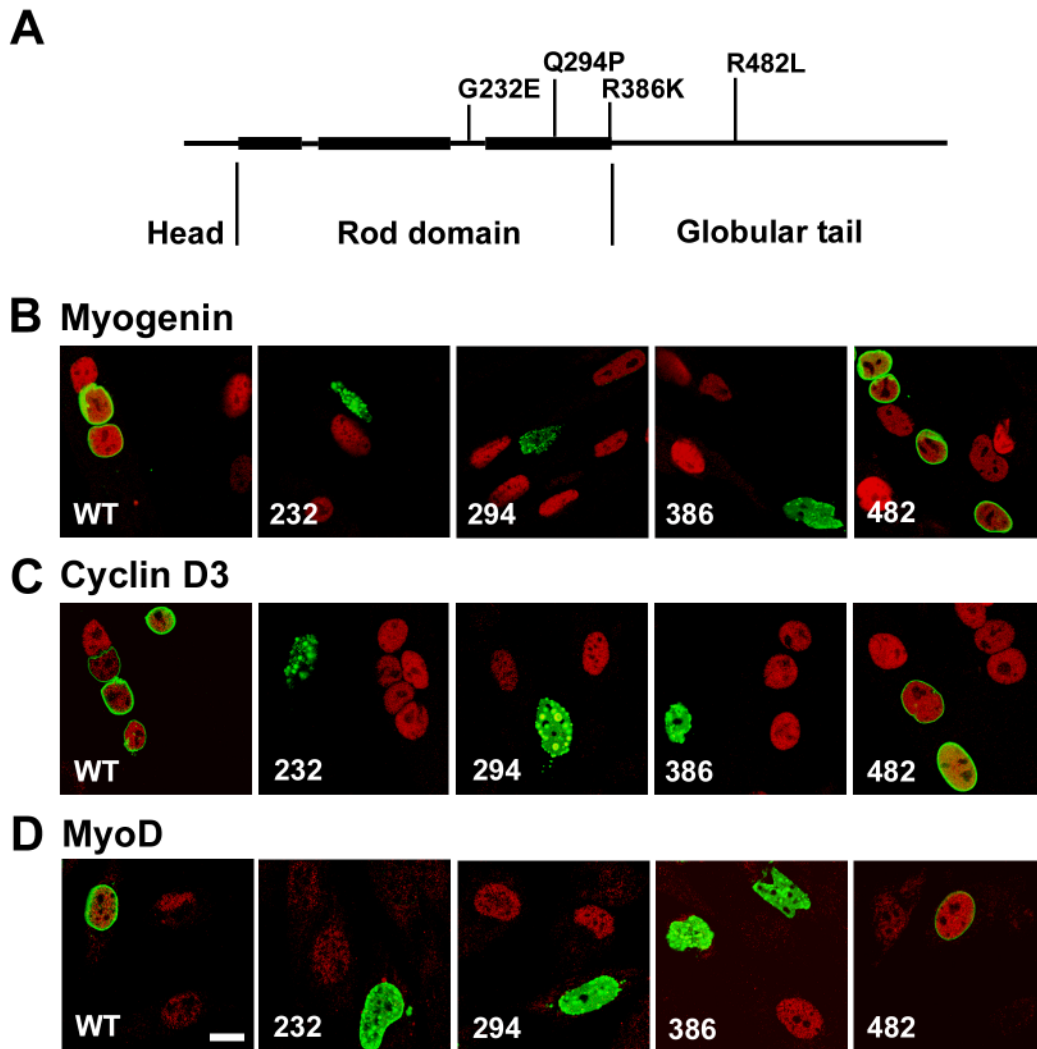
Mutation	Nuclear localization (%) <sup>a</sup>		Cell survival in DM (%) <sup>b</sup>	
	Rim	Aggregates	Rim	Aggregates
G232E	13.84	86.14	23.70	2.13
Q294P	8.46	91.54	23.19	1.86
R386K	16.45	83.55	15.52	1.04
R482L	88.66	11.34	32.00	nd <sup>c</sup>
Wild-type	95.83	4.17	43.32	nd

<sup>a</sup> Average values of 3 experiments with  $n = 100$  cells each.

<sup>b</sup> Average values of 2 experiments with  $n = 100$  cells each, normalized to transfection efficiency. DM is differentiation medium.

<sup>c</sup> Not detectable





**Figure 2.** Characterization of C2C12 cells expressing GFP lamin A constructs. (A) Domain structure of lamin A protein indicating disease-causing mutations that have been analysed in our laboratory. (B,C) C2C12 cells were induced to differentiate to multinucleated myotubes in serum-depleted medium for 36 h after transient transfection with GFP lamin A constructs for 24 h (green), and stained with antibody to myogenin (red) in (B) and cyclin D3 (red) in (C). (D) C2C12 myoblasts were transfected with GFP lamin A constructs (green) and stained with antibody to MyoD (red). All samples were counterstained with the DNA dye DAPI (not shown). WT, wild-type GFP lamin A; numbers represent GFP lamin A constructs with altered amino acid residues indicated in (A). Bar, 10  $\mu$ m.

GFP-lamin A staining at the nuclear rim survived to a higher extent (table 1). Expression of the early markers of differentiation, such as myogenin and cyclin D3, was strongly impaired in cells expressing aggregates of G232E, Q294P and R386K but nearly normal in cells with peripheral GFP-lamin A staining (figure 2B,C). To determine whether levels of muscle-specific regulators such as MyoD were altered in proliferating myoblasts, we analysed the effects of ectopic expression of lamin constructs on MyoD levels by an immunofluorescence assay. We observed that only 10–20% of myoblasts transfected with the G232E, Q294P or R386K mutants expressed MyoD, whereas ~50% of cells transfected with wild-type GFP lamin A or the R482L

mutant expressed MyoD (figure 2D). Moreover, MyoD was detectable in only ~50% of untransfected cells due to its tight regulation in dividing myoblasts. Expression of these mutants in myoblasts did not affect total RNA pol II transcription levels (data not shown). Hence dividing myoblasts expressing lamin EDMD mutants are clearly deficient in MyoD and this might explain why these cells show impaired differentiation.

The differentiation potential of primary myoblasts isolated from lamin A/C knock-out mice has been investigated by Frock and colleagues (Frock *et al* 2006). These myoblasts have delayed differentiation kinetics and impaired differentiation. Certain markers of muscle

differentiation such as MyoD and pRb were observed to be downregulated at the protein level while the muscle regulator Myf5 was upregulated several-fold. Myf5 has been earlier shown to be upregulated in mice lacking MyoD (Rudnicki *et al* 1992). Interestingly, levels of the cytoskeletal protein desmin were also reduced in ~70% of cells, as observed earlier in *Lmn*<sup>-/-</sup> cardiomyocytes (Nikolova *et al* 2004). Both MyoD and desmin transcript levels were reduced in proliferating *Lmn*<sup>-/-</sup> myoblasts but pRb transcript levels were normal, suggesting that pRb protein stability was reduced in these cells, perhaps due to the absence of lamin A/C as a binding partner for pRb. RNAi-mediated silencing of lamin A/C expression in myoblasts also caused impaired differentiation, and reduction in MyoD and desmin levels. Ectopic expression of MyoD or desmin in *Lmn*<sup>-/-</sup> myoblasts resulted in increased differentiation potential. It is thus evident that MyoD levels are sensitive to the presence of a normal lamin A network as both depletion of lamin A in *Lmn*<sup>-/-</sup> myoblasts and overexpression of lamin A constructs, in particular the EDMD mutants, can lead to lowering of MyoD and impairment of muscle differentiation. Earlier studies showing that desmin knock-out mice have post-natal defects in muscle development (Li *et al* 1996) imply that desmin is also important for muscle differentiation. Hence muscle-specific gene expression might involve cross-talk between cytoplasmic filaments and the nuclear skeleton. An interesting point to note is that *Lmn*<sup>-/-</sup> myoblasts are unable to exit the cell cycle and continue to proliferate upon serum deprivation. This property of the lamin-deficient cells is in contrast to the loss in survival of myoblasts expressing EDMD lamin mutants in serum-depleted medium (Favreau *et al* 2004; this study). A recent transcriptional profiling of EDMD muscle biopsies suggests a failure of interactions between envelope proteins and pRb and MyoD during exit from the cell cycle (Bakay *et al* 2006).

We have also been interested in understanding the role of nucleoplasmic lamins in the muscle differentiation. Our earlier studies have shown that internal lamins are antigenically masked and reorganized to a diffuse network at an early stage (prior to cell cycle arrest) during the differentiation of C2C12 myoblasts in culture (Muralikrishna *et al* 2001). This diffuse network was also observed in quiescent, satellite cells but not in non-muscle cell types. In further studies, lamin organization was observed to be linked to the myogenic programme as it occurred in fibroblasts induced to trans-differentiate to muscle cells by MyoD expression, but not in other cell types. Lamin speckles were induced to rearrange upon expression of cyclin D3 in myoblasts and this process also required pRb; both pRb and cyclin D3 were sequestered on the insoluble lamin matrix (Mariappan and Parnaik 2005). Lamin reorganization may thus be required to maintain the post-mitotic state. Dominant negative mutants such as an N-terminal deletion

of lamin A inhibited expression of early markers of muscle differentiation such as myogenin and transfected cells were not incorporated into myotubes.

### 5.3 Effects on adipocyte differentiation

The mutations in *LMNA* that result in FPLD are clustered within the exons encoding the C-terminal domain, specifically exons 8 and 11. Yeast two-hybrid and *in vitro* binding analysis have shown that this region binds specifically to SREBP1 (Lloyd *et al* 2002). SREBPs are produced as membrane-bound precursors which reside in the ER. Upon reduction in cellular cholesterol levels, SREBP1 is proteolytically cleaved and imported into the nucleus where it directly activates genes involved in cholesterol biosynthesis (Brown and Goldstein 1999). SREBP1 has been shown to be sequestered by pre-lamin A at the nuclear periphery in fibroblasts from lipodystrophy patients, thus lowering the pool of active SREBP1 and inhibiting pre-adipocyte differentiation (Capanni *et al* 2005). In this study, the levels of pre-lamin A were found to be higher in lipodystrophy fibroblasts compared to normal fibroblasts. A recent report suggests that lamin A may normally act as an inhibitor of adipocyte differentiation, as *Lmn*<sup>-/-</sup> fibroblasts accumulate more intracellular lipid and show elevated triglyceride synthesis compared to wild-type fibroblasts, and overexpression of both wild-type and mutant lamin A inhibits adipocyte differentiation (Boguslavsky *et al* 2006). The authors suggest that mutations causing FPLD might be 'gain-of-function' mutations that result in higher binding affinity to a pro-adipogenic factor, thereby sequestering it at the nuclear periphery and inhibiting expression of genes involved in adipogenesis. This pro-adipogenic factor might correspond to SREBP1, as proposed in the earlier studies (Lloyd *et al* 2002; Capanni *et al* 2005). Interestingly, misexpression of the lamin A-binding protein LAP2 $\alpha$  alters cell cycle progression and pRb-E2F signalling, and also inhibits adipocyte differentiation (Dorner *et al* 2006). The role of a "master repressor" for lamin A in regulating gene transcription is supported by a recent report of sequestration of c-Fos at the nuclear periphery by lamin A, followed by suppression of AP-1 transcriptional activity (Ivorra *et al* 2006).

### 5.4 Effects on DNA repair pathways

Cells have developed complex systems for the repair of DNA which has been damaged by external agents such as ionizing and UV-irradiation and genotoxic agents, or by internal sources such as reactive oxygen species. The initial response to DNA damage involves the chromatin-dependent activation of complex checkpoint signalling pathways in

order to delay the cell cycle and repair the defects. The major kinases that serve as damage sensors and regulate cell cycle checkpoints and DNA repair by phosphorylation of key substrates are Ataxia-telangiectasia-mutated (ATM) and ATM-and-Rad3-related (ATR) kinases that belong to the phosphoinositide 3-kinase-related family of serine/threonine protein kinases (reviewed by Zhou and Elledge 2000; Abraham 2001; Shiloh 2003; Bartek *et al* 2004). ATM and ATR activate cell cycle checkpoints and phosphorylate p53 as well as a number of other downstream targets such as the histone variant H2AX (Redon *et al* 2002). Mutations in several components of these DNA repair pathways cause premature ageing syndromes in humans and cellular senescence in cell culture models (Lombard *et al* 2005). A common premature ageing disorder, Werner's syndrome, is caused by loss of a RecQ family DNA helicase, WRN, which functions in several DNA repair pathways (Bohr 2005). As discussed earlier in this review, mutations in the lamin A gene are associated with atypical Werner's syndrome (Chen *et al* 2003) and HGPS (Eriksson *et al* 2003; De Sandre-Giovannoli *et al* 2003). Deletion of the pre-lamin A processing enzyme, *Zmpste24* in mice also leads to a progeria-like syndrome (Pendás *et al* 2002; Bergo *et al* 2002).

Two recent studies with *Zmpste24*-null fibroblasts and HGPS fibroblasts have demonstrated a link between defects in lamin A and the DNA damage response. *Zmpste24*-null fibroblasts showed genomic instability and the mice were more sensitive to DNA damaging agents (Liu *et al* 2005). Furthermore, recruitment of repair proteins such as p53 binding protein 1 (53BP1) and Rad51 to sites of DNA lesions was impaired. Chromosomal aneuploidy in HGPS cells has been observed previously (Mukherjee and Costello 1998). In the second study, an analysis of transcriptional changes in *Zmpste24*-deficient cells has revealed that several targets of the p53 signalling pathway are upregulated, suggesting a checkpoint response that is activated by abnormal nuclear architecture (Varela *et al* 2005). An earlier study with HGPS fibroblasts has shown changes in gene transcription profiles which suggest developmental defects in mesodermal and mesenchymal cell lineages (Csoka *et al* 2004b).

Parnaik and coworkers have examined the early response of cells expressing disease-causing lamin A mutants to DNA damage induced by cisplatin or UV, which primarily cause replicational stress due to stalled replication forks in S phase, and observed that several mutants impaired the formation of phosphorylated H2AX at DNA repair foci and hindered the recruitment of 53BP1 to repair sites. These mutants disrupted emerin localization and, importantly, also mislocalized ATR kinase in untreated cells (Manju *et al* 2006). Our results suggest that lamin mutants are likely to affect the response of the DNA repair machinery to DNA damage by altered interactions with chromatin.

## 6. Conclusions and perspectives

Investigations carried out in the past decade have established that lamins, which were considered to be only structural elements in the nucleus, have diverse roles in the organization of nuclear functions such as DNA replication, transcription and repair, in addition to their requirement for nuclear integrity. Observations that the mutations in human *LMNA* are associated with the group of highly degenerative genetic disorders termed laminopathies have contributed significantly towards understanding the role of lamins. Valuable insights into the role of lamins in tissue-specific signalling pathways have been obtained by analysis of animal models as well as *in vitro* differentiation models, although the potential limitations of these models in interpretation for diseases in humans need to be kept in mind. Important aspects of lamin biology that need further investigation include the precise role of lamins in the organization of DNA replication, transcription and repair, the mode of interaction of lamins with chromatin and the mechanism of formation of higher order assemblies of lamins.

From an analysis of the recent literature, it is becoming increasingly evident that disease-causing mutations in lamin A/C may be considered to be dominant negative mutations that lead to cellular toxicity and cell death. One line of possible therapies for HGPS is the use of inhibitors to reduce the amount of toxic farnesylated pre-lamin A (Fong *et al* 2006a). Since loss of lamin A is not detrimental in the mouse (Fong *et al* 2006b), the accompanying loss in mature lamin A might not be harmful. An intriguing finding is the accumulation of progerin and accompanying nuclear defects in cells from old individuals, which raises the possibility that normal ageing might be due to aberrations in nuclear architecture.

A crucial question that has not yet been answered satisfactorily is why do different mutations in *LMNA* cause such diverse pathologies. A possible clue has come from studies on the FPLD mutations, which appear to disrupt the functions of SREBP1, a tissue-specific transcription factor that plays an essential role in adipocyte differentiation. Do other mutations perturb the functions of other tissue-specific factors and thus affect distinct tissues? MyoD expression is clearly altered by downregulation of lamin A or expression of EDMD mutations but not by FPLD mutations. However, the exact role of lamin A in the expression of MyoD is not yet clear. Though initial analysis of patients had suggested that most mutations affected only specific tissues such as muscle or adipose tissue, recent reports indicate that some mutations may cause overlapping disease symptoms. Do such mutations affect interactions with more than one transcription factor? Furthermore, progeroid features appear to accompany several disorders. Do lamin mutations have pleiotropic effects on DNA repair pathways? Some of these

aspects are under active investigation by several groups, and hopefully a unifying explanation for this intriguing aspect of laminopathies would be forthcoming soon.

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