Review

Laminopathies: Multiple disorders arising from defects in nuclear architecture

VEENA K PARNAIK* and KALIYAPERUMAL MANJU
Centre for Cellular and Molecular Biology
Uppal Road, Hyderabad 500 007, India

**Corresponding author (Fax, 91-40-27160591; Email, veenap@ccmb.res.in)*

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.

[Parnaik V K and Manju K 2006 Laminopathies: Multiple disorders arising from defects in nuclear architecture; *J. Biosci.* **31** 405–421]

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 AΔ10. *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

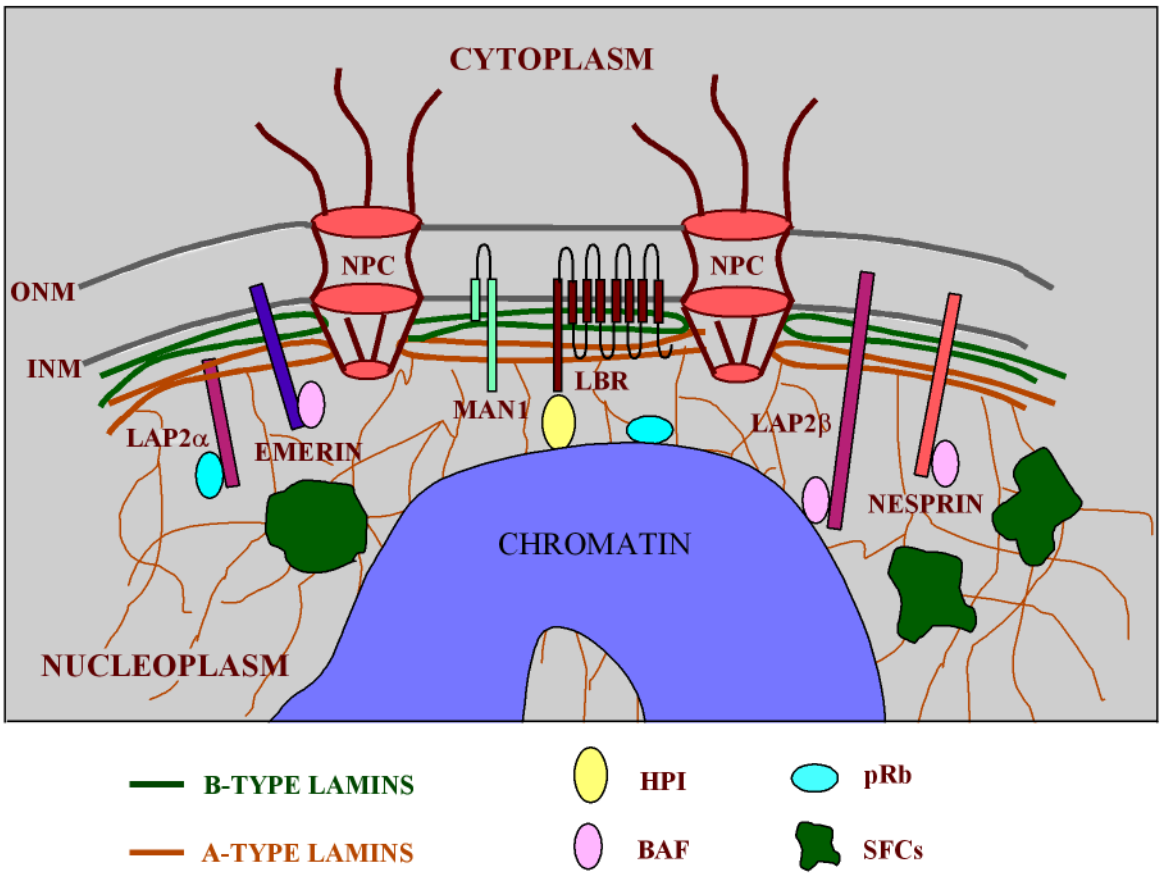

Figure 1. Schematic representation of nuclear envelope. The major components of the nuclear envelope and some of the nuclear proteins that interact with the lamins are shown here. The A-type lamins are concentrated at the periphery (thick lines) and also distributed throughout the nucleoplasm (thin lines). ONM, outer nuclear membrane; INM, inner nuclear membrane; NPC, nuclear pore complex; LAP, lamina-associated polypeptide; LBR, lamin B receptor; HP1, 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 α-helical rod domain, flanked by relatively flexible N-terminal and C-terminal segments. The C-terminii 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 α-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). LAP2α 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 (Guillemin *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 (Dreiullet *et al* 2002) and more recently, c-Fos (Ivorra *et al* 2006). Lamin A/C can bind to protein phosphatase 2A to modulate TGF-β1 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 LAP2β 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, ΔNLA, 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 (Raffaeli 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 (Hellemans *et a*l 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*$^{-/-}$ 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*$^{-/-}$ 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; Guillemin *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 $Lmn^{-/-}$ 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 $Lmn^{-/-}$ 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). $Lmn^{-/-}$ 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 $Lmn^{-/-}$ 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.

	Nuclear localization (%)[a]		Cell survival in DM (%)[b]	
Mutation	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[c]
Wild-type	95.83	4.17	43.32	nd

[a] Average values of 3 experiments with $n = 100$ cells each.
[b] Average values of 2 experiments with $n = 100$ cells each, normalized to transfection efficiency. DM is differentiation medium.
[c] Not detectable

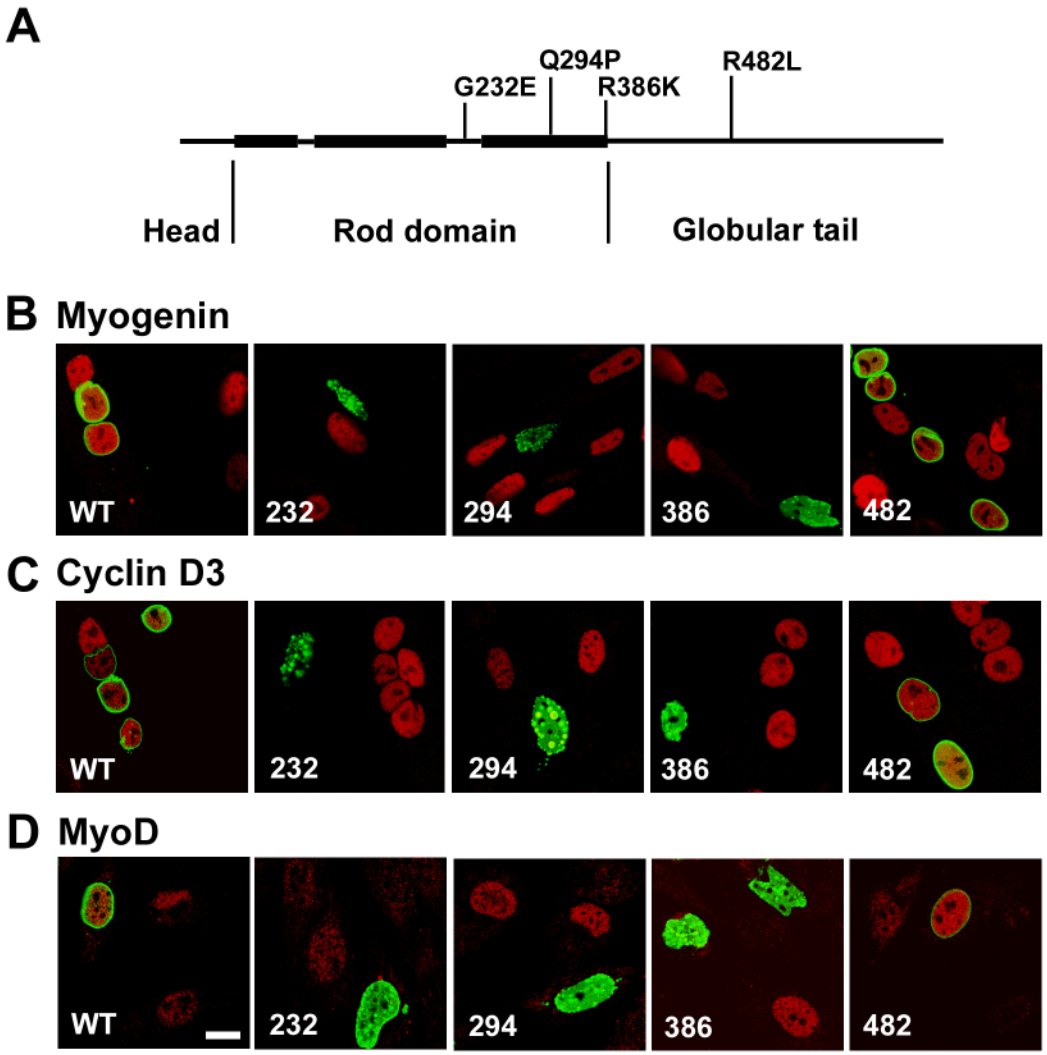

Figure 2. Characterization of C2C12 cells expressing GFP lamin A constructs. (**A**) Domain structure of lamin A protein indicating disease-causing mutations that have been analysed in our laboratory. (**B**,**C**) C2C12 cells were induced to differentiate to multinucleated myotubes in serum-depleted 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 μm.

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*$^{-/-}$ cardiomyocytes (Nikolova *et al* 2004). Both MyoD and desmin transcript levels were reduced in proliferating *Lmn*$^{-/-}$ 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*$^{-/-}$ 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*$^{-/-}$ 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*$^{-/-}$ 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*$^{-/-}$ 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 LAP2α 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.

Acknowledgements

We thank Durgadas Kasbekar for helpful comments on the manuscript and Indumathi Mariappan for providing figure 1. Financial support of the Department of Biotechnology and Council of Scientific and Industrial Research, New Delhi for this work is gratefully acknowledged. KM was supported by a senior research fellowship from the Council of Scientific and Industrial Research.

References

Abraham R T 2001 Cell cycle checkpoint signalling through the ATM and ATR kinases; *Genes Dev.* **15** 2177–2196

Agarwal A K, Fryns J P, Auchus R J and Garg A 2003 Zinc metalloproteinase, ZMPSTE24, is mutated in mandibuloacral dysplasia; *Hum. Mol. Genet.* **12** 1995–2001

Arimura T, Helbling-Leclerc A, Massart C, Varnous S, Niel F. Lacène E, Fromes Y, Toussaint M, Mura A, Keller D I *et al* 2005 Mouse model carrying H222P-*Lmna* mutation develops muscular dystrophy and dilated cardiomyopathy similar to human striated muscle laminopathies; *Hum. Mol. Genet.* **14** 155–169

Bakay M, Wang Z, Melcon G, Schiltz L, Xuan J, Zhao P, Sartorelli V, Seo J, Pegoraro E, Angelini C *et al* 2006 Nuclear envelope dystrophies show a transcriptional fingerprint suggesting disruption of Rb-MyoD pathways in muscle regeneration; *Brain* **129** 996–1013

Bartek J, Lukas C and Lukas J 2004 Checking on DNA damage in S phase; *Nature (London)* **5** 792–804

Bechert K, Lagos-Quintana M, Harborth J, Weber K and Osborn M 2003 Effects of expressing lamin A mutant protein causing Emery-Dreifuss muscular dystrophy and familial partial lipodystrophy in HeLa cells; *Exp. Cell Res.* **286** 75–86

Benedetti S, Bertini E, Iannaccone S, Angelini C, Trisciani M, Toniolo D, Sferrazza B, Carrera P, Comi G, Ferrari M *et al* 2005 Dominant LMNA mutations can cause combined muscular dystrophy and peripheral neuropathy; *J. Neurol. Neurosurg. Psychiatry* **76** 1019–1021

Bergo M O, Gavino B, Ross J, Schmidt W K, Hong C, Kendall L V, Mohr A, Meta M, Genant H, Jiang Y *et al* 2002 Zmpste24 deficiency in mice causes spontaneous bone fractures, muscle weakness, and a pre-lamin A processing defect; *Proc. Natl. Acad. Sci. USA* **99** 13049–13054

Bione S, Maestrini E, Rivella S, Mancini M, Regis S, Romeo G and Toniolo D 1994 Identification of a novel X-linked gene responsible for Emery-Dreifuss muscular dystrophy; *Nat. Genet*. **8** 323–327

Boguslavsky R L, Stewart C L and Worman H J 2006 Nuclear lamin A inhibits adipocyte differentiation: implications for Dunnigan-type familial partial lipodystrophy; *Hum. Mol. Genet.* **15** 653–663

Bohr V A 2005 Deficient DNA repair in the human progeroid disorder, Werner syndrome; *Mutat. Res.* **577** 252–259

Bonne G, Di Barletta M R, Varnous S, Becane H M, Hammonda E H, Merlini L, Muntoni F, Greenberg C R, Gary F, Urtizberea J A *et al* 1999 Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy; *Nat. Genet.* **21** 285–288

Bonne G, Mercuri E, Muchir A, Urtiziberea A, Becane H M, Reca D, Merlini L, Wehnert M, Boor R, Reuner U *et al* 2000 Clinical and molecular genetic spectrum of autosomal dominant Emery Dreifuss muscular dystrophy due to mutations of the lamin A/C gene; *Ann. Neurol.* **48** 170–180

Broers J L V, Kuijpers H J H, Östlund C, Worman H J, Endert J and Ramaekers F C S 2005 Both lamin A and lamin C mutations cause lamina instability as well as loss of internal nuclear lamin organization; *Exp. Cell Res.* **304** 582–592

Broers J L, Machiels B M, van Eys G J, Kuijpers H J, Manders E M, van Driel R and Ramaekers F C 1999 Dynamics of the nuclear lamina as monitored by GFP-tagged A-type lamins; *J. Cell Sci.* **112** 3463–3475

Broers J L, Peeters E A, Kuijpers H J, Endert J, Bouten C V, Oomens C W, Baaijens F P and Ramaekers F C 2004 Decreased mechanical stiffness in *LMNA*$^{-/-}$ cells is caused by defective nucleo-cytoskeletal integrity: Implications for the development of laminopathies; *Hum. Mol. Genet.* **13** 2567–2580

Brown M S and Goldstein J L 1999 A proteolytic pathway that controls the cholesterol content of membranes, cells and blood; *Proc. Natl. Acad. Sci. USA* **96** 11041–11048

Brown C A, Lanning R W, McKinney K Q, Salvino A R, Cherniske E, Crowe C A, Darras B T, Gominak S, Greenberg C R, Grosmann C *et al* 2001 Novel and recurrent mutations in lamin A/C in patients with Emery–Dreifuss muscular Dystrophy; *Am. J. Med. Genet.* **102** 359–367

Capanni C, Cenni V, Mattioli E, Sabatelli P, Ognibene A, Columbaro M, Parnaik V K, Wehnert M, Maraldi N M, Squarzoni S and Lattanzi G 2003 Failure of lamin A/C to functionally assemble in R482L mutated familial partial lipodystrophy fibroblasts: Altered intermolecular interaction with emerin and implications for gene transcription; *Exp. Cell Res.* **291** 122–134

Capanni C, Mattioli E, Columbaro M, Lucarelli E, Parnaik V K, Novelli G, Wehnert M, Cenni V, Maraldi N M, Squarzoni S and Lattanzi G 2005 Altered pre-lamin A processing is a common mechanism leading to lipodystrophy; *Hum. Mol. Genet.* **14** 1489–1502

Cao H and Hegele R A 2000 Nuclear lamin A/C R482Q mutation in Canadian kindreds with Dunnigan-type familial partial lipodystrophy; *Hum. Mol. Genet.* **9** 109–112

Cao H and Hegele R A 2003 *LMNA* is mutated in Hutchinson-Gilford progeria (MIM 176670) but not in Wiedemann-Rautenstrauch progeroid syndrome (MIM 264090); *J. Hum. Genet.* **48** 271–274

Capell B C, Erdos M R, Madigan J P, Fiordalisi J J, Varga R, Conneely K N, Gordon L B, Der C J, Cox A D and Collins F S 2005 Inhibiting farnesylation of progerin prevents the characteristic nuclear blebbing of Hutchinson-Gilford progeria syndrome; *Proc. Natl. Acad. Sci. USA* **102** 12879–12884

Caux F, Dubosclard E, Lascols O, Buendia B, Chazouilleres O, Cohen A, Courvalin J-C, Laroche L, Capeau J, Vigouroux C and

Christin-Maitre S 2003 A new clinical condition linked to a novel mutation in lamins A and C with generalized lipoatrophy, insulin resistant diabetes, disseminated leukomelanodermic papules, liver steatosis, and cardiomyopathy; *J. Clin. Endocrinol. Metab.* **88** 1006–1013

Chen I, Lee I, Kudlow B A, Dos Santos H G, Stervoid O, Shafeghati Y, Botha E G, Garg A, Hanson N B, Martin G M *et al* 2003 *LMNA* mutations in atypical Werner's syndrome; *Lancet* **362** 440–445

Cohen M, Lee K K, Wilson K L and Gruenbaum Y 2001 Transcriptional repression, apoptosis, human disease and the functional evolution of the nuclear lamina; *Trends Biochem. Sci.* **26** 41–47

Columbaro M, Capanni C, Mattioli E, Novelli G, Parnaik V K, Squarzoni S, Maraldi N M and Lattanzi G 2005 Rescue of heterochromatin organization in Hutchinson-Gilford progeria by drug treatment; *Cell. Mol. Life Sci.* **62** 2669–2678

Csoka A B, Cao H, Sammak P J, Constantinescu D, Schatten G P and Hegele R A 2004a Novel lamin A/C gene (LMNA) mutations in atypical progeroid syndromes; *J. Med. Genet.* **41** 304–308

Csoka A B, English S B, Simkevich C P, Ginzinger D G, Butte A J, Schatten G P, Rothman F G and Sedivy J M 2004b Genome-scale expression profiling of Hutchinson-Gilford progeria syndrome reveals widespread transcriptional misregulation leading to mesodermal/mesenchymal defects and accelerated atherosclerosis; *Aging Cell* **3** 235–243

de la Luna S, Allen K E, Mason S L and La Thangue N B 1999 Integration of a growth-suppressing BTB/POZ domain protein with the DP component of the E2F transcription factor; *EMBO J.* **18** 212–228

De Sandre-Giovannoli A, Bernard R, Cau P, Navarro C, Amiel J, Boccaccio I, Lyonnet S, Stewart C I, Munnich A, Le Merrer M and Levy N 2003 Lamin A truncation in Hutchinson-Gilford progeria; *Science* **300** 2055

De Sandre-Giovannoli A, Chaouch M, Kozlov S, Vallat J M, Tazir M, Kassouri N, Szepetowski P, Hammadouche T, Vandenberghe A, Stewart C L *et al* 2002 Homozygous defects in *LMNA*, encoding lamin A/C nuclear-envelope proteins, cause autosomal recessive axonal neuropathy in human (Charcot-Marie-Tooth disorder type 2) and mouse; *Am. J. Hum. Genet.* **70** 726–736

Dechat T, Korbei B, Vaughan O A, Vlcek S, Hutchison C J and Foisner R 2000 Lamina-associated polypeptide 2α binds intranuclear A-type lamins; *J. Cell Sci.* **113** 3473–3484

Dhe-Paganon S, Werner E D, Chi Y and Shoelson S E 2002 Structure of the globular tail of nuclear lamin; *J. Biol. Chem.* **277** 17381–17384

Dorner D, Vlcek S, Foeger N, Gajewski A, Makolm C, Gotzmann J, Hutchison C J and Foisner R 2006 Lamina-associated polypeptide 2α regulates cell cycle progression and differentiation via the retinoblastoma-E2F pathway; *J. Cell Biol.* **173** 83–93

Dreuillet C, Tillit J, Kress M and Ernoult-Lange M 2002 *In vivo* and *in vitro* interaction between human transcription factor MOK2 and nuclear lamin A/C; *Nucleic Acids Res.* **30** 4634–4642

Ellis D J, Jenkins H, Whitfield W G F and Hutchison C J 1997 GST-lamin fusion proteins act as dominant negative mutants in *Xenopus* egg extract and reveal the function of the lamina in DNA replication; *J. Cell Sci.* **110** 2507–2518

Emery A E and Dreifuss F E 1966 Unusual type of benign X-linked muscular dystrophy; *J. Neurol. Neurosurg. Psychiatry* **29** 338–342

Eriksson M, Brown W T, Gordon L B, Glynn M W, Singer J, Scott L, Erdos M R, Robbins C M, Moses T Y, Berglund P *et al* 2003 Recurrent *de novo* point mutations in lamin A cause Hutchinson-Gilford progeria syndrome; *Nature (London)* **423** 293–298

Fatkin D, MacRae C, Sasaki T, Wolff M R, Porcu M, Frenneaux M, Atherton J, Vidaillet H J Jr, Spudich S, De Girolami U *et al* 1999 Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease; *New Engl. J. Med.* **341** 1715–1724

Favreau C, Dubosclard E, Östlund C, Vigouroux C, Capeau J, Wehnert M, Higuet D, Worman H J, Courvalin J C and Buendia B 2003 Expression of lamin A mutated in the carboxyl-terminal tail generates an aberrant nuclear phenotype similar to that observed in cells from patients with Dunnigan-type partial lipodystrophy and Emery-Dreifuss muscular dystrophy; *Exp Cell Res.* **282** 14–23

Favreau C, Higuet D, Courvalin J-C and Buendia B 2004 Expression of a mutant lamin A that causes Emery-Dreifuss muscular dystrophy inhibits *in vitro* differentiation of C2C12 myoblasts; *Mol. Cell. Biol*. **24** 1481–1492

Fawcett D W 1966 On the occurrence of a fibrous lamina on the inner aspect of the nuclear envelope in certain cells of vertebrates; *Am. J. Anat.* **119** 129–145

Fong L G, Frost D, Meta M, Qiao X, Yang S H, Coffinier C and Young S G 2006a A protein farnesyltransferase inhibitor ameliorates disease in a mouse model of progeria; *Science* **311** 1621–1623

Fong L G, Ng J K, Lammerding J, Vickers T A, Meta M, Coté N, Gavino B, Qiao X, Chang S Y, Young S R *et al* 2006b Prelamin A and lamin A appear to be dispensable in the nuclear lamina; *J. Clin. Invest.* **116** 743–752

Frock R L, Kudlow B A, Evans A M, Jameson S A, Hauschka S D and Kennedy B K 2006 Lamin A/C and emerin are critical for skeletal muscle satellite cell differentiation; *Genes Dev.* **20** 486–500

Gant T M and Wilson K L 1997 Nuclear assembly; *Annu. Rev. Cell Dev. Biol.* **13** 669–695

Garg A, Speckman R A and Bowcock A M 2002 Multisystem dystrophy syndrome due to novel missense mutations in the amino-terminal head and alpha-helical rod domains of the lamin A/C gene; *Am. J. Med.* **112** 549–555

Gerasimova T I and Corces V G 1998 Polycomb and trithorax group proteins mediate the function of a chromatin insulator; *Cell* **92** 511–521

Gilchrist S, Gilbert N, Perry P, Östlund C, Worman H J and Bickmore W A 2004 Altered protein dynamics of disease-associated lamin A mutants; *BMC Cell Biol.* **5** 46

Glynn M W and Glover T W 2005 Incomplete processing of mutant lamin A in Hutchinson-Gilford progeria leads to nuclear abnormalities, which are reversed by farnesyltransferase inhibition; *Hum. Mol. Genet.* **14** 2959–2969

Goizet C, Yaou R B, Demay L, Richard P, Bouillot S, Rouanet M, Hermosilla E, Le Masson G, Lagueny A, Bonne G

and Ferrer X 2004 A new mutation of the lamin A/C gene leading to autosomal dominant axonal neuropathy, muscular dystrophy, cardiac disease, and leuconychia; *J. Med. Genet.* **41** e29

Goldman R D, Gruenbaum Y, Moir R D, Shumaker D K and Spann T P 2002 Nuclear lamins: Building blocks of nuclear architecture; *Genes Dev.* **16** 533–547

Goldman A E, Moir R D, Montag-Lowy M, Stewert M and Goldman R D 1992 Pathway of incorporation of microinjected lamin A into the nuclear envelope; *J. Cell Biol.* **119** 725–735

Goldman R D, Shumaker D K, Erdos M R, Eriksson M, Goldman A E, Gordon L B, Gruenbaum Y, Khuon S, Mendez M, Varga R and Collins F S 2004 Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome; *Proc. Natl. Acad. Sci. USA* **101** 8963–8968

Gruenbaum Y, Margalit A, Goldman R D, Shumaker D K and Wilson K L 2005 The nuclear lamina comes of age; *Nat. Rev. Mol. Cell. Biol.* **6** 21–31

Guillemin K, Williams T and Krasnow M A 2001 A nuclear lamin is required for cytoplasmic organisation and egg polarity in *Drosophila*; *Nat. Cell Biol.* **3** 848–851

Haithcock E, Dayani Y, Neufeld E, Zahand A J, Feinstein N, Mattout A, Gruenbaum Y and Liu J 2005 Age-related changes of nuclear architecture in *Caenorhabditis elegans*; *Proc. Natl. Acad. Sci. USA* **102** 16690–16695

Hellemans J, Preobrazhenska O, Willaert A, Debeer P, Verdonk P C M, Costa T, Janssens K, Menten B, Van Roy N, Vermeulen S J T *et al* 2004 Loss-of-function mutations in LEMD3 result in osteopoikilosis, Buschke-Ollendorff syndrome and melorheostosis; *Nat. Genet.* **36** 1213–1218

Hoffman K, Dreger C K, Olins A L, Olins D E, Shultz L D, Lucke B, Karl H, Kaps R, Muller D, Vaya A *et al* 2002 Mutations in the gene encoding the lamin B receptor produce an altered nuclear morphology in granulocytes (Pelger-Huet anomaly); *Nat. Genet.* **31** 410–414

Hozák P, Sasseville A M-J, Raymond Y and Cook P R 1995 Lamin proteins form an internal nucleoskeleton as well as a peripheral lamina in human cells; *J. Cell Sci.* **108** 635–644

Huang S, Chen L, Libina N, Janes J, Martin G M, Campisi J and Oshima J 2005 Correction of cellular phenotypes of Hutchinson-Gilford Progeria cells by RNA interference; *Hum. Genet.* **118** 444–450

Hutchison C J and Worman H J 2004 A-type lamins: Guardians of the soma?; *Nat. Cell Biol.* **6** 1062–1067

Imai S, Nishibayashi S, Takao K, Tomifuji M, Fujino T, Hasegawa M and Takano T 1997 Dissociation of Oct-1 from the nuclear peripheral structure induces the cellular aging-associated collagenase gene expression; *Mol. Biol. Cell* **8** 2407–2419

Ivorra C, Kubicek M, González J M, Sanz-González S M, Álvarez-Barrientos A, O'Connor J-E, Burke B and Andrés V 2006 A mechanism of AP-1 suppression through interaction of c-Fos with lamin A/C; *Genes Dev.* **20** 307–320

Jacob K N and Garg A 2006 Laminopathies: Multisystem dystrophy syndromes; *Mol. Genet. Metab.* **87** 289–302

Jagatheesan G, Thanumalayan S, Muralikrishna Bh, Rangaraj N, Karande A A and Parnaik V K 1999 Colocalisation of intranuclear lamin foci with RNA splicing factors; *J. Cell Sci.* **112** 4651–4661

Karabinos A, Schunemann J, Meyer M, Aebi U and Weber K 2003 The single nuclear lamin of *Caenorhabditis elegans* forms *in vitro* stable intermediate filaments and paracrystals with a reduced axial periodicity; *J. Mol. Biol.* **325** 241–247

Kennedy B K, Barbie D A, Classon M, Dyson N and Harlow E 2000 Nuclear organisation of DNA replication in primary mammalian cells; *Genes Dev.* **14** 2855–2868

Kirschner J, Brune T, Wehnert M, Denecke J, Wasner C, Feuer A, Marquardt T, Ketelsen U P, Wieacker P, Bonnemann C G and Korinthenberg R 2005 p.S143F mutation in lamin A/C: A new phenotype combining myopathy and progeria; *Ann. Neurol*. **57** 148–151

Kumaran R I, Muralikrishna Bh and Parnaik V K 2002 Lamin A/C speckles mediate spatial organisation of splicing factor compartments and RNA polymerase II transcription; *J. Cell Biol.* **159** 783–793

Lammerding J, Schulze P C, Takahashi T, Kozlov S, Sullivan T, Kamm R D, Stewart C L and Lee R T 2004 Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction; *J. Clin. Invest.* **113** 370–378

Lenz-Bohme B, Wismar J, Fuchs S, Reifegerste R, Buchner E, Betz H and Schmitt B 1997 Insertional mutation of the *Drosophila* nuclear *lamin* Dm_0 gene results in defective nuclear envelopes, clustering of nuclear pore complexes, and accumulation of annulate lamellae; *J. Cell Biol.* **137** 1001–1016

Li Z, Colucci-Guyon E, Pincon-Raymond M, Mericskay M, Pournin S, Paulin D and Babinet C 1996 Cardiovascular lesions and skeletal myopathy in mice lacking desmin; *Dev. Biol.* **175** 362–366

Liu J, Ben-Shahar T R, Riemer D, Treinin M, Spann P, Weber K, Fire A and Gruenbaum Y 2000 Essential roles for *Caenorhabditis elegans* lamin gene in nuclear organization, cell cycle progression, and spatial organization of nuclear pore complexes; *Mol. Biol. Cell* **11** 3937–3947

Liu B, Wang J, Chan K M, Tjia W M, Deng W, Guan X, Huang J D, Li K M, Chau P Y, Chen D J *et al* 2005 Genomic instability in laminopathy-based premature aging; *Nat Med.* **11** 780–785

Lloyd D J, Trembath R C and Shackleton S 2002 A novel interaction between lamin A and SREBP1: implications for partial lipodystrophy and other laminopathies; *Hum. Mol. Genet.* **11** 769–777

Lombard D B, Chua K F, Mostoslavsky R, Franco S, Gostissa M and Alt F W 2005 DNA repair, genome stability and aging; *Cell* **120** 497–512

Mallampalli M P, Huyer G, Bendale P, Gelb M H and Michaelis S 2005 Inhibiting farnesylation reverses the nuclear morphology defect in a HeLa cell model for Hutchinson-Gilford progeria syndrome; *Proc. Natl. Acad. Sci. USA* **102** 14416–14421

Mancini M A, Shan B, Nickerson J A, Penman S and Lee W H 1994 The retinoblastoma gene product is a cell cycle-dependent, nuclear matrix-associated protein; *Proc. Natl. Acad. Sci. USA* **91** 418–422

Manilal S, Nguyen T M, Sewry C A and Morris G E 1996 The Emery-Dreifuss muscular dystrophy protein, emerin, is a nuclear membrane protein; *Hum. Mol. Genet.* **5** 801–808

Manju K, Muralikrishna Bh and Parnaik V K 2006 Expression of disease-causing lamin mutants impairs the formation of DNA repair foci; *J. Cell Sci.* **119** 2704–2714

Markiewicz E, Dechat T, Foisner R, Quinlan R A and Hutchison C J 2002 Lamin A/C binding protein LAP2α is required for nuclear anchorage of retinoblastoma protein; *Mol. Biol Cell* **13** 4401–4413

Markiewicz E, Ledran M and Hutchison C J 2005 Remodelling of the nuclear lamina and nucleoskeleton is required for skeletal muscle differentiation *in vitro*; *J. Cell Sci.* **118** 409–420

Mariappan I and Parnaik V K 2005 Sequestration of pRb by cyclin D3 causes intranuclear reorganization of lamin A/C during muscle cell differentiation; *Mol. Biol. Cell* **16** 1948–1960

Mattout A, Dechat T, Adam S A, Goldman R D and Gruenbaum Y 2006 Nuclear lamins, diseases and aging; *Curr. Opin. Cell Biol* **18** 1–7

McClintock D, Gordon L B and Djabali K 2006 Hutchinson-Gilford progeria mutant lamin A primarily targets human vascular cells as detected by an anti-lamin A G608G antibody; *Proc. Natl. Acad. Sci. USA* **103** 2154–2159

Meier J, Campbell K H, Ford C C, Stick R and Hutchison C J 1991 The role of lamin LIII in nuclear assembly and DNA replication, in cell free extracts of *Xenopus* eggs; *J. Cell Sci.* **98** 271–279

Meune C, Van Berlo J H, Anselme F, Bonne G, Pinto Y M and Duboc D 2006 Primary prevention of sudden death in patients with lamin A/C gene mutations; *N. Engl. J. Med.* **354** 209–210

Mintz P J, Patterson S D, Neuwald A F, Spahr C S and Spector D L 1999 Purification and biochemical characterisation of interchromatin granule clusters; *EMBO J.* **18** 4308–4320

Mislow J M K, Kim M S, Davis D B and McNally E M 2002 Myne-1, a spectrin repeat transmembrane protein of the myocyte inner nuclear membrane interacts with lamin A/C; *J. Cell Sci.* **115** 61–70

Moir R D, Montag-Lowy M and Goldman R D 1994 Dynamic properties of nuclear lamins: lamin B is associated with sites of DNA replication; *J. Cell Biol.* **125** 1201–1212

Moir R D, Spann T P, Herrmann H and Goldman R D 2000a Disruption of nuclear lamin organisation blocks the elongation phase of DNA replication; *J. Cell Biol.* **149** 1179–1192

Moir R D, Yoon M, Khuon S and Goldman R D 2000b Nuclear lamins A and B1: Different pathways of assembly during nuclear envelope formation in living cells; *J. Cell Biol.* **151** 1155–1168

Mounkes I C, Kozlov S, Hernandez I, Sullivan T and Stewart C I 2003 A progeroid syndrome in mice is caused by defects in A-type lamins; *Nature (London)* **423** 298–301

Mounkes L C, Kozlov S V, Rottman J N and Stewart C L 2005 Expression of a LMNA-N195K variant of A-type lamins results in cardiac conduction defects and death in mice; *Hum. Mol. Genet.* **14** 2167–2180

Muchir A, Bonne G, van der Kooi A J, van Meegan M, Baas F, Bolhuis P A, de Visser M and Schwartz K 2000 Identification of mutations in the gene encoding lamins A/C in autosomal dominant limb girdle muscular dystrophy with atrioventricular conduction disturbances; *Hum. Mol. Genet.* **9** 1453–1459

Muchir A, Medioni J, Laluc M, Massart C, Arimura T, van der Kooi A J, Desguerre I, Mayer M, Ferrer X, Briault S *et al* 2004 Nuclear envelope alterations in fibroblasts from patients with muscular dystrophy, cardiomyopathy, and partial lipodystrophy carrying lamin A/C gene mutations; *Muscle Nerve* **30** 444–450

Muchir A, van Engelen B G, Lammens M, Mislow J M, McNally E, Schwartz K and Bonne G 2003 Nuclear envelope alterations in fibroblasts from LGMD1B patients carrying nonsense Y259X heterozygous or homozygous mutation in lamin A/C gene; *Exp. Cell Res.* **291** 352–362

Mukherjee A B and Costello C 1998 Aneuploidy analysis in fibroblasts of human premature aging syndromes by FISH during *in vitro* cellular aging; *Mech. Ageing Dev.* **103** 209–222

Muralikrishna Bh, Dhawan J, Rangaraj N and Parnaik V K 2001 Distinct changes in intranuclear lamin A/C organisation during myoblast differentiation; *J. Cell Sci.* **114** 4001–4011

Nagano A, Koga R, Ogawa M, Kurano Y, Kawada J, Okada R, Hayashi Y K, Tsukahara T and Arahata K 1996 Emerin deficiency at the nuclear membrane in patients with Emery-Dreifuss muscular dystrophy; *Nat. Genet.* **12** 254–259

Navarro C L, Cadinanos J, De Sandre-Giovannoli A, Bernard R, Courrier S, Boccaccio I, Boyer A, Kleijer W J, Wagner A, Giuliano F *et al* 2005 Loss of ZMPSTE24 (FACE-1) causes autosomal recessive restrictive dermopathy and accumulation of Lamin A precursors, *Hum. Mol. Genet.* **14** 1503–1513

Navarro C, De Sandre-Giovannoli A, Bernard R, Boccaccio I, Boyer A, Genevieve D, Hadj-Rabia S, Gaudy-Marqueste C, Smith H S, Vabres P *et al* 2004 Lamin A and ZMPSTE24 (FACE-1) defects cause nuclear disorganization and identify restrictive dermopathy as a lethal neonatal laminopathy; *Hum. Mol. Genet*. **13** 2493–2503

Newport J W, Wilson K L and Dunphy W G 1990 A lamin-independent pathway for nuclear envelope assembly; *J. Cell Biol.* **111** 2247–2259

Nikolova V, Leimena C, McMahon A C, Tan J C, Chandar S, Jogia D, Kesteven S H, Michalicek J, Otway R, Verheyen F *et al* 2004 Defects in nuclear structure and function promote dilated cardiomyopathy in lamin A/C-deficient mice; *J. Clin. Invest.* **113** 357–369

Nili E, Cojocaru G S, Kalma Y, Ginsberg D, Copeland N G, Gilbert D J, Jenkins N A, Berger R, Shaklai S, Amariglio N *et al* 2001 Nuclear membrane protein LAP2β mediates transcriptional repression alone and together with its binding partner GCL (germ-cell-less); *J. Cell Sci.* **114** 3297–3307

Novelli G, Muchir A, Sangiuolo F, Helbling-Leclerc A, D'Apice M R, Massart C, Capon F, Sbraccia P, Federici M, Lauro R *et al* 2002 Mandibuloacral dysplasia is caused by a mutation in *LMNA* encoding lamins A/C; *Am. J. Hum. Genet.* **71** 426–431

Osouda S, Nakamura Y, Saint Phalle B, McConnell M, Horigome T, Sugiyama S, Fisher P A and Furukawa K 2005 *Null* mutants of *Drosophila* B-type *lamin* Dm_0 show aberrant tissue differentiation rather than obvious nuclear shape distortion or specific defects during cell proliferation; *Dev. Biol.* **284** 219–232

Östlund C, Bonne G, Schwartz K and Worman H J 2001 Properties of lamin A mutants found in Emery-Dreifuss muscular dystrophy, cardiomyopathy and Dunnigan-type partial lipodystrophy; *J. Cell Sci.* **114** 4435–4445

Ozaki T, Saijo M, Murakami K, Enomoto H, Taya Y and Sakiyama S 1994 Complex formation between lamin A

and the retinoblastoma gene product: identification of the domain on lamin A required for its interaction; *Oncogene* **9** 2649–2653

Padmakumar V C, Libotte T, Lu W, Zaim H, Abraham S, Noegel A A, Gotzmann J, Foisner R and Karakesisoglou I 2005 The inner nuclear membrane protein Sun1 mediates the anchorage of Nesprin-2 to the nuclear envelope; *J. Cell Sci.* **118** 3419–3430

Patterson K, Molofsky A B, Robinson C, Acosta S, Cater C and Fischer J A 2004 The functions of Klarsicht and nuclear lamin in developmentally regulated nuclear migrations of photoreceptor cells in the *Drosophila* eye; *Mol. Biol. Cell* **15** 600–610

Pendás A M, Zhou Z, Cadiñanos J, Freije J M P, Wang J, Hultenby K, Astudillo A, Wernerson A, Rodriguez F, Tryggvason K and López-Otin C 2002 Defective prelamin A processing and muscular and adipocyte alterations in Zmpste24 metalloproteinase-deficient mice; *Nat. Genet.* **31** 94–99

Plasilova M, Chattopadhyay C, Pal P, Schaub N A, Buechner S A, Mueller H, Miny P, Ghosh A and Heinimann K 2004 Homozygous missense mutation in the lamin A/C gene causes autosomal recessive Hutchinson–Gilford progeria syndrome; *J. Med. Genet.* **41** 609–614

Raffaele Di Barletta M, Ricci E, Galluzzi G, Tonali P, Mora M, Morandi L, Romorini A, Voit T, Orstavik K H, Merlini L *et al* 2000 Different mutations in the *LMNA* gene cause autosomal dominant and autosomal recessive Emery–Dreifuss muscular dystrophy; *Am. J. Hum. Genet.* **66** 1407–1412

Raharjo W H, Enarson P, Sullivan T, Stewart C L and Burke B 2001 Nuclear envelope defects associated with *LMNA* mutations cause dilated cardiomyopathy and Emery-Dreifuss muscular dystrophy; *J. Cell Sci.* **114** 4447–4457

Redon C, Pilch D, Rogakou E, Sedelnikova O, Newrock K and Bonner W 2002 Histone H2A variants H2AX and H2AZ; *Curr. Opin. Genet. Dev.* **12** 162–169

Riemer D, Stuurman N, Berrios M, Hunter C, Fisher P A and Weber K 1995 Expression of *Drosophila* lamin C is developmentally regulated: analogies with vertebrate A-type lamins; *J. Cell Sci.* **108** 3189–3198

Röber R A, Weber K and Osborn M 1989 Differential timing of nuclear lamin A/C expression in the various organs of the mouse embryo and the young animal: a developmental study; *Development* **105** 365–378

Rudnicki M A, Braun T, Hinuma S and Jaenisch R 1992 Inactivation of MyoD in mice leads to upregulation of the myogenic HLH gene Myf-5 and results in apparently normal muscle development; *Cell* **71** 383–390

Sanna T, Dello Russo A, Toniolo D, Vytopil M, Pelargonio G, De Martino G, Ricci E, Silvestri G, Giglio V, Messano L *et al* 2003 Cardiac features of Emery–Dreifuss muscular dystrophy caused by lamin A/C gene mutations; *Eur. Heart J.* **24** 2227–2236

Scaffidi P and Misteli T 2005 Reversal of the cellular phenotype in the premature aging disease Hutchinson-Gilford progeria syndrome; *Nat. Med.* **11** 440–445

Scaffidi P and Misteli T 2006 Lamin A-dependent nuclear defects in human aging; *Science* **312** 1059–1063

Schulze S R, Curio-Penny B, Li Y, Imani R A, Rydberg L, Geyer P K and Wallrath L L 2005 Molecular genetic analysis of the nested *Drosophila melanogaster Lamin C* gene; *Genetics* **171** 185–196

Shackleton S, Lloyd D J, Jackson S N J, Evans R, Niermeijer M F, Singh B M, Schmidt H, Brabant G, Kumar S, Durrington P N *et al* 2000 *LMNA*, encoding lamin A/C, is mutated in partial lipodystrophy; *Nat. Genet.* **24** 153–156

Shackleton S, Smallwood D T, Clayton P, Wilson L C, Agarwal A K, Garg A and Trembath R C 2005 Compound heterozygous ZMPSTE24 mutations reduce prelamin A processing and result in a severe progeroid phenotype; *J. Med. Genet.* **42** e36

Shiloh Y 2003 ATM and related protein kinases: safeguarding genome integrity; *Nat. Rev. Cancer* **3** 155–168

Shumaker D K, Kuczmarski E R and Goldman R D 2003 The nucleoskeleton: lamins and actin are major players in essential nuclear functions; *Curr. Opin. Cell Biol*. **15** 358–366

Smith E D, Kudlow B A, Frock R L and Kennedy B K 2005 A-type nuclear lamins, progerias and other degenerative disorders; *Mech. Ageing Dev.* **126** 447–460

Spann T P, Goldman A E, Wang C, Huang S and Goldman R D 2002 Alteration of nuclear lamin organisation inhibits RNA polymerase II-dependent transcription; *J. Cell Biol.* **156** 603–608

Spann T P, Moir R D, Goldman A E, Stick, R and Goldman R D 1997 Disruption of nuclear lamin organisation alters the distribution of replication factors and inhibits DNA synthesis; *J. Cell Biol*. **136** 1201–1212

Speckman R A, Garg A, Du F, Bennett L, Veile R, Arioglu E, Taylor S I, Lovett M and Bowcock A M 2000 Mutational and haplotype analyses of families with familial partial lipodystrophy (Dunnigan variety) reveal recurrent missense mutations in the globular C-terminal domain of lamin A/C; *Am. J. Hum. Genet*. **66** 1192–1198

Spector D L 2003 The dynamics of chromosome organisation and gene regulation; *Ann. Rev. Biochem.* **72** 573–608

Starr D A and Han M 2003 ANChors away: an actin-based mechanism of nuclear positioning; *J. Cell Sci.* **116** 211–216

Stuurman N, Heins S and Aebi U 1998 Nuclear lamins : Their structure, assembly and interactions; *J. Struc. Biol.* **122** 42–46

Sullivan T, Escalante-Alcade D, Bhatt H, Anver M, Bhat N, Nagashima K, Stewart C L and Burke B 1999 Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy; *J. Cell Biol.* **147** 913–920

Taniura H, Glass C and Gerace L 1995 A chromatin binding site in the tail domain of nuclear lamins that interacts with core histones; *J. Cell Biol*. **131** 33–44

Taylor M R, Slavov D, Gajewski A, Vlcek S, Ku L, Fain P R, Carniel E, Di Lenarda A, Sinagra G, Boucek M M *et al* 2005 Thymopoietin (lamina-associated polypeptide 2) gene mutation associated with dilated cardiomyopathy; *Hum. Mutat.* **26** 566–574

Tsai M Y, Wang S, Heidinger J, Shumaker D, Adam S A, Goldman R D and Zheng Y 2006 A mitotic lamin B matrix induced by RanGTP required for spindle assembly; *Science* **311** 1887–1893

Van Berlo J H, Voncken J W, Broers J L, Duisters R, van Leeuwen R E, Crijns H J, Ramaekers F C, Hutchison C J and Pinto Y M 2005 A-type lamins are essential for TGF-β1 induced PP2A to dephosphorylate transcription factors; *Hum. Mol. Genet.* **14** 2839–2849

van der Kooi A J, Bonne G, Eymard B, Duboc D, Talim B, Van der Valk M, Reiss P, Richard P, Demay L, Merlini L *et al* 2002 Lamin A/C mutations with lipodystrophy, cardiac abnormalities, and muscular dystrophy; *Neurology* **59** 620–623

van Engelen B G M, Muchir A, Hutchison C J, van der Kooi A J, Bonne G and Lammens M 2005 The lethal phenotype of a homozygous nonsense mutation in the lamin A/C gene; *Neurology* **64** 374–376

Varela I, Cadinanos J, Pendas A M, Gutierrez-Fernandez A, Folgueras A R, Sanchez L M, Zhou Z, Rodriguez F J, Stewart C L, Vega J A *et al* 2005 Accelerated ageing in mice deficient in Zmpste24 protease is linked to p53 signalling activation; *Nature (London)* **437** 564–568

Varga R, Eriksson M, Erdos M R, Olive M, Harten I, Kolodgie F, Capell B C, Cheng J, Faddah D, Perkins S *et al* 2006 Progressive vascular smooth muscle cell defects in a mouse model of Hutchinson-Gilford progeria syndrome; *Proc. Natl. Acad. Sci. USA* **103** 3250–3255

Vergnes L, Peterfy M, Bergo M O, Young S G and Reue K 2004 Lamin B1 is required for mouse development and nuclear integrity; *Proc. Natl. Acad. Sci. USA* **101** 10428–10433

Vigouroux C, Auclair M, Dubosclard E, Pouchelet M, Capeau J, Courvalin J C and Buendia B 2001 Nuclear envelope disorganisation in fibroblasts from lipodystrophic patients with heterozygous R482Q/W mutations in the lamin A/C gene; *J. Cell Sci.* **114** 4459–4468

Vital A, Ferrer X, Goizet C, Rouanet-Larriviere M, Eimer S, Bonne G and Vital C 2005 Peripheral nerve lesions associated with a dominant missense mutation, E33D, of the lamin A/C gene; *Neuromuscul Disord.* **15** 618–621

Walter J, Sun L and Newport J 1998 Regulated chromosomal DNA replication in the absence of a nucleus; *Mol. Cell* **1** 519–529

Waterham H R, Koster J, Mooyer P, Noort Gv G, Kelley R I, Wilcox W R, Wanders R J, Hennekam R C and Oosterwijk J C 2003 Autosomal recessive HEM/Greenberg skeletal dysplasia is caused by 3-beta-hydroxysterol-delta-14-reductase deficiency due to mutations in the lamin B receptor gene; *Am. J. Hum. Genet.* **72** 1013–1017

Wilson K 2000 The nuclear envelope, muscular dystrophy and gene expression; *Trends Cell Biol.* **10** 125–129

Worman H J and Courvalin J-C 2004 How do mutations in lamins A and C cause disease?; *J. Clin. Invest.* **113** 349–351

Wydner K L, McNeil J A, Lin F, Worman H J and Lawrence J B 1996 Chromosomal assignment of human nuclear envelope protein genes *LMNA*, *LMNB1*, and *LBR* by fluorescence *in situ* hybridization; *Genomics* **32** 474–478

Yang S H, Bergo M O, Toth J I, Qiao X, Hu Y, Sandoval S, Meta M, Bendale P, Gelb M H, Young S G and Fong L G 2005 Blocking protein farnesyltransferase improves nuclear blebbing in mouse fibroblasts with a targeted Hutchinson–Gilford progeria syndrome mutation; *Proc. Natl. Acad. Sci. USA* **102** 10291–10296

Zastrow M S, Vicek S and Wilson K L 2004 Proteins that bind A-type lamins: integrated isolated clues; *J. Cell Sci.* **117** 979–987

Zastrow M S, Flaherty D B, Benian G M and Wilson K L 2006 Nuclear titin interacts with A- and B-type lamins *in vitro* and *in vivo*; *J. Cell Sci.* **119** 239–249

Zhang Q, Ragnauth C, Greener M J, Shanahan C M and Roberts R G 2002 The nesprins are giant actin-binding proteins, orthologous to *Drosophila melanogaster* muscle protein MSP-300; *Genomics* **80** 473–481

Zhang Q, Ragnauth C D, Skepper J N, Worth N F, Warren D T, Roberts R G, Weissberg P L, Ellis J A and Shanahan C M 2005 Nesprin-2 is a multi-isomeric protein that binds lamin and emerin at the nuclear envelope and forms a subcellular network in skeletal muscle; *J. Cell Sci.* **118** 673–687

Zhou B B and Elledge S J 2000 The DNA damage response, putting checkpoints in perspective; *Nature (London)* **408** 433–439

MS received 11 May 2006; accepted 7 July 2006

*e*Publication: 8 August 2006

Corresponding editor: VIDYANAND NANJUNDIAH