Eukaryotic cells are characterized by the presence of membrane-bound organelles like the nucleus, endoplasmic reticulum, golgi network, mitochondria, lysosomes and peroxisomes. These specialized organelles carry out specific cellular functions. The nucleus is central to the activities of the cell. Important functions such as DNA replication, transcription and RNA splicing are housed in the nucleus.

1.1 The nucleus

The nucleus was the first intracellular structure to be discovered and was originally described by Franz Bauer in 1802 and later investigated by Robert Brown in the 1830's (Harris, 1999). The nucleus is a highly dynamic organelle, which contains distinct compartments (Spector, 1993; Lamond and Earnshaw, 1998; Matera, 1999). These are characterized by the absence of delineating membranes, which feature distinguishes nuclear compartments from their cytoplasmic counterparts. The best studied nuclear compartments are the nucleolus, the splicing factor compartments, Cajal bodies, and the promyelocytic leukemia bodies. The nucleus is composed of three main structural elements - the nuclear envelope, nuclear lamina and nuclear pores.

1.1.1 Nuclear envelope

The nuclear envelope is composed of two concentric lipid bilayer membranes separated by an intermembrane space of about 20-40 nm. The outer membrane is continuous in many places with the rough endoplasmic reticulum (ER). Like the rough ER, the outer membrane of the nuclear envelope is dotted with ribosomes. Attached to the nucleoplasmic side of the inner membrane is a sheet-like structure of protein filaments called the nuclear lamina. This helps to give strength and support to the nuclear envelope. The nuclear envelope is perforated by about 3000-4000 nuclear pore complexes (NPCs). These pores permit molecular transport to and from the nucleus (Moir et al., 1995; Stoffler et al., 1999; Ellenberg and Lippincott, 1999; Spector, 2001). The structure of the nuclear envelope is presented in a schematic diagram in Fig. 1.1.

1.1.2 Nuclear pore complex

The NPC has a molecular mass of about 125 mDa in vertebrates and contains about 30 or more different proteins (Nakielny and Dreyfuss, 1999; Lyman and Gerace, 2001). The NPC spans the dual membrane of the nuclear envelope and acts as a gateway for macromolecular traffic between the cytoplasm and the nucleus. The basic framework of the NPC consists of a central core with a spoke-ring structure exhibiting 8-fold radial symmetry. From this central ring 50 to 100 nm fibrils extend into the
Fig. 1.1 A schematic model of a typical eukaryotic nucleus. This schematic depicts various proposed interactions of lamins with inner nuclear membrane proteins, nuclear pores and other nucleoplasmic proteins. Lamins are mainly concentrated at the periphery and also distributed throughout the nucleoplasm, where they interact with various structural and functional complexes.
nucleoplasm and the cytoplasm. The NPC is in turn anchored in the nuclear envelope by the nuclear lamina. A number of proteins called nucleoporins have been localized to discrete regions of the NPC and are often used as markers for this compartment, e.g. Nup 158 (Stoffler et al., 1999). Approximately half the nucleoporins contain a phenylalanine-glycine repeat motif.

Proteins are targeted for nuclear transport by either nuclear localization signals or nuclear export signals which are composed of specific sequences of amino acids. For transport of ribonucleoproteins, the signal either resides on the RNA or consists of a composite of signals on both RNA and protein (Nakielny and Dreyfuss, 1999). A number of nuclear transport receptors exist that recognize these signals and are variously called karyopherins, importins, or transportins. Ran-GTP is an important component of the transport cycle. The signal receptors are generally large acidic proteins that share the ability to bind components of the NPC and contain both an N-terminal Ran GTP-binding domain and a C-terminal cargo-binding domain (Fabre and Hurt 1994; Weis, 1998; Ohno et al., 1998).

1.1.3 Nuclear lamina

The nuclear lamina is a thin, filamentous network of insoluble proteins present beneath the inner nuclear membrane. The nuclear envelope and nuclear lamina are involved in maintaining the architecture and integrity of the nucleus. The lamina also helps in the organization of the higher order structure of chromatin, in the spatial organization of nuclear functions such as DNA replication and transcription, and in the regulation of nuclear disassembly and assembly during mitosis (Spector, 2001; Burke, 2001; Goldman et al., 2002). The nuclear lamina is composed of lamin proteins.

1.2 Nuclear lamins

Nuclear lamins were initially identified as the major components of the nuclear lamina (Fawcett, 1966). Lamins belong to the intermediate filament (IF) superfamily of proteins. IF proteins are expressed in almost all metazoan cells (Fuchs and Weber, 1994; Hermann and Aebi, 2000). Most of the IF proteins are present in the cytoplasm (Fuchs and Weber, 1994). The only nuclear IF proteins known are the lamins which come under the type V IF family (Aebi et al., 1986; Fisher et al., 1986; McKeon et al., 1986; Gerace and Burke et al., 1988).
1.2.1 Types of nuclear lamins

Nuclear lamins are classified into two major types namely, A and B-type lamins based on their structural and biochemical properties. The B-type lamins, B1 and B2 are ubiquitously expressed in all somatic cells and are encoded by two separate genes in mammals, namely *LMNB1* and *LMNB2* (Weber et al., 1990; Höger et al., 1990; Pollard et al., 1990; Biamonti et al., 1992). A-type lamins, namely lamins A and C are developmentally regulated and are expressed only in differentiated cell types. Both lamins A and C arise through alternative splicing of the same primary transcript encoded by the mammalian lamin A (*LMNA*) gene (Lin and Worman, 1993; Furukawa et al., 1994). Another alternatively spliced product of lamin A, lamin AΔ10 which lacks the 30 amino acids encoded by exon 10, has been identified in a variety of mammalian cell types including normal cells and carcinomas (Machiels et al., 1996). Lamins C2 and B3 are expressed in male germ cells, and are alternative splice variants of *LMNA* and *LMNB2* genes respectively (Furukawa and Hotta, 1993; Furukawa et al., 1994; Alsheimer et al., 1999). The number and complexity of lamin genes have increased during metazoan evolution. An invertebrate like *Caenorhabditis elegans* has only one gene *lwm-1* (Liu et al., 2000a). *Drosophila melanogaster* has two lamin genes coding for lamin Dm0 and lamin C (Gruenbaum et al., 1988; Bossie and Sanders, 1993). Lamin Dm0 shares similarity with B-type lamins and lamin C resembles an A-type lamin. The nuclear lamina in chicken is composed of two lamins namely, lamin B1, which is a B-type lamin, and lamin B2, which is an A-type lamin (Lehner et al., 1986). Genome sequence analysis of yeast and Arabidopsis indicates that those species do not have lamins.

1.2.2 Genomic organization of lamin genes

Lamins A, AΔ10, C and C2 are encoded by a single gene on chromosome 1 which maps to the locus 1q 21.2 – 1q 21.3 in the human genome, whereas human lamin B1 and B2 are encoded by two different genes on chromosomes 5 and 19 which map to loci 5q 23.3 – q 31.1 and 19q 13.3 respectively (Biamonti et al., 1992; Wydner et al., 1996). The lamin A cDNAs from human (Fisher et al., 1986), rat (Ozaki and Sakiyama, 1992; Parnaik et al., 1994) and mouse (Riedel and Werner, 1989) have been cloned, sequenced and analyzed. The human lamin A/C gene contains 12 exons and 11 introns spread approximately over 24 kb of genomic DNA (see in Fig. 1.2A). The first intron is about 16 kb in length, the second intron is about 2 kb and the remaining introns are less than 1 kb in length. Lamin A and C share the first nine exons. Exon 1 codes for the head domain and the first part of the rod domain. Exons 2-6 code for the remaining part of the
Fig. 1.2 Schematic representation of the LMNA gene with the positions of disease-causing mutations.
(A) Structural organization of the LMNA gene, boxes represent exons 1 to 12 and lines indicate introns; the positions marked indicate the sites of mutations in the LMNA gene. (B) Domain structure of lamin A and lamin C proteins. The highly conserved α-helical central rod domain (blue) is flanked by non-helical head and tail domains (pink), the nuclear localization signal (NLS) and the sites of proteolytic cleavage are shown as P1 and P2. (C) The diseases caused by the different lamin mutations are represented by the indicated colour codes.
rod domain. Exons 7-9 code for the tail domain common to lamin A and C. Exon 7 contains the nuclear localization signal sequence (NLS). The alternatively used 5' splice site for the generation of lamin A and C lies in exon 10 after 566 amino acids. Exon 11 and 12 code for the lamin A specific tail domain sequences. The CaaX (C, cysteine; a, aliphatic; X, any amino acid) motif encoding sequence is present in exon 12. Alternative splicing within exon 10 gives rise to pre-lamin A and lamin C mRNAs. They both share the first 566 amino acids. The codons for the amino acids 567-572 which are unique for lamin C immediately follow the codon for amino acid 566, followed by a stop codon and a polyadenylation signal in exon 10 to generate lamin C mRNA. Pre-lamin A mRNA is produced by the lamin A specific alternative splicing event wherein, the 3' end of codon 566 is joined to the 5' end of the exon 12. Exon 12 has the stop codon and polyadenylation sequence for lamin A (Lin and Worman, 1993). The germ-cell specific lamin C2 is similar to lamin C except for a separate exon 1 which codes for its unique N-terminal hexapeptide. Lamin C2 exon 1 lies in the intron 1 of the LMNA gene. It is speculated that lamin C2 is probably transcribed from a separate promoter that resides in intron 1 and not from the promoter that regulates the expression of lamin A/C in differentiated cell types (Nakajima and Abe, 1995). Another alternative splice product of the LMNA gene, lamin AΔ10 is similar to lamin A except for the internal deletion of 30 amino acids encoded by exon 10 (Machiels et al., 1996). The human lamin B1 gene LMNB1 consists of 11 exons and 10 introns spanning about 45 kb of genomic DNA (Lin and Worman, 1995). The mouse lamin B2 gene contains 12 exons and 11 introns (Zewe et al., 1991).

1.2.3 Structural organization of lamins

The lamins are conserved throughout evolution. The primary sequence and the deduced secondary structure information predict that, like all IF family proteins, lamins have a tripartite structure consisting of a central α-helical rod domain flanked by non-helical N-terminal head and C-terminal tail domains. The highest homology amongst lamins was found within the two short segments at either ends of the α-helical rod domain. The highly conserved central rod domain is about 354 amino acid long and consists of four distinct α-helical coils namely 1A, 1B, 2A and 2B. The coiled-coil domains are organized around heptad repeats and coil 1B has a 42 amino acid extension relative to that of the cytoplasmic IF proteins. The four α-helical domains are separated by linker segments L1, L12 and L2, which are predicted to be α-helical. The linker L1 also maintains the heptad repeat pattern and therefore together with the segments 1A and 1B it forms a continuous coiled coil (Heitlinger et al., 1992; Stuurman
The N-terminal head domain is relatively smaller than the C-terminal tail domain; both are of variable lengths in different lamins and play an important role in lamin assembly (Fisher et al., 1986; McKeon et al., 1986, 1991; Moir et al., 1991). The central rod domain drives the interaction between two lamin proteins to form a coiled-coil dimer, the basic unit of lamin assembly. The head-to-tail associations between the rod domains of two lamin dimers are essential for the assembly of higher order structures and lamin polymerization (Stuurman et al., 1996; 1998). The C-terminal tail domain has a nuclear localization sequence at 415-422 amino acid residues for nuclear import (see in Fig.1.2B). The crystal structure of the C-terminal domain of lamin A (amino acids 438-552) has revealed a compact, well-defined domain composed of β strands termed the immunoglobulin-like (lg) fold (Dhe-Paganon et al., 2002).

Lamins (except lamin C and C2) have a CaaX motif at the C-terminus, which undergoes post-translational modifications like cysteine isoprenylation, proteolytic cleavage of the last three amino acids, followed by methyl esterification of the carboxyl group of the isoprenylated cysteine residue. Isoprenylation appears to be required for increasing the hydrophobicity and targeting the lamins to the inner surface of the nuclear envelope (Holtz et al., 1989; Kitten and Nigg, 1991; Firnbach-Kraft and Stick, 1993; Hofemeister et al., 2000). After nuclear envelope localization, the carboxy terminal 18 residues of pre-lamin A including the isoprenylated cysteine are cleaved off by endoproteolysis to form mature lamin A (Weber et al., 1989). Lamin C and C2 lack the CaaX box and require the presence of other lamins like A and B1 for their assembly into the nuclear lamina. Lamin C2 has been shown to be post-translationally modified at its unique N-terminus by myristylation on the first glycine residue, which confers the hydrophobicity required for nuclear envelope localization (Alsheheimer et al., 2000).

1.2.4 Localization of lamins

In addition to being components of the peripheral nuclear lamina, lamins have also been detected in the interior of the nucleus in the form of foci, as well as a diffuse network. DNA replication initiation foci have been shown to co-localize with internal lamin A/C structures (Kennedy et al., 2000). Intracellular lamin foci have also been detected in G1-phase cells (Bridger et al., 1993). Lamins have been shown to form an internal nucleoskeleton during all stages of the cell cycle (Goldman et al., 1992; Hozak et al., 1995). Overexpression of GFP-tagged A-type lamins leads to the formation of an extensive network of intra and transnuclear tubular structures in addition to the peripheral lamina. These structures are present in all interphase stages of the cell cycle (Ellenberg et al., 1997; Broers et al., 1999; Moir et al., 2000b). In addition, a veil of
nucleoplasmic fluorescence has been observed in interphase cells expressing GFP-tagged lamin A or B1 (Moir et al., 2000b). Studies from our laboratory have shown a novel structural aspect of lamins in the form of speckles that are associated with RNA splicing factors in nuclear speckles, and are specifically recognized by the monoclonal antibody LA-2H10. Lamin speckles co-localize with components of splicing factor compartments such as SC-35 in various cell types (Jagatheesan et al., 1999). Studies with transcriptional inhibitors have shown that lamin speckles are sensitive to inhibitors of transcription, suggesting that lamin speckles may play an important role in the spatial coordination of RNA splicing and transcription (Kumaran et al., 2002).

1.3 Lamin binding proteins

Lamins are known to interact with many other proteins, which can be broadly grouped into nuclear membrane proteins, chromatin binding proteins and gene regulatory proteins.

1.3.1 Nuclear membrane proteins

In earlier studies, several inner membrane proteins have been shown to interact directly with nuclear lamins in vitro and to remain closely associated with the nuclear lamina after extraction of nuclei with non-ionic detergent, nucleases and/or high salt buffers (Foisner and Gerace, 1993; Stuurman et al., 1998; Fairley et al., 1999). The lamin B receptor (LBR) was the first transmembrane protein identified to interact with B-type lamins (Moir et al., 1995). LBR has an N-terminal nucleoplasmic domain and a C-terminal region that includes eight transmembrane domains. The nucleoplasmic domain interacts with B-type lamins, heterochromatin protein1 (HP1) and chromatin in vitro (Stuurman et al., 1998). The lamina-associated-polypeptides (LAPs) LAP1A, LAP1B and LAP1C are alternatively spliced products of the LAP1 gene. LAP1A and LAP1B have been shown to interact with lamin A, lamin C and lamin B1. LAP1C is anchored to the nuclear envelope as part of a multimeric complex that includes LAP1A and B-type lamins but not A-type lamins. LAP2 proteins are expressed as six isoforms namely, α, β, γ, δ, ε and ξ and are alternatively spliced products of a single gene. All these isoforms except LAP2α possess a conserved membrane-spanning domain. LAP2β binds specifically to lamin B1, chromatin (Foisner and Gerace, 1993) and barrier-to-auto-integration factor (BAF) a DNA-binding protein (Shumaker et al., 2001; Cal et al., 2001). LAP2α is the only member of the LAP family that is distributed in the nucleoplasm, where it associates with BAF, A-type lamins and chromatin (Dechat et al., 2000; Vlcek et al., 1999). LAP2α binds directly to A-type lamins during interphase (Dechat et al., 2000), but has dynamic
architectural roles during nuclear envelope assembly (Vlcek et al., 2002). It is proposed to tether A-type lamins to intranuclear sites and to cooperate with lamins in organizing chromatin and in the regulation of gene expression (Foisner, 2003).

Emerin is an inner nuclear membrane protein that interacts with lamin A. Emerin is related to the LAPs in that it is also a type-II integral membrane protein and its N-terminal nucleoplasmic domain possesses the LEM motif (domain common to LAPs, Emerin and MAN1). The distribution of emerin at the inner nuclear membrane is dependent upon the presence of lamin A (Fairley et al., 1999; Sullivan et al., 1999; Vaughan et al., 2001). Emerin also binds directly to BAF, and might thereby anchor lamins and chromatin to the nuclear envelope during interface (Lee et al., 2001).

Actin, which has been detected in the nucleus, interacts with lamin A and C at their C-terminal tail domains (Sasseville and Langelier, 1998). Actin also associates with emerin, chromatin remodelling complexes, and the tumour suppressor protein, p53. Another class of proteins that interact with lamin A are the nesprins. Human nesprins are encoded by two genes, which yield multiple protein isoforms through alternative mRNA splicing. Nesprin 1α, a nuclear membrane protein binds directly to A-type lamins and emerin (Mislow et al., 2002). All nesprin family proteins have multiple spectrin repeat domains, and many also have an actin-binding domain (Chang et al., 2001; Starr and Han, 2003). Nesprin is dependent on lamin A for proper localization in the nuclear envelope (Muchir et al., 2003). The lamin interactions are depicted schematically in Fig. 1.1.

1.3.2 Chromatin binding proteins

Lamins bind DNA directly in vitro (Luderus et al., 1992). The DNA-binding region is identical in lamin A and C and includes both the Ig-fold domain and the NLS in the tail (Stierle et al., 2003). Lamins A and C also interact with chromatin through the α-helical rod domain (Glass et al., 1993) and the C-terminal tail domain (Hoger et al., 1991). This interaction is mediated by the direct binding of A-type lamins with core histones (Taniura et al., 1995), and thereby nuclear lamins may serve as scaffolds for multiprotein complexes associated with chromatin and mediate their organization. The chromatin binding factor BAF also interacts with lamin A apart from its interaction with DNA and LEM-domain proteins (Holaska et al., 2003). BAF has been proposed to help in rapid interlinking of chromatin, lamins and LEM-domain proteins, and may play an important
role in gene expression and in establishing chromatin organization and structure during nuclear assembly.

1.3.3 Gene regulatory proteins

A-type lamins are thought to provide scaffolds for protein complexes that regulate gene expression (Cohen et al., 2001). The hypophosphorylated, active form of retinoblastoma protein (pRb) has been shown to bind to the α-helical rod domain of A-type lamins (Mancini et al., 1994; Markiewicz et al., 2002; Ozaki et al., 1994). Hypophosphorylated pRb binds to E2F-DP heterodimers and blocks E2F-dependent gene expression through a variety of mechanisms, including the recruitment of histone deacetylase complexes (HDACs) (Chau and Wang, 2003). During G1 phase, hypophosphorylated Rb is anchored to the nucleoskeleton by its pocket C domain that binds directly to A-type lamins and LAP2α (Mancini et al., 1994; Markiewicz et al., 2002). Another repressor of E2F-DP heterodimer-mediated gene expression is germ-cell-less (GCL), which is required to establish the germ cell lineage during development in Drosophila. GCL is known to bind to DP proteins and regulate the cell cycle. GCL has been shown to form a stable ternary complex with emerin and lamin A (Holaska et al., 2003). GCL also interacts with LAP2β (Nili et al., 2001). Thus, a stable lamin-based scaffold might be required to tether and stabilize the enormous chromatin-remodelling machines recruited by pRb and other gene regulators (Neely and Workman, 2002).

Sterol response element binding protein 1 (SREBP1) binds to the Ig-fold domain of lamin A/C (Lloyd et al., 2002). SREBP1α and SREBP1c are encoded by alternatively spliced mRNAs, and are both helix-loop-helix leucine zipper transcription factors. They activate genes required for cholesterol biosynthesis and lipogenesis (Horton, 2002) and promote adipocyte differentiation (Kim and Spiegelman, 1996). MOK2 is a DNA binding transcriptional repressor that interacts with lamin A and C. It represses genes activated by cone-rod homeobox protein (Crx) (Dreuil et al., 2002). MOK2 also binds RNA in vitro, and might thus influence RNA processing (Arranz et al., 1997). MOK2 binds to the coil region of A-type lamins (Dreuil et al., 2002). BAF, which is known to interact with lamin A, chromatin and LEM domain proteins (Holaska et al., 2003) also binds to several homeodomain transcriptional activators, including Crx, and represses Crx-dependent gene expression in retinal cells (Segura-Totten et al., 2002). Other inner membrane proteins like young arrest (YA) and Bicaudal-D (BICD) involved in Drosophila oocyte development have been shown to interact with the Drosophila B-type lamin, Dm0 (Goldberg et al., 1998; Stuurman et al., 1999). A recent study shows that lamin A/C can directly interact with c-Fos to suppress the activity of the transcription factor AP-1 and
thus control cell cycle progression (Ivorra et al., 2006). Thus several gene regulators bind directly to lamins and its other partners like emerin and LAPs and mediate expression of different genes in a developmentally regulated manner.

1.4 Functions of lamins

Lamins are the major components of the nuclear lamina and play an important role in the maintenance of nuclear integrity, in the assembly of the nuclear envelope, in the organization of replication and transcription, in gene regulation and in apoptosis.

1.4.1 Maintenance of nuclear integrity

Loss of nuclear lamins has been shown to affect nuclear shape and cause abnormal heterochromatin organization, unequal separation of chromosomes and abnormal distribution of nuclear pore complexes leading to embryonic lethality in C. elegans (Liu et al., 2000a). A hypomorphic mutation in Drosophila lamin Dm0 reduces lamin expression, inhibits nuclear membrane assembly and induces the formation of annulate lamellae (Lenz-Bohne et al., 1997). Another Dm0 mutant has profound effects on nuclear morphology and Drosophila development (Guillemin et al., 2001). Homozygous lamin A (Imn<sup>-/-</sup>) null mice shows reduced post-natal growth and also undergoes severe muscle wasting and die after 8 weeks. In Imn<sup>-/-</sup> cells emerin is mislocalized to the ER and nuclear integrity is severely compromised in skeletal and cardiac muscle cells (Sullivan et al., 1999). Mutations in A-type lamins lead to highly debilitating genetic disorders in humans. A common feature in cells from patient’s samples is aberrant nuclear morphology and abnormal chromatin organization, with defective interactions of lamins with inner nuclear membrane proteins (Vigourox et al., 2001). Thus nuclear lamins along with other inner membrane proteins play an important role in the maintenance of nuclear shape and integrity.

1.4.2 Nuclear envelope disassembly and assembly during mitosis

Lamin disassembly and reassembly during mitosis play an important role in the breakdown and reassembly of the nuclear envelope. At the onset of mitosis, the lamina is depolymerized and the nuclear membranes detach from the lamina and become absorbed into the ER (Tarasaki et al., 2001; Beaudouin et al., 2002). The lamin polymers are rapidly disassembled into monomers during the prophase to metaphase transition and this is mediated by the phosphorylation of lamins by cdc2 kinase at specific serine residues in the regions flanking the central α-helical rod domain (Heald and McKeon, 1990).
After mitosis lamin monomers are dephosphorylated by protein phosphatase-1 (PP1) and get reassembled into the nuclear lamina. The nuclear envelope anchorage of PP1 and lamin dephosphorylation is mediated by interaction with A-kinase anchoring protein, AKAP 149 (Steen et al., 2000). The reassembly of lamin B occurs approximately at the same time as nuclear envelope formation, whereas the A-type lamins are assembled at a later step. A and B-type lamins follow different pathways of assembly into the nuclear membrane (Steen and Collas, 2001).

1.4.3 Role of lamins in DNA replication

There is considerable evidence for the involvement of lamins in DNA replication. The existence of nucleoplasmic structures containing lamins that interact with replication factors has been reported. Nucleoplasmic lamin B foci are co-localized with PCNA at DNA replication sites (Moir et al., 1994) and lamin A foci localize with retinoblastoma protein at early S-phase replication sites (Kennedy et al., 2000). Therefore, lamins are now considered to form, besides the nuclear lamina, an internal scaffold upon which nuclear processes such as DNA replication are organized (Moir et al., 2000a). This endogenous lamin network is disrupted when lamin N-terminal deletion mutants are utilized in nuclear reconstruction experiments and, as a consequence, DNA replication is prevented. The resulting lamin aggregates contain factors involved in the elongation phase of replication, suggesting that elongation rather than initiation of replication is mediated by the lamin scaffold (Spann et al., 1997).

1.4.4 Role of lamins in organization of transcription

A role for lamins in transcription is supported by the findings that the induction of lamin A/C expression during development is dependent on cell differentiation (Rober et al., 1989). Recent studies on the insulator element gypsy (Gerasimova et al., 2000) and on the pRb/E2F system (Mancini et al., 1994) indicate that these transcriptional regulatory elements require interaction with a nuclear substrate containing lamins. The expression of a dominant negative lamin N-terminal deletion mutant has been reported to disrupt the normal organization of the lamins and to inhibit RNA pol II activity (Spann et al., 2002). Inhibition of RNA pol II transcription using specific transcriptional inhibitors leads to the reorganization of the intranuclear lamin A/C speckles to form large foci similar to those formed by the RNA splicing factors SC-35 and U5-116 kD. Removal of the inhibition resulted in reactivation of transcription and rapid and synchronous redistribution of lamin A/C speckles and splicing factor compartments (Kumaran et al.,
These findings suggest that lamins are distributed throughout the nucleoplasm, forming a scaffold upon which transcription sites are organized.

1.4.5 Role of lamins in gene regulation

The nuclear lamina may be involved in the regulation of global patterns of gene expression. In fact, the lamina exerts a profound influence on the organization of heterochromatin that is generally silent in terms of transcriptional activity. The expression of dominant negative mutants of lamins may lead to changes in nuclear organization that induce changes in gene expression programmes (Burke and Stewart, 2002). Furthermore, a series of transcription factors, including SREBP-1, Oct-1, pRb and GCL, co-localize with the nuclear lamina or interact with proteins anchored to the lamina, such as LAP 2β (Holaska et al., 2003). These findings suggest that the disruption of lamin or lamin-associated protein interactions may result in specific alterations in gene regulation that could induce altered patterns of differentiation in specific tissues (Wilson, 2000).

1.4.6 Role of lamins in apoptosis

Apoptosis is characterized by a dramatic reduction in nuclear size and chromatin condensation accompanied by chromatin fragmentation. The alteration of nuclear lamin organization is a key step in the initiation and execution phases of apoptotic cell death. During apoptosis, lamins are cleaved by caspases (Lazebnik et al., 1995; Takahashi et al., 1996). The single cleavage site of lamin A has been mapped to aspartic acid 230, which is present in the central rod domain (Takahashi et al., 1996). The cleavage of lamins and of lamina-associated proteins may be required to allow chromatin detachment from the lamina and to ensure changes in nuclear shape and strength. The onset of apoptosis is largely delayed by the expression of uncleavable mutant forms of lamins, and chromatin fails to condense although caspases are activated; this suggests that lamin degradation occurs downstream of caspase activation and that lamin proteolysis may activate nucleases (Rao et al., 1996). On the basis of information available on the function of lamins in DNA replication and in transcription, lamin degradation appears to play a critical role in shutting down vital nuclear processes during apoptosis.

1.5 Lamins in disease

Mutations in the LMNA gene cause a number of debilitating diseases, collectively termed laminopathies, that affect skeletal and cardiac muscle, adipose, bone and neural tissues, and also cause premature ageing syndromes. These diseases have been linked
to LMNA by both positional cloning and candidate gene approaches. LMNA has been mapped to chromosome 1q21.2 – q21.3 (Wydner et al., 1996). Over 100 different mutations have been identified so far spanning the entire length of the lamin gene (see Fig.1.2A). These include missense mutations, deletions leading to frameshifts, creation of a new splice site and two nonsense mutations resulting in truncated products. The diseases caused by LMNA mutations have been grouped into four major categories depending on the nature of the tissue-types affected and other physiological conditions and these are as follows.

1. Muscular dystrophies
   i) Autosomal dominant Emery-Dreifuss muscular dystrophy (AD-EDMD)
   ii) Autosomal recessive Emery-Dreifuss muscular dystrophy (AR-EDMD)
   iii) Dilated cardiomyopathy 1A (DCM)
   iv) Limb-girdle muscular dystrophy 1B (LGMD)

2. Partial lipodystrophy syndromes
   i) Dunnigan-type Familial Partial Lipodystrophy (FPLD)
   ii) Mandibulacral Dysplasia (MAD)
   iii) Lipoactrophy with diabetes, hepatic steatosis, hypertrophic cardiomyopathy and leukomelanodermic papules.

3. Peripheral neuropathy
   i) Charcot-Marie Tooth Disorder Type 2B1 (CMT2 B1)

4. Premature ageing syndromes
   i) Hutchinson-Gilford Progeria Syndrome (HGPS)
   ii) Atypical Werner’s Syndrome

5. Overlapping syndromes with progeroid characteristics
   i) Restrictive dermopathy (RD)
   ii) Seip Syndrome
   iii) Atypical cases of HGPS

At present no mutations in LMNB1 or LMNB2 have been linked to human disease, suggesting that loss 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). Mutations in the lamin B receptor gene are associated with Pelger-Huet anomaly in mice and humans (Hoffman et al., 2002; Schultz et al., 2003) as well as autosomal recessive Hydrops-ectopic calcification-"motheaten" (HEM)/Greenberg skeletal dysplasia (Waterham et al., 2003). Since the lamin B receptor may be important for B-type lamin targeting to the nuclear envelope, these diseases may result at least in part from altered nuclear lamin function.
1.5.1 **Muscular dystrophies**

1.5.1.1 **Emery-Dreifuss muscular dystrophy**

A mutation in the emerin gene located on the X-chromosome was first identified to be the cause of the X-linked form of this disease (Bione et al., 1994; Yates and Wehnert, 1999). Another group later identified mutations in the *LMNA* gene leading to an autosomal dominant form of EDMD (Bonne et al., 1999, 2000). A rare autosomal recessive form of EDMD is caused by the inheritance of two different mutant *LMNA* alleles (Raffaele Di Barletta et al., 2000). EDMD manifests in childhood and is characterized by early contractures of the elbows, Achilles tendons, and spine in addition to slowly progressive muscle wasting and weakness with a predominantly humeroperoneal distribution. Cardiac involvement occurs in nearly all patients by the third decade, initially involving arrhythmias, and progressing towards complete heart block with a substantial risk of sudden death in middle age. The clinical manifestations of all the three forms of EDMD are similar.

1.5.1.2 **Dilated cardiomyopathy**

Five novel missense mutations have been identified in *LMNA* in cases of DCM: four in the α-helical rod domain of the *LMNA* gene and one in the lamin C tail domain. These mutations show autosomal dominant inheritance (Fatkin et al., 1999). DCM is characterized by progressive conduction system disease (sinus bradycardia, atrioventricular conduction block) and cardiac failure, with no effects on skeletal muscles. Sudden death occurs frequently and can be fatal at a relatively young age.

1.5.1.3 **Limb girdle muscular dystrophy**

Mutations in *LMNA* gene leading to LGMD show an autosomal dominant pattern of inheritance. The disease is characterized by proximal muscular dystrophy with proximal muscle weakness that is greater in the legs than in the arms. Cardiac abnormalities are seen relatively late in life. Muscle weakness in the pelvic and shoulder girdles is also observed (Muchir et al., 2000).

1.5.2 **Partial lipodystrophy syndromes**

1.5.2.1 **Dunnigan-type familial partial lipodystrophy**

This is an autosomal dominant inherited disease caused by mutations in the tail domain of the *LMNA* gene (Cao and Hegele, 2000), and is characterized by loss of subcutaneous fat in the limbs and trunk, with excessive fat accumulation around the neck and shoulders, leading to a condition known as ‘buffalo hump’. The onset of the
disease is only after puberty, and patients show normal fat distribution in early childhood. Loss of subcutaneous fat causes prominence of muscles and superficial veins in these areas. Insulin resistance and type II diabetes mellitus occur by 20 years of age with hypertriglyceridemia and low concentrations of high density lipoprotein.

**1.5.2.2 Mandibuloacral dysplasia**

This is a rare autosomally recessive inherited disease caused by mutations in the LMNA gene (Novelli *et al*., 2002) and is characterized by congenital abnormalities, post-natal growth retardation, craniofacial anomalies, skeletal malformations and mottled cutaneous pigmentation, partial liposyrphosis, insulin resistance and diabetes mellitus. This disease shares several features with HGPS, although the life expectancy is not shortened. Mutations in the zinc metalloproteinase gene, ZMPSTE24 also cause MAD associated with progeroid and general lipodystrophy (Agarwal *et al*., 2003).

**1.5.3 Peripheral neuropathy**

**1.5.3.1 Charcot-Marie-Tooth Disorder Type 2B1**

This is a rare form of autosomal recessive disease caused by mutations in the rod domain of the LMNA gene (De Sandre-Giovannoli *et al*., 2002) and is characterized by axonal degeneration of peripheral nerves, abolished deep-tendon reflexes affecting mainly the lower limbs, motor deficits in upper and lower limbs, sciatica and slight or no reduction of nerve conduction velocities. Nerve biopsies show substantial loss of large myelinated fibres and abnormally myelinated axons though their nuclei appear normal.

**1.5.4 Premature ageing syndromes**

**1.5.4.1 Hutchinson-Gilford Progeria Syndrome**

HGPS is a rare form of progeria which occurs in 1 in 8 million births and displays striking features of premature ageing. HGPS recapitulates most of the pathologies of normal ageing at an accelerated rate, with sparing of the nervous system. Children with HGPS usually appear normal in early infancy, but at about six months of age begin to experience profound growth delay. Scalp hair, eyebrows and eyelashes are typically lost resulting in total alopecia. A gradual, almost complete lipodystrophy begins in infancy, and the skin acquires an abnormally aged appearance with prominent veins. In some children osteolysis may affect the clavicles, terminal phalanges, and acetabulum and sometimes even more bone deformities occur, including generalized osteoporosis leading to repeated fractures and degenerative joint changes and hip dislocation. Affected children as young as 5 years of age develop widespread atherosclerosis.
including the coronary arteries and aorta, often resulting in death by myocardial infarction or stroke in the early teens. The median age of death is 13.4 years. In the majority of cases, HGPS is caused by a silent mutation in LMNA gene at nucleotide 1824 in exon 11, which does not affect the codon for Gly608. However, this mutation activates a cryptic splice site within exon 11, resulting in the production of a truncated product with an internal deletion of 50 amino acids near the carboxy terminus of lamin A, leaving lamin C unmodified (De Sandre-Giovannoli et al., 2003; Erikson et al., 2003). The resultant truncated protein termed 'progerin' retains the CaaX motif needed for isoprenylation and nuclear membrane targeting, but lacks the site for the second endoproteolytic cleavage due to the internal deletion. Therefore, the incompletely processed progerin accumulates and acts as a dominant negative protein, affecting the lamina and heterochromatin organization. A few missense mutations such as E145K, R471C and R527C that do not affect processing of the C-terminus of lamin A have also been linked to HGPS (Cao and Hegele, 2003; Eriksson et al., 2003). A homozygous missense mutation K542N has been reported that results in recessive HGPS (Plasilova et al., 2004).

1.5.4.2 Atypical Werner's Syndrome

Werner's syndrome is another type of autosomal recessive premature ageing disease wherein the WRN gene encoding 3'-5' Rec Q DNA helicase-exonuclease is mutated. Patients with the disease show a high incidence of early onset cataracts, atherosclerosis, diabetes, premature graying of hair and early death (usually in the 40's). A subset of Werner's syndrome patients are known as atypical cases, as they do not carry any detectable mutations in the WRN gene. 15% of these atypical cases have been reported to contain mutations in the LMNA gene (Chen et al., 2003).

1.5.5 Overlapping syndromes with progeroid characteristics

1.5.5.1 Restrictive dermopathy

RD is also called tight skin syndrome, and is a rare disorder characterized by intrauterine growth retardation, tight and rigid skin with erosions, prominent superficial vasculature and epidermal hyperkeratosis, aberrant facial features, sparse eyelashes and eyebrows, mineralization defects of the skull, thin dysplastic clavicles, pulmonary hypoplasia, multiple joint contractures and an early neonatal lethal course. Live born children usually die within the first week of life. It is inherited in an autosomal recessive manner. Out of nine fetuses/newborns with RD, two were found to have a heterozygous splicing mutation in the LMNA gene, leading to a complete or partial loss of exon 11 in mRNAs encoding lamin A and resulting in a truncated prelamin A protein. In the other
seven patients, a unique heterozygous insertion led to the creation of a premature termination codon in the gene ZMPSTE24, also known as FACE-1 in humans (Navarro et al., 2004). This gene encodes a metalloproteinase specifically involved in the post-translational processing of lamin A precursor. In all patients carrying a ZMPSTE24 mutation, loss of expression of lamin A as well as abnormal patterns of nuclear size and shape and mislocalization of lamin-associated proteins were evident. Hence, a common pathogenetic pathway, involving defects of the nuclear lamina and matrix, is likely to be involved in all RD cases. RD is one of the most deleterious laminopathies identified so far in humans caused by (primary or secondary) A-type lamin defects and nuclear structural and functional alterations.

1.5.5.2 Seip syndrome

This is an autosomal dominant inherited disease caused by the missense mutation T101 in LMNA gene (Csoka et al., 2004) and is clinically characterized by hypertriglyceridemia, hyperglycemia, thinned skin lipoatrophy and progeroid features.

1.5.5.3 Atypical cases of progeria

An atypical case of progeria has been reported to be caused by the R644C mutation in the LMNA gene (Csoka et al., 2004), and is an autosomal dominant inherited disease characterized by short stature, generalized wasting, thinned skin and survival to relatively older age.

Individuals of a single family carrying the same mutation can develop different laminopathies like AD-EDMD, DCM or FPLD (Broadsky et al., 2000). Another mutation in the LMNA gene, E33D results in autosomal dominant axonal neuropathy, muscular dystrophy, cardiac disease and leuconychia (Goizet et al., 2004). The variations suggest different degrees of penetrance for the same disease, and the development of a specific pathology may be influenced by modifying genes or other environmental factors (Goldman et al., 2002; Mounkes et al., 2003a, 2003b).

1.6 Models of laminopathies

It is now clear that mutations in the LMNA gene lead to a number of heritable genetic disorders collectively termed laminopathies. But the question that remains to be answered is, why do mutations in a ubiquitously expressed nuclear envelope protein give rise to a variety of tissue-specific phenotypes? Several hypotheses have been proposed in an attempt to answer this paradox. Two major non-exclusive hypotheses have gained greater acceptance in the light of available evidence, namely, the mechanical stress...
hypothesis and altered gene expression hypothesis. The mechanical stress hypothesis is based on the assumption that skeletal and cardiac muscle cells are particularly subjected to mechanical stress (Sullivan et al., 1999; Worman and Courvalin, 2004). The abnormalities in nuclear structure that result from lamin mutations lead to increased susceptibility to cellular damage by physical stress. This hypothesis is supported by observations that fibroblasts from patients with lamin A/C mutations and transfected cells expressing the mutant proteins often have severe abnormalities in nuclear morphology (De Sandre-Giovannoli et al., 2003; Eriksson et al., 2003; Östlund et al., 2001; Raharjo et al., 2001; Vigouroux et al., 2001; Holt et al., 2001; Favreau et al., 2003). Mechanical stress is probably not a factor in the development of other laminopathies such as FPLD.

An alternative hypothesis that has been put forward is the gene expression hypothesis, which proposes that the nuclear envelope plays a role in tissue-specific gene expression which can be altered by mutations in lamins (Wilson, 2000). This hypothesis is based on the observation that the nuclear lamina exerts a profound influence on the organization of chromatin within the nucleus. Further evidence that lamins influence transcriptional activity comes from the finding that lamins interact with pRb and MOK2 proteins, both of which are potent transcriptional repressors (Dreuillet et al., 2002). Other important physiological interactions of lamins include those with SREBP1, and the splicing-associated factor YT521B, which is directly involved in correct RNA splicing in muscle. Hence, absence or disruption of the lamin A/C network might have downstream effects on gene expression that lead to specific alterations of muscle and other mesenchyme-derived tissues. A number of experimental systems have been used in an effort to understand the mechanisms of pathogenesis and these are described below.

1.6.1 Mouse models of laminopathies

Gene targeting experiments in mice have proved to be a useful tool to generate laminopathy models. *LMNA* null mice appear normal at birth but develop a form of muscular dystrophy resembling EDMD shortly thereafter (Sullivan et al., 1999). Mortality occurs by 2 months of age with cardiac failure being the principal cause of death (Nikolova et al., 2004). Examination of sciatic nerves shows non-myelinated axons and reduced axon density, consistent with CMT2B1 phenotypes in humans (De Sandre-Giovannoli et al., 2002). Although these mice lack white adipose tissue, they do not show metabolic signs of lipodystrophy, and the reduced adipose tissue is probably a secondary effect of muscle decline (Sullivan et al., 1999; Cutler et al., 2002). Mice lacking A-type lamins exhibit tissue-specific alterations to their nuclear envelope integrity. Fibroblasts from *lmn−/−* mice display ultrastructural perturbations to the nuclear envelope,
including the mislocalization of the inner nuclear membrane protein emerin. Nuclei are highly elongated and exhibit loss of B-type lamins from one pole and show herniations of the nuclear membrane. Disruption of inner nuclear membrane proteins LAP2 and nuclear pore complex protein NUP153 are also observed (Sullivan et al., 1999). Overall, the lamin A null mice develop a cardiac and skeletal myopathy bearing a striking resemblance to human EDMD (Wehnert and Mutoni, 1999).

A number of mouse models have been developed for progeria. When a human EDMD LMNA mutation L530P was introduced into the mouse locus in a homozygous manner by gene replacement, the resulting animal exhibited phenotypes associated with progeria, but not muscular dystrophy (Mounkes et al., 2003a, 2003b). The introduced LMNA allele also exhibited aberrant transcripts attributable to altered splicing of intron 9. Pathology of the heart, skin, skeletal muscle and bone of 4-week old homozygous mice suggested developmental defects consistent with progeria. In another knock-out model, zinc metalloprotease, ZMPSTE24 null mouse also develop muscular dystrophy, and dilated cardiomyopathy. Nuclei from ZMPSTE24−/− cells accumulate unprocessed pre-lamin A and are irregular in shape, with herniations and nuclear blebs (Pendas et al., 2002). It has been proposed that ZMPSTE24 may be the metalloprotease which activates the serine protease involved in pre-lamin A processing. Accumulation of unprocessed pre-lamin A might interfere with normal nuclear lamina organization, leading to phenotypes similar to those of the LMNA knock-out model and laminopathies.

A progerin Knock-in mouse model of HGPS has been generated (Yang et al., 2005) wherein heterozygous mice exhibit growth retardation and fibroblasts show aberrant nuclear morphology. Treatment with a farnesyl transferase inhibitor mislocalized progerin away from the nuclear envelope to the nucleoplasm, and resulted in a striking improvement in nuclear blebbing. This suggests a possible treatment strategy for HGPS. Another transgenic mouse model of progeria has been generated using a human bacterial artificial chromosome that harbors the common HGPS mutation (Varga et al., 2006). These mice develop progressive loss of vascular smooth muscle cells in the medial layer of large arteries, in a pattern very similar to that seen in children with HGPS. This model should prove valuable for testing experimental therapies for this devastating disorder.

Homozygous LMNA knock-in mice carrying a H222P mutation develop muscular dystrophy and dilated cardiomyopathy associated with conduction defects, reminiscent of EDMD in humans (Arimura et al., 2005). The mutant mice are normal at birth but develop disease symptoms in adulthood and die by 9 months of age. These knock-in mice show normal expression and localization of lamin A/C and emerin in myocytes but heterochromatin is mislocalized. Thus, the knock-in LMNA H222P mice represent an
important model for studying laminopathies affecting striated muscles as they develop a
dystrophic condition of both skeletal and cardiac muscle similar to the human diseases.

A mouse line, expressing the Lmna-N195K variant of the A-type lamins, which
causes DCM in humans, has been generated by homologous recombination (Mounkes
et al., 2005). This mouse line shows characteristics consistent with DCM. Electrocardiagraphic monitoring of cardiac activity demonstrated that the Lmna
mice die at an early age due to arrhythmia. By immunofluorescence and Western
analysis, the transcription factor Hf1b/Sp4 and the gap junction proteins connexin 40 and
connexin 43 were misexpressed and/or mislocalized in Lmna hearts. Desmin
staining revealed a loss of organization in the sarcomeres and intercalated disks.
Mutations in LMNA gene may therefore cause cardiomyopathy by disrupting the internal
organization of the cardiomyocyte and/or altering the expression of transcription factors
essential for normal cardiac development, aging or function.

1.6.2 Tissue culture models of laminopathies

A number of studies suggest that nuclear structural defects could contribute to
the etiology of laminopathies. Histological examination of tissue samples from
laminopathic patients show abnormal morphologies with membrane blebbing and
herniations, chromatin leakage, disorganization of heterochromatin and mislocalization of
emerin, LAPs and lamin B1 (Vigouroux et al., 2001). 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) Ectopic
expression of some of the disease-causing lamin mutants as short epitope-tagged
constructs in HeLa cells results in dominant negative effects, leading to disruption of
endogenous lamins, LAPs and NUP153, mislocalization of emerin and abnormal nuclear
morphology (Raharjo et al., 2001; Bechert et al., 2003). Expression of some of the
EDMD-causing lamin mutants in C2C12 myoblasts causes a dramatically abnormal
localization, with decreased nuclear rim staining and formation of intranuclear foci. The
distribution of endogenous lamin A/C, lamin B and emerin are also altered in cells
transfected with certain mutants (Östlund et al., 2001). Expression of R453W, an EDMD-
causing mutant, in C2C12 myoblasts affects differentiation as cells do not exit the cell
cycle properly at G0 and undergo apoptosis, whereas C2C12 myoblasts transfected with
wild-type lamin A or with the FPLD mutant R482W undergo normal differentiation
(Favreau et al., 2004). Thus, physiological and histological observations of cells from
laminopathic patients are similar to those of cultured cells expressing mutant lamins.
1.7 Objectives of the work

Although mutations in human LMNA gene cause a number of debilitating diseases collectively termed laminopathies, the cellular functions that are altered due to these mutations are not well understood. Cell culture models have been shown to be useful for analyzing the effects of disease-causing mutations in lamin A/C. Ectopic expression of various lamin A mutants upon transfection in cultured cells has been shown to affect normal lamin assembly and binding to the inner nuclear membrane protein emerin (Östlund et al., 2001; Vigorous et al., 2001; Raharjo et al., 2001). An EDMD mutant also hinders muscle differentiation and promotes cell death upon serum withdrawal (Favreau et al., 2004). Expression of progerin leads to aberrant nuclear morphology, and blocks progression of cells through S-phase (Goldman et al., 2004). Although lamin mutants cause cell division defects, it is not known whether cells expressing lamin mutants are affected in their response to DNA damaging agents. Therefore, the objectives of the present work are (1) to construct different disease-causing lamin A mutants, express them in HeLa and C2C12 cells, and study their assembly properties and effects on the endogenous lamin organization, intranuclear lamin speckles and emerin localization; (2) to examine the response of HeLa cells expressing these mutants to DNA damaging agents; and (3) to study the effects of lamin mutants on muscle differentiation using the C2C12 myoblast cell line.