A basic mystery that is not yet fully understood is how the initial prokaryotic organisms evolved into more complex eukaryotes. This succession of life forms by gradual cellular reorganization occurred approximately 850 million years ago (Cavalier-Smith, 2010). Relatively simpler prokaryotes gave rise to complex and compartmentalized membrane bound cellular machineries, with each organelle having discrete identity and function. Presence of these membrane bound organelles in the eukaryotic cell was an evolutionary prerequisite to compensate for increase in size and to maintain enzymatic complexes at higher localized concentrations for enhanced efficiency. One such key event in eukaryotic development was appearance of the nucleus, where genomic material was efficiently organized in the form of chromosomes. This provided eukaryotes with selective advantages of separating transcription from protein translation and regulating the expression of processed transcripts in response to the surrounding milieu. Avoiding cleavage of nascent transcripts is another argument favouring evolution of complex nuclear machinery and nuclear architecture (Cavalier-Smith, 1982).

1.1. Nuclear envelope

A dual membrane structure defines the boundary of the eukaryotic nucleus, separating cellular components into outer cytoplasm and inner nucleoplasm. The metazoan nuclear envelope is comprised of the following subunits: outer nuclear membrane, inner nuclear membrane, lumenal space of ~40-130 nm separating the two membranes and several nuclear pore complexes punctuating the nuclear envelope (see Fig. 1.1).

1.1.1. Outer nuclear membrane

The outer nuclear membrane (ONM) is essentially an extension of rough endoplasmic reticulum (RER), harbouring ribosomes at its cytoplasmic surface. Although RER and ONM are continuous structures, they differ significantly in their protein composition. This difference can be attributed to the presence of several trans-membrane proteins with KASH (Klarsicht, ANC-1, Syne Homology) or SUN (sad/uncoordinated-84 or UNC-84 homology) domains and their interacting partners (Starr and Han, 2005). Owing to this unique composition of ONM, the nuclear envelope not only separates the nucleus from the cytoplasm, but also acts as an active entity in regulating interactions between nucleoplasm and cytoskeleton. This function of ONM embedded proteins along with proteins tethered to the INM was initially observed by Hedgecock and Thomson (1982) in Caenorhabditis elegans. They observed that the C-terminal segment of ANC-1, a KASH domain containing protein localizes in the ONM, while its N-terminal region binds to the actin cytoskeleton (Hedgecock and Thomson, 1982). The long fibrous middle region of ANC-1 extends between these two structures and tethers the nucleus to the actin cytoskeleton (Starr and
Fig. 1.1. **Schematic representation of a typical metazoan nucleus.** The figure depicts various intranuclear structures, lamina, nuclear pores and chromatin. Inner nuclear membrane proteins and their association with lamins at the nuclear periphery are shown in the inset. Adapted from Spector, 2001.
Han, 2002; Starr and Han, 2003). Moreover, mutations in ANC-1 are known to disrupt the anchorage of the nucleus in somatic and post-embryonic cells (Starr and Han, 2002). In Drosophila embryos, the ANC-1 homologue Klarsicht has been implicated in nuclear movement during eye formation (Welte et al. 1998). In mammalian cells, similar functions of nuclear anchorage are performed by nesprins. These KASH domain family proteins are involved in several processes ranging from activity of various neurotransmitter receptors in the brain (Cottrell et al. 2004), organization and functions of focal adhesions, sarcoplasmic reticulum and Golgi apparatus, and targeting of other nuclear membrane-associated scaffolding proteins such as AKAPs (A-kinase anchoring proteins) in cardiomyocytes (Beck, 2005; Pare et al. 2005).

1.1.2. Inner nuclear membrane

The inner nuclear membrane (INM) represents the nucleoplasmic face of the nuclear envelope, being biochemically distinct from the ONM and ER. Proteomic analysis of INM in mammalian cells has shown the presence of more than 80 transmembrane proteins (Schirmer and Gerace, 2005) with varying roles such as maintenance of nuclear architecture, chromatin interaction, sequestering of transcription factors and nucleocytoplasmic interactions. The recruitment of proteins to INM takes place by lateral diffusion of proteins through ONM and the nuclear pore membrane. In addition to diffusion, active energy dependent targeting of proteins carrying an INM signal sequence has been observed in HeLa cells (Ohba et al. 2004).

Studies on oocyte nuclei of Xenopus laevis have shown the presence of the nuclear lamina beneath the INM (Aebi et al. 1986). This lamina is made up of an interwoven lattice of intermediate filaments comprised of proteins termed lamins that are associated with INM embedded lamin-binding proteins. Barring a few exceptions, the nuclear lamina of vertebrate somatic cells is approximately 20-50 nm thick and largely defines the shape of the interphase nucleus (Furukawa and Hotta, 1993; Sullivan et al. 1999). Apart from their peripheral localization, lamins have also been observed to extend into the nucleoplasm to form an internal lamina or nucleoplasmic lamina, a fraction of which is easily extractible (Goldman et al. 1992; Hozak et al. 1995; Jagatheesan et al. 1999; Barboro et al. 2002; Muralikrishna et al. 2004). Recent studies have shown that nucleoplasmic lamins are targeted to the nucleoplasm by lamin-associated polypeptide 2α (LAP2α) and regulate activity of the retinoblastoma protein (pRb) in early tissue progenitors to maintain a proper balance of proliferation, cell cycle exit and differentiation (Naetar and Foisner, 2009). Involvement of mutations in INM proteins in a wide range of disorders indicates the vital role of the nuclear envelope in regulating nuclear architecture and functions. Perturbed
cytoplasmic mechanics and defects in mechanotransduction signaling also implicates INM as a signaling node between the nucleus and cytoplasm.

The nuclear envelope is not only a hub for cellular regulatory processes, but also for assembly of infectious agents. Viruses in particular have evolved different mechanisms to overcome this nucleo-cytoplasmic barrier for their propagation. Parvovirus, for instance, transiently disrupts the nuclear membranes to import its genome into the nucleus (Cohen et al. 2006). Adenoviruses and influenza A viruses on the other hand use virally encoded adapter proteins for nuclear import (Wodrich et al. 2006; Wu et al. 2007). Assembled capsids of Herpes virus are exported out of the nucleus by budding through the lamina and INM into the perinuclear space, thereby bypassing the nuclear pores and conventional export pathways (Mettenleiter, 2006). Another example of utilization of host machinery is hijacking of leucine rich exportin-Crm1 by HIV to export its RNA to the cytoplasm using HIV Rev protein as an adapter (Fischer et al. 1995). Involvement of the nuclear envelope in diseases, both hereditary and infectious, thus reiterates its importance in metazoan cells.

1.1.3. Nuclear pore complexes

The two nuclear membranes with distinct protein composition are punctuated by nuclear pore complexes (NPCs) at the pore membrane, which contains its own discrete set of transmembrane proteins (Hallberg et al. 1993; Wozniak et al. 1989). The NPCs are macromolecular complexes containing multiple copies of about 30 different proteins termed nucleoporins (Nups), that form a symmetrical hexagonal structure perpendicular to the plane of the nuclear envelope (Mans et al. 2004). Structurally, a vertebrate NPC has two distinct functional regions: the NPC core or central structure embedded in the nuclear envelope, and the NPC peripheral structures that extend on both cytoplasmic and nucleoplasmic sides. At the cytoplasmic face, eight ~50 nm long filamentous structures extend into the cytoplasm and have been proposed to couple mRNA export to translational initiation. On the nucleoplasmic side, another set of eight filamentous spokes form a ring-like structure called the nuclear basket or fish trap structure of ~100 nm in length (Arlucea et al. 1998; Beck et al. 2004). FG-nucleoporins or FG-Nups are a family of NPC proteins that carry repeats of phenyalanine-glycine (FG) followed by characteristic spacer sequences (Rout and Wente, 1994). These Nups form almost one-third the molecular mass of the NPC, occupy peripheral, surface accessible positions and line the central core channel of the NPC. FG-Nups have been predicted to regulate functions of the NPC such as receptor-mediated transport, directionality of transport, maintenance of permeability barrier, gene gating, and chromatin association with NPCs (Terry and Wente, 2009). They also regulate targeting of INM proteins from the ONM/ER.
Apart from the well-characterized functions of nucleo-cytoplasmic trafficking, the role of NPCs in various functions on either side of the nuclear envelope has been reported. Proteins associated with the nuclear basket are shown to regulate mRNA biogenesis and surveillance, small ubiquitin-like modifier (SUMO) homeostasis, chromatin maintenance and control of cell division (Strambio-De-Castillia et al. 2010). NUP358 has been shown to sumoylate nuclear transport cargoes to facilitate protein-protein interactions; the distal end of NUP358 has microtubule binding property, suggesting a role for NPCs in microtubule dynamics during interphase (Reverter and Lima, 2005; Radtke et al. 2006). Also NPCs have been proposed to provide a microenvironment at the nuclear periphery for ubiquitination and proteasome-dependent degradation of DNA damage-stalled RNA-polymerase II (Pol II) complexes (Faza et al. 2009). Another study implicates NPCs in SUMO dependent DNA repair pathways mediated by synthetic-lethal-of-unknown-function protein (Six5-Six8) and SUMO-dependent ubiquitin ligase complex (Li et al. 2007).

1.2. Nuclear lamins

Members of the intermediate filament (IF) family of proteins are present in all metazoans except Hydra and arthropods. These filaments form the major structural framework both in the nucleus and cytoplasm of metazoan cells. Human IF proteins are encoded by at least 70 genes, although only a few of them have been studied extensively (Fuchs and Weber, 1994; Herrmann et al. 2003). Six different types or sequence homology classes of IF proteins have been identified on the basis of amino acid composition. Type I and II IF proteins represent acidic and basic keratins (expressed in epithelial cells only), whereas Type III IF proteins are mostly mesodermal proteins, for example, desmin (in skeletal, cardiac and smooth muscle cells), vimentin (in mesenchymal, endothelial and hematopoetic cells), Glial Fibrillary Acidic Protein or GFAP (in astroglial cells) and peripherin (in a subset of neuronal cells). Neurofilaments and related proteins (NF-L, NF-M and NF-H, nestin, synemin, syncoilin, α-internexin) are type IV IF proteins. The nuclear lamins are type V IF proteins and, unlike other IF proteins, lamins are expressed in all somatic cells and are nuclear in localization. Type VI group includes two eye lens IF or ‘beaded filament’ proteins, phakinin (CP49) and filensin (CP115) (Szeverenyi et al. 2008). Another classification of IF proteins is based on their mode of assembly and identifies them as keratins, vimentin-like proteins and lamins (Herrmann et al. 2007). The IF proteins impart mechanical strength to the cell and play diverse roles in various signal transduction pathways.
1.2.1. Structure of lamins

Lamins are type V IF proteins that form a meshlike structure called the nuclear lamina beneath the inner nuclear membrane, which is the major component of the nucleoskeleton or nuclear matrix (Fawcett, 1966). Lamins are considered to be the evolutionary predecessors of other vertebrate IF proteins. Strong evolutionary conservation across the species as compared to other IF proteins suggests that lamins are essential for cellular functions (Weber et al. 1989). Genome analysis of budding yeast, fission yeast and Arabidopsis indicates the absence of lamin homologues, supporting the notion that these proteins may have evolved in animal cells during the transition from a closed to an open mitosis (Cohen et al., 2001; Hutchison, 2002). Like all IF family proteins, lamins contain three distinct structural domains: a central 360 amino acid long α-helical coiled-coil rod domain flanked by an N-terminal head and a globular C-terminal tail domain (Stuurman et al. 1998). The rod domain can be subdivided into four α-helical coils, 1A, 1B, 2A and 2B, each harbouring a periodic heptad repeat. These α-helical regions are separated by linker segments called L1, L12 and L2, respectively (see Fig. 1.2). Linker L1 also has a heptad repeat sequence and hence participates in formation of a coiled-coil structure with its flanking 1A and 1B regions. The six extra heptad repeats in coil 1B region are unique to vertebrate lamins and invertebrate IF proteins, but are not present in the vertebrate IF proteins. A nuclear localization signal (NLS) is located after the C-terminal end of the rod domain at position 417-422. The corresponding region is absent in other IF proteins, which explains the differential localization patterns of members of the IF family.

The central rod domain drives the interaction between two lamin proteins to form a coiled-coil dimer, the basic structural unit of lamin assembly. The head-to-tail associations between two lamin dimers lead to the formation of protofilaments, which have the propensity to associate laterally in different configurations such as parallel, staggered or half-staggered. Eight of these protofilaments combine to form the 10 nm intermediate filamentous structures (Burke et al. 2001). Although the detailed ultrastructure of polymeric vertebrate lam in situ is not well characterized, these 10 nm filaments are proposed to be part of higher order structures seen in the lamina. In vitro experiments on C. elegans lamin (Ce-lamin) have shown the formation of 10 nm filaments, but vertebrate lamins form paracrystals at higher concentrations (Karabinos et al. 2003; Foeger et al. 2006). NMR and X-Ray crystallographic studies have shown that the conserved 116 amino acid region of the tail domain folds into compact nine β-strands that form a β-sandwich structure, the immunoglobulin-like fold (lg-fold). Similar domains or folds have been identified in immunoglobulins, growth factors, cytokine receptors and several transcription factors, and
Fig. 1.2. Schematic representation of the LMNA gene with the positions of disease-causing mutations. Structural organization of the LMNA gene, filled boxes represent exons 1 to 12 and lines indicate introns; the positions marked outside the circle indicate the sites of mutations in the LMNA gene. The mutants used in study are shown in boxes outside the circled region. Domain structure of lamin A and lamin C proteins are shown inside the circle. The highly conserved α-helical central rod domain (brown) is flanked by non-helical head and tail domains (grey), the nuclear localization signal (NLS), linker regions-L1,L12 and L2, and the sites of proteolytic cleavage are shown as P1 and P2. The CAAX sequence of prelamin A which is subsequently cleaved from mature lamin A and unique VSGSRR sequences of lamin C are shown at the C-terminus of the respective isoforms.
these domains provide docking sites for other proteins, DNA and phospholipids (Dhe-Paganon et al. 2002).

The C-termini of lamins contain a conserved sequence commonly referred to as the CaaX motif (C is cysteine, a is an aliphatic amino acid and X is any amino acid), which is post-translationally farnesylated at the cysteine residue. The last three amino acid residues of the CaaX motif are then cleaved off by the ER-associated zinc metallo-protease ZMPSTE24 (also called FACE1) for lamin A and Ras-converting enzyme 1(Rce1) for lamin B1. This cleavage is followed by addition of a carboxymethyl moiety at the farnesylated cysteine residue by another ER-associated enzyme, Isoprenylcysteine carboxymethyltransferase (lcmt). This modification possibly increases the hydrophobicity of the C-terminus of lamins, leading to association of lamins with the INM. The resultant precursor lamin A (prelamin A in this case) undergoes a second endoproteolytic cleavage by ZMPSTE24. This cleavage takes place at 14 amino acids upstream of the carboxymethylated cysteine, releasing mature lamin A (Hennekes and Nigg, 1994; Dai et al. 1998; Bergo et al. 2002). B-type lamins do not undergo the second endoproteolysis and remain constitutively farnesylated and carboxymethylated (Maske et al. 2003). A recent study has shown that inhibition of farnesylation of lamin A by farnesyl transferase inhibitors (FTIs) causes addition of a geranylgeranyl prenyl group by geranylgeranyl transferase at the CaaX motif (Varela et al. 2008).

1.2.2. Lamin Isoforms

The number and complexity of lamin proteins among metazoans increases as we move higher on the evolutionary scale. The only lamin gene of C. elegans (lmn-1) is expressed ubiquitously, except in mature sperm (Liu et al. 2000a). Drosophila melanogaster has two different lamin genes, one expressed throughout development (lamDmo), while expression of the other is restricted to differentiated cells and is initiated during late embryonic development (lamC). In humans, three genetic loci encode nuclear lamins: LMNA, LMNB1 and LMNB2. The LMNA gene is located on chromosome 1q21.2-q21.3 (Lin and Worman, 1993; Wydner et al. 1996) and encodes at least four alternatively spliced products- lamin A, AΔ10, C and C2. The LMNA gene spans over 24 kb and contains 12 exons and 11 introns interspersed at irregular intervals. The first intron is the longest covering almost 16 kb length, followed by the 2 kb long second intron. Lamin A and C are expressed at roughly equivalent amounts in most differentiated cell types and differ at the C-terminal tail region. First nine exons are common for both lamin A and C, but an alternative 3' end splice site at position 566 adds an additional 98 residues (exon 11 and 12) to lamin A, whereas lamin C has 5 unique residues from 567-572 contributed by intron 10, followed by a stop codon and polyadenylation sequence. Expression of lamin C2 is
reported to be germ cell-specific (Furukawa et al. 1994), whereas AΔ10 is observed in cell lines derived from colon, lung and breast carcinomas. Lamin AΔ10 lacks 30 amino acids, corresponding to an in-frame deletion of upstream sequences from exon 10 (Machiels et al. 1996). Expression of A-type lamins is developmentally regulated and is first observed during gastrulation stage, but lamin A/C expression in undifferentiated embryonic stem cells is a matter of dispute (Constantinescu et al. 2006; Foster et al. 2007; Hall et al. 2005).

B-type lamins are biochemically distinct from A-type lamins in terms of sequence homology, solubility, expression patterns and localization during mitosis. They are considered to be the ancestral lamins (Stick, 1992; Stick, 1994), while A-type lamins are evolutionary successors of a lamin B gene duplication event. During this process an additional exon, corresponding to ~90 residues preceding the CaaX box was acquired by the lamin A gene. Therefore, compared to lamin B1/B2, mature lamin A is larger (65 kDa and 70 kDa respectively). The B-type lamins remain associated with fragments of the nuclear envelope during mitosis and coat chromosomes during the anaphase-telophase transition (Gerace and Burke, 1988), providing a surface for formation of the nascent nuclear envelope around chromosomes. A-type lamins, on the contrary, have diffused cytoplasmic distribution throughout mitosis and assemble into the lamina independent of B-type lamins during late telophase or early G1 phase (Broers et al., 1999; Moir et al., 2000a; Steen and Collas, 2001). The biochemical differences between lamins are supported by two dimensional isoelectric focusing gel analysis which shows that B-type lamins have acidic isoelectric point, while A-type lamins have neutral isoelectric point (Gerace and Blobel, 1980). Human B-type lamins are encoded by two independent genes: LMNB1 at chromosomal location 5q23.3-q31.1 and LMNB2 at chromosomal location 19p13.3 (Lin and Worman, 1995; Wydner et al., 1996). The LMNB1 gene spans 45 kb of genomic contig and gives rise to a transcript containing 11 exons and 10 introns, which codes for lamin B1. On the other hand, LMNB2 has 12 exons and 11 introns (Hegele et al. 2006), producing two alternative spliced isoforms lamin B2 and lamin B3. All vertebrates express at least one B-type lamin throughout the developmental stages, while lamin B3 expression is restricted to germ cells only (Biamonti et al., 1992; Furukawa and Hotta, 1993; Furukawa et al., 1994; Alsheimer et al., 1999).

1.2.3. Localization of lamins

In metazoans, nuclear architecture and integrity is maintained by the dense network of nuclear lamins localized at the nuclear periphery beneath the INM. Lamins not only provide a functional interface for association of diverse sets of nuclear envelope proteins, histones and transcription factors, but also for spatio-temporal organization of chromatin at the nuclear periphery. Mature A-type lamins do not have a farnesylated residue at the C-
terminus and are probably incorporated into the lamina in both homo- and hetero- assemblies with B-type lamins. Yeast two-hybrid experiments have shown that lamin A, prelamin A, lamin B1, and lamin C can assemble into homo- and heterodimers (Ye and Worman, 1995). This observation is further supported by in vitro affinity chromatography and solid-phase binding assays (Schirmer and Gerace, 2004). On the other hand, a fraction of total lamins, also called intranuclear lamins, form foci or a relatively uniform, diffuse 'veil' throughout the nucleoplasm. FRAP studies and detergent extractions have established the paradigm that the nucleoplasmic lamins are more dynamic and less strongly associated with the nuclear envelope (Bridger et al. 1993; Goldman et al. 1992; Hozak et al. 1995; Moir et al. 1995; Jagatheesan et al. 1999; Muralikrishna et al. 2004). Intranuclear localization of both A- and B-type lamins has been documented. Lamin B is associated with mid-late S phase replication (Moir et al. 1994), and forms more stable assemblies than lamin A foci. A novel structural subset of nucleoplasmic lamin A was identified in our lab using a monoclonal antibody LA-2H10, raised against recombinant rat lamin A, which recognizes an epitope mapping to region 209-215 of lamin A corresponding to the amino acid sequence NIYSEEL. This antibody recognizes intranuclear lamin speckles, but not peripheral lamin and colocalizes with components of RNA splicing factor compartments (SFCs) such as the splicing factor SC35 in asynchronous cell populations (Jagatheesan et al. 1999; Parnaik and Manju, 2006). These nucleoplasmic lamin speckles are resistant to high salt, detergents and nucleases, but are sensitive to treatment with transcriptional inhibitors. During muscle differentiation or upon treatment with the phosphatase inhibitor, okadaic acid, these lamin speckles get redistributed to disperse, insoluble nucleoplasmic networks (Muralikrishna et al. 2001; Indumathi, 2005).

1.3. Lamin-binding proteins

Lamins are key structural determinants of nuclear architecture and provide a scaffold for a wide range of proteins, thereby orchestrating spatio-temporal regulation of nuclear functions (Taddei et al. 2004; Dechat et al. 2008; Parnaik, 2008). Several studies have shown that lamins directly or indirectly associate with proteins found in the nucleoplasm, INM, ONM and cytoplasm. This unique interactome of lamins makes them pivotal in maintenance of mechanical properties of the entire cell and not the nuclear envelope alone. Cells deficient in lamin A/C expression have defective mechanotransduction, mechanical stiffness and impaired cell migration (Houben et al. 2007; Lee et al. 2007). Structural aspects and functional implications of lamins and their interacting proteins are summarized below.
1.3.1. INM and ONM resident structural proteins

Several proteins associated with the inner nuclear membrane interact with lamins. Some of these proteins are emerin, Lamina Associated Polypeptides (LAP) 1 and 2 family members, MAN1, Lamin B Receptor (LBR) and nesprin-1α. LBR was among the first INM proteins to be identified and it binds to B-type lamins (Moir et al., 1995). It is an evolutionarily conserved protein with both chromatin binding activity and sterol reductase functions. The N-terminal 200 amino acids constitute the nucleoplasmic domain of LBR, which interacts with lamin B, Heterochromatin Protein-1 (HP1) family of chromatin modifier proteins, chromatin-associated protein HA95 and histone H3-H4 tetramer, and binds directly to dsDNA (Holmer and Worman, 2001). The C-terminal domain of approximately 450 amino acids has eight transmembrane segments and confers sterol \( \Delta^{14} \)- reductase activity (Holmer et al. 1998). Ectopic expression of human LBR in yeast retains this enzymatic activity (Silve et al. 1998). These observations suggest a role for lamin-LBR complexes in both chromatin organization and sterol dependent signaling in the nucleus.

Emerin is a type II transmembrane protein localized at the INM through an N-terminal LEM domain. The LEM domain is a 40-residue motif common to LAP-Emerin-MAN1 proteins and found in several INM and nucleroplasmic proteins (Lin et al. 2000). Proteins harbouring the LEM domain bind directly to the C-terminal tail of A- or B-type lamins (Lee et al. 2001; Bengtsson and Wilson, 2004). Another aspect of LEM family proteins is their role in chromatin organization by virtue of their ability to interact with the chromatin associated protein, Barrier to autointegration Factor (BAF). Like lamin A/C, its interacting partner emerin is also expressed in cells in a developmentally regulated manner at the time of organogenesis (Gareiss et al. 2005). Recruitment of emerin to INM is dependent on lamin A/C, since cells lacking A-type lamins show mislocalization of emerin to ER network, followed by its proteasome mediated degradation (Sullivan et al. 1999; Muchir et al. 2006). Moreover, loss of emerin protein causes X-linked Emery-Dreifuss Muscular Dystrophy (X-EMD) in humans.

The LAPs are LEM family proteins found only in vertebrates and are divided into two subgroups: LAP1 and LAP2. The former subgroup comprises of three developmentally regulated isoforms, LAP1A, LAP1B and LAP1C proteins which localize at the nuclear periphery (Foissner and Gerace, 1993). LAP1A and B bind directly to both A- and B-type lamins, while LAP1C forms a complex containing LAP1A and binds only to B-type lamins. The C-terminus of LAP1 associates with Dystonia-causing mutated torsin A (DYT1). Torsin A is a member of the AAA+ ATPase family and resides in the ER lumen, while the mutated form DYT1 predominantly binds to the nuclear envelope (Breakefield et al. 2001; Goodchild and Dauer, 2005). Six well known isoforms of LAP2 arise from alternative splicing of the
LAP2 gene in human and mice (α, β, γ, δ, ε, and ζ). All these isoforms except LAP2α possess a conserved C-terminal membrane spanning domain and a variable N-terminus. This membrane associated domain binds primarily to B-type lamin (Foisner and Gerace, 1993; Furukawa et al. 1998). LAPs are expressed throughout development and are essential for cell survival (Harborth et al. 2001). LAP2α lacks the trans-membrane domain, but has a long LAP2α specific C-terminal domain carrying an NLS. It is the only LAP2 isoform located in the nucleoplasm instead of the nuclear periphery, where it binds to intranuclear lamin A and chromatin in a phosphorylation dependent manner (Dechat et al. 2000; Markiewicz et al. 2002). LAP2α also binds to retinoblastoma protein (pRb) and regulates cell cycle progression and differentiation via pRb-E2F pathway (Dorner et al. 2006). Recently LAP2α has been shown to interact with LINT-25, a protein involved in cell cycle exit (Naetar et al. 2007). LAP2β binds specifically to lamin B1, chromatin and BAF (Cai et al. 2001; Shumaker et al. 2001).

MAN1 (also known as LEMD3) is another INM protein belonging to the LEM domain family and has two membrane spanning domains. It shares overlapping functions of lamin binding, chromosome segregation and cell division with emerin during early embryogenesis (Lin et al. 2000; Liu et al. 2003). It localizes to INM via interactions of its N-terminal segment with lamin or emerin, while the C-terminal nucleoplasmic domain of MAN1 directly interacts with transcriptional regulators such as Bcl2-associated transcription factor (Btf) and germ-cell-less (GCL), and sequesters receptor regulated SMADs to the nuclear periphery. MAN1 has been shown to antagonize transforming growth factor-β (TGFβ), activin and Bone Morphogenetic Protein (BMP) signaling by blocking phosphorylation and nuclear translocation of SMADs (Raju et al. 2003; Lin et al. 2005; Pan et al. 2005).

The Nesprin family of proteins includes nesprin-1 (also known as CPG2, syne-1, myne-1 and enaptin), nesprin-2 (also known as syne-2 and NUANCE) and nesprin-3 (Starr and Fischer, 2005), and its various splice variants. Some of the nesprins can have molecular masses up to 1 MDa. Nesprins like ANC-1 of C. elegans and Klarsicht of D. melanogaster are characterized by the presence of a 60 amino acid long C-terminal KASH domain. This domain has a single transmembrane anchor region and about 40 amino acids of it reside in the luminal space. Giant nesprin-1 and 2 isoforms link the nuclear envelope to the cytoskeleton via an ONM resident C-terminal KASH domain and an N-terminal paired calponin homology domain that binds to actin. On the other hand, shorter isoforms like nesprin-1α and 2α localize to the nucleoplasmic face of INM by virtue of binding to lamin A/C and emerin (Mislow et al. 2002; Zhang et al. 2005; Haque et al. 2010). Both emerin and nesprin-1α have been shown to delocalize into the ER in LMNA+ fibroblasts, which
suggests that LINC (Linker of the Nucleoskeleton and Cytoskeleton) complexes are dependent on lamin proteins (Muchir et al. 2003).

The KASH domain containing proteins are anchored to the nuclear envelope through SUN domain proteins in the lumen (Padmakumar et al. 2005; Crisp et al. 2006). The S. pombe genome encodes for a single SUN-domain protein Sad1, C. elegans and D. melanogaster have two SUN domain proteins each, while mammals have at least four of them- SUN1-3 and SPAG4 (Starr and Fischer, 2005). Most SUN proteins are multipass transmembrane proteins and hence predicted to be mechanical load bearing structures and force transmitters. Human SUN1 has been found to cross the membrane thrice (Crisp et al. 2006). Mammalian SUN1 and SUN2 proteins interact with lamin A at INM, but only SUN2 is dependent on lamin A for nuclear envelope localization while the anchorage requirement of SUN1 is not yet understood. Interestingly, SUN1 shows enhanced localization at the envelope in Hutchinson Gilford Progeria syndrome (HGPS) cells but not in EMD cells, suggesting preferential interaction of SUN1 with prelamin A (Haque et al. 2010). Disruption of SUN-KASH domain interaction leads to expansion of the nuclear envelope lumen, indicating the importance of this binding for nuclear integrity (Crisp et al. 2006).

Increasing evidence suggests a role for protein complexes containing lamin and its associated proteins such as actin and actin-binding proteins (e.g. nucleus-specific myosin I and VI isoforms) in providing the architectural framework to the nucleus. Furthermore, nuclear actin has been shown to bind to lamin A at two discrete regions of the lamin A/C tail corresponding to amino acids 461-536 (Ig-fold domain) and 563-646 (Sasseville and Langelier, 1998; Zastrow et al. 2004). It has been proposed that instead of long filamentous actin (F-actin), the nuclear actin may exist in different conformations such as dimers, short protofilaments, tubular or branched oligomers (Pederson and Aebi, 2002). Nuclear actin has also been identified as part of transcription, RNA processing, transport and chromatin remodeling complexes, possibly serving as a molecular scaffold for these multiprotein complexes (Bettinger et al. 2004; Parnaik, 2008).

1.3.2. Chromatin binding proteins

Several independent studies have implicated A-type lamins in higher order chromatin organization by binding to DNA, core histones and BAF (Vlcek and Foisner, 2007). This notion is strengthened by the fact that any mutation or disruption of the lamin gene in C. elegans, D. melanogaster or Mus musculus leads to aberrant heterochromatin, developmental defects, embryonic lethality, clustering of nuclear pore complexes and distorted nuclei (Sullivan et al. 1999; Liu et al. 2000a; Guillemin et al. 2001; Patterson et al. 2004; Gurudatta et al. 2010). Both lamin A and lamin C have a chromatin binding sites in the α-helical rod domain (Glass et al. 1993) and in the C-terminal tail domain (Höger et al. 2004).
Introduction

1991). *In vitro* binding assays have shown that both lamin A and lamin C bind to dsDNA in a sequence independent manner through the Ig-fold and NLS region of the tail domain (Ludérus *et al.* 1994; Stierlé *et al.* 2003). Lamin Dm0, the B-type lamin in *D. melanogaster* interacts with approximately 500 genes *in vivo*. These genes display characteristic heterochromatic features of late replication, depleted active histone modifications and a tendency to cluster in the genome to form transcriptionally repressed domains (Pickersgill *et al.* 2006). Furthermore, in a rare form of laminopathy—Hutchinson Gilford Progeria syndrome (HGPS), patients' fibroblasts display delocalized chromatin with loss of peripheral heterochromatin and alteration of other epigenetic marks (Goldman *et al.* 2004).

In addition to binding directly to DNA and LEM proteins, lamins also interact with chromatin modifying proteins such as LAPs, BAF and histones. The tail domain of lamin NC binds to core histones with an affinity in the range of 400 nm (Taniura *et al.* 1995), which is similar to the affinity of lamins for naked dsDNA.

BAF is a conserved and essential metazoan sequence-independent DNA binding protein, first identified by its role in retroviral DNA stability (Zheng *et al.* 2000a; Shumaker *et al.* 2001). It affects chromatin condensation and decondensation during nuclear assembly in *Xenopus* extracts *in vitro* (Segura-Totten *et al.* 2002) and is essential in both *C. elegans* and *Drosophila* (Zheng *et al.* 2000a; Furukawa *et al.* 2003). BAF forms dimeric assemblies that bind to the minor groove of dsDNA, histone H3, histone H1.1, lamin A, and transcription regulators, as well as emerin and other LEM-domain nuclear proteins (de Oca *et al.* 2005; Bengtsson and Wilson, 2006). In mammalian cells, BAF exists in two different complexes; one anchored to A-type lamins, emerin and MAN1 (Holaska *et al.* 2003), while the other has LAP2β tethered to B-type lamins.

**1.3.3. Gene regulatory proteins**

Lamins form both permissive and repressive environments at the nuclear periphery by providing a structural scaffold for gene regulatory complexes (Cohen *et al.* 2001; Zastrow *et al.* 2004; Wilson and Foisner, 2010). Studies on *S. cerevisiae* and *D. melanogaster* have shown that the nuclear lamina regulates gene expression by serving as an attachment site for transcription factors, altering epigenetic modifications and sequestering essential regulatory elements (Taddei *et al.* 2004; Pickersgill *et al.* 2006; Kumaran and Spector, 2008). Lamins associate with the cell cycle regulator cyclin D3 (Mariappan *et al.* 2007), and transcription factors such as pRb (Mancini *et al.* 1994; Ozaki *et al.* 1994), sterol response element binding protein 1 (SREBP1) (Lloyd *et al.* 2002), mouse Kruppel-like factor 2 (MOK2) (Dreuillet *et al.* 2002), c-Fos (Ivorra *et al.* 2006) and Octamer binding transcription factor-1 (Oct-1) (Imai *et al.* 1997), as well as the tumour suppressor inhibitor of Growth-1 (ING1) (Han *et al.* 2008), kinases such as protein kinase C α (PKCα) and JIL1 kinase (Bao
Cyclin D3 is a G1/S-phase specific cyclin and unlike other D-type cyclins, is upregulated upon differentiation in both muscle and non-muscle cells (Bartkova et al. 1998). It interacts specifically with lamin A/C at amino acid positions 383-474, corresponding to the C-terminal region of the rod domain of lamin A/C (Mariappan et al. 2007), and this interaction is important for lamina remodeling during muscle differentiation (Mariappan and Parnaik, 2005). Oct-1 is a POU domain repressor protein that is essential for transcriptional regulation of genes involved in oxidative stress response. Malhas et al. have shown that Oct-1 is sequestered away from promoters by binding to lamin B1 (Malhas et al. 2009). During senescence, Oct-1 dissociates from the nuclear periphery and induces expression of collagenase, suggesting repressive activity of the lamina on Oct-1 activity. Similarly c-Fos, an early response transcription factor is tethered away from its target promoters by A-type lamins present at the nuclear envelope (Ivorra et al. 2006). Activated ERK1/2 (component of MAP kinase signaling pathway) facilitates release of c-Fos from the envelope by interacting with lamin A/C and c-Fos complex during cell proliferation (González et al. 2008). A-kinase anchoring protein-149 (AKAP149) is another example of a regulatory protein that is sequestered at the nuclear lamina by B-type lamins. AKAP149 binds to the regulatory subunit of Protein Kinase A (PKA) and is essential for proper assembly of B-type lamins into the nuclear lamina at the end of mitosis (Steen and Collas, 2001). Lamin A has also been shown to affect differentiation of mesenchymal stem cells by tethering SKIP (Ski interacting protein), a coactivator of the Notch pathway (Scaffidi and Misteli, 2008).

MOK2 is a DNA binding transcriptional repressor that competes with cone–rod homeobox (Crx) transcription factor for DNA binding sites. It also has RNA binding property in vitro. MOK2 has been shown to bind at the coiled-coil region 2 of lamin A/C rod domain, which links lamin A/C to transcriptional and RNA processing activities (Dreuillet et al., 2002). Retinoblastoma protein (p110\textsuperscript{Rb} or pRb) is an important transcriptional regulator in cell cycle control. Hypophosphorylated pRb blocks E2F dependent gene expression by creating a repressive environment through recruitment of histone deacetylases (HDACs) and by binding to E2F-DP dimers at genes necessary for S-phase entry and progression. During late G1 phase, hyperphosphorylation of pRb by cyclin dependent kinase activity releases E2F-DP and transactivates genes necessary for S phase progression (Brehm et al. 1998). pRb also regulates apoptosis by controlling expression of Apoptosis-protease activating factor-1 (Apaf-1). Lamin A/C directly or through its interacting partner LAP2\textalpha sequesters hypophosphorylated pRb in the nuclear matrix (Mancini et al. 1994; Markiewicz et al. 2005; Vleck and Foisner, 2007) and 12(S)-lipoxygenase [12(S)-Lox] enzyme (Tang et al. 2000).
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et al. 2002). Disruption of these interactions gains physiological significance in the case of EMD and other laminopathies, where loss of muscle tissues due to apoptosis is a characteristic pathological feature (Bonne et al. 2003). To add another layer of complexity to pRb regulation, it was observed that Protein Phosphatase 2A (PP2A) interacts with the lamin A-pRb complex. According to the model proposed by the authors, the PP2A–lamin A/C complex restores pRb functionality via rapid TGF-β1–dependent pRb dephosphorylation (Van Berlo et al. 2005). Furthermore, loss of lamin A/C leads to proteasome mediated degradation of pRb, suggesting that A-type lamins are not only involved in dephosphorylation of pRb, but also protect it from degradation in order to regulate cell growth and division (Johnson et al. 2004).

SREBP1 has been identified as a basic-helix-loop-helix leucine zipper transcriptional factor involved in cholesterol biosynthesis and adipogenic differentiation (Raghow et al. 2008). SREBP1 is synthesized as an ER resident membrane-bound precursor, which undergoes intramembrane proteolysis upon depletion of cellular cholesterol, releasing its DNA binding amino-terminal domain. This domain then translocates to the nucleus and activates expression of genes such as peroxisome proliferator-activated receptor-γ (PPAR-γ) (Fajas et al. 1998). Two alternatively spliced variants SREBP1α and SREBP1c bind to farnesylated prelamin A (Lloyd et al. 2002; Capanni et al. 2005).

In addition to a large number of lamin bound transcriptional regulators, a variety of lamin interacting proteins also have specific regulatory functions. Emerin, LAP2β and MAN1 directly bind to GCL transcription factor in mammalian cells both in vitro and in vivo (Nili et al. 2001; Holaska et al. 2003; Mansharamani and Wilson, 2005). In Drosophila GCL is required for silencing germline transcription (Leatherman et al. 2002). Both LAP2β and GCL can directly repress E2F-DP in a cooperative manner (Nili et al. 2001). Interaction of emerin with the putative transcriptional regulator Lmo7 has been observed (Holaska et al. 2006). Emerin also interacts with pro-apoptotic transcription regulator Btf at the nuclear periphery in cells committed for apoptosis (Haraguchi et al. 2004). Both of these interactions are significant in the disease-causing emerin mutant S54F, where binding of Btf to emerin is compromised and Lmo7 loss leads to muscle defects in mice (Semenova et al. 2003; Haraguchi et al. 2004). Furthermore, the recruitment of chromatin regulatory proteins HP1 (Ye et al. 1997; Li et al. 2003) and methyl-CpG-binding protein (MeCP2) (Guarda et al. 2009) at the nuclear periphery occurs through interactions with LBR. An adenovirus early protein E1B 19K, which shares low sequence homology with Bcl2 blocks apoptosis in a lamin-dependent manner (Rao et al. 1997). It localizes to the ER and nuclear membrane by interacting with lamins and emerin, and thereby inactivates Btf mediated apoptosis pathway (Zastrow et al. 2004).
Among the other interacting partners of lamins are the heat shock proteins HSP70, HSP26, HSP25 and αB-crystallins (Willsie and Clegg, 2002; Adhikari et al. 2004). Association of lamins with heat shock proteins suggests a role for heat shock proteins in maintenance of lamins in intranuclear speckles and in the lamina under conditions of heat stress. In fact, mutations in HSPs are responsible for several neurodegenerative disorders such as Charcot-Marie-Tooth disease type 2F and distal hereditary motor neuropathies involving impaired neurofilament assembly (Evgrafov et al. 2004), as well as a desmin-related cardiomyopathy (Vicart et al. 1998). Furthermore, fibroblasts from HGPS patients with LMNA mutation at G608G residue are hypersensitive to heat shock (Paradisi et al. 2005). Several INM proteins in Drosophila like Young arrest (YA) and Bicaudal-D (BICD) have been shown to interact with the B-type lamin Dm0 (Goldberg et al. 1998; Stuurman et al. 1999). Thus, a multitude of gene regulatory factors interact directly with lamins or through their interacting partners to orchestrate nuclear activity in a well-coordinated manner.

1.4. Functional role of lamins

Lamins have long been considered to be immobile structural components of the nucleus, but recent discoveries have changed this paradigm and novel regulatory functions in cellular signaling, differentiation and proliferation have been ascribed to them. Some of these vital functions of lamins are summarized below.

1.4.1. Mechanical and nucleoskeletal functions

Several studies have shown the importance of stable structures formed by lamins in nuclear lamina and nucleoplasm in determining nuclear shape and size, resisting nuclear deformation and anchoring other INM proteins (Dechat et al. 2008). It has also been established that lamins play an important role in nuclear mechanics. It has been found that cells lacking A-type lamins have reduced nuclear stiffness and increased fragility, making them more vulnerable to cell death under mechanical strain. On the other hand, cells lacking only lamin C have slight nuclear abnormalities and no significant reduction of viability, while lamin B1 deficient cells have increased blebs but display normal nuclear mechanics (Broers et al. 2004; Lammerding et al. 2006). These studies propose distinct roles for A- and B-type lamins, suggesting that B-type lamins are essential for nuclear integrity whereas A-type lamins provide mechanical stiffness to the nucleus. These results are further substantiated by the fact that B-type lamins are ubiquitously expressed and appear to be essential, whereas A-type lamins are developmentally regulated and, although not essential for viability, mutations in A-type lamins can cause a variety of diseases called laminopathies (Broers et al. 2006). Furthermore, it has been found that
mice lacking A-type lamins have growth retardation as well as skeletal and cardiac abnormalities including dilated cardiomyopathy (DCM), and life expectancy is reduced to 4-6 weeks (Sullivan et al. 1999; Nikolova et al. 2004). On the contrary, mice having insertional mutations in lamin B1 that disrupt the structure in the rod domain from amino acid position 273 onwards (lacking part of rod domain, NLS and CaaX motif), show defects in bone and lung development and die immediately after birth (Vergnes et al. 2004). This severe phenotype may be attributed to broad and ubiquitous expression of lamin B1 compared to lamin A/C. Similar roles for lamin C in D. melanogaster have been identified, where lamC null cells in imaginal discs display detachment of the two nuclear membranes and chromatin leakage (Schulze et al. 2009).

Recent studies also implicate lamins in maintenance of mechanical properties of the entire cell and not only the nucleus. LMNA<sup>−/−</sup> cells display defective cell migration, impaired mechanotransduction and fragility (Houben et al. 2007; Lee et al. 2007). This observation can be explained by the role played by lamins in LINC complexes, which connect the nucleoskeleton to the cytoskeleton by virtue of binding of lamins with SUN1/2, nesprin and cytoskeletal filaments (Burke and Roux, 2009). Studies in worms, flies and mammals suggest that the LINC complex coordinates the cytoplasmic-motor driven movement of the entire nucleus within the cell as well as movement of envelope-tethered chromosomal territories to new locations in the nucleus and direct signal transduction from cell surface to the nucleus (Wilson and Foisner, 2010). These observations suggest that instead of being passive structural components, nuclear lamins have direct effects on mechanical strength of the cell as a whole, as well as nucleo-cytoplasmic force transmission and cell viability.

1.4.2. DNA replication and transcriptional regulation

Replication of DNA in metazoan cells is a complex process and involves two distinct stages: initiation phase, which needs DNA polymerase α, origin recognition complex (ORC2), minichromosome maintenance protein (MCM6) and other proteins, and elongation phase involving DNA polymerase δ, proliferating cell nuclear antigen (PCNA), replication factor C (RFC) and accessory proteins (Stillman, 1994; Waga et al. 1994). Kennedy et al (2000) have observed that primary fibroblasts have very few (5 to 20) DNA replication foci that localize around the nucleolus, while transformed cell lines have numerous dispersed DNA replication foci, although the overall process of replication is similar. They also found that replication foci colocalize with intranuclear lamin A/C in G1 and early S phase but not in late S phase (Kennedy et al. 2000), which implicates lamin A/C in organizing early replication foci. Heterochromatic replication sites, which are late replication foci in S phase localize with lamin B and it has been observed that intranuclear foci of lamin B1 colocalize
with PCNA and RFC, but not with components of DNA replication initiation complex (Moir et al. 1994; Shumaker et al. 2008). Role of lamins in DNA replication was further substantiated by studies in which addition of dominant N-terminal deletion mutants of human lamin A (ΔNLA) or Xenopus lamin B3 (ΔNLB3) to in vivo nuclear assembly reactions resulted in impaired DNA replication (Ellis et al. 1997; Spann et al. 1997; Moir et al. 2000b). Using a combination of inhibitors it was also demonstrated that introduction of human ΔNLA disrupted DNA replication during the transition from initiation to elongation phase (Moir et al. 2000b). Interestingly, interaction between two lamin partners LAP2α and HA95 (a chromatin and nuclear matrix associated protein involved in nuclear envelope-chromatin interactions) is essential for initiation, but not elongation step of DNA replication (Martins et al. 2003) Thus, intranuclear lamins can be regarded as molecular scaffolds for proper assembly of multiprotein DNA replication complexes during G1-S phase.

Lamins are important for the spatial organization of RNA Pol II mediated transcription as expression of a truncated version of lamin A that lacks the head domain disrupts lamin filament assembly and inhibits Pol II activity by causing dispersal of TATA binding protein (TBP), without affecting Pol I or Pol III activity (Spann et al. 2002). In addition, Kumaran et al. (2002) observed that intranuclear lamins (also termed lamin speckles) form enlarged aggregates upon treatment with Pol II transcriptional inhibitors such as α-amanitin or 5,6-dichlorobenzimidazole riboside (DRB); and disruption of lamin speckles by lamin A overexpression adversely affects Pol II activity and disorganizes splicing factor compartments (Kumaran et al. 2002). Splicing factor compartments or interchromatin granule clusters (IGCs) are regarded as storage and recruitment compartments for splicing factors (Spector, 2003) and lamins may be important for maintenance of these SFCs as depletion of peripheral and intranuclear lamin A/C by RNA interference (RNAi) disorganizes these compartments. Lamins have also been detected in purified fractions of IGCs (Mintz et al. 1999), which supports close association of lamins with splicing factors. Moreover, reorganization of lamin A/C speckles has been observed specifically during muscle differentiation. This event is mediated by cyclin D3 and coincides with induction of early markers of muscle differentiation, thereby indicating a specific role of A-type lamins during muscle differentiation (Muralikrishna et al. 2001; Mariappan and Parnaik, 2005; Mariappan et al. 2007). Both A- and B-type lamins associate with several transcriptional factors and regulators directly or through lamin interacting partners, and are thus able to modify the activity of specific genes, as described earlier in section 1.3.3. pRb, cFos, SREBP1, MOK2, GCL, Oct-1, cyclin D3, ING1 are a few such examples.
1.4.3. Lamins and mitosis

'Open' mitosis of metazoans as opposed to 'closed' mitosis of yeasts, filamentous fungi and some protists involves extensive changes in nuclear architecture and is accompanied by nuclear envelope breakdown (NEBD). NEBD is a tightly regulated and coordinated event between nuclear assembly-disassembly and mitotic entry-exit steps, ensuring segregation of chromosomes into a single nascent nucleus in each daughter cell. NEBD marks the entry of a cell into mitosis and precedes formation of a mitotic spindle. This is initiated in early prophase by depolymerization of the lamina by phosphorylation of serine residues in the rod domain of lamins by cyclin dependent kinase -1 (Cdk1) (Peter et al. 1990). This is in part assisted by ripping apart of the nuclear membrane into fragments at the end of prophase by dynein-microtubule proteins (Salina et al. 2002; Beaudouin et al. 2002). Mutations in lamin A/C at Cdk1 phosphorylation sites have been shown to block disassembly of the lamina at the onset of mitosis (Heald and McKeon, 1990). In mammalian cells, solubilization of lamin A/C at early prophase is the earliest sign of lamina depolymerization while B-type lamins disassemble only after NPC disruption (Georgatos et al. 1997; Beaudouin et al. 2002). During mitosis, A-type lamins disperse completely into the cytoplasm, while B-type lamins remain associated with membrane fragments, probably due to the presence of a farnesyl moiety at their C-termini (Gerace and Blobel, 1980). Phosphorylation of nucleoporins (NUP98, GP210, NUP107-160), LAP2α, LAP2β, LBR and other INM proteins by Cdk1 causes further destabilization of the nuclear envelope. During mitosis, the ER is also reorganized and it forms an exclusive tubular network without membrane sheets that harbours several INM proteins (Puhka et al. 2007).

Reassembly of the nuclear envelope starts in early telophase by binding of several INM proteins to separated sister chromatids. Decondensation of chromatins is assisted by concomitant post-translational modifications of histones and reassocation of key chromatin modifying proteins such as HP1. This binding of HP1 in turn recruits INM proteins such as LBR that anchor chromatins to nuclear membrane fragments (Collas et al. 1996; Pyrpasopoulou et al. 1996), while chromatin binding protein BAF serves a direct role in recruitment of LEM proteins to chromatins (Gorjánácz et al. 2007). Incorporation of A- and B-type lamins into these nascent nuclei differs spatiotemporally. B-type lamins can be detected in newly formed nuclei at during telophase, while A-type lamins enter after B-type lamins, NPC and other envelope components are assembled (Moir et al. 2000a; Dechat et al. 2004). Inhibition of polymerization of B-type lamins at the end of mitosis does not affect assembly of A-type lamins, suggesting that the two types of lamins assemble into the lamina independent of each other (Steen and Collas, 2001). Reassembly of the lamina involves dephosphorylation of lamins, probably by Protein Phosphatase-1 (PP1), which is
recruited to the envelope at the end of mitosis by AKAP149 (Steen et al. 2000). Dynamic modifications of lamins are essential prerequisites for efficient segregation of sister chromatids during mitosis. Growth of the nucleus during interphase is accompanied by continuous synthesis and incorporation of lamins into the nuclear lamina as cells progress from early to late G1 phase (Gerace et al. 1984; Yang et al. 1997).

1.4.4. Delamination events during apoptosis

Apoptosis or programmed cell death is an energy dependent process characterized by distinct morphological and biochemical changes both in the cytoplasm and nucleus. These morphological and biochemical changes are triggered by a family of cysteine-dependent aspartate proteases called caspases. They are present as inactive procaspases that are cleaved and activated by auto- or transactivation pathways (Hengartner, 2000). The changes include reduction in cellular volume, chromatin detachment from nuclear lamina, fragmentation of DNA by nucleases, chromatin condensation, clustering of NPCs, membrane blebbing and fragmentation of nucleus to small membrane bound structures called apoptotic bodies (Thompson, 1998). In cells committed to apoptosis, nuclear fragmentation is preceded by caspase mediated lamin proteolysis (Lauebnik et al. 1995; Rao et al. 1996). Cleavage of lamins is necessary for cell death as cells expressing uncleavable lamin mutants show delayed chromatin condensation and nuclear shrinkage, although terminal stages of apoptosis are unaffected (Rao et al. 1996). Lamins are cleaved in the α-helical rod domain (amino acid residues 227-230) by caspase-6 (Orth et al. 1996). This proteolysis probably causes disassembly of the lamina, followed by chromatin condensation and fragmentation (Ruchaud et al. 2002). Several other INM proteins have also been identified as 'death targets' of caspase-3, namely LAP2β, LBR and NUP153, and are generally degraded after cleavage of lamins (Duband-Goulet et al. 1998; Buendia et al. 1999). The cleaved fragments of lamin B1 remain anchored to the nuclear membrane, but lamin A fragments diffuse freely both in the nucleoplasm and cytoplasm (Broers et al. 2002).

Phosphorylation of lamin B by protein kinase C δ and βII isoforms is observed upon onset of apoptosis (Cross et al. 2000; Chiarini et al. 2002), suggesting that both phosphorylation and proteolysis of lamins are essential for apoptosis induction (Prokocimer et al. 2006). Interestingly, interaction between lamin A/C and p19E1b is essential for envelope localization of E1b and maintenance of its anti-apoptotic activity (Rao et al. 1997). E1b is an adenoviral early protein and Bcl2 homolog, which inhibits p53 mediated apoptosis in cells infected with adenoviruses.
1.4.5. Integrating signaling pathways

Presence of lamins and their interacting partners at the nuclear periphery makes the nuclear envelope a signaling hub. Studies have shown that lamins form a ’tensegrity element’ at the nuclear envelope with the cytoskeleton via SUN and nesprin proteins, which is capable of mechanotransduction signaling (Inbger, 2003; Hutchison and Worman, 2004). Fibroblasts lacking A-type lamins show defective mechanotransduction signaling, leading to impaired anti-apoptotic response (Lammerding et al. 2004; Nikolova et al. 2004). The increase in apoptotic cell fraction in \( LMNA^+ \) fibroblasts has been attributed to attenuated expression of the mechanosensitive gene egr-1 and anti-apoptotic gene iex-1. The latter gene belongs to the NF-\( \kappa \)B signaling pathway, which also shows attenuated expression on application of strain or chemokine stimulus in \( LMNA^+ \) fibroblasts (Lammerding et al. 2005).

The C-terminal region of lamin A/C interacts with PKC\( \alpha \), a serine-threonine kinase activated by signals such as calcium ions, diacylglycerol (DAG) and 12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE]. Upon activation, PKC\( \alpha \) translocates to the nucleus and attaches to the envelope by virtue of its binding with A-type lamins (Martelli et al. 2000). Another A-type lamin interacting protein involved in signaling is 12(S)-lipoxygenase [12(S)-Lox]. This enzyme catalyzes the conversion of arachidonic acid to 12(S)-HETE and thus acts upstream of the PKC\( \alpha \) mediated lipid signaling cascade (Tang et al. 2000). The anti-apoptotic factor p19E1b, interacts with lamins in cells infected with adenoviruses. It has also been shown to bind to and possibly inactivate the death promoting repressor Btf (Kasof et al. 1999). Association of lamins with hypophosphorylated pRb and SREBP1 are important for maintaining proper balance between proliferation and differentiation of precursor cells to myotubes and adipocytes, respectively (Lloyd et al. 2002; Favreau et al. 2004). In primary myoblasts from \( LMNA \) null mice, differentiation is impaired due to degradation of pRb and decreased levels of myogenic factors (Frock et al. 2006). These studies implicate A-type lamins in signaling involved in terminal differentiation to various lineages.

Several studies have shown an indirect involvement of lamins in modulation of signaling pathways through LEM domain partners. Lamin B binding protein LAP2\( \beta \) directly interacts with transcriptional regulators such as GCL and epigenetic modifiers such as HDAC3, and in turn represses transcription leading to germline specification (Nili et al. 2001). Lamin A/C-MAN1-R Smad complex antagonizes BMP signaling in \( Xenopus \) embryogenesis (Osada et al. 2003) and mammalian cells (Raju et al. 2003; Pan et al. 2005). In addition to indirect roles, interaction of A-type lamins and protein phosphatase 2A is shown to have a direct effect on TGF-\( \beta_1 \) signaling (Van Berlo et al. 2005). A-type lamins are also involved in suppressing activity of AP-1 transcription factor by sequestering c-Fos.
at nuclear periphery and inhibiting heterodimerization of c-Fos and c-Jun (Ivorra et al. 2006). During MAP kinase signaling, activated ERK1/2 interacts with lamin A/C and releases c-Fos to facilitate cell proliferation (González et al. 2008). Disruption of this cascade in laminopathic cells might be the reason for upregulated ERK-mediated pathways (Muchir et al. 2009). Emerin is found to associate with at least six distinct multiprotein complexes (Holaska and Wilson, 2007), which include proteins such as lamins, actin, nuclear myosin1c, nuclear α1l-spectrin, Lmo7, pRb and β-catenin (Lammerding et al. 2005; Holaska et al. 2006; Markiewicz et al. 2006). Lamins are also known to modulate Notch signaling by sequestering SKIP, an essential coactivator of Notch target genes (Scaffidi and Misteli, 2008). This pathway is essential for differentiation of mesenchymal stem cells to bone, adipose tissue and vascular epithelium (Hennekam, 2006). Involvement of lamin and associated proteins in these diverse signaling pathways might therefore explain the reason for disrupted balance between proliferation-differentiation and tissue regeneration-apoptosis in laminopathic patients.

1.4.6. Regulation of NPC organization and nucleocytoplasmic trafficking

The NPCs are anchored to the lamina at the nucleoplasmic face by Nup153, and associate with nuclear membranes through two nucleoporins- POM121 and gp210 (Akey, 1989). Additionally, nucleoporins have been shown to associate directly or indirectly with lamins (Smythe et al. 2000) or colocalize with mutant lamin A aggregates (Hübner et al. 2006; Pan et al. 2007). Nup153 interacts with import and export receptors and shuttles between the nuclear and cytoplasmic sides of NPC (Moroianu et al. 1997; Shah and Forbes, 1998). During early nuclear assembly it associates with chromosomes (Bodoor et al. 1999), while its C-terminus binds to B-type lamins (Smythe et al. 2000). Disruption of lamina organization by dominant negative mutant lamins causes depletion of Nup153, but not other Nups, thereby leading to clustering of NPCs within the envelope (Walther et al. 2001). Similarly, reduction in levels of C. elegans Ce-lamin, Drosophila lamin Dm0 and mouse lamin A lead to abnormal distribution of NPCs (Lenz-Bohme et al. 1997; Harel et al. 1998; Sullivan et al. 1999; Liu et al. 2000a). Organization of NPCs in the envelope is primarily dependent on A-type lamins as exogenous expression of lamin C in Drosophila lamin Dm0 null brain cells restored the normal distribution of NPCs. It should be noted that lamin C is not normally expressed in brain cells of Drosophila and hence lamin Dm0 null brain cells are essentially devoid of all types of lamins (Furukawa et al. 2009). These findings show that lamins are essential components of the nuclear envelope and maintain NPC organization for efficient nucleo-cytoplasmic transport processes.
1.5. Nuclear Envelopathies

The term nuclear envelopathies is a general expression defining the diseases and anomalies arising due to mutations in components of the nuclear envelope and lamina (Worman and Courvalin, 2002). Mutations in an X-linked gene leading to Emery-Dreifuss muscular dystrophy was the first report of a nuclear envelope protein in human disease and the protein was subsequently named as emerin (Bione et al. 1994; Manilal et al. 1996). Later studies have identified disease-causing mutations in different components of the nuclear envelope. Heterozygous mutations in LBR cause Pelger Huet anomaly, while homozygous mutations cause HEM/Greenberg skeletal dysplasia (Hoffmann et al. 2002; Waterham et al. 2003). Heterozygous loss-of-function mutations in MAN1 are associated with Buschke-Ollendorff syndrome and non-sporadic melorheostosis (Hellemans et al. 2004). Mutations in Syne-1 are implicated in autosomal recessive myogenic arthrogryposis multiplex congenital and autosomal recessive adult onset cerebellar ataxia (Attali et al. 2009). Polymorphisms in LAP2 have been reported for cardiomyopathy in two subjects, while early onset torsion dystonia is caused by mutations in the ER/nuclear envelope associated protein torsinA (Ozelius et al. 1997). Nuclear pore complexes have also been implicated in nuclear envelopathies, where mutation in a nuclear pore protein Aladin causes triple A syndrome characterized by adrenal-insufficiency, achalasia and alacrima (Cranshaw and Matunis, 2003).

Mutations in the LMNA gene encoding the A-type lamins in autosomal dominant EMD was observed in 1999 by Bonne et al. (Bonne et al. 1999) and subsequently several other human diseases have been linked to mutations in LMNA gene. Diseases arising due to defects in lamins have been grouped as primary and secondary laminopathies (Somech et al. 2005; Shaklai et al. 2007; Worman and Bonne, 2007). The diseases arising due to mutations in LMNA have been grouped as primary laminopathies. A second class of related diseases due to mutations in ZMPSTE24 gene, which encodes for an enzyme involved in post-translation processing of lamins is termed as secondary laminopathies. So far more than 333 disease-causing mutations have been reported for LMNA, with the mutations spanning over the entire length of the gene (see Fig. 1.2). This represents the highest number of recorded mutations in a single intermediate filament protein gene and these mutations have been implicated in 18 of the 20 known laminopathies. These mutations include missense mutations, insertion or deletion mutations leading to frameshifts, generation of cryptic splice sites and nonsense mutations causing truncated proteins (Szeverenyi et al. 2008). Most striking feature about these diseases is the occurrence of tissue-specific phenotypes, principally involving striated muscles, cardiac tissue, adipose
tissues, sensory and motor neurons. Depending on the nature of the tissue involved, primary laminopathies have been grouped into four major categories. These are as follows:

i) Muscular dystrophies
   a. Autosomal dominant Emery-Dreifuss muscular dystrophy
   b. Autosomal recessive Emery-Dreifuss muscular dystrophy
   c. Dilated cardiomyopathy type 1A
   d. Limb-girdle muscle dystrophy type 1B

ii) Lipodystrophies
   a. Dunnigan-type familial partial lipodystrophy
   b. Polycystic ovary syndrome with insulin resistance

iii) Peripheral neuropathy
   a. Charcot-Marie-Tooth syndrome type 2B

iv) Systemic laminopathies: Premature ageing syndromes
   a. Mandibuloacral dysplasia
   b. Hutchinson-Gilford progeria syndrome
   c. Atypical Werner syndrome
   d. Restrictive dermopathy
   e. Lethal fetal akinesia

v) Overlapping laminopathies

1.5.1. Muscular dystrophies

1.5.1.1. Emery-Dreifuss muscular dystrophy (EMD):

   EMD is a degenerative form of myopathy with characteristic weakening and atrophy of muscles without involvement of the nervous system. Mutations in \textit{LMNA} gene cause autosomal forms of Emery-Dreifuss muscular dystrophy (AD-EMD), whereas mutations in \textit{STA} gene encoding emerin are associated with X-linked EMD (Bione \textit{et al.} 1994; Bonne \textit{et al.} 1999). AD-EMD displays symptoms of dystrophy in specific muscles with associated dilated cardiomyopathy (DCM) and atrioventricular conduction defects. Muscle weakness at first affects lower extremities, and by teenage symptoms such as weakening of shoulder girdle and lumbar lordosis leading to waddling gait become prominent. Cardiac conduction defects and DCM have been observed to cause most serious, life threatening complications in EMD patients. \textit{LMNA} mutations causing AD-EMD display diverse phenotypes and penetrance ranging from asymptomatic to typical EMD phenotypes (Morris, 2001). These mutations generally tend to cluster in the tail domain and 2A rod domain of lamin, suggesting the importance of binding of nuclear envelope components to these regions in skeletal and cardiac tissues. A rare form of EMD having autosomal recessive mode of inheritance (AR-EMD) and severe atypical EMD without cardiac
involvement has also been attributed to mutations in LMNA gene (Di Bartella et al. 2000). In addition, mutations in the SYNE1 gene and SYNE2 gene, which encode for ONM proteins with KASH domains, have also been shown to cause AD-EMD (Zhang et al. 2007). Involvement of three different nuclear envelope proteins emerin, A-type lamins and nesprins 1/2 (syne-1 and syne-2) in various forms of EMD with overlapping symptoms underscore the importance of the LINC complex in basic physiological functions of muscle and cardiac tissues (Muchir et al. 2003).

1.5.1.2. Dilated cardiomyopathy (DCM):

DCM is a progressive disease characterized by cardiac dilation, hypertrophy and systolic dysfunction. DCM-1A is caused by mutations in LMNA gene and out of more than 20 individual genes identified in DCM cases, this represents the largest subset of dilated cardiomyopathies. In a study on families with autosomal dominant DCM with conduction defects, Fatkin et al. (1999) identified 5 novel LMNA missense mutations responsible for DCM-1A; 4 of them in the α-helical rod domain of lamin A and one in the tail domain of lamin C. None of the patients displayed skeletal myopathy or elevated serum creatine kinase levels. Sudden cardiac arrest leading to death was observed in these families. Later studies have indicated that mutations in the LAP2 gene (R690C mutation in LAP2α) are also responsible for DCM (Taylor et al. 2005).

1.5.1.3. Limb Girdle muscular dystrophy (LGMD):

LGMD was identified as a non-congenital muscular dystrophy displaying progressive proximal weakening without primary dystrophin deficiency. Depending on mode of inheritance, the disease is referred as LGMD-1 for the autosomal dominant form and LGMD-2 for the autosomal recessive form (Bushby and Beckmann, 1995). Mutations in LMNA gene have been implicated in the autosomal dominant form termed LGMD1B (van der Kooi et al. 1996). Most of the causative mutations in LMNA tend to cluster in the Ig-fold and coiled-coil 2 region of lamin A. Involvement of mutated forms of several other proteins has also been reported in LGMD cases, eg. calpain-3, FKRP (Fukutin Related Protein), dysferlin, myotilin, TRIM-32, titin, and α,β,δ and γ sarcoglycans. LGMD is characterized by progressive muscle weakness with onset in the limb girdle muscles and pronounced tendon contractures. The age of appearance of phenotype may vary from early childhood to late adulthood (Bushby, 1995). Autosomal dominant LGMDs are 10 times more prevalent than recessive ones and have slower onset, lesser elevation of serum creatine kinase levels and more heterogeneous symptoms. Varying degrees of cardiac conduction defects seen in EMD cases are also observed in LGMD, and hence early prognosis and follow-up becomes necessary.
Another laminopathy affecting striated muscles and resembling LGMD-1B has been described as quadricipital myopathy associated with dilated cardiomyopathy (Charniot et al. 2003). Members of a French family were found to carry the mutation R377H, previously reported to be responsible for DCM where neuromuscular symptoms preceded cardiac involvement. However, in this case it was observed that the same mutation had resulted in cardiomyopathy preceding neuromuscular defects and only the quadriceps muscle was affected. This suggests that modifier genes or environmental factors may also be involved in manifestation and penetrance of pathological effects of mutations causing laminopathies. Interestingly, lamin mutations affecting striated muscles account for approximately 60% of all the cases of laminopathies (Burke and Stewart, 2006).

1.5.2. Lipodystrophies

1.5.2.1. Dunnigan-type familial partial lipodystrophy (FPLD):

Lipodystrophies, both genetic and acquired, are a heterogeneous group of disorders with generalized or partial loss of body fat accompanied by insulin resistance. FPLD caused by mutations in LMNA gene is inherited as an autosomal dominant trait. Majority of the mutations reported for FPLD (approximately 230 to date) are localized at amino acid position Arg482 in exon 8 of LMNA gene which encodes for a part of the C-terminal globular tail domain of lamin A and C (Shackleton et al. 2000). Recurrent involvement of R482W mutation in FPLD with no apparent common ancestry among the patients indicates a possible deamination of cytosine to thymidine at position 1444 or transition of guanosine to adenosine residue at position 1445 in LMNA gene (Shackleton et al. 2000; Szeverenyi et al. 2008). FPLD patients have a normal fat distribution during childhood, but with onset of puberty the symptoms become pronounced. These symptoms include loss of subcutaneous adipose tissues from upper and lower extremities leading to prominent muscles and superficial veins. A concomitant accumulation of adipose tissues in face and neck region is observed, giving rise to a double-chin appearance and fat neck. Affected patients display insulin resistance, glucose intolerance and diabetes mellitus usually after the age of 20 years, with hypertriglyceridemia and low levels of high-density lipoprotein cholesterol (HDL-cholesterol). The phenotypes are more distinct in affected females with severe metabolic manifestations, while in males these are difficult to recognize probably due to increased musculature and reduced fat distribution (Garg, 2000; Vigouroux et al. 2000). The dystrophy can be fatal due to hypertriglyceridemia, liver cirrhosis and premature atherosclerosis leading to coronary defects (Al-Shali and Hegele, 2004). The patients can also have cardiac or muscular dystrophies such as LGMD and cardiac conduction defects (Vantyghem et al. 2004).
1.5.2.2. Polycystic ovary syndrome with insulin resistance:

Although polycystic ovary syndrome (PCOS) is considered to be an endocrine disorder, which affects 4-7% of women in reproductive age group, the underlying mechanisms are still unclear. One of the pathological basis of PCOS involves a heterozygous missense mutation, R482Q in LMNA gene, which is also implicated in FPLD cases (Cao and Hegele, 2000). The symptoms include insulin resistance, hyperandrogenism, oligomenorrhoea and moderate acanthosis nigricans on neck, axillae and inguinal areas with or without hirsutism (Gambineri et al. 2008).

1.5.3. Peripheral neuropathy

1.5.3.1. Charcot-Marie-Tooth syndrome type 2B (CMT-2B):

Charcot-Marie-Tooth disorder or hereditary motor and sensory neuropathy (HMSN) is an autosomal recessive form of laminopathy with adult onset pattern. Prevalence of CMT is usually 1-4 in 1 million individuals. CMT is a heterogeneous group of neuropathies, where type 1 refers to demyelination of nerves leading to reduced conductivity and type 2 is associated with axonal form with slightly reduced or normal nerve conduction velocity (Garcia, 1999). Most of the mutations causing CMT-2B have been mapped to R298 amino acid position of coil 2b region in the rod domain of lamin A/C (De Sandre-Giovannoli et al. 2002; Bouhouche et al. 2007). The affected individuals have wasting and weakness of distal lower limb muscles, foot deformities (pes cavus), difficulty in walking and reduced or absent tendon reflexes.

1.5.4. Systemic laminopathies: Premature ageing syndromes

1.5.4.1. Mandibuloacral dysplasia type A (MAD-A):

It is a rare autosomal recessive disorder characterized by postnatal growth retardation, skeletal malformation, delayed closure of cranial sutures, premature loss of teeth, extreme insulin resistance, joint contractures, partial lipodystrophy, mottled cutaneous pigmentation, marked hypermetabolism and signs of accelerated ageing (Novelli et al. 2002). Most patients of MAD-A are reported to harbour a R527H homozygous mutation in LMNA gene, although several other mutations such as A529T, A529V and K542N have also been reported. Interestingly, mutations at the same amino acid residue R527 have been implicated in different phenotypes. Homozygous R527H substitution mutations have been implicated in typical MAD-A phenotype (Shen et al. 2003), while R527C substitution causes MAD associated with progeriod symptoms (Cao and Hegele, 2003; Agarwal et al. 2008) and R527P is responsible for AD-EMD with or without lipodystrophy (Makri et al. 2009). The amino acid R527 is situated on the exposed surface
of the C-terminal Ig-fold domain common to both lamin A and C, and mutations in it may possibly disrupt important protein-protein interactions.

1.5.4.2. Hutchinson-Gilford progeria syndrome (HGPS):

HGPS was first described by Jonathan Hutchinson (1828-1913) more than a century ago as a case of congenital absence of hair, with atrophic condition of the skin and its appendages, in a boy whose mother had been almost wholly bald from alopecia areata from the age of six (Hutchinson, 1886). Few years later in 1897 Hastings Gilford termed the disorder as progeria (Greek- progeria means ‘prematurely old’) and later it was called Hutchinson-Gilford syndrome (Gilford, 1904; Debusk, 1972).

HGPS is an extremely rare (1 in 4-8 million live births) dominantly inherited trait with symptoms appearing as early as the first year of life. More than 90% of deaths occur at an average age of 13 years, frequently from coronary artery disorders. The prominent phenotypic features include severe growth retardation with short stature and reduced weight, incomplete sexual maturation, sclerotic skin, joint contractures, severe and progressive atherosclerosis, prominent eyes, beaked nose, alopecia, prominent scalp veins, craniofacial abnormalities, loss of subcutaneous fat and other skeletal deformities as delayed and crowded dentition, short dystrophic clavicles, generalized osteodysplasia with osteolysis and pathologic fractures.

In the majority of cases, HGPS is caused by a de novo single base substitution, a C- to -T transition (GGC to GGT) at position 1824 within exon 11. The mutation does not alter the amino acid sequence (G608G), but generates a cryptic donor-splice site that results in 150 bp deletion in the mature mRNA, resulting in a concomitant 50 amino acid truncation (pVal608-Gln656:del) at C-terminus in lamin A isoform only. This deletion leads to removal of internal proteolytic cleavage site, which is used during second proteolytic processing of farnesylated prelamin A by ZMPSTE24 enzyme, resulting in a constitutively farnesylated truncated isoform of lamin A called progerin or L.Ab.50. This mutation does not affect processing and maturation of the lamin C isoform. The distorted nuclear appearance in HGPS cells has been attributed to presence of this progerin protein, which acts as a dominant negative lamin protein (De Sandre-Giovannoli et al. 2003; Erikson et al. 2003; Mounkes et al. 2003a). Autosomal recessive inheritance of R527C (Liang et al. 2009), and other mutations such as S143F, E145K or R435C have also been shown to cause HGPS, albeit with associated myopathy and scleroderma-like features (Kirschner et al. 2005; Madej-Pilarczyk et al. 2008; Madej-Pilarczyk et al. 2009). Involvement of mutations such as P4R, T10I, E111K, D136H, E159K, and C588K has also been reported in atypical progeria cases, where symptoms are milder than those in classical HGPS (Garg et al. 2009).
Fibroblasts from HGPS patients show lobulated nuclei, thickened lamina, clustering of nuclear pores and loss of peripheral heterochromatin.

1.5.4.3. Atypical Werner syndrome:

Werner syndrome is an autosomal recessive disorder characterized by premature ageing symptoms, but unlike HGPS it is involved in increased risk of neoplasms (Mohaghegh and Hickson, 2001). The symptoms usually start appearing in second to third decade of life. The key pathological features are high incidence of cancers, early-onset cataracts, premature arteriosclerosis, scleroderma-like skin change especially in extremities (tight and atrophic skin, altered pigmentation, hyperkeratosis, ulceration), short stature, subcutaneous calcification, osteoporosis, diabetes mellitus, wizened and prematurely aged bird-like face, slender limbs, premature graying and/or thinning of scalp hair and death usually in the late 40s from myocardial infarction (Oshima, 2000; Fry, 2002; Hickson, 2003). Most of the cases (83%) of Werner syndrome are due to mutations in 3'-5' DNA helicase-like (RECQL2) gene, which is a homolog of E. coli RecQ helicase (Yu et al. 1996). However, some cases (15%) of 'atypical Werner syndrome' or 'non-WRN syndrome' have been identified with mutations in LMNA gene. These atypical Werner patients have more severe phenotype as compared to those with RECQL2 mutations (Chen et al. 2003).

1.5.4.4. Restrictive dermopathy (RD):

Restrictive dermopathy or tight skin contracture syndrome is a rare disorder characterized by intrauterine growth retardation, tight and rigid skin with erosion, hyperkeratosis of skin, prominent superficial vasculature, mineralization defects of skull, thin clavicles, multiple joint contractures and early neonatal lethality within few hours or few weeks of birth. In two of the nine observed fetuses, a heterozygous splicing mutation in LMNA gene leading to partial or complete loss of exon 11 was observed. In the other 7 fetuses, a heterozygous 1bp insertion in the ZMPSTE24 gene leading to a premature stop codon was observed (Navarro et al. 2004). Therefore, accumulation of prelamin A can be envisaged as a possible reason for the defect in nuclear lamina formation and pathophysiology of restrictive dermopathy. It can be classified as both primary and secondary laminopathy because of involvement of de novo mutations in LMNA and ZMPSTE24 genes.

1.5.4.5. Lethal fetal akinesia:

This disorder was observed in a newborn deceased child with a homozygous LMNA nonsense mutation (Y259X). The prominent features were facial dysmorphism with retrognathia, contracture of skin and toes, long bone fractures, almost complete absence of
fibres in intercostal muscles and immediate post-natal death due to respiratory failure. The parents had the heterozygous mutation Y259X and belonged to a large Dutch family with history of LGMD-1B (van Engelen et al. 2005; Broers et al. 2006).

1.5.5. Overlapping laminopathies

Apart from the mutations implicated in abovementioned disorders, a large number of mutations have been identified in overlapping, multi-tissue disorders. A frameshift mutation at amino acid 536 in lamin A was recently identified in a Slovenian family to cause Heart-hand syndrome, characterized by congenital cardiac disorder and limb malformations (Renou et al. 2008). Severe weakness of neck extensor muscles as seen in case of 'dropped head syndrome' can be a part of generalized neuromuscular disorders like myasthenia gravis, amyotrophic lateral sclerosis (ALS), mitochondrial disease, hypothyroidism etc. or can be caused by mutations in LMNA or SEPN1 encoding for selenoprotein N. Several mutations causing dropped head syndrome with overlapping features of EMD or LGMD-1B have been mapped to LMNA gene, for example, K32Del, R50P, L302P, E358K, R455P (D'Amico et al. 2005; Quijano-Roy et al. 2008). Another laminopathy referred to as metabolic syndrome laminopathy has increased risk of cardiovascular diseases and diabetes. The syndrome is sometimes accompanied by polycystic ovary syndrome, acanthosis nigricans and hemochromatosis. This syndrome is caused by a C-to-T transition at terminal nucleotide of exon 10, the site of alternate splicing of lamin A and C mRNA. It is a silent mutation (H566H), possibly altering the relative levels of lamin A and lamin C under pathological conditions (Steinle et al. 2004). Furthermore, LMNA mutations have also been associated with generalized muscular dystrophy with/without axonal neuropathy, lamin-related rigid spine muscular dystrophy, lone atrial fibrillation, distal motor neuropathy, and cardiovascular disorders such as arrhythmia, conduction defects and dilated cardiomyopathy (Szeverenyi et al. 2008).

1.5.6. B-type lamins and laminopathies

A few hundred mutations in LMNA have been implicated in laminopathies, but very few disorders have been attributed to mutations in B-type lamins. The underlying reason for this may be the essential function of B-type lamins, since such mutations would be detrimental for proper nuclear envelope assembly. Mutations in B-type lamins have been linked to two entirely different disorders: autosomal dominant adult onset leukodystrophy (ADLD) and Barraquer-Simons syndrome or acquired partial lipodystrophy (APL). ADLD is a slow progressive neurological disorder characterized by symmetric loss of myelin in the CNS. This disorder is caused by a tandem duplication of the genomic region containing the lamin B1 gene, thereby generating an additional lamin B1 copy (Padiath et al. 2006). It is the first disease attributed to B-type lamins. On the other hand, APL is associated with two
missense mutations (R215Q, A407T) and one polymorphism at intron-exon boundary (intron 1–6C→T) in the lamin B2 gene. The key features of APL are gradual loss of subcutaneous fat from head, neck, upper extremities, and thorax, but not from lower extremities (Hegele et al. 2006). Furthermore, a mouse model expressing an insertional mutant of lamin B1 has been generated, which has truncated lamin B1 lacking part of rod domain, NLS and CaaX motif. Homozygous mice survived the entire embryonic development period, but died neo-natally with defects in lung and bone, suggesting the essential and non-redundant function of B-type lamins (Vergnes et al. 2004). Additionally, downregulation of lamin B1 by shRNAs causes dramatic changes in nuclear envelope structure and affects localization of lamin A and lamin B2 meshworks. Also, these lamin B1 depleted nuclei had two-three blebs containing lamin A/C and nuclear pore complexes but not lamin B2, implicating B-type lamins in maintenance of lamin microdomains for proper chromatin organization and gene regulation (Shimi et al. 2008). In a recent report, knock-out mouse for lamin B2 was described, which carries an insertion of lacZ reporter in its first exon. This causes knockout of lamin B2, but not the testis-specific isoform lamin B3 (which uses a separate exon located in intron 4 of LMNB2 gene). The mice died immediately after birth with severe defects in brain, resembling lissencephaly. The authors observed that this abnormality was caused by defective neuronal migration during late stages of embryonic development (E16.5 onwards), which can be attributed to defective nucleo-cytoplasmic LINC complex formation (Coffinier et al. 2010).

1.6. Animal and cell culture models for laminopathies

Since laminopathies are rare in prevalence and exhibit varying degrees of tissue involvement and overlapping symptoms, case studies on patients can provide information mostly about the disease progression and systemic etiology. To gain better insights into the pathological alterations in cellular and tissue organization, various model systems have been adopted. For analyzing ultrastructural deformities and pathways involved in laminopathies, cell culture models derived from patients' fibroblasts and cell lines transiently expressing mutant lamins have been used. On the other hand, transgenic animal models of laminopathies have been useful in studying mechanisms of disease progression and effects of therapeutic interventions. Several animal models using C. elegans, D. melanogaster and mouse have been developed for this purpose, some of which are discussed here.

1.6.1. Animal models

Sullivan et al (1999) reported a lamin A knock-out mouse model with a deletion in the lamin A gene from exon 8 to the middle of exon 11. At birth the homozygous offspring were
indistinguishable from heterozygous and wild-type littermates. But, by 3-4 weeks of birth the LMNA\textsuperscript{+} offspring had an abnormal gait with stiff walking posture, scoliosis and other symptoms of cardiac and muscular dystrophy resembling EMD. The average life-span of these homozygous lamin A null mice was 7-8 weeks, while heterozygous mice were apparently normal (Sullivan et al. 1999). However, heterozygous (LMNA\textsuperscript{+}) mice at 1 year of age displayed atrioventricular conduction defects with defective cell and sarcoplasmic contractility, similar to those observed in laminopathy patients (Wolf et al. 2008). So far, no human patient has been reported to be completely null for lamin A and C, although presence of premature termination codons (Q6X and Y259X) has been identified in two independent reports, where heterozygous mutations lead to DCM and LGMD-1B, respectively, and homozygous Y259X leads to lethal fetal akinesia (Bonne et al. 1999; Bécane et al. 2000; van Engelen et al. 2005).

Since lamins are extensively modified at their C-termini and last 18 amino acids are proteolytically cleaved by ZMPSTE24, any perturbation in this gene may cause accumulation of prelamin A, leading to features of laminopathic cells. Transgenic mice null for this gene (Zmpste24\textsuperscript{-/-}) were independently reported by two groups. In both the studies, authors documented multiple bone fractures, splayed lower incisors, retarded growth, excessive hair loss and muscle weakness in upper and lower extremities. By 6-8 weeks after birth, the Zmpste24\textsuperscript{-/-} mice developed kyphosis of spine and died by the age of 6-7 months (Bergo et al. 2002; Pendas et al. 2002). It is worth mentioning that elimination of ZMPSTE24 protein effectively reduces levels of lamin A/C directly and emerin in an indirect way, since emerin stability and localization are dependent on A-type lamins. Therefore, Zmpste24\textsuperscript{-/-} null mice are practically null for ZMPSTE24, lamin A/C and emerin, and serve as a useful model for studying laminopathies (Pendas et al. 2002). Moreover, mice lacking emerin (emd\textsuperscript{-/-}) were found to have very mild phenotypic anomalies in skeletal muscles and cardiac tissues (Ozawa et al. 2006). Similar results have been obtained in studies involving knock-down of C. elegans emerin gene (emr1). Only potential caveat in the study is the possible interference of trace amounts of leftover emerin in rescuing the effect of emerin knock-down (Gruenbaum et al. 2002).

Mounkes et al. reported a knock-in mouse model for the L530P mutation, which is implicated in AD-EMD in humans. Surprisingly, homozygous knock-in (LMNAL530P/L530P) mice displayed characteristic symptoms of HGPS with severe post-natal growth retardation and death within 4-5 weeks after birth. As observed in case of mice carrying heterozygous LMNA\textsuperscript{+} mutations, the heterozygous LMNAL530P/wt mice had a phenotype similar to wild-type siblings (Mounkes et al. 2003a). This mouse model was later found to have a serendipitous deletion of exon 9 and subsequent in frame elimination of 40 amino acids.
from C-terminal region of lamin A and is now designated as $LMNA^{AG/A9}$. This mutation resulted in a truncated version of lamin A with constitutive farnesylation. Homozygous mice were normal during the gestation period, but developed HGPS-like symptoms post-natally. Loss of subcutaneous fat, alopecia, abnormal dentition, hyperkeratosis and severe growth retardation were observed before death by 4 weeks of age (Hernandez et al. 2010). Furthermore, two more human laminopathic mutations H222P (AD-EMD) and N195K (DCM) have been established in mouse models. The $LMNA^{H222P/H222P}$ homozygous mice develop both muscular and cardiac dystrophies, and mimic most of the symptoms observed in human patients (Arimura et al. 2005). Homozygous mice for the N195K knock-in die within 3 months after birth due to arrhythmia, but do not show any sign of muscular dystrophy (Mounkes et al. 2005). Intriguingly, all the three knock-in mouse models show a phenotype only in homozygous condition, while most of the laminopathy cases in human patients are heterozygous in nature.

To study the genotype-phenotype correlation in HGPS cases, two mouse models carrying HGPS mutations were created. One study involved creation of HGPS mice which carry a deletion in intron 10 and last 150 nucleotides of exon 11 and intron 11 from $LMNA$ gene, abolishing wild-type lamin A and lamin C expression, with exclusive yield of progerin. The mice having $LMNA^{HGI+}$ genotype exhibited retarded growth and bone deformities within 4 months after birth (Yang et al. 2006), but did not exhibit arterial lesions that are characteristic of HGPS (Yang et al. 2006). In another progeric mouse model, a human 164 kb artificial bacterial artificial chromosome (BAC) clone carrying the G608G mutation of HGPS was expressed. Although the level of progerin in these mice was very low, some of the phenotypic features of HGPS like loss of smooth muscle cells in aorta were observed after 1 year age (Varga et al. 2006). Some studies have employed expression of mutant lamins in a regulated manner using tissue-specific promoters. Mutant M371K (which causes EMD) was expressed in mouse heart using cardiac specific promoter $\alpha$-myosin heavy chain ($\alpha$-MHC) promoter. A transgenic line could not be established from these mice as they did not survive beyond 2-7 weeks of age (Wang et al. 2006). Using similar strategies, transgenic mice expressing progerin under regulation of keratin-5 and -14 promoters were established. Expression of progerin using keratin-5 promoter led to loss of dermal fat, hair follicles and sweat glands, abnormal incisors and skin (Sagelius et al. 2008). However, mice with progerin expressed under keratin-14 promoter did not display any tissue abnormality, barring misshapen nuclei (Wang et al. 2008).

Schulze et al. have studied nuclear envelope organization in transgenic Drosophila lacking lamin C. They observed separation of inner and outer nuclear membranes, chromatin leakage, clustering of nuclear pores and presence of ring like clusters of actin in
nuclei of cells lacking A-type lamins. Additionally, transgenic flies expressing rod domain mutations of A-type lamins (modeled from human laminopathy mutations) also displayed muscle abnormalities and lethality (Schulze et al. 2009). A tightly regulated expression of lamin C is essential for viability and normal development during post-embryonic stages in larval tissues as wings, thorax, legs and eyes. Our group has demonstrated that down-regulation or over-expression of lamin C in wings, thorax and muscle tissues gives rise to various deleterious phenotypes with aberrant nuclear and chromatin organization (Gurudatta et al. 2010). On the other hand, targeted misexpression of lamin C in CNS of Drosophila, which is usually negative for lamin C expression, does not alter nuclear envelope organization.

1.6.2. Cell Culture models

Several interdisciplinary studies involving fibroblasts derived from patients and cells transiently expressing mutant lamins have provided valuable insights in understanding pathological features of laminopathies at the molecular level. Exogenous expression of various 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; Manju et al. 2006). Aberrant nuclear morphology results in cellular senescence, downregulation of transcription, impaired repair of DNA damage and apoptosis (Capanni et al. 2003; Alsheimer et al. 2004; Goldman et al. 2004; Lammerding et al. 2004; Manju et al. 2006). Since a prominent feature of laminopathies is progressive loss of muscle mass leading to myopathy, several studies have employed in vitro differentiation of myoblasts to myotubes in presence of mutant lamins to ascertain whether the defects in differentiation are involved in adult onset mechanism or loss of nuclear integrity leading to enhanced cell death is the cause. In one study, long term expression of EMD causing mutant R453W in C2C12 myoblasts was observed to affect expression of myogenic markers and cell cycle progression, followed by apoptosis (Favreau et al. 2004). In addition to cell death, reduced expression of myogenic markers in presence of lamin A/C rod domain EMD mutants also impairs differentiation of these myoblasts to myotubes (Markiewicz et al. 2005; Parnaik and Manju, 2006). Delayed differentiation kinetics with reduced protein levels of MyoD and pRb have also been reported in primary fibroblasts obtained from lamin A/C knockout mice (Frock et al. 2006). Similarly, downregulation of lamin A/C by RNAi mediated silencing in myoblasts also adversely affects differentiation, which can be restored by ectopic expression of myogenic markers (Frock et al. 2006). It has been shown earlier in our lab that internal lamina is reorganized into a diffuse, insoluble network during differentiation of C2C12 myoblasts, but not in non-muscle cells.
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(Muralikrishna et al. 2001). This reorganization is dependent on expression of cyclin D3 and requires pRb (Mariappan and Parnaik, 2005; Mariappan et al. 2007), and probably maintains the quiescent state in differentiated myotubes or satellite muscle cells by sequestering pRb-cyclin D3 complexes. Thus, a likely scenario for impaired differentiation in laminopathies may be due to defects in this reorganization of internal lamins. Lamins have been suggested to suppress adipocyte differentiation, and ectopic expression of wild-type or mutant lamin A inhibits this process (Boguslavsky et al. 2006). Based on these findings, the authors propose FPLD mutations to be 'gain-of-function' mutations, which predispose the cells harbouring these mutations to aberrant adipogenesis (Boguslavsky et al. 2006).

Cells from HGPS patients and normally aged individuals have striking similarity in terms of accumulation of progerin and other nuclear deformities, suggesting the role of prelamin A in the normal ageing process (Scaffidi and Misteli, 2006). Interestingly, downregulation of progerin by using shRNAs targeting the mutated pre-spliced or mature lamin A/C mRNAs was seen to restore normal nuclear morphology, reduce number of senescent cells and improve proliferative potential (Huang et al. 2005). Similar improvement in nuclear morphology was observed in cells expressing GFP-tagged progerin upon exposure to farnesyltransferase inhibitor (FTI), PD-169541 (Columbaro et al. 2005; Glynn and Glover, 2005). In a recent study, treatment of HGPS cells with both statins and aminobisphosphonates showed restoration of nuclear morphology and reduction in accumulation of prelamin A. The treatment inhibits farnesylpyrophosphate synthesis and cross-prenylation of prelamin A. The treatment inhibits farnesylpyrophosphate synthesis and cross-prenylation of prelamin A, and has been shown to increase longevity in mice deficient in ZMPST24 (Varela et al. 2008). Accumulation of prelamin A in FPLD, MAD and atypical Werner syndrome also correlates with impaired differentiation of pre-adipocytes, reduced SREBP binding to DNA and decreased PPARγ activity (Lloyd et al. 2002; Hutchinson and Worman, 2004; Capanni et al. 2005; Maraldi et al. 2007). Furthermore, a recent study has identified reduced nuclear import and impaired dynamics of Nup153 in fibroblasts expressing human lamin A mutants causing restrictive dermopathy and HGPS (Busch et al. 2009).

1.7. Hypotheses on the role of lamins in genetic diseases

One of the most pertinent questions about laminopathies is what are the mechanisms underlying adult onset and pleiotropic phenotypes of these disorders. Although A-type lamins are expressed in most terminally differentiated cells, the severity of disease is observed more frequently in certain groups of tissues. To make the situation even more complex, the mutations are scattered all over the LMNA gene and are implicated in more than 10 different diseases without any domain-specific pathological pattern. However,
based on extensive in vitro and in vivo studies and clues obtained from symptoms displayed by patients some plausible mechanisms have been proposed. These models are not mutually exclusive and have various common attributes. Some of the models are as follows:

1. Disruption of mechanical function of lamina
2. Altered gene regulatory patterns
3. Toxic effects of prelamin A

1.7.1. Disruption of mechanical function of lamina

According to this model, the nuclear lamina is essential for maintaining structural integrity of the nucleus and hence, loss of lamin and/or its-interacting proteins leads to architectural deformities associated with fragile nuclei and decreased nuclear stiffness. These mechanically destabilized nuclei have impaired nucleo-cytoplasmic connections resulting in defective force transmission. Furthermore, attenuation of mechanosensitive genes egr-1 and anti-apoptotic gene iex-1 in LMNA<sup>−/−</sup> fibroblasts has been attributed to increased apoptosis upon application of external stimuli (Lammerding et al. 2005). Another evolving idea in the mechanical hypothesis is the disruption of LINC complexes due to disease-causing lamin mutants (Muchir et al. 2003). Intact LINC complex at the nuclear envelope is important for maintaining nuclear position within the cell and transducing signals directly from the cell surface to the nucleus (Wilson and Foisner, 2010). Consequently, deleterious mutations in lamin A or any other constituent protein of this LINC complex may be envisaged to disrupt envelope-cytoplasmic connectivity, making the cell more vulnerable to mechanical loads. In accordance with this model, disruption of cytoskeletal organization in LMNA<sup>−/−</sup> cells is seen to cause cardiomyopathies. Recent studies have also shown the role of nesprin-1 in positioning of nuclei at the post-synaptic membrane at neuromuscular junctions (Grady et al. 2005). Expression of dominant negative mutant lamin causes loss of nuclear positioning, and hence breakdown of the LINC complex by mutant lamins might be responsible for certain aspects of CMT1, DCM or CMT2B (Wilson and Foisner, 2010).

This hypothesis provides an attractive explanation for cardiac, neuromuscular and skeletal pathologies seen in EMD, DCM, LGMD and CMT. Since a skeletal muscle fibre is a syncitium, the proportion of undamaged to damaged nuclei in a fibre dictates the functionality of the muscle fibre. Myopathic condition is observed only after a particular stage (depending upon extent of tissue usage) when most of the nuclei in a fibre are senescent or apoptotic. The situation is slightly different in cardiac muscles, which are not syncitial and hence damage to the nucleus will lead to immediate cell death, hampering cardiac functions. Therefore, it can be hypothesized that lamin A mutations that disrupt
dimerization, filament assembly or membrane localization might lead to compromised nuclear integrity and ultimately to disease phenotypes (Burke and Stewart, 2002).

1.7.2. Altered gene regulatory patterns

According to the gene expression hypothesis, A-type lamins are essential for gene regulation and certain mutations can disrupt association of lamins with transcription factors and other chromatin modifiers. These altered interactions in turn might deregulate tissue-specific expression patterns of several downstream genes and thereby promote disease phenotypes. A great deal of available literature suggests the direct or indirect involvement of A-type lamins in regulation of gene expression. These regulatory functions can occur during histone modification, chromatin organization, recruitment/sequestration of transcriptional activators or repressors, mRNA biosynthesis and splicing etc. Furthermore, A-type lamins bind directly to dsDNA under in vitro conditions (Ludérus et al. 1994; Stierle et al. 2003). Also studies in D. melanogaster have identified in vivo interaction of lamin Dm0 with approximately 500 genes (Pickersgill et al. 2006).

In a study pertaining to global gene expression profiles in three fibroblast samples from HGPS patients, it was observed that approximately 361 genes (1.5% of total human genes) showed more than 2-fold altered patterns. The prominent candidates in this study were 39 transcription factors and about 30 extracellular matrix proteins, many of which are involved in embryonic development and tissue differentiation. Vascular and atherosclerosis related pathways were represented by 31 genes, followed by 22 genes involved in skeletal, limb and cartilage functions (Csoka et al. 2004). The alterations in global gene expression profiles in HGPS fibroblasts underscore the importance of lamins in the coordinated expression of genes involved in embryonic development and tissue homeostasis.

The role of lamins in regulation of muscle differentiation and adipogenesis (as discussed earlier) also support the gene expression hypothesis. For instance, deregulation of SREBP1 activity in the backdrop of lamin mutants may explain the abnormal distribution of body fat in FPLD and MAD patients (Capanni et al. 2005). The disruption of pRb-lamin A or cyclin D3-lamin A interactions by lamin mutants in differentiating myoblasts may contribute to dystrophic muscle phenotypes in EMD and LGMD patients (Favreau et al. 2004; Bakay et al. 2006; Parnaik and Manju, 2006; Emerson et al. 2009). Apart from direct involvement in gene expression, lamins also provide a structural scaffold for transcription and splicing complexes that may be disrupted by lamin mutations (Kumaran et al. 2002; Spann et al. 2002; Capanni et al. 2003).
1.7.3. Prelamin A toxicity

Homozygous mice with genotypes Zmpste24<sup>+/+</sup>, LMNA<sup>Δ9/Δ9</sup> and LMNA<sup>HG+/</sup> display several features of progeria including growth retardation, retarded growth, alopecia, osteolytic lesions, micrognathia and osteoporosis (Bergo et al. 2002; Pendas et al. 2002; Mounkes et al. 2003b; Yang et al. 2006). A hallmark feature observed in all these mouse models is the accumulation of farnesylated prelamin A in the nucleoplasm and nuclear periphery. These nuclei have abnormal morphology and blebbings due to accumulation of farnesylated prelamin A in the nuclear membrane, which interferes with normal lamina formation as observed in HGPS cells. Downregulation of ZMPSTE24 protease by siRNA in HeLa cells also leads to accumulation of partially processed prelamin A both in the nucleoplasm and nuclear periphery. This accumulation results in cell cycle arrest and apoptosis, indicating that accumulation of the farnesylated form of prelamin A is toxic (Gruber et al. 2005). Farnesylated prelamin A also blocks global transcription leading to growth arrest (Candelario et al. 2011). Treatment of cells with FTIs prevents this farnesylation and restores normal nuclear morphology (Toth et al. 2005) and restores heterochromatin (Columbaro et al. 2005). Similarly, it has been reported that treatment of HGPS cells with a combination of statins and aminobisphosphonates ameliorates nuclear morphology defects and reduces accumulation of prelamin A. Furthermore, this treatment also increases longevity in mice deficient in ZMPSTE24 (Varela et al. 2008), reaffirming the fact that prelamin A accumulation in cells is toxic and may be a major player in laminopathies that display progeroid features.

1.8. Epigenetics: A language beyond the four nucleotides

Until the advent of this decade, the genome was considered to be a unidimensional entity that was solely dependent on the DNA sequence. But in the post human genome sequencing era, the study of information beyond the genome has gained pace. In abstract terms this study can be referred to as ‘epigenetics’. The inherent complexity of the field is truly reflected by the ways the term epigenetics is defined by various scholars. To Conrad Waddington, ‘epigenetics is studying how phenotypes are determined by genotype during development’ (Waddington, 1957). Arthur Riggs considered that the inheritability aspect of epigenetic modification was more important and defined epigenetics as the ‘study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by DNA sequence alone’ (Russo et al. 1996). In a simplistic and unified view, epigenetic events are associated with both stable and transient changes at DNA and chromatin levels to establish patterns of gene expression or repression during development. The multifold epigenetic regulatory functions can be orchestrated by several components including DNA methylation, histone modifications, non-coding RNAs and transcriptional factors. These
apparently different pathways intersect frequently and depend on each other for their maintenance and propagation. For instance, methylation of a cytosine residue at a CpG dinucleotide frequently coincides with heterochromatic loci, whereas unmethylated CpG-rich chromatin has hyperacetylated histones (Bird and Wolffe, 1999; Fuks, 2005).

Initially, Heitz categorized chromatin into two distinct domains, euchromatin and heterochromatin. In an interphase nucleus, euchromatin appears light while heterochromatin is seen as a darker structure under the light microscope (Heitz, 1928). This staining pattern of euchromatin is due to its open and unfolded conformation, which is accessible to transcriptional and replication complexes. On the contrary, much of the heterochromatin is a transcriptionally repressive compartment of chromatin by virtue of its closed conformation, which restricts access of trans-acting factors to regulatory DNA sequences. Euchromatin replicates earlier than heterochromatin and has higher density of genes, most of which are generally active. Furthermore, heterochromatin can be subdivided into facultative heterochromatin and constitutive heterochromatin. In lower eukaryotes, most of the chromatin remains as euchromatin and only telomeres and centromeres constitute constitutive heterochromatin, while in higher eukaryotes due to increased genome size a larger proportion of the genome is heterochromatized. In higher eukaryotes constitutive heterochromatin is present mostly at telomeres, centromeres, repetitive and non-coding sequences (Talbert and Henikoff, 2006; Grewal and Jia, 2007). However, facultative heterochromatin of higher eukaryotes represents a transitive phase between euchromatin and constitutive heterochromatin, whose expression is context dependent, for instance, temporal (developmental or cell-cycle specific genes), spatial (nuclear localization to centre or periphery) or inherited (monallelic genes).

1.8.1. Histone code or an evolving language of chromatin?

Peptide mass finger printing has shown that histones can be modified at more than 60 individual amino acid positions in their tail regions by at least eight different covalent post-translational modifications (PTMs) such as phosphorylation, acetylation, methylation, sumoylation, ubiquitination, ADP ribosylation, deimination and proline isomerization (Kouzarides, 2007). These modifications are essentially dynamic and reversible in nature, allowing coordinated regulation of chromatin compaction and relaxation. Various studies have shown 13 such sites for histone H2A, 12 for histone H2B, 21 for histone H3 and 14 for histone H4 (Zhang et al., 2003). In addition, the lysine residues can have mono-, di- or tri-acetylation or methylation. Methylation has been observed on the arginine residue also, but unlike lysine methylations only mono- and di- methylations of arginine have been reported. The combinatorial repertoires of these histone modifications are numerous and constitute a set of instructions for higher order chromatin assembly and efficient regulation of gene
expression. This pattern of histone modifications is called the histone code (Strahl and Allis, 2000). The histone modifications are carried out by a battery of enzymes called 'writers of PTMs' (Allis et al. 2007), which are specific for each PTM event. Some of these writers and modifiers are: histone acetyltransferases (HATs) for acetylation of specific lysines (e.g. HAT1, CBP/p300, pCAF/GCN5), deacetylases (e.g. SirT2), lysine methyltransferases (e.g. SUV39H1, G9a, ESET/SETDB1, RIZ1), lysine demethylases (e.g. LSD1, JMJD2B, JMJD2D), arginine methyltransferases (e.g. CARM1, PRMT4, PRMT5), serine/threonine kinases (e.g. Haspin, CKII, Mst1), ubiquitin ligases (e.g. Bmi/Ring1A, RNF20/RNF20) and proline isomerases (e.g. ScFPR4).

1.8.2. Histone modifiers: Gatekeepers of chromatin state

Histone modifications may affect chromatin assembly by altering interactions between histones of adjacent nucleosomes or DNA present in the same nucleosome. In general, acetylation is associated with transcriptionally accessible state of chromatin or euchromatin, while methylation is responsible for closed state or heterochromatization, although several exceptions have been identified. Similarly, phosphorylation of histones has been attributed to specialized functions in mitosis, apoptosis and gametogenesis (Kouzarides, 2007). Studies on individual gene loci have identified that active chromatin is enriched in acetylated histones H3, H4 and H2A and methylated lys4 of H3. Conversely, hypoacetylation coupled with trimethylation of H3lys9 (H3K9me3) is characteristic of inactive chromatin (Litt et al. 2001). Interestingly, a plethora of reports have converged on the fact that the 'histone code' is far more complex than the combinatorial histone modifications. The so called ‘bivalent chromatin domain’ shows simultaneous presence of both repressive (H3K27me3) and activating (H3K4me3) marks. These bivalent domains represent transcriptionally poised genes such as developmental genes, which are repressed at a particular stage and reactivated later on (Azuara et al. 2006; Bernstein et al. 2006).

According to a theoretical prediction, combination of all the known histone modifications would yield ~256 X 10^6 distinct patterns, which would need an equally efficient set of histone code ‘readers’ to de-code this voluminous information. Some of the identified proteins contain discrete structural features called bromo, chromo or 14-3-3 domains, which bind to specific modifications of acetylated lysine, methylated lysine or phosphorylated serine/threonine residues respectively. Several other domains have also been identified that are found predominantly in chromatin associated proteins, including tudors, PhDs, SANTs, SWIRMs, MBTs and PWWPs. Among these, chromo-, tudor, MBT and PWWP domains share functional similarity and are called the ‘royal family’ domains (Maurer-Stroh et al. 2003). Proteins harboring these domains act as adapters between histone or DNA and large enzymatic complexes (Daniel et al. 2005).
1.8.3. HP1 protein family

A family of chromatin modifier organizer (chromo)-domain containing proteins referred to as Heterochromatin Protein-1 (HP1) presents a classic case of histone code readers. It is a structurally and functionally conserved protein family with orthologs found in all eukaryotes ranging from fission yeast to human (Eissenberg et al. 1990). *Neurospora crassa* has a single HP1 gene (hpo-1), while *C. elegans* harbours two HP1 isoforms (HPL-1 and HPL-2). No HP1 gene has yet been identified in budding yeast (*S. cerevisiae*) while *S. pombe* has three (Swi6, Chp1 and Chp2). Five HP1 proteins have been documented in *Drosophila*, out of which HP1a, HP1b and HP1c are ubiquitously expressed, and the other two (HP1d/Rhino and HP1e) are predominantly expressed in the germline (Vermaak et al. 2005). Mammals have been shown to have three HP1 isoforms (HP1α, HP1β and HP1γ) and more than ten HP1 pseudogenes (Norwood et al. 2004). It is worth mentioning that the genomic structure of HP1-encoding genes is conserved from *Drosophila* to humans. The gene encoding *Drosophila* HP1 designated as *Su(var)2-5*, genes coding for mouse HP1 isoforms α, β and γ (*Cbx5, Cbx1* and *Cbx3*, respectively) and human HP1 proteins (*Cbx5, Cbx1* and *Cbx3*) each contain five exons and four introns (Jones et al. 2001). Moreover, the human *Cbx* genes are located on chromosomes 12, 17 and 7 for the respective isoforms, which are syntenic in mouse at chromosomes 15, 11 and 6, respectively (Norwood et al. 2004). This stringent conservation of genomic structure and location of HP1 family during the course of evolution corroborates with their essential non-redundant functions. The HP1 variants differ in their subcellular localization, post-translational modifications, biochemical properties, binding partners and genetic redundancy (Sadaie et al. 2008). The HP1α and β mammalian isoforms are found to be localized, although not exclusively, in pericentric heterochromatin (*Nielsen et al. 1999*) and telomeric heterochromatin, and in minor cases in euchromatin. In contrast, HP1γ is found in both euchromatin and heterochromatin (Hiragami and Festenstein, 2005; Hediger and Gasser, 2006). These HP1 isoforms share conserved functions across species from flies to humans with approximately 50% sequence identity between them. Overall sequence identity between the three human isoforms of HP1 is around 66% (Furuta et al. 1997). Contrary to popular notion that HP1 proteins are equally abundant in all cells, a recent study has shown existence of 'distinct HP1 repertoires' in various tissues (Ritou et al. 2007). Generalized reduction in HP1 levels is known to occur during erythropoiesis and granulopoiesis (Gilbert et al. 2003; Lukássová et al. 2005), while during lymphocyte differentiation HP1α mRNA levels are increased significantly (Klein et al. 2003). Reduction in HP1α and HP1β isoforms also correlates with increased metastatic ability of cancerous cells (Kirschmann et al. 2000; Nishimura et al. 2006).
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Structurally, HP1 proteins are small, non-histone chromosomal proteins, made up of a conserved N-terminal chromo-domain (CD), a less conserved hinge or intervening region (IVR) and conserved C-terminal chromoshadow domain (CSD) (see Fig. 1.3). The CD binds to methylated lysine 9 residue of histone H3 (Lachner et al. 2001; Nakayama et al. 2001) and histone fold motif of histone H3 (Nielsen et al. 2001). Dimerization of two HP1 molecules creates a hydrophobic pocket in the CSD, where the proteins carrying a consensus pentapeptide motif PXVXL (X = any amino acid) called the HP1 box can bind (Le Douarin et al. 1996; Thiru et al. 2004). Several HP1 interacting proteins such as p150 large subunit of chromatin assembly factor 1 (CAF1), TATA binding protein (TBP)-associated factor of 130 kDa (TAF1130) and Transcription Intermediary Factors (TIFs) carry this canonical HP1 box (Hiragami and Festenstein, 2005). Presence of a non-canonical or variant PXVXL motif has been reported in some proteins such as LBR, ATRX and Sp100a (Lechner et al. 2005). Additionally, the HP1 hinge region can bind directly to RNA, linker histones and telomeric DNA in a sequence independent manner (Muchardt et al. 2002; Perrini et al. 2004).

1.8.4. Mechanisms of heterochromatin formation and maintenance

The most common function ascribed to HP1 proteins is formation and maintenance of heterochromatin along with histone methyltransferases (HMTases). According to a widely accepted model, HP1 gets recruited to H3K9me3 through the action of methyltransferases-Suv39H1 and Suv39H2 (Stewart et al. 2005). In turn, HP1 recruits histone deacetylases and methyltransferases by direct binding and creates a positive feedback loop for the spreading of heterochromatin in cis, along the chromosome unless a boundary element is encountered. This recruitment of HP1 and methylated H3K9 subsequently causes tight crosslinking of nucleosomes and formation of highly compacted chromatin structure at pericentric or telomeric heterochromatin (Bannister et al. 2001; Lachner et al. 2001). In euchromatic loci, this repressive effect of HP1 is short range (upto 2 kb) with or without involvement of heterochromatin formation. Several lines of evidence indicate interaction of individual HP1 isoforms with sequence specific DNA binding proteins in silencing of target genes. Additionally, dimethylation of histone 3 lysine 9 (H3K9me2) catalyzed by G9a HMTase, can facilitate recruitment of HP1 at euchromatic genes. This bound HP1 then acts as molecular ‘beacon’ for methylation of neighbouring CpG residues by DNA methyl transferase (DNMT1). Presence of HP1 for translation of H3K9 methylation to DNA methylation is indispensable in Neurospora and mammals, but not in Arabidopsis (Freitag et al. 2004). The CpG methylation in turn, propagates the methyl H3K9 mark by recruiting HDAC complexes and serves as positive feedback loop for shutting down gene expression
<table>
<thead>
<tr>
<th>Domain Function</th>
<th>HP1α</th>
<th>HP1β</th>
<th>HP1γ</th>
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<tr>
<td>Heterochromatization, Telomere silencing</td>
<td>Chromodomain</td>
<td>Chromodomain</td>
<td>Chromodomain</td>
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<tr>
<td></td>
<td>Hinge</td>
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<td></td>
<td>1-14</td>
<td>1-15</td>
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<td>71</td>
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<td>71</td>
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<td></td>
<td>Chromo Shadow domain</td>
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<td>Chromo Shadow domain</td>
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<td></td>
<td>116-171</td>
<td>112-172</td>
<td>106-173</td>
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<td></td>
<td>191</td>
<td>185</td>
<td>166-173</td>
</tr>
<tr>
<td>Nuclear targeting, Dimerization domain</td>
<td>H3K9me3 binding</td>
<td>RNA, chromatin</td>
<td>HP1, LBR, ATRX, CAF1, Dnmt1, Dnmt3a</td>
</tr>
<tr>
<td>Binding Partners</td>
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Fig. 1.3. A schematic representation of human Heterochromatin Protein-1 (HP1) isoforms. The domain organization of individual HP1 isoforms α, β and γ are shown with their common binding partners and their functional domains. The numbers above the domains represent amino acid positions.
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(Smallwood et al. 2007). Thus, the code embedded in methylation marks flow from histone to DNA and back for quasi-stable inheritance of silencing information.

Expression of several genes involved in endodermal differentiation in F9 cells is regulated by association of HP1 with transcriptional repressor (TIF1β/KAP1) proteins (Cammas et al. 2004). Interaction of HP1γ isoform with E2F-6 complex at the promoters of E2F- and c-myc responsive genes such as E2F-1, c-myc and cdc25A has been identified in quiescent cells (Ogawa et al. 2002). Direct binding of LXCXE (X=any amino acid) motif in HP1 to pocket domain of pRb and SUV39H1 HMTase silences pRb regulated-cyclin E promoter (Nielsen et al. 2001). Human TAFii130 (and also Drosophila TAFii110) are essential activators of the general transcription complex TFIID. Isoform specific interaction of HP1α and HP1γ with TAFii130 may either prevent access of TFIID to its cofactors or may stall the progression of promoter-bound ‘preloaded’ TFIID complex, thereby repressing the genes poised for rapid activation (Vasallo and Tanese, 2002). The propagation of heterochromatization in these genes may involve interaction of HP1 with euchromatic HMTase G9a and HDACs (Hiragami and Festenstein, 2005). Although the role of HP1 proteins in gene regulation and chromosomal structure maintenance has been studied extensively, the specific gene cluster occupancy of HP1 proteins is largely unknown. In a report employing DamID method, target genes for HP1β in mouse and human cells were identified. The authors observed that in both the species a large gene family of Krüppel-associated box containing zinc finger (KRAB-ZNF) transcriptional repressors are bound by HP1β and SUV39H1 (Vogel et al. 2006). This family encodes for approximately 800 putative transcription factors in human (Knight and Shimeld, 2001). More than half of these human KRAB-ZNF genes are located as clusters on chromosome 19 (Grimwood et al. 2004). HP1β was found to be enriched at the 3’ end of KRAB-ZNF genes, but absent from the promoters (Vogel et al. 2006). This implicates HP1 proteins in gene regulatory functions mediated by KRAB-ZNF transcriptional factors.

1.9. Objectives of the study

Mutations in genes encoding nuclear lamins cause pleotropic, debilitating disorders with adult-onset and tissue specific phenotypes. Several studies have suggested abnormal heterochromatin organization in fibroblasts derived from HGPS and MAD-A patients (Goldman et al. 2004; Filesi et al. 2005). Along with perturbation in heterochromatin organization, defects in positioning of specific chromosomes are also evident in laminopathic cells (Meaburn et al. 2007). Importance of lamins in chromatin maintenance is further supported by studies where fragmented heterochromatin is seen in Lmna−/− mouse cardiomyocytes (Nikolova et al. 2004) and developmental defects in lamin null mutants in C. elegans (Liu et al. 2000a). Interestingly, a homozygous nonsense LMNA mutation
Y259X causes mislocalization of emerin and nesprin-1α, which are subsequently degraded by the proteasomal machinery (Muchir et al. 2006). Similarly, lamin A knock-out mice have reduced stability of pRb leading to developmental disorders (Johnson et al. 2004). Thus, laminopathic cells present an interesting system to study the role of lamins in maintenance of nuclear architecture and chromatin organization, and in stabilization of nuclear proteins. Several studies have established transient overexpression of tagged lamin A mutant constructs in mammalian cell lines to overcome the problem of availability of patient fibroblasts. Incorporation of these mutant lamins into the nuclear lamina mimics the nuclear envelope organization in patient fibroblasts (Östlund et al. 2001; Raharjo et al. 2001; Vigoroux et al. 2001; Bechert et al. 2003; Favreau et al. 2003). Using similar approaches, previous studies in our lab have shown that lamin A mutants that cause EMD assemble in abnormal intranuclear aggregates and impair DNA repair and differentiation pathways (Manju et al. 2006; Parnaik and Manju, 2006). One key investigation in our lab showed that misexpression or depletion of A-type lamins in Drosophila caused depletion of HP1 levels, with a concomitant alteration in nuclear morphology (Gurudatta, 2006). Therefore, the present study was planned to investigate the role of lamin mutants in heterochromatin organization in mammalian cells as described below:

1. To examine the expression patterns of different disease-causing lamin mutants in mammalian cell lines.
2. To study the effects of ectopic expression of mutant lamins on markers of heterochromatin such as HP1 and modified histones such as H3K9\textsuperscript{me3}, H4K20\textsuperscript{me3} and H3K9-14\textsuperscript{ac2}.
3. To identify the mechanisms underlying the reduction of the heterochromatin marker-HP1.
4. To ascertain whether this perturbation of HP1 isoforms has any effect on target genes.