1. INTRODUCTION

The principal feature that distinguishes a eukaryotic cell from its prokaryotic counterpart is the presence of distinct compartments. The two major compartments in a eukaryotic cell are the cytoplasm, which is bounded by the plasma membrane, and the nucleus which is demarcated from the cytoplasm by the nuclear envelope. The nuclear compartment communicates with the cytoplasmic compartment through specialized pores termed nuclear pore complexes. The cytoplasmic compartment contains internal membrane-bound compartments or organelles such as mitochondria, endoplasmic reticulum (ER), Golgi apparatus, lysosomes and peroxisomes that carry out specialized functions like ATP-synthesis, protein and lipid synthesis, secretion and sorting of proteins, protein degradation and detoxification respectively.

1.1 Nucleus

The nucleus harbours the machinery for processes such as DNA replication, transcription and RNA processing. The boundary of the nucleus is marked by a well-defined nuclear envelope, which regulates macromolecular transport in and out of the nucleus in a temporal and spatial manner. The nucleus contains domains or sub-compartments that are enriched in specific proteins and carry out distinctive functions, for example, the nucleolus and splicing factor compartments.

The nuclear envelope has a complex organization consisting of three prominent components: (i) the inner and outer nuclear membranes that are separated by a lumenal space; (ii) the nuclear pore complexes (NPCs) which are macromolecular assemblies embedded in the nuclear envelope and mediate bi-directional transport between the cytoplasm and the nucleus of interphase cells; and (iii) the nuclear lamina, a filamentous protein network that lines the nucleoplasmic face of the inner nuclear membrane and also extends into the interior of the nucleus (Fig. 1.1).

1.1.1 Nuclear membranes

The nuclear envelope consists of two lipid bilayers termed the outer and inner nuclear membranes. The outer nuclear membrane exhibits continuity with the rough endoplasmic reticulum to which it bears a functional resemblance. The inner membrane
Fig. 1.1. Schematic diagram of a typical eukaryotic nucleus depicting lamin B, lamin A, chromatin and the proteins interacting with lamin A and the inner nuclear membrane. Lamins are mainly concentrated beneath the inner nuclear envelope and also distributed throughout the nucleoplasm, where they interact with many proteins and associate with splicing factor compartments (SFC).
is lined by a network of lamin filaments on the nucleoplasmic face (Goldberg and Allen, 1995). Attachment of the lamina to the inner nuclear membrane has been proposed to be mediated by at least three integral membrane proteins (Gerace and Foisner, 1994), lamina-associated polypeptide 1 (LAP1) and its isoforms LAP1A, LAP1B and LAP1C; lamina-associated polypeptide 2 (LAP2) and the lamin B receptor (LBR). LAP1C and LAP2 are transmembrane proteins and have an N-terminal segment residing in the nucleoplasm, a single transmembrane segment and a C-terminus localized in the lumen. On the other hand, LBR has eight membrane spanning domains near its C-terminus. Other inner nuclear membrane proteins that have been identified include emerin, nurim, MAN1, otefin, nesprins 1 and 2, Sun1 and Sun2 ((Schirmer, 2002; Crisp et al., 2006). Emerin shares 40% homology with LAP2 and otefin. Otefin, a Drosophila protein, is a peripheral membrane protein having an N-terminal nucleoplasmic segment (Ashery-Padan et al., 1997). The outer and inner nuclear membranes enclose the nuclear envelope lumen. The lumen provides a specialized environment for the lumenal domains of the outer and inner nuclear membrane proteins, which anchor the NPCs and the lamina.

The nuclear membrane is a dynamic structure exhibiting growth in S-phase of the cell cycle and vesicularization at mitosis. In ‘open mitosis’ the nuclear envelope and NPCs break down as cells enter the mitotic phase of the cell cycle and then reform after chromosomal segregation takes place in a Ran-GTPase-dependent manner. Open mitosis occurs in higher eukaryotes like plants and animals. But in ‘closed mitosis’ chromosomal segregation occurs with an intact nuclear envelope. Closed mitosis occurs in lower eukaryotes like yeast and other fungi (Sazer, 2005).

1.1.2 Nuclear pore complex

Structural characterization of the NPC by electron microscopy has revealed that it is a large and complex structure of 120 million daltons, having a diameter of 1200 Å. It has a thickness of 500 Å and spans the outer and inner nuclear membranes. 3D structures of NPC have been derived from electron tomography analysis. The NPC consists of a central framework with eight-fold symmetry called the ‘spoke complex’. The central framework is a ring-like assembly around a central pore which is the exclusive gateway for nucleocytoplasmic transport pathways. The central pore has a
length of about 90 nm and maximum diameter of 25-30 nm. This central framework is surrounded by a cytoplasmic ring and a nuclear ring. The cytoplasmic ring consists of eight kinked cytoplasmic filaments and the nuclear ring is capped by a nuclear basket of filaments (Fahrenkrog et al., 2004). The NPC consists of a set of proteins which are collectively termed nucleoporins (Nups). Lamin filaments have been proposed to interact with the nuclear ring of NPCs. In Drosophila mutants that are null for lamin Dm0, the NPCs do not have fixed positions and are clustered together (Lenz-Bohme et al., 1997). In Xenopus egg extracts supplemented with a dominant-negative lamin mutant, the nuclear pore basket protein Nup153 is not incorporated in NPCs, and in Xenopus egg extracts depleted of Nup153 NPCs show increased mobility and cluster together in the nuclear envelope (Smythe et al., 2000; Walther et al., 2001).

1.1.3 Nuclear sub-compartments

The mammalian cell nucleus is a highly organized organelle with morphologically distinct compartments. The individual chromosomes have been suggested to occupy distinct positions within the nucleus (Cremer et al., 1982). Apart from spatial organization of chromosomes the nucleus also contains numerous, distinct sub-compartments. The best characterized nuclear sub-compartment is the nucleolus, the site of rRNA synthesis.

1.1.3.1 The nucleolus

The nucleolus is a large nuclear domain and is also known as the 'ribosome factory'. Ribosome biogenesis involves the transcription of ribosomal genes (rDNA) by RNA pol I. The human rDNA genes are located in approximately 400 copies of a 43 kb human ribosomal gene repeat, and are distributed among the short arms of human acrocentric chromosomes (chromosomes 13, 14, 15, 21 and 22). Head-to-tail tandemly repeated gene clusters representing on an average 3 mb of DNA are termed NORs (nucleolar organizer regions) (Heliot et al., 1997). Formation of the nucleolus is both transcription and cell cycle dependent. In higher eukaryotes, the nucleolus is assembled at the transition of mitosis and interphase (Hernandez-Verdun, 2004). During mitotic events the rDNA transcription machinery remains associated with rDNA. When cells exit from
mitosis, reactivation of rDNA transcription and nucleolar assembly around the rDNA occurs (Roussel et al., 1996).

In yeast more than 60 transcription factors are necessary for efficient assembly of ribosomes (Kressler et al., 1999). These factors follow a specific order of assembly on the pre-rRNA followed by processing and modification of precursor rRNA transcripts, leading to the formation of a 90S pre-ribosomal particle. The 90S pre-ribosomes are subsequently processed to yield large 66S (28S and 5.8S rRNAs) and small 43S particles (20S rRNA) which are the precursors of mature 60S and 40S subunits (Kressler et al., 1999; Fatica and Tollervey, 2002).

1.1.3.2 Splicing factor compartments

Splicing factor compartments are domains that are involved in the storage of splicing factors and their recruitment to sites of transcription. Splicing factor compartments are also known as speckles. ‘Splicing speckles’ are 0.2 – 0.5 μm in diameter. Each speckle is composed of 5-50 sub-speckles (Mintz and Spector, 2000). Speckles are enriched in pre-messenger RNA splicing factors, snRNAs, transcription factors, 5’ and 3’ processing factors, ribosomal proteins, nascent transcripts, mature polyadenylated mRNAs, splicing factor specific protein kinases, phosphatases, and large subunit of RNA pol II (Lamond and Spector, 2003). A reversible protein phosphorylation mechanism regulates the movement of speckle components between speckles and transcription sites where spliceosomes are assembled. A spliceosome consists of five small nucleoprotein particles (snRNPs) designated as U1, U2, U4, U5 and U6 snRNPs and a large number of non-snRNP proteins, which include the essential SR family proteins. SR proteins have either a single RNA recognition motif (e.g. SC-35, SRP20) or two RNA recognition motifs (e.g. SF2/ASF) at the N-terminus and an RS domain (arginine and serine residues) at the C-terminus (Zahler et al., 1992). SR proteins affect the selection of the 5’ splice site (Ge and Manley, 1990) and stimulate the binding of snRNP particles to the 5’ splice site (Eperon et al., 1993).
### 1.1.3.3 Cajal bodies

Cajal bodies (CBs) are sub-nuclear domains that appear as a tangle of coiled threads, and are also referred to as coiled bodies (Carmo-Fonseca, 2002). The size of CBs varies from 0.1 – 2 μm in diameter and the number of CBs varies from 0-10 per nucleus. Cajal bodies contain many factors involved in transcription, RNA processing and regulation of cell cycle (Ogg and Lamond, 2002). The major proteins present in CBs are coilin, SMN (survival of motor neuron), fibrillarin, Nopp140, GAR1, Gemini3 and Tgs. Cajal bodies are involved in post-transcriptional modification of newly assembled snRNA and in the maturation of snRNPs. snRNPs or snoRNPs (small nucleolar RNPs) from the cytoplasm enter into Cajal bodies where 2’-0-methylation and pseudouridylation occurs. Mature snoRNPs move from Cajal bodies to the nucleolus to participate in rRNA modification, while snRNPs accumulate in nuclear speckles and function in splicing of pre-mRNA (Ogg and Lamond, 2002). Proteins that promote the assembly of U4/U6 and U4/U6/U5 snRNPs are enriched in CBs (Makorova et al., 2002).

### 1.1.3.4 Gems

The SMN complex localizes in separate coiled body-like structures called ‘gems’ (Liu and Dreyfuss, 1996). The SMN protein plays a crucial role in coordinating the assembly of Sm proteins onto newly synthesized snRNAs which are exported into the cytoplasm (Paushkin et al., 2002). The U1, U2, U4 and U5 snRNAs transcribed by RNA pol II have a monomethylated guanosine cap. The association of Sm proteins with a given snRNA is a prerequisite for 3’ end trimming and hypermethylation of the cap, generating the characteristic 2,2,7-trimethylated guanosine form (Will and Luhrmann, 2001). Loss of function mutations in SMN gene are predominantly responsible for the severe inherited disorder spinal muscular dystrophy (SMA) which is characterized by degeneration of motor neurons.

### 1.1.3.5 PML bodies

PML (promyelocytic leukemia) bodies are small spherical domains scattered throughout the nucleoplasm. Cells typically express 5-30 PML bodies per nucleus,
ranging in size from 0.2 – 1 μm (Melnick and Licht, 1999). PML bodies play a crucial role in a wide variety of cellular processes such as cell cycle regulation, apoptosis, proteolysis, tumour suppression, DNA repair and transcription (Ching et al., 2005). PML protein is a constituent of PML bodies (Dyck et al., 1994). PML body associated proteins include BLM (Blot Asyndrome Protein), CBP (CREB-binding protein, a histone acetyl transferase), Daxx, Hipk2, Mdm2, NBS, p53, Sp100, SUMO-1, TRF1 and TRF2 (Ching et al., 2005). The association of PML bodies with chromatin brings together specific gene loci that are regulated by PML associated proteins. PML bodies are of clinical interest in acute promyelocytic leukaemia since they are disrupted in cell lines derived from patients owing to the formation of a fusion protein between PML protein and retinoic acid receptor α, as a consequence of a chromosomal reciprocal translocation (Dyck et al., 1994; Koken et al., 1994).

1.2 Lamin structure and gene organization

1.2.1 Lamin isoforms

Nuclear lamins are karyoskeletal proteins which form a filamentous meshwork underlying the inner nuclear membrane. Lamins belong to the intermediate filament (IF) gene super family. Lamins are involved in the organization and functions of the nucleus (Gerace and Burke, 1988; Goldman et al., 2002). In mammalian somatic cells (including those of human, rat and mouse), the lamina is composed of four major lamin proteins A, B1, B2 and C having molecular masses of 70, 68, 66 and 60 kDa (Gerace et al., 1978). The A-type lamins include lamin A and lamin C which have a neutral isoelectric point and get completely solubilized and dispersed when the nuclear lamina transiently disassembles during mitosis. In contrast, B-type lamins are more acidic and remain associated with membranes during mitosis (Kaufmann et al., 1983). The expression of lamins is developmentally regulated (Krohne and Benavente, 1986). Both undifferentiated embryonal carcinoma cells and early embryos express B-type lamins whereas the expression of A-type lamins has been detected in differentiating tissues of the mouse embryo when development of organs commences (Lebel et al., 1987; Rober et al., 1989). In mammalian spermatocytes, three germ-cell specific lamin isoforms have been identified. Lamin C2, is a 52 kDa protein bearing structural homology to somatic
lamin C (Furukawa et al., 1994). Lamin B3, is a 53 kDa variant of somatic B-type lamins (Furukawa and Hotta, 1993) and a third 60 kDa lamin, is closely related to somatic B-type lamins (Sudhakar et al., 1992; Vester et al., 1993). Chickens express two kinds of lamins, lamin B1, a minor protein which has a molecular mass of 68 kDa and is immunologically related to mammalian lamin B, and a major protein lamin B2 which is related to lamin A (Lehner et al., 1987). The amphibian Xenopus has at least five different types of lamins. LI and LII are the Xenopus homologs of mammalian lamins B1 and B2 respectively. LA is a homolog of lamin A. LIII is a lamin that is considered to be a B-type lamin. LIV is a sperm-specific lamin (Benavente and Krohne, 1985). Invertebrates typically have few lamins. Drosophila melanogaster expresses two lamin genes termed lamin Dm0 and lamin C (Gruenbaum et al., 1988). Lamin Dm0 is expressed essentially in all cells throughout development and shares similarities with B-type lamins. Lamin C shows a regulated pattern of expression and resembles an A-type lamin (Bossie and Sanders, 1993). Lamin C is expressed late in embryonic development in many differentiated cell types. Caenorhabditis elegans expresses a single B-type lamin, LMN-1 (also known as Ce-lamin) (Riemer et al., 1993). Genomic sequence analysis of yeast and Arabidopsis indicates that these species do not have lamin genes.

1.2.2 Structure of lamins

The lamin proteins are highly conserved throughout evolution. Like all IF proteins lamins possess a tripartite structure consisting of a central $\alpha$-helical rod domain flanked by a non $\alpha$-helical N-terminal head and C-terminal tail domain. The $\alpha$-helical rod domain contains a characteristic heptad repeat pattern (McLachlan, 1978). The rod domain of nuclear lamins is 42 amino acids longer than the rod domain of cytoplasmic vertebrate IF proteins (Weber et al., 1989b). The $\alpha$-helical rod domains of two lamin molecules can wrap around each other in parallel, unstaggered fashion with hydrophobic side chains of one $\alpha$-helix interacting with hydrophobic side chain of the other, leading to the formation of a two stranded $\alpha$-helical coiled-coil structure. The $\alpha$-helical rod segments (1A, 1B, 2A and 2B) are separated by linker segments L1, L12 and L2 (Conway and Parry, 1990). The linker regions are highly conserved with respect to amino acid sequence and length. The length of the rod domain is 354 amino acids. Segments of 16 amino acids at the N-
terminal end and 30 amino acids at the C-terminal end of the rod domain are highly conserved among lamins and IF proteins. Lamins contain a nuclear localization sequence (NLS) at their C-terminal tail domain which is homologous to the NLS of SV40 large T-antigen. The NLS consists of an invariant lysine, followed by three other basic residues (Loewinger and McKeon, 1988). There are phospho acceptor sites within the two regions flanking either end of the α-helical rod domain. Serine or threonine residues are phosphorylated during mitosis by the mitotic specific Cdc2 kinase (Maller et al., 1989). Phosphorylation is a critical step for controlling longitudinal association of lamin dimers to form head-to-tail polymers (Geiffers and Krohne, 1991). Phosphorylation of the N-terminal phospho-acceptor sites but not the C-terminal phospho-acceptor sites inhibits head-to-tail assembly of lamins (Peter et al., 1991). Cdc2 kinase acts directly on nuclear lamins. Other kinases too can phosphorylate lamins at M-phase specific sites and may thus be involved in controlling lamina assembly/disassembly (Peter et al., 1992). Phosphorylation of sites in the lamin tail domain by protein kinase C βII is responsible for disassembly of nuclear lamins in certain cell types (Goss et al., 1994).

All known members of the lamin protein family except mammalian lamin C contain a C-terminal motif commonly referred to as the CaaX box (C: cysteine, a: aliphatic amino acid, X: any amino acid) which is the target for post-translational modification. This motif is also found in members of the ras family of small GTP binding proteins and yeast mating pheromones (Magee and Hanley, 1988). CaaX tetrapeptide is the substrate for a series of post-translational modifications, namely, isoprenylation of cysteine, followed by proteolytic removal of the three C-terminal amino acids, and carboxyl methylation of the resulting C-terminal isoprenylated cysteine residue (Glomset et al., 1990; Rine and Kim, 1990). Isoprenylation is required for increasing hydrophobicity to enable targeting of lamins to the inner surface of the nuclear envelope.

The structure of the globular tail of lamin A reveals a compact, well defined domain composed entirely of β strands. Two large β sheets form a β sandwich. One sheet has five β strands and the other has four. A second smaller β sheet lies perpendicular and adjacent to the plane of the β sandwich. This type of β sandwich is referred to as the immunoglobulin (Ig) domain. The lamin A tail represents the prototype for a new class of
Ig-related domain, which is called the L sub-type or lamin sub-type (Dhe-Paganon et al., 2002).

1.2.3 Lamin gene organization

Gene structures of human and mouse lamins A and C, mouse lamin C2 and B3, human and mouse lamin B1 and mouse B2 have been analyzed. The cloning and sequencing of lamin complementary DNAs have been carried out in humans (Fisher et al., 1986) rat (Ozaki and Sakiyama, 1992; Parnaik et al., 1994) and mouse (Riedel and Werner, 1989). Human lamin A and C are identical for the first 566 amino acids. Proteolytical cleavage of 18 amino acids from pre-lamin A gives rise to lamin A which has 98 unique C-terminal amino acids, whereas lamin C has six unique C-terminal amino acids. The sequence similarities and data obtained from Southern blotting indicate that lamin A and lamin C arise from the same genomic locus by alternative splicing (Lin and Worman, 1993). These are referred to as lamin A/C in this thesis, according to current convention in the literature.

The lamin A genomic locus contains 12 exons which span approximately 24 kb of contiguous genomic DNA. The first intron is approximately 16 kb in length, the second intron is about 2 kb and remaining introns less than 1 kb in length. Exon 1 codes for the amino-terminal head domain and the first part of the central rod domain. Exons 2 to 6 encode the rest of the central rod domain. Exons 7 to 9 code for carboxy terminal tail domain sequences common to both lamins A and C. Exon 10 contains alternatively used 5' splice site for generating lamins A and C mRNAs and exons 11 and 12 are lamin A specific exons. The lamin C mRNA is derived from exons 1 to 9, and a part of exon 10. The mouse lamin A and C mRNA sequences are coded by 12 and 10 exons respectively which are contained within a 22 kb segment of genomic DNA. The germ line specific lamin C2 mRNA (which is identical to the lamin C mRNA except for the first 71 bases) is generated from the somatic A-type lamin gene by differential splicing during spermatogenesis. The first exon of lamin C2 resides in the first intron of the lamin A gene, about 4 kb upstream of exon 2. The A, C and C2 lamins therefore share 8 exons between them. The mouse lamin B1 gene codes for a 2.9 kb lamin B1 cDNA (Maeno et al., 1995). The mouse B1 gene spans about 43 kb of the genome and consists of 11 exons.
and 10 introns. The exon/intron structure of lamin B1 gene clearly shows the conserved organization shared among IF family genes. (Fig. 1.2)

1.2.4 Evolution of lamins

IF proteins constitute a large family of proteins encoded by 65 functional genes in the human genome which are expressed in specific patterns during embryogenesis (Fuchs and Weber, 1994). IFs have a diameter (10-12 nm) in between microtubules (25 nm) and microfilaments (7-10 nm). All IF subunits share a common characteristic tripartite domain organization. A central α-helical coiled-coil forming segment consisting of coil 1A, 1B, 2A and 2B is flanked by non-α-helical N-terminal (head) and C-terminal (tail) domains of varying lengths (Herrmann and Aebi, 2000). cDNA sequencing as well as structural analysis shows remarkable similarities between lamins and IF proteins (McKeon et al., 1986; Fisher et al., 1986). According to their sequence similarities, IF proteins can be classified into five major types. Acidic and basic keratins form type I and II. Vimentin, desmin, glial fibrillary acidic protein (GFAP), and peripherin form type III. The neurofilament proteins (NF-L, NF-M, NF-H) and internexin form type IV. IF protein types I to IV are present in the cytoplasm. The nuclear lamins belong to the type V class of IF proteins (Strelkov et al., 2003). Both nuclear lamins and IF proteins from mollusks and nematodes (i.e. invertebrates) have an extra segment of six heptads (i.e. 42 amino acids) in the rod domain. Sequence similarities between the tail domains of invertebrate IF proteins and nuclear lamins have been observed (Weber et al., 1988; 1989). However nuclear lamins possess a nuclear localization signal (Loewinger and McKeon, 1988) and CaaX motif (Hancock et al., 1989) which are absent in IF proteins.

Conservation of intron positions in the genomic sequences coding for the central rod domain in the nuclear lamin genes and vertebrate IF genes suggests a common ancestry between lamin and IF proteins. Xenopus lamin III possesses a tail domain which is homologous to the tail domain of invertebrate IF proteins but not like vertebrate IF proteins. This suggests that IF proteins might have evolved from a lamin-like ancestor in eukaryotic evolution (Dodemont et al., 1990). Moreover, interaction between B-type lamins and IF proteins is observed in vertebrates, suggesting the occurrence of convergent evolution in these proteins (Georgatos and Blobel, 1987). IF proteins are
Fig. 1.2: Schematic representation of the protein structure, cDNA map and genomic locus of the lamin A gene. 

A. Structure of the A-type lamin proteins. The highly conserved α-helical central rod domain is flanked by non-helical head and tail domains. The helical segments 1A, 1B, 2A and 2B are separated by linker segments (L1, L12 and L2). The hatched region in coil 1B represents the 42-amino-acid insertion in the rod domain that is present in lamin but not cytoplasmic IFs of vertebrates. The black boxes at the ends of the rod segment represent the segments highly conserved in all the IF proteins. The circles with P represent essential phosphorylation sites, though many more are actually present in the protein. The NLS is the nuclear localization signal. Only lamin A has the CaaX motif, which undergoes 2 proteolytic cleavages at positions C1 and C2. Lamins C and C2 do not contain CaaX motif.

B. Comparison of the three A-type lamin mRNAs. The shaded boxes represent exons, regions between dotted vertical bars are common to all three A-type lamins, and white boxes denote non-coding regions. Hatched regions are coding sequences unique to a single lamin (adapted from Nakajima and Abe, 1995).

C. Structural organization of mouse lamin A gene. Shaded boxes represent exons 1 to 12 and straight lines represent the introns.
differentially expressed during development and cell differentiation and are probably involved in maintaining the differentiated state of cells. Lack of IF proteins in certain cells (Venetianer et al., 1983) implies that they do not serve housekeeping functions, whereas nuclear lamins (particularly lamin B) is constitutively expressed suggesting that nuclear lamins carry out house-keeping functions (Benavente et al., 1985). Thus nuclear lamins represent ancestral members of the IF protein family. A fresh water sponge hydra expresses an IF protein with considerable similarity to human lamin B suggesting that lamins are the oldest group of IF proteins (Erber et al., 1999).

1.2.5 Localization of lamins

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

One of the features that distinguishes A and B-type lamins from each other is their expression profile. Lamin expression has been studied in several organisms ranging from Drosophila to human. Earlier studies have been carried out primarily by immunoblotting of lamin proteins with a wide panel of antibodies to the various lamin isoforms. In later studies, the expression of lamin transcripts has been analyzed in a few systems.

The developmental regulation of nuclear lamin expression was first studied in *Xenopus laevis* where at least five different lamins, LI, LII (homologues of mammalian lamins B1 and B2), LIII (considered to be a B-type lamin as it possesses a CaaX box), LA (homologue of lamin A) and LIV (a sperm-specific lamin) have been identified by immunochemical procedures. Expression of lamins was monitored during gonadal differentiation and embryonic development (Benavente and Krohne, 1985; Benavente *et al.*, 1985; Stick and Hausen, 1985), and it was observed that Xenopus oocytes expressed only LIII, which was also the major lamin found in early Xenopus embryos. LI and LII, the adult specific lamins were expressed from mid-blastula and gastrula stages onwards respectively and thereafter continued to be maintained in all somatic tissues. The levels of LIII, on the other hand, decreased as development proceeded till it became undetectable in the larval stages. LIII was again expressed later in development in the diplotene stage of developing oocytes and was also shown to be synthesized in specific somatic cell types of the adult animal such as the neural and muscle cells. Unlike the above three lamins, LIV was expressed exclusively in male germ cells during spermatogenesis and LA, the A-type lamin was expressed late in embryonic development and was found in all somatic cell types except for erythrocytes (Wolin *et al.*, 1987).

Differential expression of nuclear lamin proteins has also been demonstrated in chicken (Lehner *et al.*, 1987), with lamins A, B1 and B2 being expressed in a developmentally controlled and tissue-specific manner. There was no difference in the patterns of expression of any of the three lamin proteins during gametogenesis in either female or male germ cells, but early chicken embryos contained only lamins B1 and B2. Lamin A became increasingly prominent during development, whereas the amounts of
B1 decreased in many tissues. Lamin B2 continued to remain constant in almost all cell types investigated.

An analysis of lamin expression in Drosophila at selected time points of development has revealed that expression of lamin C (analogous to mammalian lamin C in that it lacks the CaaX box) (Bossie and Sanders, 1993) was developmentally controlled, while lamin Dm0, proposed to be analogous to B-type lamins (Gruenbaum et al., 1988), was constitutively expressed (Riemer et al., 1995). Transcription of lamin C was initiated 6 to 9 hours after embryonic development and continued to increase till it reached a maximum in 19 to 22-hr old embryos. Immunostaining of embryos confirmed that lamin C was acquired during late embryogenesis and also revealed a tissue-specific pattern of expression.

Expression of lamins in mammalian cells has been studied in extensive detail in a wide variety of systems including mouse embryos, rat tissues, normal and cancerous human tissues and cell lines, several undifferentiated cell lines and embryonal carcinoma cell lines derived from mouse and rat. Investigation of lamin expression in mammalian cells was initiated by (Stewart and Burke, 1987) who employed a variety of immunochemical and biochemical techniques to demonstrate that early mouse embryos expressed only a single lamin species that closely resembled lamin B. In contrast, lamins A and C were not synthesized in early preimplantation embryos, but were detected only 8 days after implantation, indicating that early mouse embryos do not have a functional requirement for lamins A and C. These results were confirmed and further extended by (Rober et al., 1989) who systematically monitored lamin A and C expression in the various organs of the mouse embryo and young animal at different stages of development. The results indicated that the timing of onset of murine lamin A and C expression was tissue-dependent and that these lamins appeared at embryonic day 12 in muscle cells of the trunk, head and appendages, and only after birth in lung, liver, kidney, heart and brain. Thus, the results from studies on Drosophila, Xenopus and mouse embryos highlighted a common underlying feature, which was the late acquisition of A-type lamins during embryonic development, and provided evidence for the developmental regulation of these lamins.
Analysis of normal human tissues to explore the tissue distribution of lamins using a panel of antibodies to lamins A, C, B1 and B2 (Broers et al., 1997) has demonstrated that lamin B2 is expressed in almost all tissues. In comparison, the expression of lamin B1 was not as widespread and it appeared to be preferentially detected in proliferating cells. Expression of A-type lamins was reported to be most prominent in well differentiated epithelial cells, but undetectable in most cells of neuroendocrine and hematopoietic origin. Taken together, these data are suggestive of a correlation between lamin expression and the differentiated state of a cell. This hypothesis is supported by experimental evidence derived from the analysis of lamin expression in undifferentiated cells such as embryonal carcinoma cells and cells of the hematopoietic lineage. Earlier studies have shown that undifferentiated F9 (Lebel et al., 1987; Stewart and Burke, 1987; Worman et al., 1988a), PCC-4 (Hamid et al., 1996) and PCC-3 embryonal carcinoma cells (Rober et al., 1989) express only lamin B and not lamins A or C transcripts. Lamin A and C transcripts were synthesized only upon retinoic acid-induced differentiation of these cells (Mattia et al., 1992). More recent data using RT-PCR analysis has confirmed that undifferentiated mouse and human embryonic stem cells express only lamins B1 and B2, and not A or C (Constantinescu et al., 2006).

Examination of T and B lymphocytes, granulocytes and monocytes directly isolated from spleen, thymus, blood and bone marrow tissues has indicated that unlike the vast majority of somatic cells, these cells express only lamin B, but not lamins A and C (Rober et al., 1990b). In accordance with this, several murine hematopoietic cell lines such as EL4 (thyoma cell line), BW5147 and HK22 (lymphoma cell lines) and PAI (a myeloma cell line) were also found to express only lamin B, demonstrating that hematopoietic cells in vivo as well as in vitro, lack lamins A and C. In a subsequent study, rat bone marrow precursor cells and human monocytes were cultured under conditions which allowed these cells to differentiate into accessory cells and mature macrophages. A dramatic increase in lamin A and C expression paralleled the emergence of the differentiated cell type (Rober et al., 1990a) suggesting that the acquisition of lamins A and C in cells of hematopoietic origin occurs after commitment of cells to a particular differentiation pathway. Lamins A and C were also found to be absent in KE37, a human T lymphoblastic cell line, but were induced upon transformation of KE37.
with Epstein Barr virus and expression was found to be regulated at the transcriptional level (Guilly et al., 1987). This study was extended further by a systematic analysis of the lamin content of human B and T lymphoid cells at different stages of differentiation, where the early stages of differentiation were represented by pre-B leukemia cells and K37 rat thymocytes, the intermediate stages by T and B lymphocytes prepared from peripheral blood smear and the fully differentiated cells by lymphoblastoid RPM1-8866 and U266 plasctoma cell lines. Results indicated that while lamin B was constitutively expressed at all stages, A-type lamin expression was restricted to the later stages of differentiation (Guilly et al., 1990). Studies by Paulin-Levasseur et al., (1988) on several tumour cell lines of hematopoietic origin, such as the murine plastocytoma cells MPC-11 and MOPC-316, the human promyelocytic leukemia cell line HL-60 and murine myeloma cells X63 – Ag 8.6.5.3 showed that these cell lines are devoid of lamin A and C. TPA (12-O-tetradecanoylphorbol-13-acetate) induction of differentiation resulted in enhanced expression of lamins A and C in HL-60 cells though no change was noted in the MPC-11 cell line (Paulin-Levasseur et al., 1989a, 1989b). The correlation between the differentiation status of a cell and A-type lamin expression was further strengthened by studies conducted by Hass et al. (1990) wherein U937 human monoblastoid tumour cells which expressed lamins A and C upon being stimulated to differentiate along the monoblastoid pathway, showed a decline in lamin A and C levels when these cells underwent retrodifferentiation (a reversal of the differentiation process).

Induction of lamins A and C in lymphocytes has also been observed in response to mitogenic stimulation (Kaufmann, 1987). Similar results were obtained by Stadelmann et al., (1990) who reported that lamin transcripts were absent in resting normal peripheral blood lymphocytes, but accumulated after mitogenic stimulation. Decrease in lamin A and C transcripts concomitant with attainment of cellular quiescence has been observed in Swiss 3T3 and NIH 3T3 cultured cells (Parnaik et al., 1994; Pugh et al., 1997) as well as terminally differentiated liver cells (Hamid et al., 1996). These studies suggest that transcription of the lamin gene is related to cell proliferation in differentiated cells.

Differential expression of lamins A and C has been reported in human lung cancer cell lines, where Calu-3, H157 and SK-MES-1 non-small cell lung cancer cell lines displayed abundant levels of lamin A, B and C, but the OH-1, OH-3, NCL-H82, NCI-
H209 and NCI-H249 small cell lung cancer lines showed diminished expression of lamins A and C (Kaufmann et al., 1991). The relationship between the lung cancer phenotype and lamin expression was explored further by transformation of the NCI-H429 small cell line with the v-ras<sup>H</sup> oncogene. This gave rise to the NCI-H429 ras<sup>H</sup> cell line which manifested several features of the large cell carcinoma of the lung. Concomitant with the v-ras<sup>H</sup> induced change in phenotype, a dramatic increase in levels of lamin A and C was observed, which provided evidence for differential lamin expression in histologically different neoplastic cells derived from the same epithelial cell system. Neoplastic cells derived from patients with acute lymphoblastic leukemia or non-Hodgkin’s lymphoma did not exhibit lamin A and C expression (Stadelmann et al., 1990). Since it is widely accepted that lymphoid neoplasias represent clonal expansion of cells unable to differentiate beyond a certain level, this finding is in accordance with the hypothesis that lamins A and C are activated in response to cell differentiation.

### 1.4 Lamin interacting proteins

Lamins can associate with a number of proteins present in the nuclear membrane and chromatin, as well as various gene regulatory factors.

#### 1.4.1 Lamin interactions with nuclear membrane proteins

After targeting of lamins to the nuclear envelope, lamina assembly and association is stabilized by interactions with integral nuclear membrane proteins such as LAP1, LAP2 and LBR. The LAP1 family of proteins includes LAP1A, 1B and 1C which are alternately spliced products of the same gene. LAP1A and 1B can associate with lamins A, B and C (Foisner and Gerace, 1993). LAP1A and 1B are abundant in differentiated cells whereas LAP1C is present both in non-differentiated and differentiated cells (Martin et al., 1995). The LAP2 family of proteins shows tissue-specific expression and include six isoforms that are alternatively spliced products of the same gene, LAP2<sub>α</sub>, <sub>β</sub>, <sub>γ</sub>, <sub>δ</sub>, <sub>ε</sub>, <sub>ξ</sub>. LAP2<sub>β</sub>, <sub>ε</sub>, and <sub>δ</sub> have lamin-binding domains whereas other members do not have a lamin-binding domain. LAP2<sub>β</sub> gene is the best characterized member of the LAP2 family and is selectively expressed in highly proliferative tissues (Alsheimer et al., 1998). LAP2<sub>β</sub> binds to lamin B1 and interacts also
with chromatin. LAP2β interaction with the lamina is needed for nuclear growth following mitosis (Furukawa and Kondo, 1998). LAP2α is localized in the nucleoplasm, where it associates with A-type lamins, chromatin and barrier-to-autointegrated factor (BAF) (Dechat et al., 1998; Vlcek et al., 1999; Dechat et al., 2000). LAP2α becomes hyperphosphorylated during mitosis, coinciding with higher solubility. LAP2α phosphorylation might regulate its association with chromosomes, thereby controlling early steps of post-mitotic nuclear envelope assembly (Dechat et al., 1998).

LBR, also called p58, interacts with B-type lamins. The N-terminal first globular domain of LBR (1-60 amino acids) is sufficient for lamin binding, and the second globular domain (105-210 amino acids) associates with heterochromatic protein 1 (HP1) (Pyropasapolous et al., 1996; Ye et al., 1997). LBR contains an N-terminal RS domain with several phospho acceptor sites for Cdc2 kinases (Ye and Worman, 1994). RS kinase and Cdc2 kinase phosphorylate LBR during mitosis; phosphorylation by RS kinase controls the interaction of LBR with other subunits of the LBR complex and with chromatin (Nikolakaki et al., 1997). Phosphorylation by Cdc2 kinase does not prevent binding of LBR to lamin B. The LEM domain is a conserved region of 43 amino acids which is found in LAP2β, emerin, MAN1 and otefin (Dechat et al., 2000). Vertebrates express MAN1 and a smaller MAN1-related proteins called Lem2 (Berger et al., 1996; Osada et al., 2003; Raju et al., 2003). From in vitro experiments it is known that emerin binds directly to BAF and other LEM domain proteins (Lee et al., 2001). The combination of lamin / LEM domain protein / BAF interactions with chromatin, can effect processes like replication and transcription (Cai et al., 2001; Laguri et al., 2001).

Drosophila YA (young arrest) protein is a nuclear lamina component that is essential for the transition from meiosis to mitosis during initiation of embryogenesis (Goldberg et al., 1998).

Another group of lamin binding proteins include the nesprins which are located in the nuclear envelope (Malone et al., 1999). Human nesprins are encoded by two genes which yield multiple protein isoforms through alternative mRNA splicing. Nesprin 1α is a large nuclear membrane protein that binds directly to A-type lamins and emerin (Mislow et al., 2002; Zhang et al., 2004). Nesprins have multiple spectrin repeat domains
and many isoforms also have an actin-binding domain (Zhang et al., 2001; Starr and Han, 2003).

1.4.2 Lamin interactions with chromatin

Lamins bind to DNA directly in vitro (Luderus et al., 1992) and this binding is mediated by the Ig-fold domain and the NLS region (Stierle et al., 2003). Lamins also associate with specific DNA segments called matrix associated regions (MARs). MARs have a length of 300 bp – 1000 bp and are 70% A + T rich. Lamins interact with the minor groove of an A+T rich duplex and to a lesser extent with the minor groove of G+C rich segments. Vertebrate lamins A and B and Drosophila lamin Dm0 can bind to MARs. The rod domain of the lamin protein is sufficient for MARs binding provided that it assembles into a polymer (Zhao et al., 1996). Binding of MARs with A and B-type lamins from rat liver is saturable and of high affinity (Luderus et al., 1992). Apart from interacting with MARs, lamins A and C have been shown to bind to mitotic chromosomes in vitro through the α-helical rod domain (Glass et al., 1993) and the C-terminal tail domain (Hoger et al., 1991). In addition, a second motif in the tail domain of A and B-type lamins mediates binding to core histones (Taniura et al., 1995). The chromatin binding factor BAF interacts with lamin A as well as LEM-domain proteins and DNA (Holaska et al., 2003). BAF has been proposed to help in rapid interlinking of chromatin, lamins and LEM-domain proteins, and may play an important role in chromatin organization (Zastrow et al., 2004).

1.4.3 Lamin interactions with gene regulatory factors

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

Sterol response element binding protein 1 (SREBP1) binds to the Ig-fold domain of lamin A/C (Lloyd et al., 2002). SREBP1a and SREBP1c are encoded by alternatively spliced mRNAs, and are both helix-loop-helix leucine zipper transcription factors. They activate genes required for cholesterol biosynthesis and lipogenesis (Horton, 2002) and promote adipocyte differentiation (Kim and Spiegelman, 1996). MOK2 is a DNA binding transcriptional repressor that interacts with lamin A and C. It represses genes activated by cone-rod homeobox protein (Crx) (Dreuillet et al., 2002). MOK2 also binds RNA in vitro, and might thus influence RNA processing (Arranz et al., 1997). MOK2 binds to the coil region of A-type lamins (Dreuillet et al., 2002). BAF, which is known to interact with lamin A, chromatin and LEM domain proteins (Holaska et al., 2003) also binds to several homeodomain transcriptional activators, including Crx, and represses Crx-dependent gene expression in retinal cells (Segura-Totten et al., 2002). Other inner membrane proteins like young arrest (YA) and Bicaudal-D (BICD) involved in Drosophila oocyte development have been shown to interact with the Drosophila B-type lamin, Dm0 (Goldberg et al., 1998; Stuurman et al., 1999). A recent study shows that lamin A/C can directly interact with c-Fos to suppress the activity of the transcription factor AP-1 and thus control cell cycle progression (Ivorra et al., 2006). Thus several gene regulators bind directly to lamins and other partners like emerin and LAPs and mediate activation or expression of different genes in a developmentally regulated manner.
1.5 Functions of lamins
Lamins are crucial for maintenance of nuclear integrity and are involved in several processes like assembly and disassembly of the nuclear envelope, DNA replication, transcription and apoptosis.

1.5.1 Maintenance of nuclear integrity
Loss of nuclear lamins has been shown to affect nuclear shape and cause abnormal heterochromatin organization, unequal separation of chromosomes and abnormal distribution of nuclear pore complexes leading to embryonic lethality in C. elegans (Liu et al., 2000a). A mutation in Drosophila lamin Dm0 reduces lamin expression, inhibits nuclear membrane assembly and induces the formation of annulate lamellae (Lenz-Bohme et al., 1997). Another lamin Dm0 mutant has profound effects on nuclear morphology and Drosophila development (Guillemin et al., 2001). Homozygous lamin A (lmn-1) null mice show reduced post-natal growth and also undergo severe muscle wasting and die after 8 weeks. In lmn- cells emerin is mislocalized to the ER and nuclear integrity is severely compromised in skeletal and cardiac muscle cells (Sullivan et al., 1999). Mutations in A-type lamins lead to highly debilitating genetic disorders in humans. A common feature in cells from patient’s samples is aberrant nuclear morphology and abnormal chromatin organization, with defective interactions of lamins with inner nuclear membrane proteins (Vigourox et al., 2001). Thus nuclear lamins along with other inner membrane proteins play an important role in the maintenance of nuclear shape and integrity.

1.5.2 Assembly and disassembly of lamins during cell cycle
In addition to providing integrity to the nuclear envelope, lamins are involved in mitotic disassembly and reassembly of the nuclear envelope. The nuclear lamina is a dynamic structure and undergoes structural reorganization concomitant with nuclear envelope breakdown, which leads to its reversible disassembly. The A-type lamins are released as soluble dimers and tetramers, whereas B-type lamins remain associated with nuclear membrane vesicles. Disassembly of the nuclear lamina occurs during the
transition from late prophase stage to metaphase stage at the beginning of cell division and it occurs by phosphorylation of the residues flanking the rod domain by Cdc2 kinase (Peter et al., 1990; Dessev et al., 1991). The disassembly process starts with shrinkage of lamin in size followed by corresponding decrease in nuclear diameter (Dessev and Goldman, 1988). The timing and pattern of lamin disassembly varies with lamin isoforms. Lamins A and C are released into the nucleoplasm in early prophase, whereas lamin B remains associated with the nuclear periphery until prometaphase (Georgatos et al., 1997).

Following mitosis, the reassembly of the lamina occurs by dephosphorylation of residues which were phosphorylated during lamin disassembly (Peter et al., 1990). The exact role of nuclear lamins in post-mitotic reassembly of nuclear envelope has remained controversial for several years. One model suggests a lamin-dependent pathway of nuclear envelope reassembly. In this model, lamins interact with nuclear envelope components very early in the assembly process and these interactions are critical for nuclear envelope formation. Support for this model largely comes from a report that when lamins are immunodepleted from mitotic extracts of CHO cells, they do not initiate nuclear assembly in vitro (Burke and Gerace, 1986). Similarly in vitro studies using antibodies to deplete lamins from extracts of Xenopus eggs and Drosophila embryos revealed the blockage of nuclear envelope assembly (Dabauvalle et al., 1991). In contrast to the first model, the second model supports a lamin-independent pathway of nuclear envelope reassembly. Support for this model comes from studies showing that immunodepletion of lamins from Xenopus nuclear assembly extracts does not prevent nuclear envelope formation (Newport et al., 1990; Meier et al., 1991). Furthermore, immunofluorescence and scanning electron microscopy studies have shown that the lamin accumulation occurs only after the nuclear membrane forms (Wiese et al., 1997).

One model that can reconcile these apparently contradictory results suggests that only a small fraction of total lamin is directly involved in nuclear envelope formation (Lourim and Krohne, 1994). It has been observed that small quantities of lamins begin to associate with chromatin as early as late anaphase (Yang et al., 1997). Lamins bind to a number of proteins that are associated with membrane vesicles that serve as precursors for the nuclear membrane (Maison et al., 1997).
A-type and B-type lamins follow different pathways of assembly during cell division. In live cells, determinations of the timing of initial assembly of lamin B1 on chromosomes by using GFP constructs of lamin B1 is consistent with earlier reports that lamin B1 appears to bind and assemble on the periphery of chromosomes early in the process of nuclear envelope assembly (Moir et al., 2000b). Lamin A does not assemble in the peripheral region of decondensing chromosomes, but accumulates in the nucleoplasm early after the envelope and pores have assembled, and, as G1 phase progresses, lamin A is gradually incorporated into the lamina (Dechat et al., 2000; Moir et al., 2000b).

1.5.3 Role of lamins in DNA replication

It is widely accepted that nuclear lamins have a role in DNA replication. When lamin B3 is immunodepleted from Xenopus egg extracts the resulting nuclei do not replicate their DNA, though their nuclear envelopes appear to be capable of transporting proteins normally (Newport et al., 1990). Addition of lamin B3 to lamin B3-depleted extract partially restored the phenotype, nuclei expanded and DNA replication commenced (Goldberg et al., 1995). The lamina has been proposed to form an internal nuclear scaffold to which replication foci attach (Hozak et al., 1995). Studies in Xenopus have revealed that the disruption of endogenous lamin organization using a dominant negative lamin mutant causes the arrest of replication and aggregation of elongation phase specific replication factors like PCNA and RFC with lamins (Spann et al., 1997). Furthermore, lamin B foci have been observed to colocalize with PCNA at DNA replication sites (Moir et al., 1994).

1.5.4 Lamins as regulators of transcription

The association of the lamina with chromatin suggests that lamins might organize the interphase chromatin and thereby regulate transcriptional activity of the cell (Moir et al., 1995). Studies with a dominant negative N-terminal deletion lamin mutant ΔNLA have shown that disruption of lamin organization coincides with inhibition of synthesis of RNA pol II dependent transcripts and disruption of TATA-binding protein, TFIID (Spann et al., 2002). Inhibitors of RNA pol II cause the reorganization of intranuclear lamin A/C
speckles to form large foci, together with inhibition of transcription (Kumaran et al., 2002). The retinoblastoma protein binds to transcription factor E2F and represses transcription by recruiting histone deacetylases. The transcriptional repression by pRb correlates with lamin binding activity. Moreover the active form of pRb colocalizes with lamin A/C at the nuclear periphery in vivo and in vitro (Mancini et al., 1994). A growing number of transcription factors like Oct-1 which contains a POU domain that represses the collagenase gene (Imai et al., 1997) and germ-cell-less (GCL) protein, which is essential for establishment of germ cell lineage during Drosophila are found to be associated with lamins at the nuclear envelope (Leatherman et al., 2000). The nuclear periphery also tethers transcriptional activators like insulin activator IPF-1/PDX-1 that regulates the transcription of insulin gene (Rafiq et al., 1998), as well as SREBP1 which is involved in adipocyte differentiation (Lloyd et al., 2002). The interaction of hsMOK2 with lamin A/C and nuclear matrix may be important for its ability to repress transcription (Dreuillet et al., 2002).

1.5.5 Role of lamins in apoptosis

Apoptosis or programmed cell death can regulate cell number, sculpt tissues during development and eliminate damaged cells. Apoptotic nuclei resemble nuclei in lamin-deficient cells, which have clustered nuclear pore complexes, detached chromatin and odd shapes (Lenz-Bohme et al., 1997). Both A-type and B-type lamins, LAP2α and LAP2β are early targets for caspase-mediated degradation before DNA cleavage or chromatin condensation occurs (Lazebnik et al., 1993; Gotzmann et al., 2000). Lamins are cleaved in the α-helical rod domain probably by caspase-6 (Rao et al., 1996; Orth et al., 1996). Lamins are degraded before LBR and LAP2. LAP2β is cleaved by caspase-3 (Buendia et al., 1999), and the cleaved fragments of lamins and LAP2β remain associated with the nuclear envelope. Direct evidence for the importance of lamins in apoptosis is provided by the delayed onset of apoptosis due to expression of an uncleavable mutant form of lamin in cultured cells (Rao et al., 1996). Both A-type and B-type lamins are degraded during apoptosis in many different cell types and in response to different apoptotic stimuli (Cohen et al., 2001).
1.6 Laminopathies

Nuclear lamins have been ascribed structural roles such as maintaining nuclear integrity and assisting in nuclear envelope formation after mitosis and have also been linked to nuclear activities including DNA replication and transcription. Recent evidence shows that A-type lamin mutations have been linked to a variety of human diseases like muscular dystrophy, lipodystrophy, cardiomyopathy, neuropathy and progeroid syndromes which are collectively called laminopathies (Smith et al., 2005).

1.6.1 Emery-Dreifuss muscular dystrophy

Emery-Dreifuss muscular dystrophy (EDMD) was first described as an X-linked recessive disorder. Mutations in STA gene encoding the nuclear envelope protein emerin (34 kDa) are responsible for X-linked EDMD (Bione et al., 1994; Yates and Wehnert, 1999). The majority of the emerin mutations are nonsense or frameshifts, producing a null phenotype but about 15% express modified forms of emerin (Nigro et al., 1995; Bione et al., 1995; Fairley et al., 1999). Recently autosomal recessive and autosomal dominant forms have also been recognized. EDMD manifests in childhood and is characterized by early contractures of elbows, achilles tendons, slowly progressive muscle wasting and weakness with a predominantly humeroperoneal distribution. Mutations in the LMNA gene mapped to chromosome 1q 21.2 – 21.3 have been found in patients with autosomal dominant, autosomal recessive and sporadic forms of EDMD (Bonne et al., 1999). The majority of the mutations in the lamin gene are missense mutations that occur throughout the length of the common region of lamin A and C (Felice et al., 2000). Targeted disruption of the mouse lamin A gene primarily results in EDMD (Sullivan et al., 1999).

1.6.2 Dilated cardiomyopathy

This is also one of the laminopathies associated with missense mutations in LMNA (Fatkin et al., 1999). Dilated cardiomyopathy (DCM) is a myocardial disorder characterized by dilation of the cardiac chamber and impaired systolic contraction and heart failure. Although this disorder can be transmitted as a recessive or X-linked trait, autosomal dominant inheritance occurs most frequently and exhibits both clinical
variability and genetic heterogeneity. Inherited mutations can cause approximately 35 per cent of cases of dilated cardiomyopathy. In a mouse knock-in model for DCM, mice homozygous for the N195K mutation, which causes DCM in humans, die at early age due to arrhythmia (Mounkes et al., 2005).

1.6.3 Limb-girdle muscular dystrophy

Limb-girdle muscular dystrophy with atrioventricular conduction disturbances (LGMD1B) is an autosomal dominant form of inherited disease with slow progressive limb girdle muscular dystrophy with age-related atrioventricular cardiac conduction disturbances and also absence of early contractures. Lamin mutations which cause LGMD1B are an inframe deletion in LMNA exon 3 (del K208), a missense mutation in LMNA exon 6 (R377H) and a mutation at amino acid position 536 that can cause transversion of splice donor site of intron 9, which may lead to a truncated lamin A/C protein lacking globular tail domain (Muchir et al., 2000).

1.6.4 Charcot-Marie Tooth Disease

The Charcot-Marie tooth (CMT) disorders are also known as “hereditary motor and sensory neuropathies”. CMT disorders can be divided into two main sub-types. Type 1 includes demylinating neuropathy (CMT1) and Type-2 includes axonal neuropathy (CMT2) (Garcia, 1999). Using linkage analysis, a single homozygous (892 C>T) missense mutation in exon 5 of LMNA gene which causes an amino acid substitution R298C (De Sandre-Giovannoli et al., 2002; Tazir et al., 2004) has been identified in individuals with autosomal recessive CMT2.

1.6.5 Hutchinson-Gilford progeria syndrome

Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder characterized by features of marked premature ageing. A single-base substitution G608G (GGC > GGT) within exon 11 of LMNA gene has been observed in most cases of HGPS (Erikson et al., 2003; De Sandre-Giovannoli et al., 2003). This mutation causes the activation of a cryptic splice site within exon 11 resulting in the production of a truncated protein that has a deletion of 50 amino acids near the C-terminus but retains the C-
terminal CaaX box (i.e. 607-656 of prelamin A). Thinning of skin, hypoplasia and degeneration of cardiac and skeletal muscle, osteoporosis and abnormal dentition observed in knock-in mice with a homozygous L530P mutation are all phenotypes consistent with those seen in progeria patients (Mounkes et al., 2003). A progerin knock-in model has been produced wherein heterozygous mice exhibit growth retardation (Yang et al., 2005). Two other mutations in the C-terminus of lamin A, R471C and R527C are associated with HGPS but do not affect splicing or processing of lamin A (Cao et al., 2003).

1.6.6 Mandibuloacral dysplasia

Mandibuloacral dysplasia (MAD) is a rare autosomal recessive disorder, characterized by post-natal growth retardation, cranio-facial anomalies, skeletal malformations and mottled cutaneous pigmentation. MAD disease is caused by a homozygous missense mutation (R527H) in LMNA and leads to abnormal distribution of lamin A/C and a dysmorphic envelope (Novelli et al., 2002).

1.6.7 Dunnigan type familial partial lipodystrophy

Dunnigan-type familial partial lipodystrophy (FPLD) is a rare autosomal dominant disease, which is part of heterogeneous group of disorders characterized by complete or partial absence of adipose tissue. Profound insulin resistance with diabetes can develop later in life. LMNA R482Q mutation is a common molecular basis for FPLD (Cao and Hegle, 2000). Disease-causing mutations at the C-terminus of LMNA have been suggested to alter the binding of SREBP1 (an adipocyte differentiation factor) thereby leading to abnormalities in adipocyte differentiation observed in MAD and FPLD (Lloyd et al., 2002).

1.6.8 Restrictive dermopathy

Restrictive dermopathy is one of the most deleterious laminopathies identified so far in humans and is also called tight contracture syndrome. It is mainly characterized by intrauterine growth retardation, tight and rigid skin with erosions, prominent superficial vasculature and epidermal hyperkeratosis facial features. Sparse or absent eyelashes and
eye brows and mineralization defects of the skull are also observed. Two kinds of mutations cause restrictive dermopathy in fetuses or newborn children. The first type includes a heterozygous splicing mutation in the \textit{LMNA} gene which causes a complete or partial loss of exon 11 of lamin A mRNA leading to a truncated pre-lamin A protein. The second type is a heterozygous insertion that leads to a premature termination codon in \textit{ZMPSTE24} gene which encodes a zinc metaloproteinase that is specifically involved in the post-translational processing of lamin A precursor (Navarro \textit{et al.}, 2004).

1.7 Eukaryotic transcription

In eukaryotic organisms processes like development, growth and survival require proper regulation of thousands of genes. The expression of each of these genes is controlled by a wide variety of mechanisms including transcription, splicing, post-transcriptional processes and post-translational mechanisms (Lefstin and Yamamoto, 1998; Roeder, 1998; Struhl, 1999).

Transcription in eukaryotes occurs by the involvement of three different RNA polymerases, RNA pol I, pol II and pol III. RNA polymerases cannot recognize the target promoter directly. Specific transcription factors recognize the basal promoter elements, and the correct RNA polymerase is recruited subsequently (Hernandez, 1993). TFIID is a factor that binds to the TATA box present in a large number of RNA pol II promoters (Sawadogo and Sentenac, 1990). TFIID is a large complex consisting of TATA-binding protein (TBP) and a number of TBP associated factors or TAFs (Dynlacht et al., 1991). TBP is encoded by a single gene but organisms like Arabidopsis have two TBP genes that encode highly similar proteins (Gasch \textit{et al.}, 1990).

1.7.1 Core promoter

This is the minimal stretch of contiguous DNA sequence that is sufficient to direct accurate initiation of transcription by the RNA pol II machinery (Struhl, 1987; Weis and Reinberg, 1992; Smale, 1994, 1997, 2001). A typical core promoter consists of sites of transcription initiation and extends either upstream or downstream for an additional \(~35\) nt. Thus in many instances the core promoter will comprise only about 40 nucleotides. There are several sequence motifs such as TATA box, initiator (Inr), TFIIB recognition
element (BRE) and downstream core promoter element (DPE) that are commonly found in core promoters. Each of these motifs has specific functions in the transcription process.

In addition to core promoter, other cis-acting DNA sequences that regulate RNA pol II transcription include proximal promoter motifs, enhancers, silencers and boundary / insulator elements (Blackwood and Kadonaga, 1998). These elements contain recognition sites for a variety of sequence-specific DNA-binding factors that are involved in transcriptional regulation. The proximal promoter is the region in the immediate vicinity of the transcription start site (roughly from -250 to +250 nt). Enhancers and silencers can be located many kilobase pairs from the transcription start site and act either to activate or to repress transcription. Boundary/insulator elements separate the regulatory elements of a gene from its neighbouring genes.

The first step in transcription is pre-initiation complex assembly which proceeds in a step-wise manner where TBP and its associated factor TFIID first recognizes the TATA element and then factors such as TFIIA, TFIIIB, TFIIIF, RNA pol II, TFIIIE and TFIIIH enter the promoter to form a complex which is competent for transcription. The recognition of TFII-D-A-B complex at the TATA box by RNA pol II-TFIIF is considered to be the earliest checkpoint (Finkelstein et al., 1992). From structural studies it has been shown that TFIIIE appears to bind directly to RNA pol II at the jaw position which closes around the DNA (Cramer et al., 2001; Gnatt et al., 2001). A model has been suggested for the entry of TFIIIE into a stable TFII-(D-A-B-F)-RNA pol II complex on the promoter, which gives correct conformation to the jaw and thereby establishes the RNA pol II configuration and prepares it for subsequent consequences of open complex formation/promoter melting (catalyzed by TFIIH helicase activity), and transcript initiation (Bushnell et al., 1996).

The phosphorylation status of the C-terminal repeat domain (CTD) of the largest sub-unit of RNA pol II has long been known to play an important role in transcription cycle. Hypophosphorylated polymerases enter promoters whereas a hyperphosphorylated form is responsible for elongation (Dahmus, 1996; Lin et al., 2002). CTD phosphorylation triggers promoter clearance and thereby defines initiation to elongation transition. The mediator complex interacts with the CTD of RNA pol II forming a large complex, referred to as a holoenzyme. The CTD is heavily phosphorylated on Ser-2 by
pTEFB and Ser-5 by TFIIH in the Y-S-P-T-P-S repeat sequence. Recent studies have shown that mRNA capping is coupled to transcription elongation via physical and functional interactions between cap forming enzymes, CTD and Spt-5. Spt-5 induced arrest of early elongation might thus allow time for proper recruitment and action of capping enzymes. Such an early elongation checkpoint might avoid wasteful rounds of transcription of uncapped pre-mRNAs (Pei et al., 2003).

1.7.2 Histone modification and gene regulation

The basic repeating unit of chromatin is the nucleosome, which is composed of an octamer of the four core histones H2A, H2B, H3 and H4 on which 146 base-pairs of DNA is wrapped (Luger et al., 1997). Histone tails undergo post-transcriptional modifications such as acetylation, phosphorylation, methylation, ubiquitination and sumoylation and these modifications have profound effects on the regulation of gene expression. Moreover, these modifications determine the interactions of histones with other proteins which may in turn regulate the chromatin structure (Wu and Grunstein, 2000). Mass spectrometry analysis has shown that in mammals histones undergo more than 60 modifications, of which 31 are acetylations at different sites of histones (Zhang et al., 2003). Histone acetylation is carried out by a class of enzymes known as histone acetyl transferases (HATs), which catalyze the transfer of acetyl group from acetyl CoA to the lysine ε-amino groups on the N-terminal tails of histones. Hyperacetylated histones are associated with transcriptional activation, whereas hypoacetylated histones are present in inactive chromatin (Allfrey et al., 1964).

1.7.2.1 Histone acetylation and chromatin remodeling

The enzymatic activities which make DNA more accessible for the transcription machinery are the ATP-dependent remodeling complexes and histone acetyl transferases (Kingston and Narlikar, 1999; Cheung et al., 2000). The molecular activity responsible for histone acetylation was identified for the first time in *Tetrahymena thermophila* (Brownell et al., 1996). In transcriptionally active chromatin, histones H2A and H2B are more dynamic and are readily exchanged out of transcribed chromatin. The removal of H2A/H2B from nucleosomal arrays enhances gene activity, at least partly by decreasing
the level of chromatin folding. Moreover, acetylation may promote the binding of transcriptional factors to DNA in chromatin, possibly by neutralizing the positive charge associated with the lysine ε-amino group, and may weaken internucleosomal interactions and destabilize higher order chromatin structure, thereby promoting processivity of RNA polymerase through nucleosomal arrays (Chan and La Thangue, 2001).

Molecular evidence for a direct link between acetylation and transcription was provided when Gcn5 was found to have histone acetyl transferase (HAT) activity (Brownell et al., 1996). The highly conserved lysine residues that can be acetylated on histone H3 are 9, 14, 18, 23 and those on histone H4 are 5, 8, 12, 16. However, acetylation of lysine 12 in histone H4 is associated with transcriptional silencing in yeast and Drosophila (Turner et al., 1992; Braunstein et al., 1996; Roth et al., 2001).

Histone acetyl transferases are divided into five families. The GNAT family (Gcn5-related N-acetyl transferases) includes enzymes involved in transcription initiation (Gcn5 and PCAF), elongation (EIP3), histone deposition and telomeric silencing (Hat1). The MYST related HAT family includes MOZ, Ybf2/Sas3, Sas2 and Tip60. The p300/CBP HATs family includes p300 and CBP proteins, which share homology with GNATs. The general transcription factor HAT family includes TFIID sub-unit TAF250. Nuclear hormone-related HAT family includes enzymes like SRC1 and ACTR (SRC3) (Torchia et al., 1998; Roth et al., 2001).

1.7.2.2 p300/CBP as histone acetyl transferases

The family of mammalian HATs includes CBP (CREB-binding protein) and its parologue p300. CBP and/or p300 homologues are present in many multicellular organisms like flies, worms and plants but are absent in lower eukaryotes such as yeast (Arany et al., 1994; Bordoli et al., 2001; Yuan and Giordana, 2002). CBP and p300 histone acetyl transferases interact with basal transcription factors TATA-binding protein and TFIIB, and form a complex with RNA pol II (Kwok et al., 1994; Yuan et al., 1996; Neish et al., 1998). By interacting with the basal transcription machinery and with one or more upstream transcription factors, CBP and p300 function as physical bridges or scaffolds and thereby stabilize the transcription complex. Moreover, the CBP and p300 co-activators have the ability to acetylate proximal promoter nucleosomal histones,
resulting in increased accessibility of the DNA for other essential regulators (Ogryzko et al., 1996; Bannister and Kouzarides, 1996; Kundu et al., 2000). In the HAT domain of CBP there are two functionally important regions. One region extends from residues 1459-1541 and is the coenzyme binding site. The second important region is the PHD type zinc-finger, which is an integral part of the CBP acetyl transferase domain. CBP also acetylates proteins like transcription factors and co-activators like activator of thyroid and retinoid receptors (ACTR), high mobility group protein HMG I(Y), hepatocyte nuclear factor HNF4 and E2F. So CBP and p300 are also called FATs (Factors acetyl transferases) (Sterner and Berger, 2000; Yang, 2004).

1.7.2.3 Histone deacetylation

The enzymes which remove acetyl groups from histones are called histone deacetylases (HDACs). HDACs are expressed in almost all tissues (De Ruijter et al., 2003). The catalytic domain of HDAC is formed by a stretch of ~390 amino acids consisting of conserved amino acids (Finnin et al., 1999). The removal of acetyl group occurs via a charge-relay system consisting of two adjacent histidine residues, two aspartic acid residues located approximately 30 amino acids from the histidines and separated by approximately six amino acids, and one tyrosine residue located approximately 123 amino acids downstream from the aspartic acid residues (Finnin et al., 1999; Buggy et al., 2000). The essential component of this charge-relay system is the presence of a zinc ion at the active site. HDAC inhibitors function by displacing the zinc ion and thereby inhibit the charge-relay system. HDACs are known to be associated with a number of cellular oncogenes and tumor suppressor genes like Mad and Rb, which can lead to an aberrant recruitment of HDAC activity and change in gene expression (Cress and Seto, 2000; Timmermann et al., 2001).

HDACs have been identified in yeast by genetic screens and/or reverse genetic approaches. In Saccharomyces cerevisiae there are three class I HDACs (i.e. Rpd3, Hos2 and Hos1), two class II HDACs (Had1 and Hos3), and one group of class III HDACs (sirtuins). In Schizosaccharomyces pombe, two class I HDACs (Clr6 and Hos2), a single class II HDACs (Clr3) and one group of class III HDACs (sirtuins) have been identified.
From phylogenetic analysis it is evident that yeast have fewer HDACs than humans (Ekwall, 2005)

Human HDACs have been classified into class I HDACs (HDAC 1, 2, 3 and 8) and class II HDACs (HDAC 4, 5, 6, 7, 9, 10) (Bjerling et al., 2002). Class I HDACs are exclusively nuclear localized, whereas class II HDACs shuttle in and out of the nucleus in response to a stimulus. HDAC1 and HDAC2 are highly similar enzymes with 82% sequence homology. The catalytic domain is located in the N-terminal part of these proteins (Wade, 2001). HDAC1 and 2 can bind to DNA binding proteins such as Ying Yang 1 (YY1), Rb, and specificity protein 1 (Sp1) (Ito et al., 2000; Yoshida et al., 2001). HDAC3 is evolutionarily conserved and is most closely related to HDAC8. HDAC3 has only 68% identity with HDAC1 and HDAC2. HDAC8 is most similar to HDAC3 and the function of HDAC8 is regulated by a co-repressor complex of proteins.

1.7.2.4 Histone deacetylase inhibitors

Histone deacetylase inhibitors are small molecules that have the capacity to interfere with HDAC activity and have significant biological effects in the treatment of cancer (Minucci and Pelicci, 2006). HDAC inhibitors function by blocking the access to the active site of HDAC. The most potent HDAC inhibitor discovered so far is trichostatin A (TSA). TSA is a fermentation product of streptomyces, which has both antifungal and anti-proliferation properties. TSA belongs to the hydroxamic acids group of HDAC inhibitors and is effective at nanomolar concentration in vitro. The first report that TSA inhibits cell proliferation was by Yoshida et al., 1990. HDAC inhibitors are classified into four classes. Class I consists of hydroxamic acids, which includes TSA and suberoyl anilide bis hydroxamide (SAHA). Class II includes short chain fatty acids like butyrate phenyl butyrate and valproic acid. Class III includes cyclic-tetrapeptides/epoxides like trapoxin, depudesin, depsipeptide, apicidin and chlamydocin. Class IV includes benzamides like N-acetyl dinaline. All HDAC inhibitors inhibit HDACs in a reversible fashion except for trapoxin and depudesin (De Ruijter et al., 2003).
1.8 Lamin A/C promoter regulation

Lamins A and C are encoded by a single genomic locus in mammals and are expressed by differential processing of a primary transcript (Nakajima and Sado, 1993; Lin and Worman, 1993). Primer extension analysis and northern blot analysis in mouse (McKeon et al., 1986; Fisher et al., 1986; Lin and Worman, 1993) have shown that lamin A and C genes are transcribed from the same transcription site and transcription initiated at this site gives 3.1 kb and 2 kb long mRNAs for lamin A and C respectively. For initiation of lamin A and C transcripts, a major extension band was seen at -203 position and less strong band was seen at -241 (Nakajima and Abe, 1995).

In our laboratory, an 11 kb genomic clone encompassing the 5’ region of the lamin A gene was isolated from a rat genomic library (Tiwari et al., 1998). The transcription start site for the rat lamin A gene was mapped to 203 bases upstream of the ATG codon by primer extension analysis, which was identical to that observed for the mouse gene. The 5’ flanking sequences of the mouse, rat and human lamin A genes were observed to be highly homologous, with total conservation of the GC box, TATA box and AP-1 motif in all three species (Lin and Worman, 1993; Nakajima and Abe, 1995; Tiwari et al., 1998). Luciferase reporter gene assays of deletion fragments of the promoter region in HeLa, NIH-3T3 and COS-1 cells identified a minimal promoter segment which included the GC box at -101, TATTA box at -33 and AP-1 motif at -7. A double mutation at the GC box and AP-1 motif lead to 60% decrease in activity in HeLa cells. DNase I footprinting analysis of the minimal promoter segment showed protected regions at the GC box as well as a putative GT box at -55, and a hypersensitive site at -15 which was attributed to binding at the AP-1 motif. Electrophoretic mobility shift assays (EMSAs) and supershift assays with specific antibodies provided evidence for the binding of specific transcription factors at the GC box and AP-1 motif. Sp1 and Sp3 transcription factors were bound to the GC box at -101, whereas c-Fos and c-Jun proteins were bound to the AP-1 motif at -7 (Tiwari et al., 1998).

Binding of Sp factors has been shown to be essential for rat lamin A proximal promoter activity in Drosophila SL2 cells (Muralikrishna and Parnaik, 2001). Mutation of the GC box abolished the binding of Sp proteins. The importance of the AP-1
transcription factor for lamin A/C proximal promoter activity was shown in the PCC4 embryonal carcinoma cell line where the expression of both c-Fos and c-Jun activated the wild-type promoter but did not increase activity of an AP-1 mutant (Muralikrishna and Parnaik, 2001). The lamin A/C promoter was observed to be upregulated about 2-fold by retinoic acid in P19 embryonal carcinoma cells and a retinoic acid response element (L-RARE) was located in the region from -54 to -36 of the lamin A/C promoter, specifically between -42 to -38 (Okumura et al., 2000). EMSAs suggested that Sp1 proteins might bind to this sequence. This sequence was also transactivated by overexpression of c-Jun (Okumura et al., 2004).

The factors involved in downregulation of the lamin A promoter in quiescent hepatocytes have been investigated (Tiwari and Parnaik, 1999). DNase I footprinting analysis, EMSAs and UV crosslinking experiments have revealed that the downstream region of promoter showed three distinct footprints in adult hepatocyte extract which were not observed with fetal hepatocyte extracts. Specific proteins were bound at these sites, which may function as transcriptional repressors of lamin A proximal promoter. The AP-1 complex consisting of c-Jun and c-Fos could bind to the AP-1 motif in fetal hepatocyte extract but not in adult hepatocyte extract. On the other hand, binding of Sp1 family of proteins at the GC box, GT box and a TCC motif appeared to be almost comparable in extracts of both dividing and quiescent hepatocytes.

The first intron of the lamin A gene harbours the transcription start site for the lamin C2 gene which is male germ cell specific (Nakajima and Abe, 1995). Lamin C2 is expressed specifically at the pachytene stage, whereas lamin A and C expression is downregulated during spermatogenesis (Furukawa et al., 1994; Alsheimer and Benavente, 1996). In the activated state, the lamin A/C gene shows the appearance of DNase I-hypersensitive sites in the first intron of the gene (Nakamachi and Nakajima, 2000). Arora et al. (2004) have identified a 420 bp enhancer fragment upstream of the translation initiation site of lamin C2 which increased promoter activity in differentiated cell lines but not in an undifferentiated embryonal carcinoma cell line. DNase I footprinting and EMSAs revealed two regions, namely FPRA, an AT-rich fragment which bind to hepatocyte nuclear factor β (HNFβ) in somatic cell extract. This motif had an inhibitory effect on promoter activity. Retinoic X receptor β (RXRβ) family of
proteins were bound near the second region, FPRB with extracts from lamin A or C2 expressing cells and this site enhanced promoter activity (Arora et al., 2004).

1.9 Objectives of study

The A-type lamins (A and C) are expressed primarily in differentiated cells, in a stage-specific manner depending upon the cell lineage, whereas the B-type lamins (B1 and B2) are found in nearly all somatic cells. The differential regulation of lamin expression in different tissues has important implications for lamin function, since mutations in the human lamin A gene lead to complex disease phenotypes, chiefly affecting muscle, adipose and bone tissues. In earlier work from the group, the proximal promoter of the rat lamin A gene has been delineated by reporter assays in differentiated cell lines that express lamin A and by in vitro DNA-protein binding studies. Two important motifs that have been identified in the 130-bp proximal promoter are a GC box at -101 and an AP-1 motif at -7 (Tiwari et al., 1998). The GC box binds to the Sp family of transcription factors, and the AP-1 motif binds to c-Jun and c-Fos. The importance of these motifs for promoter activity was supported by mutational analysis and overexpression studies with recombinant transcription factors (Muralikrishna and Parnaik, 2001). In vitro studies suggested that a GT box at -55 could also bind to Sp factors (Tiwari and Parnaik, 1999), but the functional importance of this site has not been determined. Sequence analysis has also revealed a TATTA sequence at -33; the importance of this motif for promoter initiation is not clear as it does not have strong homology to the canonical TATA box.

The objectives of the present study were (1) to determine the functional importance of the GT and TATTA motifs by mutational analysis in mammalian cells as well as in Drosophila Schneider line 2 cells, which lack Sp proteins; and (2) to identify additional factors involved in promoter regulation using the multifunctional adenoviral E1A oncoprotein and its mutants in functional assays.