In studies carried out by several groups in the past decade, >150 mutations in the human lamin A gene have been linked to at least 10 highly degenerative, heritable, rare disorders that primarily affect muscle, adipose, bone or neuronal tissues and also result in premature aging syndromes. Though we are far from understanding how lamin mutations result in disease, the phenotypes of the various diseases have given important clues about the functional roles of lamins, especially in nuclear organization and tissue-specific differentiation. In this section, the implications of the results of Chapter 3 are discussed with respect to the mechanistic basis of laminopathies.

4.1 Importance of lamin-emerin interactions in chromatin organization

The inner nuclear membrane protein emerin is an important binding partner for lamin, and also binds to barrier-to-autointegration factor (BAF), a DNA-binding chromatin protein (Lee et al., 2001; Haraguchi et al., 2001). Emerin and BAF, together with other lamin binding proteins such as lamin-associated-polypeptide 2α (Dechat et al., 2000) have been proposed to cooperate with lamin in the spatial organization of chromatin (Zastrow et al., 2004). Aberrant nuclear morphology and defective chromatin organization are commonly seen in cells from laminopathic patients as well as in cells from model organisms expressing lamin mutants. Aberrant heterochromatin organization has been observed in various conditions of lamin misexpression, for example, expression of lamin A del50 leads to the depletion of heterochromatin and abnormal nuclear morphology, which have been attributed to the accumulation of pre-lamin A (Goldman et al., 2004). Also, cardiomyocytes from lamin A/C-deficient mice display relocalization of heterochromatin from the periphery to the interior of the nucleus (Nikolova et al., 2004). As described in Section 3.1.7, cells expressing lamin mutants E203G, G232E, Q294P, R386K, R471C, R527C, L530P and lamin Adel50 show mislocalization of emerin from the nuclear periphery. Thus cells expressing these mutants are likely to be defective in their chromatin organization. Emerin also plays an important role in gene regulation by binding to transcriptional regulators, signalling intermediates and other structural proteins like actin and myosin I (Bengtsson and Wilson, 2004).

4.2 Importance of chromatin organization in DNA repair

Stalled replication forks lead to activation of ATR kinase pathway (Zhou and Elledge, 2000; Abraham, 2001; Bartek et al., 2004). ATR normally resides in the nucleus bound to chromatin and directly complexed with ATR-interacting protein. In response to DNA damage, the single-stranded DNA binding protein, replication protein A bound at
the DNA lesion recruits the ATR-interacting protein / ATR complex, thus facilitating the phosphorylation of downstream checkpoint kinase Chk-1, and leading to DNA repair. These events are schematically represented in Fig. 4.1A.

As shown in Section 3.1.7 and in Section 3.2, cells expressing lamin mutants E203G, G232E, Q294P, R386K, R471C, R527C, L530P and lamin Adel50 mislocalize emerin from the nuclear periphery and are impaired in the formation of γ-H2AX and 53BP1 foci after DNA damage. Furthermore, cells expressing lamin mutants that are impaired in the DNA damage response to UV and cisplatin are shown to mislocalize and/or misexpress ATR as shown in Section 3.2.6. These findings have been incorporated into the following model. As the mutants showing mislocalization of ATR also displayed altered emerin localization, this implies that lamin-chromatin interactions are required for the correct nuclear localization of ATR and subsequent activation of the ATR signaling pathway. By expression of lamin mutants, ATR association with chromatin is disrupted, leading to the release of ATR and its translocation to the cytoplasm, where it gets degraded. The proposed effects of lamin mutants on the localization of ATR is schematically represented in Fig. 4.1B. Though there is no evidence for this at present, a further possibility is that ATR normally shuttles between the nucleus and the cytoplasm, and lamin mutants may disrupt this pathway by blocking sites of nuclear anchorage of ATR in chromatin.

Thus this study provides a mechanistic basis for the defects in DNA repair caused by mutations in lamin A. Several lamin mutants that show altered emerin localization also cause mislocalization and/or misexpression of ATR prior to DNA damage, resulting in defects in the ATR pathway such as defective phosphorylation of H2AX or inadequate recruitment of 53BP1 to repair sites in response to DNA damage. This behaviour is not observed with an FPLD mutant and an EDMD mutant, both of which assemble normally and show typical peripheral localization of emerin. This result suggests that altered lamin-chromatin interactions leading to impairment of DNA repair processes might be a more general mechanism that could contribute to pathogenesis, in addition to the well-documented effects on tissue differentiation.

4.3 Role of lamins in muscle differentiation pathways

Since the majority of mutations in LMNA affect muscle tissue, there is considerable interest in understanding the role played by A-type lamins in muscle development and effects of mutations on this process. Based on the mechanical stress hypothesis, it is possible that muscle cells under high mechanical stress are unable to survive due to loss in nuclear integrity, leading to loss in cells and a dystrophic
Fig 4.1 Schematic representation of events during activation of ATR kinase after DNA damage and effects of lamin mutants on chromatin organization and ATR localization. (A) Events after exposure of cells to UV radiation or cisplatin treatment are represented in a serial order. (B) Lamin mutants disrupt interactions of emerin, BAF and chromatin and thus fail to maintain proper chromatin organization, leading to the release of ATR from anchorage site; free ATR then translocates to the cytoplasm where it gets degraded.
phenotype. The second possibility is that lamin A/C plays a specific role in muscle differentiation, and mutations in lamin A/C may affect the muscle differentiation programme leading to aberrant or incomplete differentiation due to misregulation of muscle-specific gene expression, which eventually leads to a dystrophic syndrome.

As shown in Section 3.3, myoblasts expressing certain lamin mutants showed reduced cell survival, and decreased expression of early differentiation markers like myogenin and cyclin D3. Cells expressing these constructs were not incorporated into myotubes, whereas cells expressing wild-type lamin A, and EDMD mutant H222P or FPLD mutant R482L, expressed the early differentiation markers myogenin and cyclin D3 and were incorporated into myotubes. Myoblasts expressing EDMD mutants which formed aggregates like G232E, Q294P and R386K showed reduced expression of MyoD, an important muscle regulatory factor, whereas myoblasts expressing the FPLD mutant R482L expressed MyoD at a level comparable to that of untransfected myoblasts. This suggests that those cells, which fail to express MyoD, are unable to induce differentiation markers like myogenin and cyclin D3 and are defective in myogenesis. MyoD has also been shown to be downregulated in the lamin A-deficient mouse, which displays an EDMD phenotype (Frock et al., 2006). Furthermore, pRb and MyoD signalling pathways are defective in the emerin knock-out mouse which shows defects in muscle regeneration (Melcon et al., 2006). These findings together with the results described in this thesis highlight an essential role for lamin A and emerin in the process of muscle differentiation, but their precise role is not yet understood.

Our group has been interested in understanding the role of nucleoplasmic lamins in muscle differentiation. Earlier work from our group has shown that internal lamins are antigenically masked and reorganized to a diffuse network at an early stage (prior to cell cycle arrest) during the differentiation of C2C12 myoblasts in culture (Muralikrishna et al., 2001). This diffuse network was also observed in quiescent, satellite cells but not in non-muscle cell types. In further studies, lamin reorganization was observed to be linked to the myogenic programme as it occurred in fibroblasts induced to trans-differentiate to muscle cells by MyoD expression, but not in other cell types. Lamin speckles were induced to rearrange upon expression of cyclin D3 in myoblasts and this process also required pRb; both pRb and cyclin D3 were sequestered on the insoluble lamin matrix (Mariappan and Parnaik, 2005). Dominant negative mutants such as an N-terminal deletion of lamin A inhibited expression of early markers of muscle differentiation like myogenin and transfected cells were not incorporated into myotubes. In another report, muscle differentiation in C2C12 myoblasts was accompanied by changes in the levels of lamin B2 and LAP2α, which could be correlated with redistribution of A-type lamins (Markiewicz et al., 2005). Lamin reorganization has been proposed to be required to
maintain the post-mitotic state of the differentiated myocyte (Mariappan and Pamaik, 2005). In summary, the requirement of a normal lamin A network for expression of MyoD, deduced from studies with disease-causing lamin mutants and lamin A-deficient myoblasts, and studies on lamin reorganization during muscle differentiation suggests an essential role for the nuclear lamina in the muscle differentiation programme.

4.4 Limitations of mouse models and tissue culture models

Since 1999, a few mouse models of laminopathies have been created which have provided useful clues into the mechanism as well as possible treatment strategies for laminopathies. But these mouse models have some limitations when compared with human laminopathies. For instance most of these mouse models show disease symptoms only in the homozygous state, unlike the autosomal dominant nature of the human diseases, where only one allele is mutated. Thus the mouse models might not faithfully reproduce all features of the human diseases.

The main limitations of tissue culture models are that the expression and localization of lamin mutants may vary from one cell model to the other. Hence it is important to compare the properties of the mutants in more than one cell type. The findings from this thesis indicate that the assembly of the mutants is comparable in HeLa and C2C12 cells. In case of transient transfection of mutants in cultured cells, experiments have to be optimized to express minimal levels of exogenous proteins as higher levels of wild-type lamin A are also deleterious for the cell. Thus this may not truly represent the conditions seen in vivo, where equivalent levels of mutant lamin and wild-type lamins are expressed from each allele in the autosomal dominant diseases. Due to the deleterious effects of expression of lamin mutants, it is also difficult to establish a stable cell line expressing a lamin mutant, even under inducible conditions. Maintenance of cells from laminopathic patients may represent situations similar to in vivo conditions, but here again the culture conditions may affect expression of mutant lamins. Moreover, as the laminopathies are rare disorders, the amount of tissue that can be obtained from patients is limiting.