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Towards understanding lamin gene regulation

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Abstract. The lamins are components of the nuclear lamina, which forms a fibrous meshwork lining the inner nuclear membrane. Lamina-membrane interactions play a crucial role during nuclear disassembly and reassembly at mitosis, whereas lamina-chromatin association has been proposed to be essential for chromatin organization. The composition of the lamina changes considerably during embryonic development and cell differentiation. Recent studies have provided insights into the regulation of the lamin genes.

Keywords. Nuclear lamina; lamin gene expression; nuclear envelope.

1. The nuclear lamina

The nuclear and cytoplasmic compartments of a eukaryotic cell are segregated by the double membrane of the nuclear envelope. The inner nuclear membrane is associated with a filamentous network of proteins called the nuclear lamina, whereas the outer membrane is contiguous with the endoplasmic reticulum. The inner and outer membranes are periodically joined at nuclear pore complexes, through which nucleocytoplasmic transport of molecules occurs. Lamina-membrane interactions play a crucial role in nuclear assembly and disassembly during cell division. On the other hand, lamina-chromatin interactions have been postulated to be involved in DNA replication and transcription, in addition to a possible role in nuclear assembly.

The lamina has been visualized in cells from various species. Pioneering electron microscopic studies on nuclear envelopes manually dissected from *Xenopus laevis* oocytes revealed a meshwork of 10-nm lamina filaments organized in a roughly orthogonal array with a crossover spacing of ~ 50 nm (Aebi *et al* 1986). This classical view of a regular lamina organization has been challenged in recent studies with other cell types. In *Drosophila* early embryos and cultured mammalian cells, the lamina is apparently a highly discontinuous, fibrillar network, as viewed by three-dimensional fluorescence microscopy (Paddy *et al* 1990). In studies aimed to elucidate the role of the lamina in the spatial reorganization of the chromatin during the cell cycle, Bridger *et al* (1993) have shown that during the Gl phase in human fibroblasts, A-type lamins form discontinuous filaments which may associate with partially decondensed chromatin deep within the nucleus. By S phase, the lamins relocate to the nuclear periphery, in possible association with heterochromatin.

2. Lamin structure

The nuclear lamina is composed of one or more proteins called the lamins, which

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are highly homologous to the intermediate filament (IF) family of proteins. The vertebrate lamins have been classified as either A-type or B-type lamins, based primarily on biochemical criteria. During mitosis, when the lamina depolymerises, the B-type lamins remain associated with nuclear envelope vesicles, whereas the A-type lamins become solubilized (Gerace *et al* 1984). In mammals, birds and amphibians, two structurally related B-type lamins (Bl and B2, encoded by two distinct genes) are expressed in nearly all somatic cells, whereas the A-type lamins (lamins A and C in mammals) appear only late in embryonic development. Lamins A and C have almost identical amino acid sequences, except for their C-terminal domains, and arise by alternative RNA splicing from the same gene. Germ cells are characterized by a unique complement of lamins. In *Drosophila,* a precursor lamin (Dmo) has been identified, which undergoes extensive post-translational modifications to give rise to two A-type lamins, Dml and Dm2 (Gruenbaum *et al* 1988). Recently, a second lamin, related to lamin C, has been identified in *Drosophila* (Bossie and Sanders 1993), and a B-type lamin has been characterized from *Caenorhabditis elegans* (Riemer *et al* 1993). So far, no lamin gene has been isolated from yeast, although fission yeast can incorporate an exogenous avian lamin into a lamina-like structure (Enoch *et al* 1991). Isolation of a yeast lamin gene would advance our understanding of lamin function by providing a powerful genetic tool.

Lamins contain a central, α-helical, heptad-rich rod domain, which is a highly conserved feature in the IF family of proteins. The heptad repeats of hydrophobic amino acids drive the formation of coiled-coil α-helical dimers between two lamin polypeptides, with the carboxy terminii protruding as globular heads. The dimmers initially associate longitudinally to form head to tail polymers, which then assemble laterally to form 10 nm filaments resembling the lamina fibrils seen *in vivo* (Heitlinger *et al* 1991). The rod domain is flanked by N- and C-terminal sequences of variable size. The lamins contain a nuclear localization sequence (VTKKRKLE) and a C-terminal CaaX motif (see figure 1), which is also present at the C-terminii of *ras* proteins and certain yeast mating factors. In all these cases, the CaaX motif is the target for a series of post-translational modifications, namely, isoprenylation of the cysteine, proteolytic removal of the three terminal amino acids (aaX), and

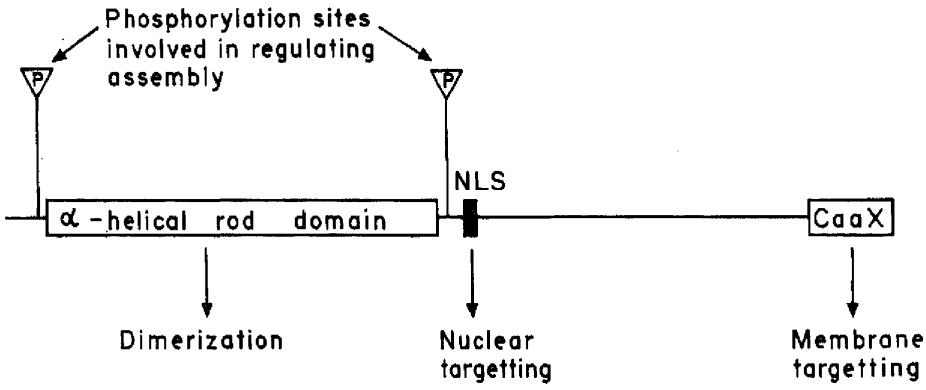

Figure 1. Diagramatic representation of the primary structure of lamins, indicating the known functional domains. NLS denotes nuclear localization signal.

carboxy-methylation of the isoprenylated cysteine (reviewed by Glomset *et al* 1990). The use of mutant lamin proteins has demonstrated that the above changes, which increase the hydrophobicity of the C-terminus, are required for targeting newly synthesized lamins to the inner nuclear membrane. However, it is possible that these changes by themselves are not sufficient for a strong membrane interaction, and association with integral membrane proteins may be required. In this regard, a lamin B membrane receptor has been identified and characterized in detail (Worman *et al* 1988). We have also shown by cross-linking techniques that the lamins from mouse liver nuclear envelopes are associated with a group of membrane proteins (Fatima and Parnaik 1991). A surprising finding is that the hydrophobic C-terminus of lamin A is proteolytically removed after incorporation of lamin A in the lamina. This may facilitate solubilization of the lamina at mitosis but poses the problem of how lamin A is reincorporated into the lamina of the reassembled envelope. The process of envelope reassembly is discussed in detail in a later section.

3. Lamins and evolution of IF proteins

Structural analysis of the lamins revealed that they contained elements common to the IF family of proteins; more specifically, the tripartite rod domain containing the α-helical coils characterized by a heptad repeat of hydrophobic amino acids. Cytoplasmic IF proteins of invertebrates show a closer relationship to lamins than do the IF proteins of vertebrates. The distinguishing features of the lamins are the NLS sequence and CaaX motif, both of which are absent in cytoplasmic IF proteins. The similarities between the cytoplasmic IF proteins and lamins may have arisen through common ancestry or could be explained by convergent evolution of these two types of filament-forming proteins. The similarities in amino acid sequence between them suggest common ancestry. Gene structure analysis by two groups (Doring and Stick 1990; Dodemont *et al* 1990) has further strengthened this model (see figure 2). There is a striking conservation of intron positions between the *Xenopus laevis* lamin LIII gene and the IF genes in the central rod domain. Furthermore, there is extensive conservation in the gene structure of the tail domains of the invertebrate non-neuronal IF protein and lamin LIII but not those of vertebrate IF proteins. The IF proteins in invertebrates can be divided into two sub-types, neuronal and non-neuronal; whereas the vertebrate IF proteins are considerably more complex and can be divided into four sub-types: type I-acidic epidermal keratins, type II-basic epidermal keratins, type III-vimentin, desmin, peripherin glial fibrillar acidic protein, type IV- neurofilaments. It has been proposed that the archetype cytoplasmic IF gene arose from a nuclear lamin-like ancestor by the loss of two signal sequences, the NLS and the CaaX motif.

The similarities in the gene structure of the lamins and cytoplasmic IF proteins may help in understanding the regulation of the lamin genes. Several IF genes have been isolated and the regulation of certain genes, in particular vimentin and keratins, has been well-characterized (Zehner 1991). Studies with transgenic mice have indicated that the sequences required for the tissue-specific expression of the vimentin gene are all at the 5' end of the gene. Four main elements have been defined. The proximal promoter element has the general regulatory motifs, that is,

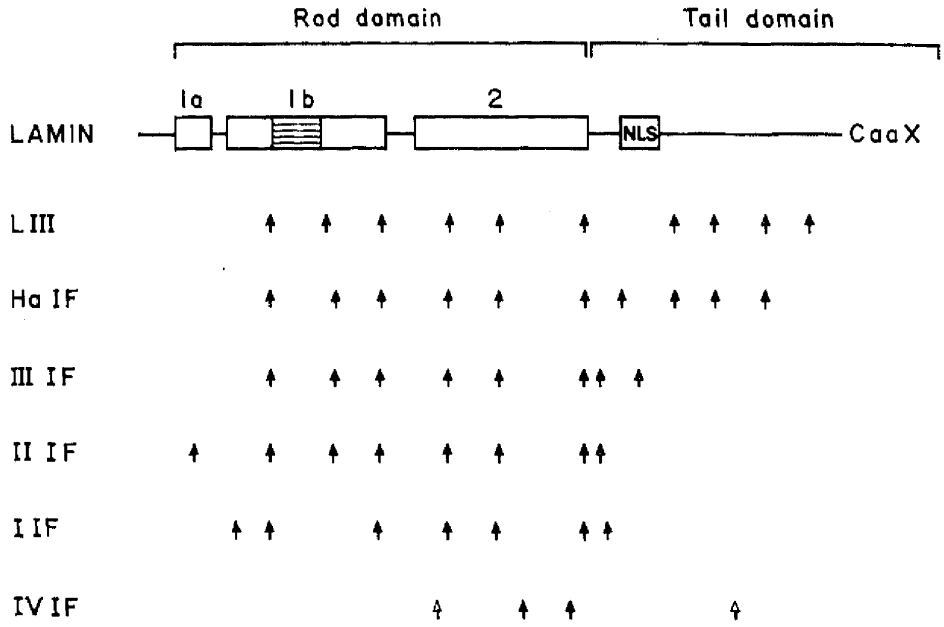

Figure 2. Comparison of intron positions in IF family of proteins. LIII, *X. laevis* lamin LIII; Ha IF, *Helix aspersa* non-neuronal IF protein; I–IV IF, type 1–IV vertebrate IF proteins. Hatched box region contains a seven heptad repeat sequence present only in lamins and invertebrate IF proteins, la, lb and 2 denote the three helical segments of the rod domain. The arrows with closed arrow heads indicate positions of introns; the two open arrow heads indicate intron positions present only in one out of three neurofilament genes that have been analysed. The data in this figure has been taken from Doring and Stick (1990) and Dodemont *et al* (1990). The human lamin A/C gene has been recently shown to contain the same intron positions as the *Xenopus* lamin LIII gene, except for an additional intron in the extreme 3' region (Lin and Worman 1993).

the CAAT, TATA and multiple GC-boxes. Tissue-specific expression is modulated by two down-regulatory sequences which function to switch the gene off during myogenesis, a proximal enhancer element and a silencer element; and one sequence, the distal enhancer dement, which probably mediates serum-inducibility of the vimentin gene.

4. Lamina reorganization during the cell cycle

4.1 *Role of phosphorylation in lamina disassembly*

Based on earlier data on the higher levels of phosphorylation of lamins during mitosis than during interphase, models have been proposed supporting the role of phosphorylation in lamina depolymerization (Gerace *et al* 1984). Several recent studies have strengthened this hypothesis. Two conserved serine residues flanking the central rod domain in lamins A and C have been shown to be phosphorylated in a mitotic extract (Ward and Kirschner 1990). Phosphorylation was accompanied by disassembly of the lamin filaments. Mutations in these serine residues which

prevent their phosphorylation, block the disassembly of the lamina during mitosis (Heald and McKeon 1990). Furthermore, these serine residues are phosphorylated *in vitro* by $p34^{cdc2}$ kinase, a major regulatory kinase of the cell cycle, and these sites are also specifically phosphorylated *in vivo* in lamin B2 during mitosis (Peter *et al* 1990; Luscher *et al* 1991). However, it should be noted that lamin disassembly is not sufficient for nuclear envelope breakdown at mitosis. Moreover, purified $p34^{cdc2}$ kinase cannot directly cause envelope breakdown, though total mitotic extracts are able to do so (Peter *et al* 1990). Other nuclear membrane components or kinases may be involved in controlling envelope breakdown, possibly by changes in phosphorylation-dephosphorylation levels of key proteins.

Lamins can be phosphorylated on other sites also. Protein kinase C phosphorylation sites occur close to the NLS of lamin B2 and phosphorylation of these sites interferes with nuclear transport of lamin B2 (Hennekes *et al* 1993). Such effects may modulate the organization of the nuclear lamina.

Recent studies on mitotic CHO cells have revealed that vimentin filaments are specifically associated with nuclear envelope vesicles containing lamin B and inner nuclear membrane proteins, in a phosphorylation-dependent manner (Maison *et al* 1993; Meier and Georgatos 1994). These findings suggest that mitotic vesicles containing lamin B may transiently dock on intermediate filaments during mitosis, whereas lamins A and C become solubilized in the cytoplasm.

4.2 *Lamins and nuclear reassembly*

The mechanism of nuclear reassembly after mitosis has been extensively studied using *Xenopus* egg extracts (Newport *et al* 1990) and mitotic cultured cells (Burke and Gerace 1986; Glass and Gerace 1990). During envelope reassembly, membrane vesicles bind to the surface of chromosomes and fuse to form the double membrane, the pore complexes reassemble from preexisting pools of solubilized proteins and the lamina repolymerizes. However, there is some controversy about the exact role of the lamins in the process. Basically, two models have been proposed. In one model, lamin proteins initially bind to the surface of the chromosomes followed by binding of membrane vesicles and envelope assembly (Burke and Gerace 1986; Glass and Gerace 1990). In the second model, the binding of membrane vesicles to chromosomes has been proposed to occur without a requirement for lamin binding (Newport *et al* 1990). These two models are not incompatible if one considers that different mechanisms may operate in different cell types. In mitotic extracts from mammalian cells, immunodepletion of lamins A and C inhibits envelope assembly significantly (Burke and Gerace 1986). Lamins A and C can also associate with the surface of condensed chromosomes (Glass and Gerace 1990). In a recent study, a class of integral membrane proteins of the envelope has been identified which can bind to lamins as well as mitotic chromosomes, and this interaction is blocked by mitotic phosphorylation (Foisner and Gerace 1993). (Lamina-chromatin interactions are dealt with in more detail in §8 of this article). In *X. laevis,* the egg contains a single lamin protein, LIII, which is solubilized during mitosis. Depletion of LIII from the egg extract does not block initial formation of an envelope around added chromatin but is essential for maintaining the structural integrity of the envelope (Newport *et al* 1990). Membrane vesicles are targeted to chromosomes *via* a

vesicle-bound receptor when components of the vesicles are in a dephosphorylated state (Pfaller *et al* 1991). Thus in both systems dephosphorylation of key proteins modulates envelope reassembly.

5. Tissue-specific expression of lamin proteins

The composition of the nuclear lamina changes significantly during development. This was initially shown in *X. laevis* by immunocytochemical studies (Stick and Hausen 1985; Benavente *et al* 1985). Lamin LIII, the only lamin present in the oocyte, is present in embryos up to the tail bud stage, and is thereafter restricted to neuronal and muscle cells. The adult specific lamins, LI and LII, appear at the midblastula transition and gastrula respectively. Translation of LI at least is from maternal mRNA stocks. Lamins LI and LII are related to the B-type mammalian lamins, whereas LIII falls into a unique class by itself. Thus the appearance of the different lamin classes during embryonic development in amphibians does not strictly coincide with that found in avian and mammalian systems. A lamin A-like protein has also been identified in *Xenopus* somatic tissues but its developmental profile has not been reported (Wolin *et al* 1987).

Early chicken embryos have been found to express substantial amounts of lamins Bl and B2 but little, if any, lamin A (Lehner *et al* 1987). Furthermore, the expression of lamin B2 remains nearly constant in all tissues whereas that of lamin A and Bl appears to be inversely related. Lamin A is induced at different stages of development in different tissues and its expression correlates with a reduced expression of lamin Bl.

Detailed immunological and biochemical studies have been carried out on lamin expression during mouse embryogenesis (Stewart and Burke 1987; Rober *et al* 1989). Embryos up to eight days of gestation express only lamin B. Embryonal carcinoma cells too express only lamin B, whereas their differentiated derivatives contain lamins A and C also. (Since the lamin B subtypes, Bl and B2, were identified later, a single lamin B type has been referred to in these studies). In the developing embryo, the timing of acquisition of lamins A and C depends on the tissue. Using immunocytochemical studies, it has been observed that lamins A and C appear by day 12 in muscle (except for heart muscle); by day 14 in the epidermis; but only after birth in several other epithelia and in heart myocytes.

Thus the fundamental organization of the lamina changes during development from a homopolymer of B-type lamins to a heteropolymer of A- and B-type lamins. Since in the adult, B-type lamins do not self-associate (Georgatos *et al* 1988) an important question is how is a lamin homopolymer formed in early embryos. We have addressed this by studying the binding of radiolabelled lamin B to fetal and adult mouse liver lamins *in vitro* (Pandey and Parnaik 1991). We have observed that the labelled lamin B binds to fetal lamin B but only adult lamins A and C. However, interaction with fetal lamin B is disrupted if the lamins are digested with phosphatase, suggesting that the fetal lamin B is hyperphosphorylated. No other difference is discernible between fetal and adult lamin B, using biochemical and immunological criteria.

6. Germ cell-specific lamins

The germ cells contain a unique complement of lamins. In *Xenopus* oocytes, a

single lamin, LIII, is expressed. In spermatogenic cells, a male germ line-specific lamin, LIV, has been detected in spermatids and sperms (Stick and Hausen 1985; Benavente *et al* 1985). Spermatogenesis-specific lamins have also been found in mammals. A lamin Bl-like lamin has been identified in rat spermatocytes, based on immunoblot analysis and two-dimensional tryptic peptide mapping (Sudhakar and Rao 1990; Vester *et al* 1993). Furthermore, this lamin can be isolated as a component of synaptonemal complexes in spermatocytes and has been postulated to play an important role in meiosis (Sudhakar and Rao 1990).

In a recent study, the gene structure of a mouse spermatocyte-specific lamin B3 has been reported (Furukawa and Hotta 1993). The cDNA and amino acid sequences of lamin B3 indicate that it is generated by differential splicing and alternative polyadenylation from lamin B2. Lamin Bl and B2 could also be detected in mouse testes but at much lower levels than in somatic cells. At the protein level, the C-terminal tail and coil 2 sequences of lamin B3 and B2 are identical. However, lamin B3 has a unique N-terminal sequence, rich in proline residues. This results in major functional differences in the two lamins, as revealed by studies involving transfection of cDNAs into cultured cells. Lamin B3-expressing somatic cells were found to undergo a significant change in nuclear morphology from spherical to hook-shape, dependent on the presence of the unique N-terminus of B3. Thus lamin B3 may play an essential role in reorganization of pachytene chromosomes during spermatogenesis in the mouse. The presence of lamin B3 in the rat or other mammalian species has not yet been reported.

We have found that in adult testes, a unique 2·0 kb RNA hybridizing to rat lamin A cDNA replaces the somatic lamin A and C RNA (figure 3), indicating

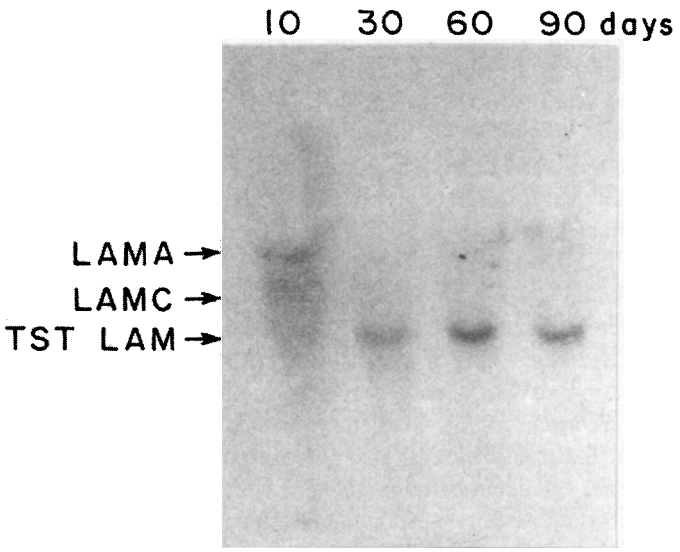

Figure 3. Northern analysis of lamin transcripts in rat testes. Total RNA (10 μ*g* each) from testes of 10-day, 30-day, 60-day and 90-day old rats was analysed by a Northern blot probed with nick-translated prlamA DNA, as described in Sambrook *et al* (1989). Bands corresponding to transcripts of lamin A, lamin C and testes-specific lamin are indicated.

that a testes-specific lamin A-like protein may exist in rat testes. This testes-specific RNA is barely detectable in the 10-day testes, which also exhibits both the somatic lamin A and C RNA species. In testes of 30-day old rats, wherein meiosis has commenced, the level of the unique RNA is significantly higher and reaches maximal levels by 60 days. The somatic RNAs are not detectable in 30-90-day testes samples. Hybridization of this testes-specific lamin A-like RNA to probes corresponding to distinct regions of the lamin A protein showed that the RNA hybridized strongly to the non-helical common regions of lamins A and C, poorly to the N-terminal helix 1, and not at all to the unique C-terminal region of lamin A. As discussed above, mouse lamin B3 has a unique N-terminus but is identical to lamin B2 from helix 2 till the C-terminus. Our preliminary data suggests that our putative testes-specific lamin A-like protein may also differ from lamin A at the N- and C-terminii, but can be similar or identical in the central regions. However, we would have to initially confirm that the lamin A-like transcript is actually translated *in vivo*.

7. Regulation of lamin genes

The cDNAs for the lamin A and C genes have been isolated from human (Fisher *et al* 1986; McKeon *et al* 1986) and rodent sources (Riedel and Werner 1989; Ozaki and Sakiyama 1992; Nakajima and Sado 1993). We have isolated a 2·9 kb rat lamin A cDNA (referred to as prlamA) from a rat cDNA library, using a 30-mer oligodeoxynucleotide probe (derived from a 5' consensus sequence in the mouse and human genes). Comparison of the sequence of our rat lamin A cDNA with those of human and mouse sequences showed that the rat cDNA started at codon 26 and continued through the coding region to end in a stretch of 28 A residues after 3'UTR of 916 bp. At the nucleotide level, the coding region showed identity of 95% and 92% with the mouse and human sequences respectively, whereas the 3'UTR showed identity of 87% and 78% with the mouse and human sequences respectively. At the protein level, most of the amino acid substitutions (figure 4) were observed in the non-helical C-terminal region. There was also a deletion of one amino acid and an insertion of two amino acids in the rodent sequences with respect to the human sequences. Overall, the rat protein showed homology of 99% and 96% to the mouse and human lamin A sequences, whereas the mouse and human proteins shared a 95% homology with each other. Thus the conservation of the lamin sequence is seen both at the amino acid as well as nucleotide levels, even in the 3'UTRs, suggesting a very slow rate of divergence or evolution. The structural organization of the gene for human lamin A and C has been described recently (Lin and Worman 1993). Sequences analysis of genomic clones has proved the hypothesis that the transcripts for these two proteins arise by alternative splicing from the same gene, by demonstrating the presence of alternative splice sites. The available 5' clone contains only ~ 400 bp of sequence upstream of the start codon, including TATA and CCAAT boxes and G+C-rich segments. A longer promoter sequence may have to be analysed in order to study the differential expression of the A-type lamins.

Avian, mouse and human cDNAs are available for lamins Bl and B2 (Vorburger *et al* 1989; Hoger *et al* 1988, 1990; Pollard *et al* 1990). These two genes share

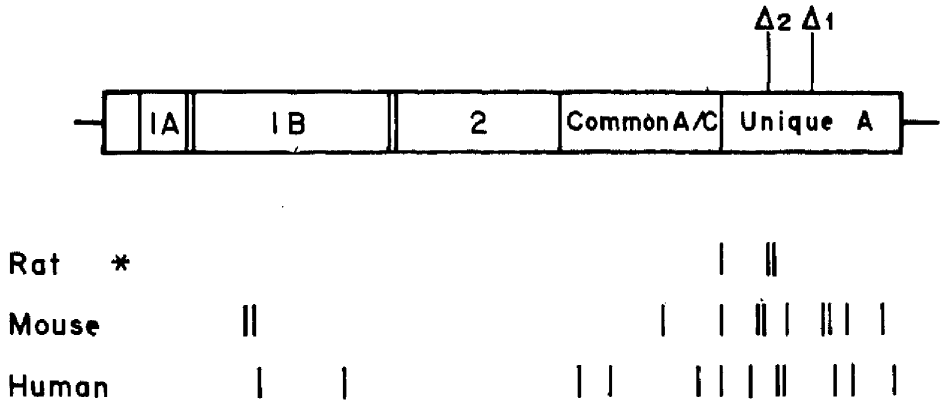

Figure 4. Comparison of rat, mouse and human lamin A protein sequences. Boxed region indicates the coding sequences; an asterisk in the rat sequence indicates the region of 25 amino acids that is absent in our cDNA sequence. Δ1 and Δ2 represent a deletion of one amino acid and insertion of two amino acids respectively in the rodent sequences with respect to the human sequence. Vertical bars represent amino acid substitutions with respect to the other two species.

only 76% homology (in mouse). A genomic clone for the mouse lamin B2 gene has been isolated and characterized (Zewe *et al* 1991). This clone spans a 15 kb segment, including 700 bp of sequence upstream of the ATG start codon. The gene has eleven introns, some as small as ~ 70 bp. The 5' region does not contain a classical TATA box but two TATA-like elements. This region also contains three putative CAAT boxes, a CCCCACCCC box and a particularly G+C-rich region with two putative Sp 1 binding sites. These promoter motifs are similar to those of other housekeeping genes. This is consistent with the observation that the B-type lamins are expressed ubiquitously.

We have analysed the expression of the rat lamin A and C genes in different tissues (figure 5). The rat lamin A cDNA probe hybridized to both lamin A and C RNAs, as expected due to their extensive homology. Tissues that contain a large population of dividing precursor cells and have small amounts of lamin A and C protein, such as thymus and spleen, did not have detectable amounts of lamin A and C RNAs, although histone H4 mRNA was expressed abundantly as expected in these dividing cells. Tissues that contain normal amounts of lamin proteins but have stopped dividing, such as adult liver and kidney, also had undetectable amounts of. lamin A and C RNAs. These findings suggest that the lamina proteins undergo a very slow turnover, as would be expected for structural proteins, and minimal amounts of steady state levels of lamin RNAs are sufficient to maintain the lamina. Dividing cultured cells such as 3T3 and F-lll fibroblasts had high levels of lamin A and C RNAs. When 3T3 cells were allowed to reach quiescence, lamin RNA levels were reduced dramatically. Our data implies that cells need to be both differentiated and dividing to express appreciable amounts of lamin A and C RNAs. This idea was strengthened by the observation that upon partial hepatectomy in the rat, the expression of lamin RNAs was reactivated in the regenerating liver.

There are very few reports on the transcriptional status of the lamin genes, and

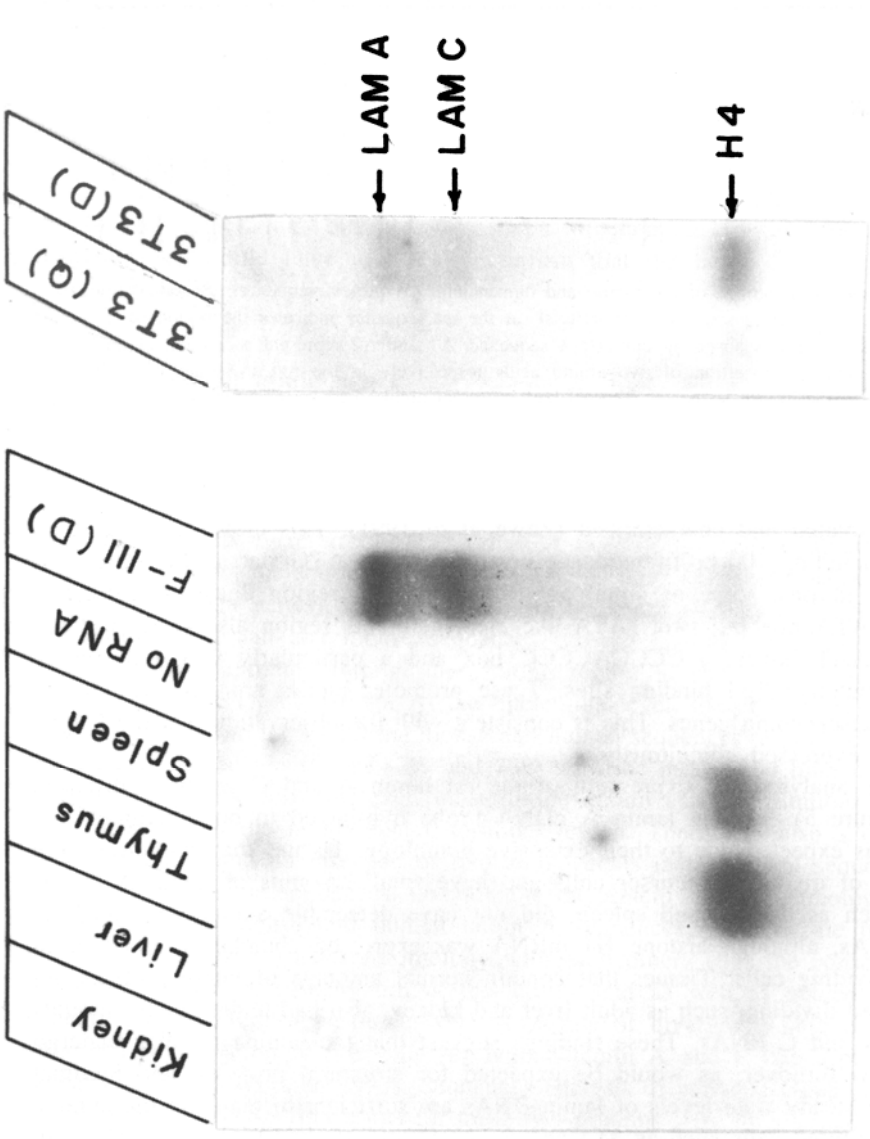

Figure 5. Northern analysis of lamin transcripts in tissues and dividing (D) and quiescent (C) cultured cells. Total RNA (10μg each) from the indicated cells was analysed by a Northern blot probed with nick-translated prlam A DNA and pHh4A-SP3 DNA (containing 840 bp of the human histone H4 gene cloned in the pSP65 vector), as described in Sambrook *et al* (1989).

the mechanisms contributing to lamin gene regulation during embryonic development or the functional significance of this regulation are not known. Towards understanding these aspects, we have studied the expression of lamin A and C during rat fetal liver development. Although significant amounts of lamin A and C proteins are present only in the newborn animal (Rober *et al* 1989; Pandey and Parnaik 1991), we have observed that lamin A and C transcripts are detectable in the fetus also. Expression levels gradually taper off after birth and lamin RNAs are undetectable in the adult. In hepatocytes from the fetus and newborn animal, the chromatin structure of the lamin A gene is highly sensitive to DNase I and is thus in an unfolded, 'active' configuration. However, in adult hepatocytes, the gene is in a DNase I-insensitive state. These preliminary findings suggest that the mechanisms contributing to lamin A regulation are complex, and it would be necessary to analyse the 5' promoter sequences of the gene in order to gain insights into its regulation. Towards this end, our current studies are aimed at isolating genomic clones for the rat lamin A gene.

In a development study on *Drosophila* lamin gene expression, it has been reported that the lamin precursor protein Dmo (which is processed to lamins Dml and Dm2) is coded by two RNAs which differ only in their 3' untranslated segments and are differentially expressed during embryogenesis (Gruenbaum *et al* 1988). The shorter transcript (2·8 kb) appears to be the oocyte "storage form", whereas the longer message (3·0 kb) predominates at other developmental stages. These RNAs are encoded by a single gene on chromosome 2. These studies may be able to provide insights into the mechanism of alternative splicing of lamin A and C in mammals, though functionally the two systems appear to be distinct.

8. Lamina-chromatin interactions

The lamins interact with chromatin in most cell types (Glass and Gerace 1990; Paddy *et al* 1990). The lamina provides attachment sites for interphase chromosomes and may be necessary for the higher level organization of chromatin. We have earlier discussed the importance of lamin association with integral membrane proteins in nuclear envelope disassembly and reformation during mitosis. Specific association of lamins and chromatin has been documented in a few studies. Mammalian lamins A and C, upon dephosphorylation, bind to mitotic chromosomes (derived from CHO cells) *in vitro,* as shown by immunofluorescence techniques (Glass and Gerace 1990), This interaction requires the chromosomes to be in their native conformation. *Xenopus* lamins A and LII have also been shown to bind to chromosomes *in vitro,* and this association is mediated by a sequence at the carboxy terminal of lamin A which is rich in serine, theonine and glycine residues (Hoger *et al* 1991). Furthermore, certain DNA sequences have been proposed to form nuclear lamina and matrix attachment regions (MARS) for chromosomes. *In vitro* binding studies have identified lamin Bl as a MARS-binding protein (Luderus *et al* 1992). Thus it is conceivable that the lamins may bind to specific DNA sequences in chromosomes.

9. Future perspectives

The principles governing lamina assembly and disassembly are now well-established,

especially the role of phosphorylation-dephosphorylation events during the cell cycle. However, the significance of and the mechanisms regulating the tissue-specific and developmental changes in lamin expression are not yet understood. Our observations on the expression of the lamin genes in developing liver provide a basis for studying the role of transcription factors in determining tissue-specific lamin expression. Future studies in the field should hopefully provide insights into the functional significance of lamin-chromatin interactions and the spatial organization of the chromosomes during cell differentiation and growth.

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