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Lamins and Other Nuclear Architectural Proteins: Emerging Roles in the Spatial Organisation of Nuclear Events

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Eukaryotic nuclei have discrete compartments or domains enriched for specific proteins involved in specialised functions such as DNA replication, transcription and RNA splicing. Chromosomes are also organised into defined domains or territories in the nucleus. A major issue concerning nuclear organisation is the identity of the underlying structure or nucleoskeleton and the extent to which the components of this structure are involved in nuclear functions. Important advances have been made in the past few years in understanding the functions of the major architectural proteins in the nucleus. The lamins play essential roles in DNA replication and transcription. Other structural proteins such as nuclear actin are components of chromatin remodelling complexes and mRNA processing complexes. These recent findings open up the exciting possibility that lamins and actin might act to modulate the organisation and functions of nuclear activities.

Key Words: Lamin A, Nuclear Compartments, Lamin Speckles, Laminopathies, Transcription

Introduction

In the eukaryotic nucleus crucial functions such as DNA replication and RNA transcription are performed in a highly organised and efficient manner despite the tremendous complexity of the genome. In studies carried out primarily with mammalian cells, molecules involved in the above processes have been localised in spatially distinct nuclear domains or compartments, such as transcription sites, DNA replication centres, speckled domains enriched in RNA splicing factors termed splicing factor compartments or interchromatin granule clusters (IGCs), Cajal bodies (CBs), and many others. Nuclear compartments contain a distinct set of proteins, can be visualised by microscopic techniques, and have been purified in some cases. These domains are not membrane-bound, unlike cytoplasmic organelles. The association of proteins with specific compartments can be highly dynamic, and the compartmentalisation of nuclear factors represents a steady state rather than a static situation. A consideration of the functions of nuclear compartments must necessarily include information on the spatial organisation of the genome.

Interestingly, the majority of nuclear compartments are resistant to extraction by detergents and nucleases - treatments which remove membranes, soluble proteins and chromatin and leave behind a nucleoskeletal substructure. Based on ultrastructural studies carried out over the past forty years, this substructure has been viewed as a nucleoplasmic network of branched 10-nm filaments that connect to the nuclear lamina that lies just beneath the inner nuclear membrane. The major components of the nuclear lamina are a group of proteins called the lamins which belong to the intermediate filament superfamily of proteins (reviewed in Goldman et al. 2002). However, the proteins that comprise the internal nucleoskeleton have not been definitively identified. Recent reports suggest that nucleoskeletal proteins such as lamins as well as nuclear actin have important functional roles in various nuclear processes including DNA replication and transcription.

In this article, I will first briefly review information about nuclear compartments, chromosome positioning and the nucleoskeleton, and then highlight new findings on the functions of lamins, lamin-binding proteins and nuclear actin, especially with respect to their role in organisation of nuclear events. This review is not an exhaustive coverage of the literature, and I recommend some excellent recent reviews for detailed information as well as citations to the original literature on nuclear compartments (Lamond & Earnshaw 1998, Misteli 2000, Spector 2003, Jackson 2003), chromosome territories (Cremer & Cremer 2001,

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Parada & Misteli 2002, Chubb & Bickmore 2003), and the nucleoskeleton (Nickerson 2001). Although the focus of this review is on the mammalian cell nucleus, other organisms are mentioned wherever appropriate.

Nuclear Compartments

The Nucleolus

The most obvious compartment of the nucleus that can be visualised by light microscopy alone is the nucleolus, which is formed by clustering of repeats of ribosomal RNA genes from several chromosomes. The nucleolus is the site of transcription and processing of 28 S, 18 S and 5.8 S rRNAs by RNA polymerase I and the site of biosynthesis of the precursor particles for ribosomes, termed the pre-90S, pre-60S and pre-40S ribosomal units (reviewed in Shaw & Jordan 1995, Scheer & Hock 1999). The nucleolus itself is spatially organised into the fibrillar centre and dense fibrillar component where pre-rRNAs are synthesised, processed and modified, and a granular component which is the site of ribosome assembly. Like most other compartments, the nucleolus is a dynamic structure that is disassembled during entry into mitosis and reassembled upon exit from mitosis in higher eukaryotes.

RNA Polymerase II Transcription Sites

Eukaryotic gene expression is a complex process involving several steps such as chromatin remodelling, transcription, 5′ capping of mRNA, pre-mRNA splicing and 3′ processing, mRNA export and translation in the cytoplasm. In classical studies, Fakan and his colleagues showed by ^{3}H-uridine incorporation into nascent mRNA that non-nucleolar transcription occurred throughout the nucleoplasm (reviewed in Fakan 1994). Using shorter pulses of precursor labelling in actively transcribing HeLa cells, Cook and coworkers showed that RNA polymerase II (pol II) transcription occurred at approximately 1000-2000 discrete sites, and at each active centre or transcription site, groups of genes were transcribed and processed (Iborra et al. 1996). The authors proposed that a transcription site or factory was composed of the gene template and RNA pol II together with associated transcription factors, and was attached to the nucleoskeleton. A typical pattern of RNA pol II transcription sites is shown in figure 1. Several studies have reported that active genes and many transcription factors are bound to the nuclear substructure in mammalian cells (reviewed in Stein et al. 1995). But as transcription factors are likely to diffuse throughout the nucleus, is their association with immobile transcription sites dynamic in nature, and how is this regulated? To address this issue, the localisation of the runt-related transcription factors (RUNX1 and RUNX2) that play important roles in cell differentiation and fetal development was studied in living cells, and the RUNX proteins were observed to be dynamically localised to discrete immobile foci, most of which coincided with active transcription sites (Harrington et al. 2002). Removal of the domain responsible for the association of RUNX proteins with subnuclear foci led to lethal abnormalities, indicating that this association was crucial for RUNX proteins to be functionally active. In studies on the distribution of estrogen receptor, it has been shown that upon ligand binding, transcriptionally active receptor exhibits a dynamic association with the nucleoskeleton in living cells (Stenoien et al. 2000). Transcription inevitably needs to be examined in the context of the chromatin template and this is discussed in the section on chromosome positioning and gene regulation.

Nuclear Speckles or Splicing Factor Compartments

RNA splicing factors are present in high concentrations in compartments or speckles that correspond at the electron microscopic level to interchromatin granule

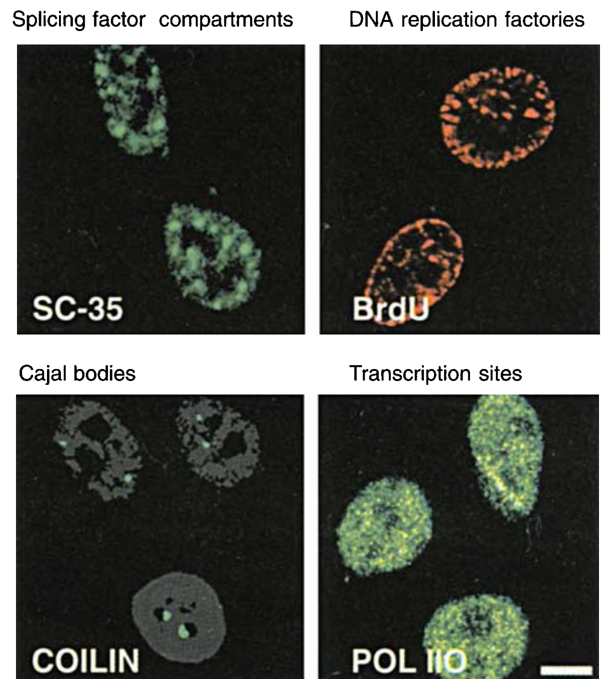

Figure 1: Examples of nuclear compartments in HeLa cells viewed by confocal laser scanning microscopy. Splicing factor compartments, stained with antibody to the non-snRNP splicing factor SC-35; DNA replication factories, labeled with antibody to bromodeoxyuridine (BrdU) to mark nascent DNA that has incorporated bromodeoxyuridine; Cajal bodies, stained with anti-coilin antibody (coilin in green and nucleus filled in grey); transcription sites, labeled with an antibody to the active hyperphosphorylated form of RNA pol II termed pol IIO. Bar, 5 mm.

clusters (IGCs), and are also dispersed in the nucleoplasm on perichromatin fibrils which contain nascent transcripts (reviewed in Spector 1993, Fakan 1994, Lamond & Spector 2003). Approximately 20-50 nuclear speckles of varying size (0.5-3 μm) are found in a mammalian cell nucleus, and each is comprised of clusters of discrete particles with dimensions of 20-25 nm. HeLa cell nuclei stained with an antibody to the splicing factor SC-35 are shown in figure 1. Biochemical purification of IGCs and identification of their constituent proteins by mass spectrometry has indicated that this compartment contains about 150 proteins (Mintz et al. 1999). These include pre-mRNA splicing factors, small nuclear ribonucleoproteins (snRNPs), transcription factors and 3′-processing factors, as well as putative structural proteins. The splicing of some pre-mRNAs occurs concomitantly with transcription on perichromatin fibrils, and away from or at the periphery of splicing factor domains (Wansink et al. 1993, Jackson et al. 1993, Cmarko et al. 1999). However, cotranscriptional splicing is unlikely to occur for all genes, especially those with large introns or genes undergoing alternative splicing (Neugebauer 2002). In a landmark study, Misteli et al. (1997) directly demonstrated that splicing factors could be recruited from splicing factor compartments to transcription sites, by using time-lapse microscopy to visualise pre-mRNA splicing factors in living cells. The association and dissociation of splicing factors from their compartments are controlled by phosphorylation-dephosphorylation of splicing factors. These compartments are dynamic and their size can change depending on RNA splicing or transcription levels in the cell; for example, they become considerably enlarged due to reduced dissociation of splicing factors in the presence of transcriptional inhibitors, in pathological conditions or upon inhibition of splicing. The exact functions of splicing factor compartments are not clear. In addition to their importance in the storage and recruitment of splicing factors, these domains may be required for the recycling of splicing factors (by phosphorylation) or for selection of alternative splice sites for specific transcripts. An interesting observation is that components of phosphoinositide signalling pathways such as phosphatidylinositol phosphate kinases and phosphatidylinositol bisphosphate have been located in these domains (Boronenkov et al. 1998), but their precise role has not yet been elucidated.

Cajal Bodies and Nuclear Gems

The Cajal bodies (CBs) are a highly conserved nuclear domain, ranging from 0-10 bodies per mammalian cell nucleus with an approximate size of 0.1 – 2 μm in diameter. CBs were originally called coiled bodies but have been recently renamed Cajal bodies in honour of Ramón y Cajal, a Spanish cytologist who discovered these structures in 1903. A specific marker for the CB is the protein coilin (CBs stained with anti-coilin antibody are shown in figure 1). CBs contain a large number of nuclear factors such as snRNPs and small nucleolar RNPs (snoRNPs), certain nucleolar proteins and pol II transcription factors (Gall 2000). The presence of CBs is correlated with gene expression, and pol II inhibitors can disrupt the structure of CBs. But as CBs do not contain DNA or non-snRNP splicing factors they are unlikely to be sites of transcription or pre-mRNA splicing. Recent evidence suggests that CBs play an important role in the biogenesis and maturation of snRNPs and snoRNPs (reviewed in Ogg & Lamond 2002). CBs have been suggested to have dynamic interactions with chromatin and possibly with specific gene loci, although their movements appear to be restricted due to interactions with subnuclear structures. The survival-of-motor-neurons (SMN) protein is an important nucleocytoplasmic shuttling protein that is involved in the biogenesis of snRNPs, and is localised in nuclear 'gems' which are bodies that coincide with CBs in many cell types though they are kinetically independent structures, as shown by studies in living cells (Dundr et al. 2004).

Promyelocytic Leukemia Nuclear Bodies, OPT Domains, Stress Granules, Omega Speckles

Promyelocytic leukemia (PML) bodies were first identified by autoantibodies recognizing the Sp100 transcription factor. PML bodies have been suggested to have roles in growth control, suppression of transformation, apoptosis and senescence (reviewed in Borden 2002). In studies on acute PML, it was observed that these bodies were disrupted due to the formation of a fusion protein by a chromosomal translocation between the PML protein and the retinoic acid receptor a. PML bodies are also disrupted in certain cases of viral infection. PML bodies have been found to contain specific transcription factors, and in a recent study these domains have been localised near transcriptionally active gene loci, though their exact role is not clear (Wang et al. 2004).

Other nuclear domains that have been identified are the Oct1/PTF transcription domain or OPT domain observed in certain mammalian cell types, which is enriched for the transcription factors Oct1 and PTF (PSE-binding transcription factor) involved in transcription of snRNA and related genes (Pombo et al. 1998), and stress granules which are formed upon heat stress and are enriched in the heat shock factor HSF1 (Jolly et al. 1997, Chiodi et al. 2000). Novel compartments

termed omega speckles contain hnRNPs associated with noncoding hsr-omega RNA in Drosophila, and have been observed to coalesce into large clusters upon heat stress (Prashanth et al. 2000). Some of these domains associate with specific chromosomes and might be involved in gene-specific transcription.

DNA Replication Factories

Sites of DNA replication in the eukaryotic nucleus are termed DNA replication factories. Using immunochemical detection of the DNA precursor bromodeoxyuridine or of replication proteins, it has been observed that DNA synthesis in S-phase occurs at discrete foci which correspond to clusters of replication forks containing nascent DNA (Nakamura et al. 1986, Nakayasu & Berezney 1989). The number and pattern of foci gradually change as S phase proceeds. Initially few small, discrete foci are observed in euchromatic regions which rapidly increase in number and intensity till mid-S phase. At this stage, the foci become larger and fewer in number and are localised towards the nuclear periphery. In late S phase few large domains are seen in the interior of the nucleus. A typical mammalian cell nucleus that has incorporated bromodeoxyuridine at DNA replication sites is shown in figure 1. In live cell imaging studies with green fluorescent protein (GFP) fused to the replication protein proliferating cell nuclear antigen (PCNA), which is a component of DNA polymerase δ and colocalises with DNA replication sites, changes in the pattern of foci have been attributed to a gradual assembly and disassembly of foci as S phase proceeds (Leonhardt et al. 2000, Somanathan et al. 2001). There are several studies which indicate that DNA replication sites are attached to the nuclear substructure (reviewed in Berezney et al. 1995, Cook 1999), the role of lamins in DNA replication is discussed in a later section.

Chromosome Positioning and Gene Regulation

Interphase chromatin is not randomly organised but occupies distinct and exclusive positions which are termed chromosome territories. The organisation of chromosomes into distinct territories was proposed by Rabl and Boveri more than a century ago, and elegantly established in the 1980s (reviewed in Manuelidis 1990, Cremer & Cremer 2001). The territories occupied by specific chromosomes have been confirmed by fluorescent in situ hybridisation (FISH) techniques using chromosome-specific painting probes. There is a strong evolutionary conservation of chromosome positions, suggesting a functional role for chromosome organisation. Generally the more gene-dense chromosomes are towards the interior of the nucleus. The preferred arrangement observed in some cell types appears to be a radial positioning of chromosomes (reviewed in Parada & Misteli 2002, Chubb & Bickmore 2003). But the above features do not hold good for all organisms or all cell types in a given organism. Chromosome positioning is probably initiated in the later stages of mitosis and chromosomes appear to be free to move to some extent till early G1 when chromatin decondenses; thereafter only limited motion occurs (Walter et al. 2003, Gerlich et al. 2003, Thomson et al. 2004). A possible consequence of the specific positioning of chromosomes relative to each other is that chromosomal translocations might occur more frequently between gene loci of chromosomes in close proximity and this has been observed with specific translocations in certain leukemias (Roix et al. 2003).

Chromosome positioning and its functional significance for gene expression are current topics of considerable interest. Studies on the location of active genes in a territory using FISH suggest that active transcription sites are scattered throughout a territory and not always limited to the surface of the territory, and certain transcribing gene loci are presented in "holes" in the chromosome territory which are likely to represent regions of decondensed chromatin (Mahy et al. 2002). A major advance in analysis of chromosome dynamics in living cells by Belmont and colleagues has been the development of the *lac* operator-GFP-*lac* repressor gene array for tagging chromosomes and directly viewing chromatin dynamics. Using this system large scale chromatin decondensation has been observed when strong transcriptional activators are targeted to the *lac* operator domain (Tumbar et al. 1999). Spector and coworkers have recently described an elegant system to study events during gene activation at a single locus in live cells, and they have visualised decondensation of the transgene array, depletion of heterochromatin protein 1, histone exchange and progressive mRNA synthesis in real time (Janicki et al. 2004).

Chromosomes are constrained in their motion by interactions with structural proteins such as lamins as well as proteins of the inner nuclear envelope, and have been observed to be organised into loops that are anchored to the nuclear matrix or chromosome scaffold in mammalian nuclei (Ma et al. 1999). In a distinct example of chromosomal organisation, the ends of chromosomes termed telomeres are positioned near the nuclear envelope in yeast. This clustering of telomeric chromatin in foci near the envelope is thought to facilitate transcriptional silencing of these regions by a family of proteins called silent information regulators (SIR) (Palladino et al. 1993). As yeast lack a nuclear lamina, it has been

suggested that yKu and SIR proteins mediate telomere attachment, though other mechanisms might also operate (Laroche et al. 1998, Andrulis et al. 1998, Taddei et al. 2004). Further studies on gene silencing and chromatin structure have been reviewed earlier (Spector 2003).

The dynamic nature of nuclear organisation is emphasised in studies on the positioning of nuclear compartments with respect to chromosomes. A distinct case of chromosome organisation leading to efficient gene expression is that of the nucleolus where the rDNA repeats of different chromosomes are brought together in a domain with high local concentration of necessary factors to ensure rapid processing of these genes. A physical proximity of nuclear domains to specific chromosomal sites has been observed with the OPT domain which is often found in association with chromosomes 6 and 7 (Pombo et al. 1998), the heat shock granules that are found near chromosomes 9, 12 and 15 (Denegri et al. 2002), the CBs that are often associated with U2 and histone genes (Ogg & Lamond 2002), and PML bodies which are localised near the major histocompatibility locus on chromosome 6 (Borden et al. 2002). It has been suggested that gene-rich regions might cluster around splicing factor compartments to form local "euchromatic neighbourhoods" (Shopland et al. 2003).

The Nucleoskeleton

The nucleoskeleton was originally defined by Fawcett as the non-chromatin structures of the nucleus readily observed in unextracted cells under the electron microscope, which were predominantly composed of a fibrogranular ribonucleoprotein network (Fawcett 1966). Berezney and Coffey isolated a proteinaceous matrix that was stable to nucleases (Berezney & Coffey 1974), thus revealing an RNase-resistant scaffolding beneath the fibrogranular ribonucleoprotein network. Electron microscopic studies have shown that this nucleoskeletal structure is connected to the nuclear lamina towards the periphery of the nucleus and a branched network of 10-nm fibres in the interior of the nucleus (He et al. 1990, Nickerson et al. 1997, Nickerson 2001). Interestingly, removal of chromatin by electroelution of intact cells also yields a structure of underlying filaments that is similar to the matrix (Jackson & Cook 1988). The groups of Penman and Cook have defined the nuclear matrix as an underlying network of branched 10-nm filaments that are connected to the peripheral lamina, and other proteins including RNPs are attached to this matrix by direct or indirect interactions. Nuclear domains such as speckles, CBs and replication factories are retained upon extraction of cells, and are observed in a precise spatial relationship with respect to each other, suggesting their attachment to an underlying structure (Nickerson et al. 1997). Though the ultrastructure of the nucleoskeleton has been relatively well characterised, a major shortcoming in the field has been an insufficient biochemical characterisation of matrix components, primarily due to problems associated with matrix isolation procedures. This has led to skepticism about whether the nuclear matrix fraction is truly representative of the *in vivo* structure (Pederson 2000). Due to the controversies in the field I will generally refer to the nuclear substructure as the nucleoskeleton. Although the protein components of the internal nucleoskeleton have not been definitively identified, the major candidate proteins are the lamins and nuclear actin. Various aspects of their functions in relation to nuclear organisation will be considered in the following sections.

Nuclear Architectural Proteins

Lamins

Lamins are the major components of a filamentous network underlying the inner nuclear membrane termed the nuclear lamina, and have more recently been observed in the interior of the nucleus also (Goldman et al. 1992, Bridger et al. 1993, Hozák et al. 1995, Jagatheesan et al. 1999, Barboro et al. 2002). The lamina plays an essential role in maintaining the integrity of the nuclear envelope and provides anchoring sites for chromatin, and is hence considered to be an important determinant of interphase nuclear architecture (reviewed in Stuurman et al.1998). Recent evidence indicates that lamins have additional functions in the organisation of DNA replication, transcription and RNA splicing, as well as in apoptosis (reviewed in Goldman et al. 2002). Two major kinds of lamins are present in vertebrate cells: B-type lamins (B1 and B2) that are found in nearly all somatic cells, and A-type lamins (A and C) which are expressed primarily in differentiated cells. The lamin genes are highly conserved across species. Lamins A and C are alternatively spliced products of the lamin A gene, *LMNA*, whereas lamins B1 and B2 are coded by two separate genes, *LMNB1* and *LMNB2*. Additional splice variants are germ cell-specific lamins C2 and B3 which are encoded by *LMNA* and *LMNB2* respectively. Their differential expression suggests tissue-specific functions for the lamins. *Drosophila melanogaster* has two lamin genes, lamin Dm0, which is expressed in most cells and lamin C, whose expression is developmentally regulated. *Caenorhabditis elegans* has only one lamin gene which is expressed in all cells except for mature sperm.

Genome sequence analysis of yeast and Arabidopsis indicates that these species do not have lamins and, though functional homologs are likely to exist, these have not yet been definitively identified. Thus lamins appear to have evolved in animal cells.

Lamins belong to the intermediate filament family of proteins and contain a characteristic central α helical rod domain, flanked by relatively flexible N-terminal and C-terminal segments. *In vitro* studies have shown that all three domains are essential for lamin assembly and functions. The rod domains of two lamin monomers associate to form a two-stranded coiled-coil α-helix, which is the basic dimeric subunit involved in formation of the higher order structure of the lamin filament. Head-to-tail interactions of lamin dimers lead to the formation of protofilaments which can associate laterally in different configurations such as parallel, staggered or half-staggered to give rise to the 10-nm lamin filament (Stuurman et al. 1998). As the formation of the 10-nm filament has not been observed *in vitro*, it has been suggested that higher order lamin assembly might require interactions with other molecules *in vivo*. Lamins interact with inner nuclear membrane proteins such as the lamina- associated polypeptides (LAPs)1 and 2β and emerin, as well as intranuclear LAP2α (Gerace & Foisner 1994, Dechat et al. 2000, Zastrow et al. 2004).

Assembly and Disassembly of Lamins

During mitosis, phosphorylation of essential serine residues on either end of the rod domain by p34/cdc2 kinase results in depolymerisation of the lamina into dimers and tetramers. The lamina is reassembled towards late telophase and in early G1 phase of the cell cycle; lamin B appears to be incorporated when the nuclear membranes and pores are assembling in the nascent nuclear envelope, whereas lamin A is imported through the nuclear pores towards the end of cytokinesis (reviewed in Gant & Wilson 1997). However the stage of lamina reformation appears to vary in different species or cell types. Genetic and biochemical evidence indicate that lamins are essential for the reformation of the nuclear envelope, but their precise role has not been clearly defined. In addition to the typical nuclear rim pattern, lamin A and B tagged with GFP have been observed to form a veil of fluorescence in the nucleoplasm of interphase cells, which is a relatively stable structure (Broers et al. 1999, Moir et al. 2000b); and more sensitive nucleoplasmic structures, likely to be assembly intermediates, have been identified in early G1 cells by FRAP analysis (Moir et al. 2000b). We have isolated and characterised a monoclonal antibody LA-2F9 produced against recombinant rat lamin A which predominantly stains a G1 subpopulation of various cell types in a pattern of small nucleoplasmic foci (see figure 2) that are unusually susceptible to mild detergent/salt extraction and might represent assembly intermediates (Muralikrishna et al. 2004). The detection of these unstable structures following G1 phase suggests that a population of lower order lamin filaments might persist in interphase. In agreement with this view, Barboro et al. (2002) have observed 3 nm and 4 nm lamin protofilaments as constituents of a thin fibrillar web within the nuclei of rat hepatocytes, which are non-dividing cells. It has been proposed that the assembly states and functions of the lamins at the nuclear periphery and in the nucleoplasm may be different (Moir et al. 2000b). Although the elements that control the different polymeric states of lamins are not known at present, their identification would be important for understanding the role of lamins in many nuclear processes.

Lamins and DNA Replication

Several studies indicate that a normal lamina organisation is required for DNA replication in intact nuclei. When assembly of nuclei was carried out *in vitro* in lamin-depleted Xenopus interphase extracts, the resulting nuclei were unable to replicate their DNA (Newport et al. 1990, Meier et al. 1991). Although it has been possible to replicate DNA *in vitro* with purified

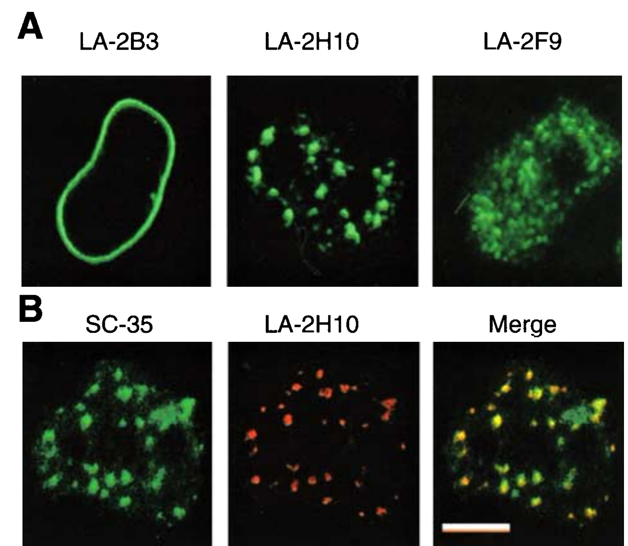

Figure 2: A, Different structural aspects of lamins A/C in HeLa cells. Typical nuclear rim (mAb LA-2B3), lamin speckles (mAb LA-2H10), and detergent-susceptible lamin foci (mAb LA-2F9); **B,** Colocalisation of lamin speckles with splicing factor compartments (SFCs) viewed by confocal laser scanning microscopy. Dual staining of a HeLa nucleus with anti-SC-35 antibody and mAb LA-2H10. The yellow spots in the Merge panel indicate colocalised speckles. Bar, 5 μm.

factors in the absence of lamins (Walter et al. 1998), an intact lamina is essential for DNA replication in assembled nuclei and cells. When dominant negative lamin mutants obtained by deletion of the N-terminal domain of human lamin A (Spann et al. 1997, Moir et al. 2000a) or Xenopus lamin B1 (Ellis et al. 1997) were added to assembled nuclei, they disrupted lamin organisation and inhibited DNA synthesis. A direct role for lamins in the spatial organisation of DNA replication has been suggested by evidence of colocalisation of lamin B with replication foci and replication factors such as PCNA in NIH 3T3 cells (Moir et al. 1994), and lamin A with replication factors at specific stages of S phase in primary mammalian cells (Kennedy et al. 2000). Although precise colocalisation between lamins and replication foci has not been observed in other studies (Dimitrova & Berezney 2002), this could be due to the different cell lines or lamin antibodies that have been used. Hence the majority of the evidence suggests that the lamina provides a platform for regulating the sequence of events in S phase in mammalian cells.

Lamins and Organisation of Transcription and Splicing

The developmental and tissue-specific expression of A-type lamins in mouse and Drosophila points to a possible involvement of lamins in transcription (Röber et al. 1989, Riemer et al. 1995). A role for lamins in controlling gene expression in muscle precursors has been proposed (Wilson 2000). The binding of lamin A to the retinoblastoma protein, an important transcriptional regulator, supports a function for lamins in gene regulation (Mancini et al. 1994, Ozaki et al. 1994). Direct evidence of a role in transcription has been provided by studies with an N-terminal deletion mutant of lamin A, ΔNLA. The expression of this mutant in cells led to disruption of the lamina and inhibition of RNA pol II transcription, without affecting pol I or pol III transcription (Spann et al. 2002).

We have identified a novel structural aspect of the internal lamina in the form of lamin A/C speckles (see figure 2) using a monoclonal antibody (mAb) raised to recombinant rat lamin A that has certain unique properties (Jagatheesan et al. 1999). This antibody, mAb LA-2H10, exclusively stains intranuclear speckles in interphase cells without labeling the peripheral lamina, and these speckles have been observed to colocalise with splicing factor compartments in a variety of cell types. MAb LA-2H10 specifically recognises only lamins A and C in immunoblots of cellular fractions and does not cross-react with other proteins. As the exclusive reactivity of mAb LA-2H10 towards speckles is retained when cells are detergent and nuclease-treated to reveal the nucleoskeletal framework, we have attributed this reactivity to differences in lamin protofilament interactions at the periphery and at internal sites. But as lamin speckles are highly insoluble structures they do not copurify with IGCs in stoichiometric amounts, and only small quantities of lamins have been detected in purified IGCs by mass spectrometry (Mintz et al. 1999). The possibility of a direct correlation between the organisation of lamin A/C speckles, splicing factor compartments and transcriptional events has been investigated (Kumaran et al. 2002). In the presence of transcriptional inhibitors, both lamin A/C speckles and splicing factor domains were reorganised to form coincident large foci; removal of inhibitor resulted in rapid, synchronous redistribution of lamin A/C speckles and splicing factors with reactivation of transcription. Furthermore, overexpression of lamins A/C was observed to disrupt lamin speckles and splicing factor compartments, accompanied by inhibition of transcription, without discernible effects on the incorporation of lamin A/C into the nuclear periphery or disruption of the endogenous lamina at the nuclear rim. These results suggest a close association between the internal lamin A/C network and spatial organisation of splicing factors and transcriptional events, and also imply that lamin speckles might play a structural role in assembly of splicing factor compartments. At present we do not have ultrastructural data for lamin speckles and are unable to comment on whether speckles are interconnected by filamentous lamin or other structural proteins. However, lamin speckles are redistributed to form a disperse, insoluble nucleoplasmic network during muscle differentiation (Muralikrishna et al. 2001) or upon treatment of cells with the phosphatase inhibitor okadaic acid (unpublished observations); we speculate that such a structure might be a component of the nucleoskeleton. It is clear that a substantial amount of work needs to be carried out on the structure, dynamics and regulation of lamin speckles.

Lamins in Disease and Stress

Inherited disorders are generally caused by a specific mutation in a gene. It is rare to find different mutations in the same gene giving rise to distinct, possibly unrelated, diseases. Different mutations in human *LMNA* cause at least 8 debilitating diseases that affect specific tissues. Most mutations affect skeletal and cardiac muscle, causing autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD), dilated cardiomyopathy and limb-girdle muscular dystrophy.

Other mutations cause Dunnigan's familial partial lipodystrophy (FPLD) leading to loss of white fat, also seen in the bone disorder mandibular dysplasia, and a peripheral neuropathy termed Charcot Marie tooth neuropathy type II (reviewed in Hutchison 2002, Mounkes et al. 2003a). The majority of mutations are missense mutations; those affecting muscle tissue occur throughout the gene whereas mutations causing abnormalities in fat tissue tend to be clustered towards the carboxy terminus. A recently described conservative mutation at the 3′ end of human *LMNA* results in aberrant splicing and removal of about 50 amino acid residues, and has been linked to the premature ageing disease, Hutchinson-Gilford progeria syndrome (De Sandre-Giovannoli et al. 2003, Eriksson et al. 2003, Mounkes et al. 2003b). Valuable insights into lamin A function have been obtained by the knockout of mouse *LMNA* (Sullivan et al. 1999). Mice that lack *LMNA* develop severe muscle wasting similar to human EDMD, and also loss of white fat, by 3-4 weeks and die by 8 weeks after birth. Furthermore, mutations in emerin, an inner nuclear membrane protein that associates with lamin A also result in EDMD of the X-linked form. It is not clear why lamin A or emerin mutations should predominantly affect muscle tissue when emerin is ubiquitously expressed and lamin A is present in almost all differentiated cells. It has been suggested that the forces generated during muscle contraction might exacerbate physical damage to muscle cell nuclei (Sullivan et al. 1999), or that lamins might influence gene expression in progenitor cells (Wilson 2000). Examination of cells cultured from disease samples in a recent report suggests that lamin deficiency is associated with defective nuclear mechanics as well as impaired gene transcription (Lammerding et al. 2004). An important possibility that needs to be examined is whether lamins play an active role in regulatory events during muscle differentiation.

Differentiation of myoblasts into myotubes is coordinated by two families of transcription factors, MyoD, which includes the muscle-specific transcription factors MyoD, Myf5, myogenin, and MRF4 (Rudnicki &Jaenisch 1995), and the MEF2 family of transcription factors (Black & Olson 1998). Muscle differentiation follows a highly ordered, temporally distinct sequence of events. Myoblasts are first committed to the differentiation pathway in a step marked by the expression of the transcription factor myogenin, which is followed by expression of cell cycle regulators such as the inhibitor p21 and irreversible, asynchronous cell cycle withdrawal (Andrés & Walsh 1996). The cells then differentiate phenotypically, express contractile genes and finally fuse into multinucleated myotubes. In order to explore the possibility of changes in internal lamin organisation during muscle differentiation, we have examined the appearance of A-type lamin speckles in C2C12 mouse myoblasts and myotubes. Lamin speckles were observed in dividing myoblasts but disappeared early during the course of differentiation in postmitotic myocytes and were absent in myotubes and muscle fibres. However, the typical organisation of peripheral lamins A/C and B1, and splicing factor domains was preserved. Lamin speckles were also absent in quiescent myoblasts but reappeared as cells were reactivated to enter the cell cycle. These changes were not observed in other quiescent cell types. When myotube or quiescent myoblast nuclei were extracted with nucleases and detergent, a uniformly stained internal lamina was revealed, indicating that lamins A/C were antigenically masked in these cells, probably due to structural reorganisation of the lamina during differentiation or quiescence. These studies suggest that muscle cell differentiation is accompanied by regulated rearrangements in the organisation of the A-type lamins that are likely to be controlled by key muscle regulatory factors (Muralikrishna et al. 2001, Mariappan & Parnaik submitted). These rearrangements in the internal lamina might be part of the overall process of nuclear architectural changes during muscle differentiation. If this is hindered by mutant lamins or absence of lamins, it could lead to muscle-specific disease symptoms.

Upon ectopic expression in cultured cells, certain lamin mutant proteins cause gross defects in the peripheral lamina and also assemble aberrantly (Östlund et al 2001, Raharjo et al. 2001, Vigouroux et al. 2001). In a recent report, ectopic expression of a mutant lamin A bearing the R453W mutation, which is known to cause EDMD in humans, has been shown to inhibit differentiation of C2C12 myoblasts and lead to apoptosis (Favreau et al. 2004). Interactions between lamin A and sterol response element binding protein 1 have been reported to be reduced *in vitro* in lamin mutants found in FPLD (Lloyd et al. 2002). In fibroblasts from FPLD patients carrying the R482L mutation, abnormal lamin aggregates were observed that partially colocalised with lamin speckles and resulted in decreased transcription together with absence of interaction with emerin (Capanni et al. 2003). These studies collectively point to the conclusion that mutations in *LMNA* might block critical nuclear functions in a tissue-specific manner.

During apoptosis, lamins are cleaved by caspases at a single site mapped to asp230 (Takahashi et al. 1996), and this may help to disassemble the lamina and allow the nuclear events of apoptosis such as reduction in nuclear size and chromatin condensation to

proceed. In light of recent findings that specific mutations in human *LMNA* lead to premature ageing syndromes (De Sandre-Giovannoli et al. 2003, Eriksson et al. 2003, Mounkes et al. 2003b, Chen et al. 2003) it would be worthwhile to determine whether the lamina plays a more direct role in the cellular response to DNA damage or stress. Levels of lamin B have been found to increase upon heat stress (Dynlacht et al. 1999). In studies on the effects of heat stress in mouse myoblasts we have observed that the integrity of lamin speckles appears to be maintained during heat stress by association with small heat shock proteins (Adhikari, Rao, Rangaraj, Parnaik & Rao submitted). Mice that are deficient in the enzyme Zmpste24, which is a metalloproteinase involved in processing of prelamin A, also exhibit pathologies that are similar to the lamin-deficient mice (Pendás et al. 2002).

Lamin-Binding Proteins and Gene Regulation

The majority of proteins associated with the inner nuclear membrane interact with lamins and/or chromatin. Prominent lamin-binding proteins are emerin, lamin B receptor, and LAPs 1 and 2 family members. Emerin, LAP2 and another envelope protein MAN1 possess a 40-residue folded motif called the LEM domain (derived from LAP, emerin, MAN1) that binds directly to barrier-to-autointegration factor (BAF), a conserved DNA binding protein that is involved in higher-order chromatin structure and is important for nuclear assembly (reviewed in Zastrow et al. 2004). LAP2α is an important binding partner for lamin A in the interior of the nucleus (Dechat et al. 2000, Markiewicz et al. 2002). These findings suggest that interactions between lamins and its binding partners might influence chromatin structure and gene activity. Moreover, many of the mutations that cause laminopathies also result in reduced amounts of emerin at the nuclear envelope.

There is evidence that localisation of specific genes at the nuclear periphery may lead to transcriptional silencing, which is in addition to studies on telomeric silencing in yeast discussed in an earlier section. In Drosophila, insertion of the *gypsy* insulator into a gene sequence causes its translocation to the nuclear envelope (Gerasimova & Corces 1998). Association of the transcription factor Oct-1 with lamin B is correlated with its repressor activity (Imai et al. 1997). The nuclear envelope protein LAP2β interacts with germ-cell-less transcriptional repressor and their association with E2F-DP can downregulate its activity (de la Luna et al. 1999, Nili et al. 2001).

A newly described class of proteins termed the nesprins (also called Syne/ANC-1 proteins) span the nuclear envelope and are able to bind to lamin A as well as emerin. The nesprins are large proteins with several spectrin repeats, and exist in many forms with tissue specific expression patterns due to alternate splicing (Mislow et al. 2002, Zhang et al. 2002). Some of the nesprins contain a calponin homology domain which is known to bind to actin. Genetic studies suggest that the nesprins are required for the anchorage of the nucleus to the cytoskeleton (Starr & Han 2002), and may thus be involved in correct nuclear positioning as well as transmission of regulatory signals from the cell surface to the nucleus. Loss-of-function mutations in Drosophila lamin Dm0 suggest that lamins might be involved in additional functions such as cytoplasmic organisation and cell polarity (Guillemin et al. 2001).

Nuclear Actin and Actin-Binding Proteins

Actin is a major component of the cytoskeletal network in most cells. The presence of actin in the nucleus in association with chromatin remodelling complexes, hnRNPs and mRNA has now been established by extensive experimentation (reviewed in Rando et al. 2000). The association of actin with hnRNPs as well as mRNA suggests that actin might play a role in mRNA processing and transport. Important insights have been obtained in recent studies on the mechanism of RNA export, but these are outside the scope of the present review. The demonstration of actin in chromatin remodelling complexes provides strong evidence for a role for actin in gene regulation. A direct role for actin in transcription has been suggested in a recent study describing the involvement of an actin-myosin complex in rRNA transcription (Fomproix & Percipalle 2004). A number of studies have shown that actin-binding proteins are also present in the nucleus. A noteworthy finding is the identification of the RNA-binding protein hrp65 as an actin-binding protein, and its association with actin has been suggested to be important for RNA pol II transcription in the dipteran *Chironomus tentans* (Percipalle et al. 2003). Actin-binding proteins generally mediate the nucleation and branching of actin filaments; their exact role in chromatin remodelling is not clear, though it has been suggested that these proteins might be involved in binding to the nuclear matrix; moreover, actin might not be present in the filamentous form in the nucleus (Pederson & Aebi 2002). Profilins, which are involved in actin polymerisation, have been detected in nuclear gems (Giesemann et al. 1999), and more recently profilin I has been localised in splicing factor compartments and CBs, suggesting that it might have a role in pre-mRNA splicing or recruitment of splicing factors (Skare et al. 2003). Proteins containing both

actin-binding and lamin-binding domains might be able to mediate functional interactions or crosstalk between lamins and actin (Shumaker et al. 2003). Moreover, direct binding of the carboxy terminal domain of lamin A to actin has been shown *in vitro* (Sasseville & Langelier 1998).

Spatial Coordination of Transcription and Splicing

A close coordination between the processes of RNA splicing and transcription is indicated by several lines of evidence such as the cotranscriptional splicing of nascent transcripts and the association of the carboxy terminal domain of the largest subunit of RNA pol II with a number of multiprotein complexes involved in transcription and pre-mRNA splicing (reviewed in Hirose & Manley 2000, Neugebauer 2002). In this section I would like to discuss the available evidence for the spatial coordination of these processes *in vivo* and the role of the nucleoskeletal framework in its organisation. Lawrence and colleagues have observed a clustering of select groups of active genes and their nascent transcripts around specific splicing factor compartments while inactive genes are mostly situated towards the nuclear rim away from these domains (Smith et al. 1999). In the case of muscle-specific genes, myogenin and myosin heavy chain become repositioned at the periphery of a compartment concomitant with or before initiation of mRNA synthesis in terminally differentiated muscle nuclei, but not in myoblasts or non-muscle cells where these genes are not expressed (Moen et al. 2004). The authors propose that the specific positioning of active genes with respect to splicing factor compartments would allow access to large amounts of RNA splicing factors, especially important for processing of abundant transcripts during differentiation and development. It may be noted that there were no significant changes in the positions of these genes from the periphery of chromosome 14 territory in which they were located in non-expressing and expressing cells.

The movement of splicing factor assemblies from splicing factor compartments towards specific transcription sites upon viral gene activation is suggestive of a directed targeting of the splicing machinery to required locations *in vivo* (Misteli et al. 1997). When transcription is inhibited there is a decrease in speckle dynamics, and budding and transport of smaller domains derived from speckles are no longer seen. There is a concomitant reorganisation of speckles to larger and more spherical foci. Removal of a reversible transcriptional inhibitor causes restoration of transcription by pol II, and simultaneously splicing factors such as SC-35 redistribute within the nucleus to their normal organisation. A coordinate redistribution of lamin A/C and SC-35 has been observed on reactivation of transcription beginning within minutes of removal of the inhibitor. Thus lamins in association with splicing factor compartments can move relatively rapidly in response to signals that also cause relocalisation of splicing factors to sites of transcription, and might be regulated by similar signalling events. Overexpression of Clk/STY, a cdc-like protein kinase, has been shown to disperse IGCs under conditions where splicing factors were phosphorylated, leading to a block in splicing but not transcription, thus affecting the coordination between these two processes (Sacco-Bubulya & Spector 2002). Lamin A and actin also dissociate from IGCs under these conditions, and the authors interpret this to mean that lamin and actin do not represent underlying architectural proteins in IGCs and may play an alternate role.

Substantial mobility of GFP-tagged nuclear proteins within the nucleoplasm of live cells is indicated by fluorescence recovery after photobleaching and fluorescence loss in photobleaching techniques, and is indicative of the dynamic nature of the exchange between proteins in the nucleoplasm and in compartments (Phair & Misteli 2000, Kruhlak et al. 2000). Kruhlak et al. (2000) suggest that splicing factor-containing domains behave as though they are physically impeded by frequent and transient associations with structural elements. This conclusion is supported by single particle tracking studies with the splicing factor U1 snRNP whose dynamics within splicing factor compartments is much slower than in aqueous solution, suggesting transient binding to immobile sites (Kues et al. 2001). Moreover, Hendzel et al. (1999) have demonstrated, by energy transmission electron microscopy in intact cells, the presence of an underlying protein architecture in IGCs that physically connects the relatively dispersed granules within the cluster.

In light of the above evidence and our data on lamin speckles, we propose that lamin A/C speckles are part of a dynamic structure that can be rapidly modulated by specific signalling events to spatially coordinate mRNA splicing and RNA pol II transcription. In the putative model presented in figure 3, upon gene activation the decondensed genomic locus binds to chromatin remodelling complexes and transcriptional activators; concomitantly there is a repositioning of splicing factor compartments and lamin speckles near the locus to facilitate splicing. We propose that lamins provide a platform for this process as the association of factors with a structural framework would undoubtedly allow for greater coordination of regulatory events. At present we can only speculate that specific targeting of a splicing factor compartment

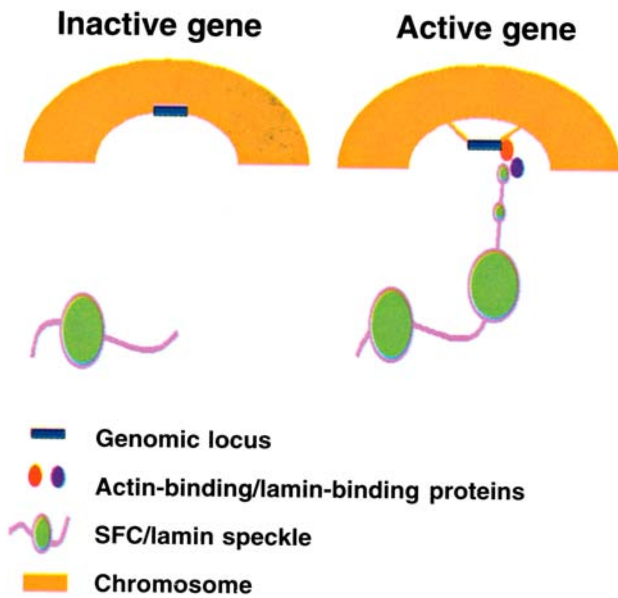

Figure 3: A hypothetical model for the spatial coordination of transcription and splicing. For an inactive gene, the genomic locus is in a condensed state. Upon gene activation, the locus is decondensed and binds to chromatin remodelling complexes and transcriptional activators; concomitantly there is a repositioning of splicing factor compartments (SFCs) and lamin speckles near the locus to facilitate splicing and might involve direct or indirect interactions of lamins (through lamin-binding proteins) with actin and actin-binding proteins in chromatin remodelling complexes.

to a genomic locus might involve direct or indirect interactions of lamins with actin and actin-binding proteins in chromatin remodelling complexes and definitive proof of this is needed. Interactions of lamins with lamin-binding proteins such as LAP2α (Dechat et al. 2000, Markiewicz et al. 2002) might facilitate this process. The role of A-type lamins is likely to be more important in differentiated cells where there is coordinate regulation of groups of genes. Other structural proteins might perform functions similar to those proposed for lamins A/C; for example, lamin B might compensate for the lack of A-type lamins in undifferentiated cells, under pathological conditions, or in situations where lamin A/C expression has been knocked down. A crucial requirement to support the above model is a system to study the dynamics of lamin speckles in living cells; this has not been feasible so far as GFP-lamin A does not form speckles.

Conclusions and Future Perspectives

The organisation of the nucleus into defined compartments involved in specific functions is now clearly established. New insights into roles for nucleoskeletal proteins such as lamins in DNA replication and transcription have been obtained. The detection of actin and actin-binding proteins in the nucleus bring up the exciting possibility of functional interactions between nucleoskeletal elements. Studies with lamin speckles, a novel structural aspect of internal lamins, suggest that the nuclear framework can be dynamic and sensitive to cell-type specific signalling pathways. This has significant implications for understanding the tissue-specific roles of A-type lamins. An important organism which has been minimally exploited to understand tissue-specific lamin functions is Drosophila and further work in this area should be forthcoming. Functional studies on Drosophila lamin Dm0 suggesting the involvement of lamins in cytoplasmic organisation and the identification of nesprins as lamin-binding proteins present the possibility of cross-talk between the cytoplasm and the nucleus via the lamina. A related aspect which needs detailed investigation is the role of phosphoinositide signalling components that are present in splicing factor compartments. Insights into normal lamin functions will undoubtedly have important implications for understanding the complex diseases resulting from mutations in *LMNA*.

Abbreviations

CB, Cajal body; EDMD, Emery-Dreifuss muscular dystrophy; FPLD, familial partial lipodystrophy; GFP, green fluorescent protein; IGC, interchromatin granule cluster; LAP, lamina-associated polypeptide; mAb, monoclonal antibody; PCNA, proliferation cell nuclear antigen; PML, promyelocytic leukemia; pol, polymerase

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Goldman and colleagues have recently shown that cells expressing mutant lamin A from Hutchinson-Gilford progeria syndrome patients display significant alterations in nuclear architecture, including loss of peripheral heterochromatin (Goldman RD, Shumaker DK, Erdos MR, Eriksson M, Goldman AE, Gordon LB, Gruenbaum Y, Khuon S, Mendez M, Varga R and Collins FS 2004 Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome; *Proc. Natl. Acad. Sci. USA* **101** 8963-8968). The paper referred to as Adhikari, Rao, Rangaraj, Parnaik & Rao, submitted, is now in press as Adhikari A S, Rao K S, Rangaraj N, Parnaik V K and Rao C M 2004 Heat stress-induced localization of small heat shock proteins in mouse myoblasts: intranuclear lamin A/C speckles as target for alpha B-crystallin and Hsp 25; *Exp. Cell Res.*

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