Gene Ther Mol Biol Vol 4, 143-158. December 1999.

Functional organization of the nuclear lamina

Research Article

Michal Goldberg[1], Einav Nili[2], Gady Cojocaru[2], Yonatan B. Tzur[1], Raanan Berger[2], Michael Brandies[1], Gideon Rechavi[2], Yosef Gruenbaum[1] and Amos J. Simon[2]

[1]Department of Genetics, The Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, 91904
[2]Institute of Hematology, Chaim Sheba Medical Center, Tel-Hashomer and the Sackler School of Medicine, Tel-Aviv University, 52621 Israel.

Correspondence: Amos J. Simon, Ph.D., Institute of Hematology, Chaim Sheba Medical Center, Tel-Hashomer 52621, Israel. Tel: 9723-5302397; Fax: 9723-5302377; E-mail: wsamos@dapsas1.weizmann.ac.il

Abbreviations: NE, nuclear envelope; **IF**, intermediate filaments; **IMPs**, integral nuclear membrane proteins; **LBR**, lamin B receptor; **LAP**, Lamina Associated Protein; **NPC**, nuclear pore complex; **INM**, inner nuclear membrane; **ONM**, outer nuclear membrane

Key words: Apoptosis, nuclear assembly, histone, thymopoietin

Received: 2 June 1999, accepted: 12 June 1999

Summary

In eukaryotic cells, DNA replication, RNA processing and ribosome assembly all occur in the nucleus, while protein synthesis occurs in the cytoplasm. These activities are physically separated by the nuclear envelope. The inner nuclear membrane and the nuclear lamina are involved in organizing nuclear structure and regulating nuclear events including: nuclear organization, DNA replication, nuclear assembly and disassembly, apoptosis, and correct spacing of nuclear pores. In order to perform these activities the inner nuclear membrane and the nuclear lamina contain a unique set of proteins, including lamin type A and B, otefin, Young Arrest, LAP1, TMPO/LAP2, emerin, LBR and MAN. The proteins of the inner membrane form a complex network of interactions between themselves and with chromatin. Mutations in several of these proteins also result in known diseases. In this paper we review these interactions and discuss their possible roles in normal cell activity and in apoptosis. In addition, we demonstrate that specific regions in lamins can interact with the core histones H2A and H2B. We also show that Thymopoietin (TMPO)/ Lamina Associated Polypeptide 2 (LAP2) gene is alternatively spliced to form a large family of proteins with different size and different distribution within the nucleus. These results are discussed in relationship to the biological roles of the nuclear lamina.

I. Introduction

The main feature of eukaryotic cells is the presence of nuclear and cytoplasmic compartments that are separated by the nuclear envelope (NE). The NE is a large complex structure composed of outer and inner lipid bilayer membranes, nuclear pores, nuclear lamina and perhaps chromatin (**Figure 1**). The outer nuclear membrane is continuous with the endoplasmatic reticulum and is covered with ribosomes. The two membranes are separated by a 20-40 nm perinuclear space and are connected at the nuclear pore complexes (reviewed in Burke, 1990a). The nuclear pore complexes are large protein structures that mediate the controlled transport of macromolecules between the cytoplasm and the nucleus (reviewed in Corbett and Silver, 1997; Davis, 1995; Fabre and Hurt, 1997; Gorlich and Mattaj, 1996). The nuclear lamina is a proteinaceous meshwork of intermediate filaments (IF) that is located underneath the inner nuclear membrane and abuts the peripheral chromatin (reviewed in Harel et al, 1998; Moir et al, 1995; Stuurman et al, 1998). The inner nuclear membrane and nuclear lamina contain unique proteins. Among them are the integral nuclear membrane proteins (IMPs) p58/lamin B receptor (LBR) (Worman et al, 1990; Worman et al, 1988), LAP1 (Martin et al, 1995), TMPO/LAP2 (Berger et al, 1996; Furukawa et al, 1995; Harris et al, 1994), emerin (Manilal et al, 1996), p34 (Simos and Georgatos, 1994) and p18 (Simos et al, 1996). The peripheral nuclear membrane proteins include type A and type B lamins (Fisher et al, 1986; McKeon et al, 1986), otefin (Harel et al, 1989; Padan et al, 1990), and Young Arrest (YA) (Lin et al, 1991; Lin and Wolfner, 1991). It is yet to be determined whether MAN protein(s) are integral or a peripheral proteins (Paulin Levasseur et al, 1996).

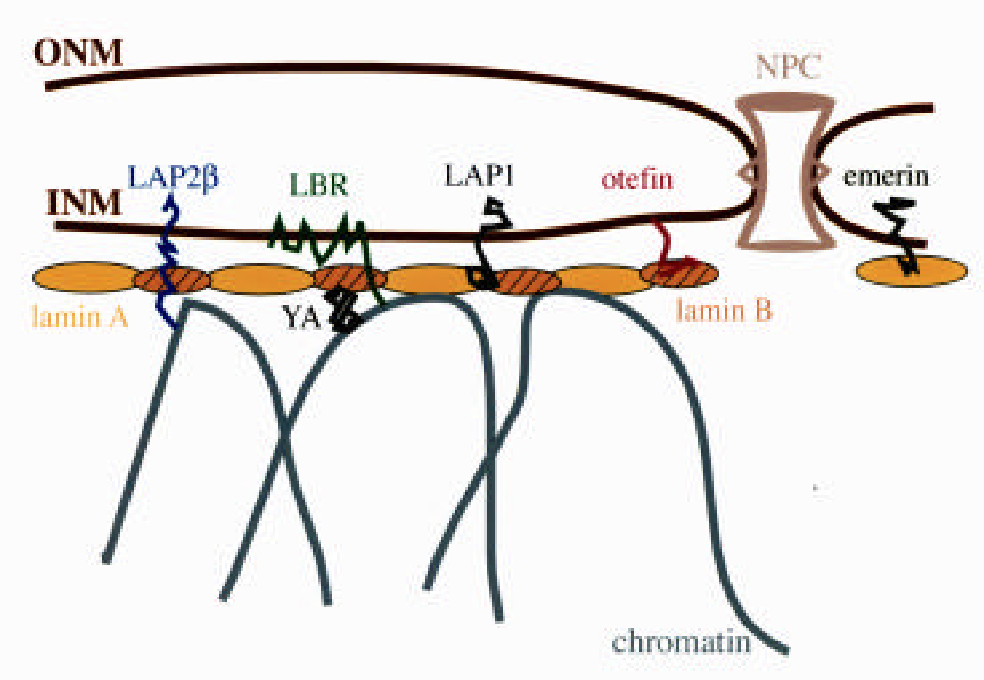

Figure 1. Schematic view of the molecular organisation of the nuclear envelope. ONM, outer nuclear membrane; INM, inner nuclear membrane; NPC, nuclear pore complexes; LAP, Lamina Associated Protein; YA, Young Arrest.

The proteins of the NE form a complex network of interactions (reviewed in Qian et al, 1998). Lamin seems to interact with LBR (Worman et al, 1988), LAP1 (Foisner and Gerace, 1993), TMPO/LAP2 (Foisner and Gerace, 1993), p18 (Simos et al, 1996), otefin and YA (Goldberg et al, 1998). p18 and p34 are associated with LBR (Simos and Georgatos, 1992). LBR (Foisner and Gerace, 1993; Pyrpasopoulou et al, 1996), YA (Lopez and Wolfner, 1997), and lamin (Glass et al, 1993; Glass and Gerace, 1990; Goldberg et al, 1999; Hoger et al, 1991; Taniura et al, 1995; Yuan et al, 1991) are all found to interact with chromatin. The inner nuclear membrane and the nuclear lamina are involved in several biological functions, including: (**a**) regulation of the NE shape and structure (Furukawa and Hotta, 1993; Furukawa et al, 1994; Newport et al, 1990); (**b**) disassembly, at the beginning of mitosis, and reassembly, at the end of mitosis of the NE (Ashery-Padan et al, 1997b; Burke and Gerace, 1986; Gant et al, 1999; Gant and Wilson, 1997; Heald and McKeon, 1990; Ulitzur et al, 1992, 1997; (**c**) providing anchoring sites for chromatin and affecting higher order chromatin organization (Belmont et al, 1993; Gerace and Foisner, 1994; Hutchison et al, 1994; Liu et al, 1997); (**d**) lamins are required for the elongation phase of DNA replication (Ellis et al, 1997; Gant et al, 1999; Goldberg et al, 1995; Moir et al, 1995; Newport et al, 1990); (**e**) lamins are very early targets for caspases in apoptosis, and inhibition of lamins apoptotic degradation delays apoptosis (Neamati et al, 1995; Oberhammer et al, 1994; Rao et al, 1997; Shimizu et al, 1998); and (**f**) the abnormal arrangement of nuclear pores in cells mutated in their lamin gene revealed that lamins have also a central role in spacing the nuclear pores (Lenz-Bohme et al, 1997).

A. Lamins: the main components of the nuclear lamina

Lamins are the major and best studied proteins of the nuclear lamina. They are type V intermediate filament (IF) proteins, and, like all IF proteins, contain an α helical rod domain flanked by a nonhelical amino terminal "head" and carboxy terminal "tail" domains. The lamin rod domain contains three α−helices, each composed of heptad repeats. These helices form coiled-coil interactions between lamin monomers, resulting in lamin dimers. The lamin dimers form longitudinal polar head-to-tail interaction, leading the formation of higher order structures. The outcome polymers further associate laterally to form 10 nm thick filaments. These 10 nm thick filaments can further associate to form the 50-200 nm thick lamin fibers (reviewed in Krohne, 1998; Stuurman et al, 1998). Lamins are divided into type A and type B lamins, according to their sequence and expression patterns. Type A lamins are essentially expressed in differentiated cells, have a neutral isoelectric point and are soluble during mitosis. Type B lamins are expressed in all somatic cells, have an acidic isoelectric point and remain associated with membrane vesicles during mitosis (reviewed in Moir et al, 1995; Stuurman et al, 1998). Metazoan cells contain between one to six lamin proteins. Mammalian lamin A, C (Fisher et al, 1986; McKeon et al, 1986), A delta10 (Machiels et al, 1996) and C_2 (Furukawa et al, 1994)

are all products of one alternatively spliced gene. Lamin B_1 and B_2 are encoded by two separate genes, and lamin B_3 is a germ cell-specific spliced variant of lamin B_2 (Furukawa and Hotta, 1993). In *Xenopus laevis*, five lamin proteins were identified, lamin B_1, B_2, B_3, B_4 and A (Benavente et al, 1985; Benavente, 1985; Stick, 1992, 1994). In chicken, there are three known lamin genes, termed lamin A, B_2 and B_1 (Peter et al, 1989; Vorburger et al, 1989). *Drosophila melanogaster* has two lamin genes, lamin Dm_0 (Gruenbaum et al, 1988) and C (Bossie and Sanders, 1993). One lamin gene is present in the completely sequenced *Caenorhobditis elegans*, termed lamin CeLam-1 (Riemer et al, 1993). Interestingly, lamins are not present in the yeast *Saccharomyces cerevisiae* genome. All nuclear lamins, except lamin C, contain a CaaX box (C is cysteine, a is an aliphatic amino acid residue and X is any amino acid) at their carboxy terminus. The CaaX box undergoes specific post-translation modifications, which include farnesylation of the cysteine residue, endoproteolysis of the last three amino acids and carboxymethylation of the cysteine residue (reviewed in Moir et al, 1995). These modifications are essential, but not sufficient, for lamin association with the NE (Firmbach Kraft and Stick, 1995; Firmbach-Kraft and Stick, 1993; Krohne et al, 1989; Mical and Monteiro, 1998). Lamins are phosphorylated by several protein kinases in a cell-cycle regulated manner (Ottaviano and Gerace, 1985). Phosphorylation and dephosphorylation of lamins regulate their polymerization disassembly (Heald and McKeon, 1990; Ottaviano and Gerace, 1985; Peter et al, 1991), and their import to the nucleus (Haas and Jost, 1993; Hennekes et al, 1993). In mitotic cells, lamins are probably phosphorylated by cdc2 kinase (Dessev et al, 1991; Eggert et al, 1993; Heald and McKeon, 1990; Peter et al, 1991; Peter et al, 1990; Ward and Kirschner, 1990), the β isoform of protein kinase C (Fields et al, 1988; Goss et al, 1994; Hocevar et al, 1993; Hocevar and Fields, 1991), and mitogen-activated protein (MAP) kinase (Peter et al, 1992). In interphase cells, lamins are also phosphorylated by protein kinases A and C (Collas and Alestrom, 1997; Eggert et al, 1993; Eggert et al, 1991; Hennekes et al, 1993; Peter et al, 1990), indicating a separate role for lamin phosphorylation during interphase. The rod domain of lamins can bind otefin (Goldberg et al, 1998), LAP2 (Furukawa et al, 1998) and DNA (Baricheva et al, 1996; Christova et al, 1989; Galcheva-Gargova and Dessev, 1987; Luderus et al, 1992; Luderus et al, 1994; Rzepecki et al, 1998; Shoeman and Traub, 1990; Wedrychowski et al, 1989; Zhao et al, 1996), while the tail domain can bind actin (Sasseville and Langelier, 1998) and histones (Goldberg et al, 1999; Taniura et al, 1995).

Lamins are located mainly at the nuclear periphery. However they are also localized internally in the nucleoplasm (Bridger et al, 1993; Goldman et al, 1992; Hozak et al, 1995; Lutz et al, 1992; Moir et al, 1994; Sasseville and Raymond, 1995). The lamin nucleoplasmic foci may represent sites for lamin assembly or modification, prior to their incorporation into the peripheral lamina (Goldman et al, 1992; Lutz et al, 1992; Sasseville and Raymond, 1995) or may be involved in DNA replication (Moir et al, 1994).

B. Lamin-chromatin interaction: implication for NE assembly and disassembly

In higher eukaryotes, the NE disassembles during the prophase stage of mitosis and starts to reassemble around daughter chromosomes at late anaphase. Both lamins and lamin-associated proteins seem to be involved in this process. The NE disassembly is driven by phosphorylation of its major structural components, i.e. lamins (reviewed in Marshall and Wilson, 1997; Moir et al, 1995), proteins of the nuclear pore complex (Favreau et al, 1996; Macaulay et al, 1995), and probably LBR, LAP1 and TMPO/LAP2 (reviewed in Chu et al, 1998; Qian et al, 1998). In *Drosophila* embryos and *Xenopus* oocytes, the NE is fragmented into small vesicles, while the nuclear pore complexes disperse as soluble complexes and the nuclear lamina is depolymerized. NE assembly includes nuclear vesicles binding to chromatin, fusion of these vesicles, flattening onto the chromatin surface and assembly of the nuclear pore complexes (reviewed in Marshall and Wilson, 1997; Wilson and Wiese, 1996). In mammalian cells, the NE loses its identity as a subcompartment of the endoplasmic reticulum (ER) during mitosis. IMPs are probably resorted to the NE during late anaphase by diffusion through the ER and subsequently association with binding sites on the chromosomes. This process requires cooperative assembly of lamins and IMPs of the inner membrane (Gant et al, 1999; Ulitzur et al, 1997; Yang et al, 1997), LAP2α (Dechat et al, 1998) and chromatin. Both in vivo and in vitro studies proved that lamins have a major role in the assembly and disassembly of the NE, probably via their interaction with chromatin. Injection of anti-lamin antibodies to PtK2 cells resulted in cells that were not able to form normal daughter nuclei. The chromatin in the daughter nuclei remained arrested in a telophase-like configuration, and the telophase-like chromatin remained inactive as judged from its condensed state and by the absence of nucleoli (Benavente and Krohne, 1986). Three-dimensional in vivo studies in *Drosophila* and mammalian cells revealed that lamin filaments are closely associated with chromatin (Belmont et al, 1993). Genetic analysis also revealed that lamins are important for an intact NE and that lamins interact with chromatin. Mutations in the *Drosophila* lamin Dm_0 gene revealed that it is an essential gene. Flies homozygous for a strong mutation in the *Drosophila* lamin Dm_0 gene died following 9-16 hours of development. These flies demonstrate aberrant nuclear structure, dissociation of chromatin from the nuclear membranes and accumulation of annulate lamellae-like structures (Harel et al, 1998). Flies that carry a weak mutation in the lamin Dm_0 gene (<20% lamin expression) show retardation in development, reduced viability, sterility, and impaired locomotion. These flies demonstrate enrichment in nuclear pore complexes in cytoplasmic annulate lamellae and in NE clusters and defects in the nuclear envelopes of several types of cells (Lenz-Bohme et al, 1997).

The role of lamins in NE assembly was studied in vitro in several ways. Incubation of CHO cell extracts with anti-lamin A/C or anti-lamin B antibodies inhibited NE assembly (Burke and Gerace, 1986). Similarly, addition of anti-lamin

Dm_0 to a *Drosophila* extracts, in a cell-free nuclear assembly system, blocked the binding of nuclear vesicles to chromatin. The NE reformed upon supplementation of purified interphase lamin to the assembly extract (Ulitzur et al, 1992; Ulitzur et al, 1997). In the *Xenopus* nuclear assembly system, antibodies directed against both *Xenopus* lamins B_2 and B_3 inhibited the assembly of the nuclear membranes around chromatin (Dabauvalle et al, 1991). Interestingly, addition of antibodies directed only against *Xenopus* lamin B_3 resulted in nuclei that were smaller and fragile, contained high density of nuclear pore complexes and failed to replicate their DNA (Goldberg et al, 1995; Jenkins et al, 1993; Meier et al, 1991; Newport et al, 1990; Spann et al, 1997). The latter experiments strongly suggest a role for lamins in nuclear organization.

Lamins can bind specific DNA sequences in vitro. These include matrix/scaffold attachment regions (MAR/SARs, Luderus et al, 1992; Luderus et al, 1994), which lamins bind through their rod domain in a way that depends on lamins polymerization (Zhao et al, 1996). Lamins can also bind telomeric sequences (Shoeman and Traub, 1990), as well as, an A+T-rich DNA fragment that is located in the centromeric regions of *Drosophila* chromosomes and is organized similar to a MAR/SAR (Baricheva et al, 1996). Photo-crosslinking of *Drosophila* K_c cells that were grown in the presence of bromodeoxyuridine revealed that interphase lamins, but not mitotic lamins, are associated with both DNA and RNA in vivo (Rzepecki et al, 1998).

Lamins also bind chromatin fragments and interphase chromatin (Goldberg et al, 1999; Hoger et al, 1991; Taniura et al, 1995; Yuan et al, 1991), in vitro assembled chromatin (Ulitzur et al, 1992), mitotic chromosomes (Glass et al, 1993; Glass and Gerace, 1990; Goldberg et al, 1999) and specific chromosomal proteins (Burke, 1990b; Goldberg et al, 1999; Taniura et al, 1995; Yuan et al, 1991). Avian type A lamin binds in vitro to polynucleosomes with a dissociation constant of about 1 nM (Yuan et al, 1991). Mammalian type A and B lamins (Glass et al, 1993; Glass and Gerace, 1990) and *Drosophila* lamin Dm_0 can bind mitotic chromosomes in vitro (Goldberg et al, 1999). The chromatin binding activity of the mammalian lamins is localized at their tail domain, is mediated by core histones and possesses a dissociation constant in the range of 0.12-0.3 μM (Taniura et al, 1995). The binding activity of *Drosophila* lamin Dm_0 is also localized at its tail domain, has a dissociation constant in the range of 1 μM and is mediated by histones H2A and H2B (see below). The actual association of lamins to histones may be stronger since lamins form large polymers in vivo.

The *Drosophila* otefin is also involved in the process of NE assembly (Ashery Padan et al, 1997a). Trypsin treatment of membrane fractions of *Xenopus* and *Drosophila* extracts abolishes the ability of nuclear vesicles to bind demembranated sperm chromatin (Ulitzur et al, 1997; Wilson and Newport, 1988), indicating that NE assembly also depends on the activity of IMPs. IMPs, such as LBR, LAP1 and TMPO/LAP2, are possible targets for this trypsinization. It is likely that protein complexes containing lamin(s), otefin and IMPs mediate this NE assembly activity.

C. The family of Thymopoietin (TMPO)/ Lamina Associated Polypeptide 2 (LAP2) proteins

Thymopoietins (TMPOs) are a family of proteins that are highly conserved in mammals and *Xenopus* and are putatively involved in functional nuclear architecture and cell cycle control. In mammalian cells, six alternatively spliced TMPO isoforms, designated α, β, β', γ, δ, ε and ζ were isolated and characterized (Berger et al, 1996; Harris et al, 1994). All of them share an identical N-terminal 186 amino acid domain. One member of this family, TMPOβ is the homolgue of the rat Lamina Associated Polypeptide 2 (LAP2) (Berger et al, 1996; Furukawa et al, 1995; Harris et al, 1995). TMPOβ/LAP2is a type II integral protein of the inner NE, which binds lamin B1 and chromosomes in a phosphorylation dependent manner (Foisner and Gerace, 1993). It can be divided roughly into four domains: a hydrophilic C-terminus domain, a hydrophobic trans-membrane domain, a NE targeting and lamina-binding domain and a chromatin-binding domain (**Figure 2**). As NE disassembly begins at prophase TMPOβ/LAP2undergoes phosphorylation by mitotic factors, possibly by p34cdc2 kinase. This has been found to abolish its interphase binding to both lamin B1 and chromosomes. Consistently, during late anaphase, TMPOβ/LAP2, as well as lamin-associated and non-associated vesicles, associates independently around chromatin to bind the surface of the decondensing chromosomes in order to complete NE reassembly (Foisner and Gerace, 1993; Yang et al, 1997). Thus, it is suggested that TMPOβ/LAP2plays a key role in NE disassembly and reassembly during mitosis and linking chromatin to the NE in interphase.

The specific binding of TMPOβ/LAP2to lamin was confirmed using the two-hybrid system (Furukawa et al, 1998; Furukawa and Kondo, 1998). Amino acids 78-258 of lamin B_1 rod domain are sufficient for the direct binding of lamin B_1 to TMPOβ/LAP2 (Furukawa and Kondo, 1998). Interestingly, the lamin binding region of TMPOβ/LAP2 coincides with TMPOβ/LAP2 NE targeting domain and includes residues 298-373. The chromosomes binding site of TMPOβ/LAP2resides within residues 1-85 (Furukawa et al, 1998; Gant et al, 1999; Yang et al, 1997), a region which is common to all TMPO isoforms. By transfecting truncated clones of TMPOα/LAP2, Yang et al (1997), showed that TMPOα/LAP2 has a role in regulating the dynamics of the nuclear lamina and the interaction between TMPOβ/LAP2 and the nuclear lamina is required for nuclear growth after mitosis (Yang et al, 1997).

Three other isoforms of the TMPO family, TMPOs ε, δ and γ, are splicing variants of TMPOβ/LAP2. All four isoforms share a putative hydrophobic transmembrane domain near their C-terminus via which TMPOβ/LAP2 was suggested to bind to the NE (Berger et al, 1996; Furukawa et al, 1995). However, whereas TMPOβ/LAP2 contains two putative p34cdc2 kinase phosphorylation sites, TMPOs δ and ε contain only one such site and TMPOγ lacks both sites (Berger et al, 1996), suggesting alternative regulating roles to these isoforms at the NE.

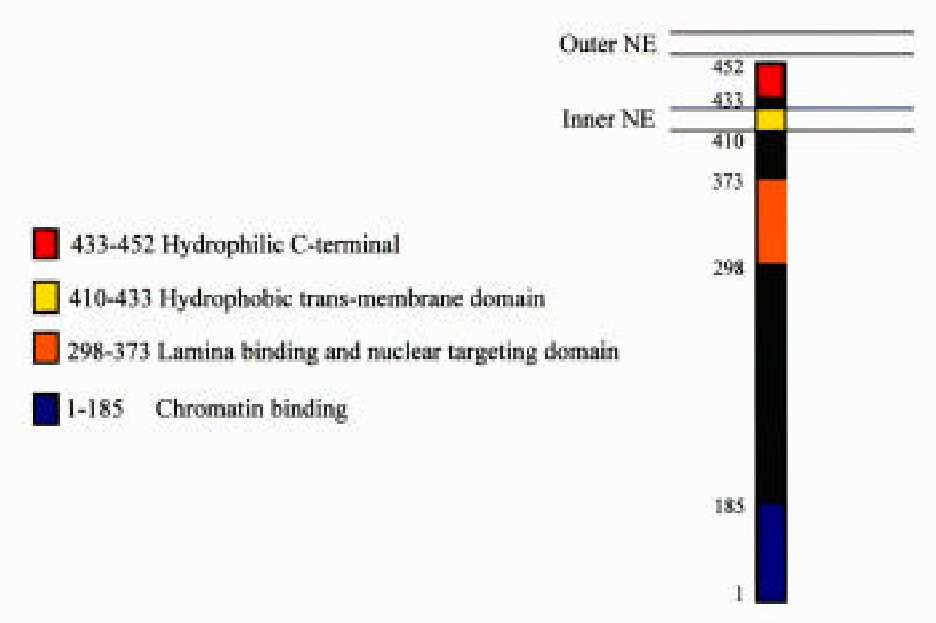

Figure 2. The molecular organisation of TMPOβ/LAP2 protein. The positions of the different domains are marked.

Another isoform, TMPOα has a completely different C-terminus, lacking a trans-membrane domain but containing a putative nuclear localization signal (NLS) and a putative tyrosine and cdc2-specific phosphorylation sites. It is localized throughout the nuclear interior in interphase cells. During metaphase it dissociates from chromosomes and becomes concentrated around the spindle poles, probably due to specific phosphorylation. The protein is relocated to chromosomes at early stages of nuclear reassembly during telophase (Dechat et al, 1998; this study-see results). It is therefore suggested that, similar to the TMPOβ/LAP2-type isoforms, TMPOα has a major role in the process of NE reassembly but in a different manner. For example, it will be interesting to reveal whether TMPOα is a true LAP that binds one of the lamins either in interphase or during mitosis like its TMPOβ/LAP2 family member. TMPOζ, the shortest isoform, lacks both the trans-membrane domain of the TMPOβ/LAP2-type isoforms and the NLS of TMPOα. The expression of the various TMPO isoforms is ubiquitous, with higher levels in proliferative tissues (Berger et al, 1996; Harris et al, 1994; Ishijima et al, 1996; Theodor et al, 1997; Zevin-Sonkin et al, 1992).

D. Interactions between lamins and other nuclear lamina and inner nuclear membrane proteins.

1. Lamin interaction with IMPs

The interactions between lamins and IMPs are discussed below. These interactions are required for lamins to exert their biological roles. The interaction between lamins and TMPOβ/LAP2 is discussed above. The abnormal arrangement of nuclear pores in cells mutated in their lamin gene indicates an additional interaction between lamins and nuclear pore proteins, possible gp210.

a. Lamin-LBR/p58 interaction: LBR/p58 is an IMP that binds lamin B in a saturable and specific fashion, with a dissociation constant of about 200 nM (Worman et al, 1988). LBR is composed of a nucleoplasmic amino terminal domain followed by a hydrophobic domain that contains eight putative transmembrane segments (Worman et al, 1990). LBR's hydrophbic region has high sequence homology to the yeast sterol c-14 reductase (Gerace and Foisner, 1994; Shimanuki et al, 1992), and it was shown to posses c-14 reductase activity in *Saccharomyces cerevisiae* (Silve et al, 1998). The amino terminal domain of LBR has three DNA-binding motifs (Worman et al, 1990) and was suggested to bind DNA (Ye and Worman, 1994). LBR is phosphorylated in a cell cycle dependent manner, on serine residues in interphase and on serine and threonine residues in mitosis (Courvalin et al, 1992; Nikolakaki et al, 1997; Nikolakaki et al, 1996; Papoutsopoulou et al, 1999; Simos and Georgatos, 1992; Worman et al, 1990). It is degraded at late stages of apoptosis, interacts with chromatin (Pyrpasopoulou et al, 1996), and with several nuclear proteins including lamin B (Worman et al, 1988), p34, p18 (Simos and Georgatos, 1992) and the human protein homologous to the *Drosophila* heterochromatin associated protein, HP1 (Ye and Worman, 1996; Ye et al, 1997). Though its name, there is a recent work, utilizing biochemical fractionation analysis, that suggests that LBR and lamin B do not bind directly to each other in vivo (Mical and Monteiro, 1998). By using in vitro reconstituted vesicles assay it was shown that LBR can provide chromatin docking sites for nuclear vesicles (Pyrpasopoulou et al, 1996). An LBR-related protein from sea urchin can be

involved in providing chromatin docking sites for nuclear vesicles and can bind to the nuclear lamina (Collas et al, 1996).

b. Lamin-LAP1A-C interaction: LAP1A-C are related IMPs, which are presumably alternatively spliced transcripts of the same gene (Foisner and Gerace, 1993; Martin et al, 1995). LAP1A and LAP1B, but not LAP1C, can bind to both lamin A and lamin B in vitro. However, LAP1C associates with type B lamin in vivo as a component of a protein complex, albeit a direct LAP1C-lamin interaction has not been demonstrated (Maison et al, 1997). Cloning of LAP1C revealed that it is a type II IMP with a single membrane spanning region and a hydrophilic amino terminal domain that is exposed to the nucleoplasm. LAP1 isotypes are differentially expressed during development. LAP1C protein is present both in non-differentiated and differentiated cells whereas LAP1A and LAP1B proteins are abundant only in differentiate cells (Martin et al, 1995).

c. Lamin-emerin interaction: Mutations in the emerin gene result in the X-linked Emery-Dreifuss muscular dystrophy (Bione et al, 1994). Emerin is a ubiquitous IMP of the inner nuclear membrane (Manilal et al, 1996; Nagano et al, 1996) with sequence and structural homology to TMPOβ/LAP2 (Manilal et al, 1998). It is reasonable to speculate that emerin and lamin A/C interact with each other, directly or indirectly, since mutations in the gene encoding the human lamin A/C cause the autosomal dominant Emery-Dreifuss muscular dystrophy (Bonne et al, 1999) and both proteins are associated with the inner nuclear membrane.

2. Lamin interaction with peripheral proteins

a. Lamin-YA interaction: YA is a *Drosophila* NE protein that is present in the nuclear lamina during the first two hours of zygotic divisions and whose function is essential for initial embryonic development (Lin et al, 1991; Lin and Wolfner, 1991; Lopez et al, 1994). YA molecules are present in complexes with each other (Goldberg et al, 1998; Liu and Wolfner, 1998), and embryonic YA can associate with decondensed chromatin in vitro (Lopez and Wolfner, 1997). The carboxy terminal 179 amino acids of YA are necessary to target and retain YA in the NE. The condensation state of the chromosomes in YA-deficient eggs and embryos is abnormal (Liu et al, 1995) and ectopically expressed YA associates with polytene chromosomes in vivo (Lopez and Wolfner, 1997). YA interacts with *Drosophila* lamin Dm_0 in the yeast two-hybrid system. This interaction requires the rod and tail domains and part of the head domain of lamin Dm_0 and the C-terminal domain of YA (Goldberg et al, 1998).

b. Lamin-otefin interaction: Otefin is a peripheral nuclear membrane protein, characterized in *Drosophila*, which has no apparent homology to other known proteins (Padan et al, 1990). In *Drosophila*, otefin is present in the NE of essentially all cells examined during *Drosophila* development, except for sperm cells. In eggs and young embryos, otefin is also associated with the maternal fraction of membrane vesicles (Ashery Padan et al, 1997a). Otefin is a phosphoprotein in vivo and is phosphorylated by cdc2 protein kinase and cAMP-dependent protein kinase in vitro (Ashery Padan et al, 1997a). The otefin protein contains a large hydrophilic domain and a C-terminal hydrophobic domain of 17 amino acids. The C-terminal domain of otefin is essential but not sufficient for the targeting of otefin to the nuclear periphery. Sequences at the hydrophilic domain of otefin are required for both the localization and stabilization of otefin in the NE (Ashery Padan et al, 1997b), and for interaction with lamins. Otefin and lamin interact with each other in the yeast two-hybrid system and are present in vivo in the same protein complex (Goldberg et al, 1998). This interaction requires the rod domain of lamin Dm_0 and two hydrophilic regions in otefin (Goldberg et al, 1998).

Otefin was shown to be involved in NE assembly as the inhibition of otefin activity in *Drosophila* cell-free system, with anti-otefin antibodies, blocked the attachment of the nuclear vesicles to chromatin (Ashery Padan et al, 1997a). The similar phenotype observed in the inhibition studies of both lamin Dm_0 (see above) and otefin is probably due to their co-localization to nuclear vesicles in the same protein complex (Goldberg et al, 1998).

E. Lamin and apoptosis

During apoptosis, lamins are major targets for the caspase family of proteases, which trigger nuclear lamina breakdown. Lamin degradation has been reported in different cell types and in response to different apoptosis-inducing stimuli (Anjum and Khar, 1997; Antoku et al, 1997; Fraser et al, 1997; Kaufmann, 1989; Kawahara et al, 1998; Kluck et al, 1997; Lazebnik et al, 1993; Neamati et al, 1995; Oberhammer et al, 1994; Orth et al, 1996; Rao et al, 1996; Shimizu et al, 1998; Shimizu and Pommier, 1997; Takahashi et al, 1996a; Takahashi et al, 1996b; Ucker et al, 1992; Zhivotovsky et al, 1997). Lamin B is phosphorylated by PKCαduring apoptosis before its degradation (Shimizu et al, 1998). The degradation of lamin B_1 precedes DNA fragmentation in apoptotic thymocytes (Neamati et al, 1995), HeLa cells (Mandal et al, 1996) and HL60 cells (Shimizu et al, 1998).

Lamins may be involved directly in the regulation of apoptosis since ectopic expression of uncleavable mutant lamin A or B caused a 12 hours delay in the onset of apoptosis. The cells expressing the mutant lamins failed to undergo chromatin condensation and nuclear shrinking, which is a hallmark of apoptotic cell death. However, in these cells the NE collapsed and the nuclear lamina remained intact. However, the late stages of apoptosis were not affected (Rao et al, 1996). Thus, the proteolysis of lamin facilitates the nuclear events of apoptosis, perhaps by facilitating nuclear breakdown. A possible role for lamin cleavage during apoptosis is to enable the dissociation of the chromatin from the nuclear lamina and thereby to affect nuclear condensation.

II. Results and discussion

A. Lamin Dm0 binds chromosomes through two separate regions within its tail domain.

Lamin Dm_0 can bind specifically to decondensed sperm chromatin in *Drosophila* in vitro nuclei assembly assay, as well as to mitotic chromosomes (Goldberg et al, 1999; Ulitzur et al, 1992). The binding assay of lamin Dm_0 to mitotic chromosomes includes incubation of purified lamin Dm_0 protein with isolated CHO mitotic chromosomes for 30-60 min at 22^0C in the presence of excess amount of non-relevant proteins. Lamin binding to the surface of the chromosomes was observed following immunofluorescence analysis with either monoclonal anti-lamin Dm_0 antibody or monoclonal anti-^{RGS}His antibody (Qiagen, Germany) that recognises a RGS(H)$_4$ epitope, as primary antibodies, and Cy3-conjugated goat-anti-mouse antibodies, as secondary antibodies. Alternatively, it was detected directly by a fusion between lamin Dm_0 and the green fluorescence protein (GFP) (**Figure 3**).

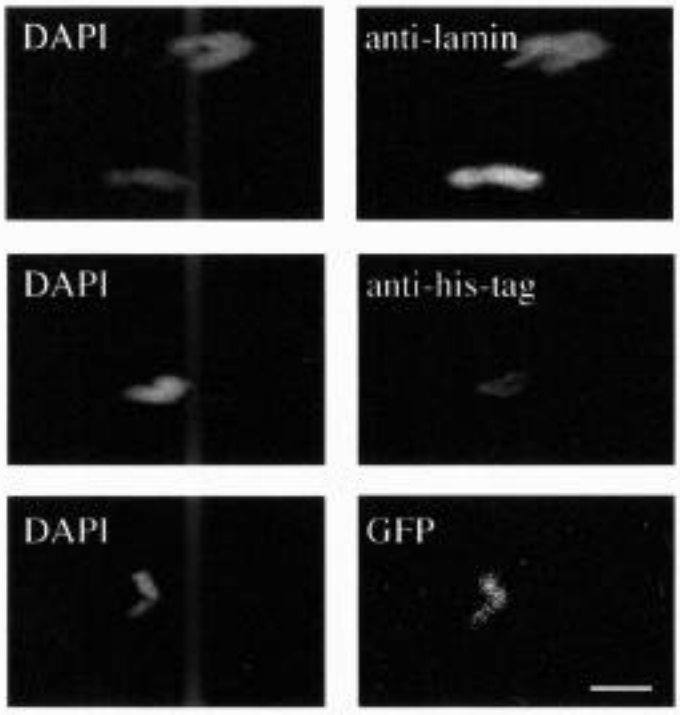

Figure 3. The binding of the tail domain of lamin Dm_0 to mitotic chromosomes can be observed with different detection systems. Upper panel – Detection was with monoclonal anti-lamin Dm_0 antibody, as primary antibody, and Cy3-conjugated goat-anti-mouse antibodies, as secondary antibodies. Middle panel - Detection was with monoclonal anti-^{RGS}His antibody (Qiagen, Germany), as primary antibody, and Cy3-conjugated goat-anti-mouse antibodies, as secondary antibodies. Lower panel – The tail domain of lamin Dm_0 was fused in frame to the Green Fluorescent Protein (GFP). Chromatin was stained with the DNA-specific dye DAPI. In all three cases, viewing was with a Leitz microscope equipped with epifluorescence. (Bar = 5μ).

The binding of lamin Dm_0 to chromosomes did not require lamin polymerization, since lamin Dm_0 mutated in Arginine 64 (R64>H), which is unable to polymerize (Zhao et al, 1996), still bound chromosomes in a similar way to that of wild type lamin Dm_0 (**Figure 4**). Further mapping of the regions that are involved in lamin Dm_0 binding to mitotic chromosomes, using deletion mutants of lamin Dm_0 protein, revealed that the lamin Dm_0 tail domain (amino acids 425-622) mediates this binding (**Figures 3, 4**). The isolated lamin Dm_0 tail domain bound to the periphery of mitotic chromosomes with similar intensity to that of wild type lamin Dm_0 (**Figure 4**). The dissociation constant of lamin tail domain binding to chromatin is approximately 1 μM (Goldberg et al, 1999). The existence of a chromatin binding site in the lamin tail domain was also reported for *Xenopus* type A and B_2 lamins (Hoger et al, 1991) and for mammalian type A and B lamins (Taniura et al, 1995).

Deletion mutants of the lamin Dm_0 tail domain were designed in order to map the region in this domain that mediates the binding of lamin Dm_0 to chromosomes. Each deletion mutant was expressed in bacteria, purified to near homogeneity and subjected to the binding assay. The ability of the different protein constructs to bind mitotic chromosomes is summarized in **Figure 4**. The T425-522 protein bound chromosomes with lower immunofluorescence intensity as compared to the complete lamin tail domain, but with significantly higher intensity as compared to the T473-622, T523-622 and T473-572 proteins. In addition, the intensity of T473-572 binding to chromosomes was close to background levels (**Figure 4**). Taken together, these data indicate that there are two regions that are required for efficient binding of lamin Dm_0 to chromosomes. The major binding region includes amino acids 425-473 of lamin Dm_0 and the minor binding region includes amino acids 572-622 of lamin Dm_0. T425-572 protein that contains the first binding region binds to chromosomes with similar efficiency to the complete tail domain, while T523-622 and T473-622 proteins that contain the second binding region bind to chromosomes with significantly weaker intensity. The lower signal obtained with T425-522, as compared to T425-572, can be explained by a difference in protein folding or requirement of additional residues.

B. Lamin Dm0 tail domain binding to chromosomes can be displaced with histones H2A and H2B

In order to detect the molecules that are required for the binding of lamin Dm_0 to chromosomes, we analyzed the ability of various chromosomal components to displace the binding of the lamin Dm_0 tail domain to mitotic chromosomes. MAR/SAR DNA sequences were previously found to bind lamin Dm_0 in vitro (Baricheva et al, 1996; Luderus et al, 1992; Luderus et al, 1994), through the rod domain of lamin Dm_0 (Zhao et al, 1996). MAR/SAR DNA sequences, as well as yeast ARS sequences and plasmid DNA, are not the target of lamin tail binding, since they could not displace the binding of the lamin Dm_0 tail domain

to chromosomes (Goldberg et al, 1999). This is expected since it is the tail domain that mediates the binding of lamin Dm0 to chromosomes. In contrast, isolated nucleosome core particles and commercially crude preparation of core histones and histone H1 displaced lamin tail domain binding to chromosomes (Goldberg et al, 1999).

To identify specific histone(s) that interact with the lamin Dm0 tail domain, the individual core histones (**Figure 5**) or histone H1 (Goldberg et al, 1999) were tested for their ability to displace the binding of the lamin Dm0 tail domain to chromosomes. A 30-120-fold molar excess of purified histones H2A or H2B could each displace the binding of lamin Dm0 tail domain to chromosomes (**Figure 5**). In contrast, a 30-120-fold molar excess of individual histones H3 or H4 did not displace the lamin Dm0 tail domain binding to chromosomes (**Figure 5**). Similarly, a 32-fold molar excess of purified histone H1 (amino acids 1-142) did not displace the binding of lamin Dm0 tail domain to chromosomes (Goldberg et al, 1999). It is, therefore, likely that the interaction between lamin Dm0 and chromosomes is mediated by histones H2A and H2B. The biological significance of this lamin-histones interaction is probably in the processes of nuclear envelope assembly and nuclear organization. The significance of this interaction in vivo is currently under investigation..

C. Analysis of the mouse TMPO α, β, β', ε, δ, γ and ζ isoforms.

The first mouse TMPO isoforms that was cloned was TMPOζ (Berger et al, 1996; Theodor et al, 1997). Its cDNA clone was isolated from thymus cDNA library and characterized, using a 126 bp fragment, encoding thymopoietin amino acids 1-42, from the bovine TMPO cDNA (Zevin-Sonkin et al, 1992), as a probe. The same library was subsequently screened with a 790 bp probe, derived from the N-terminus of the TMPOζ cDNA. Repeated screenings and restriction enzyme analysis revealed at least six distinct TMPO transcripts (**Figure 6**).

Examination of the mouse TMPOα sequence revealed a short region of basic amino acids (amino acid 188-194) suggestive of a NLS (Kalderon et al, 1984), and a possible tyrosine phosphorylation site (amino acids 618-625) (Patschinsky et al, 1982). The sequence S/T-P-X-X, a potential recognition sequence for cdc2-related kinases (Nigg, 1993), is found 10 times throughout the TMPOα sequence. Hydropathy analysis revealed that similar to the human isoforms, all mouse TMPOs lack an N-terminal hydrophobic signal sequence typical of secreted polypeptides (not shown). However, this analysis revealed that TMPOs β, ε, δ and γ contain, like the human TMPOβ and γ, a hydrophobic transmembrane domain near their C-terminus (amino acid 409-432 of TMPOβ), suggesting their association with cellular membranes such as the nuclear envelope.

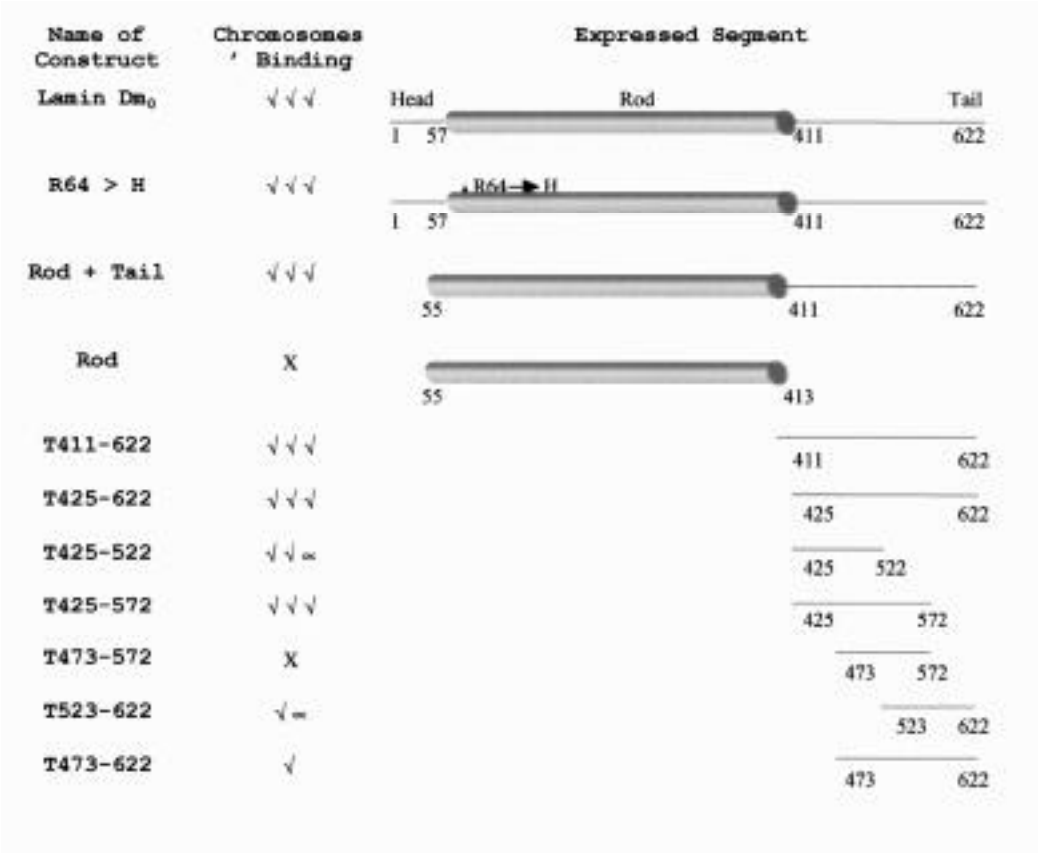

Figure 4. Binding of different lamin Dm_0 constructs to mitotic chromosomes derived from Chinese Hamster Ovaries (CHO) requires specific sequences in their tail domain. R64>H is a point mutation in lamin Dm_0 in Arginine 64 that inhibits its ability to form filaments. The numbers in the name of the construct represent the amino acids of lamin Dm_0 that are included in the construct and their position is shown below the map of the construct. √√√ indicates strong binding; √√_indicates medium binding, √ indicates weak binding and X indicates lack of detectable binding. indicates detection with the monoclonal anti-^{RGS}His antibody or with GFP.

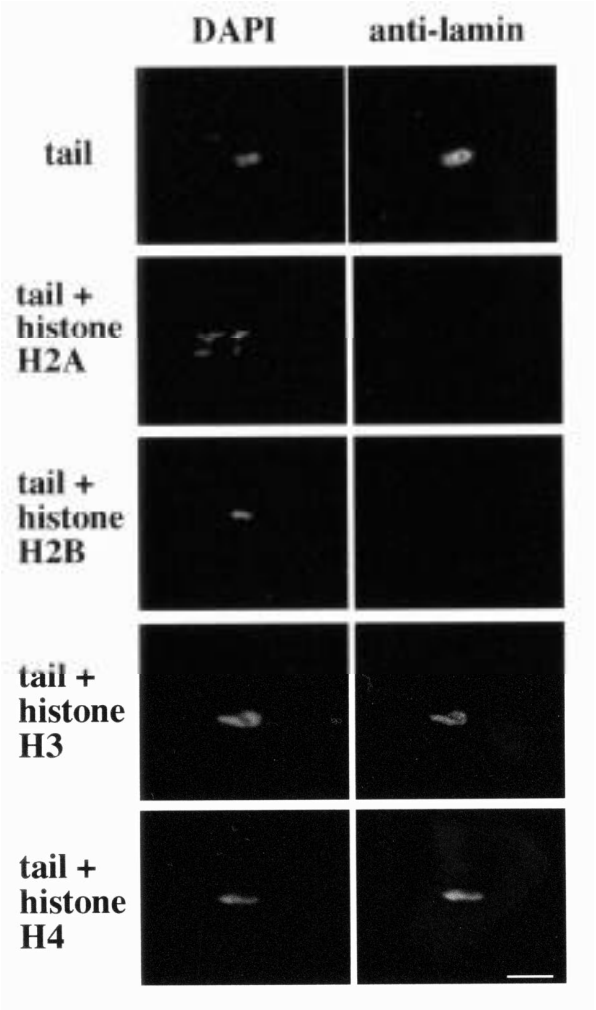

Figure 5. The binding of lamin Dm_0 to mitotic chromosomes can be displaced with histones H2A and H2B. The tail domain of lamin Dm_0 was used to bind the mitotic chromosomes in the presence or absent of histones. DAPI staining – left panwels; MAb anti-lamin Dm_0 antibody as primary antibody and Cy3-conjugated goat-anti-mouse antibodies as secondary antibodies – right panels. (Bar=5μ).

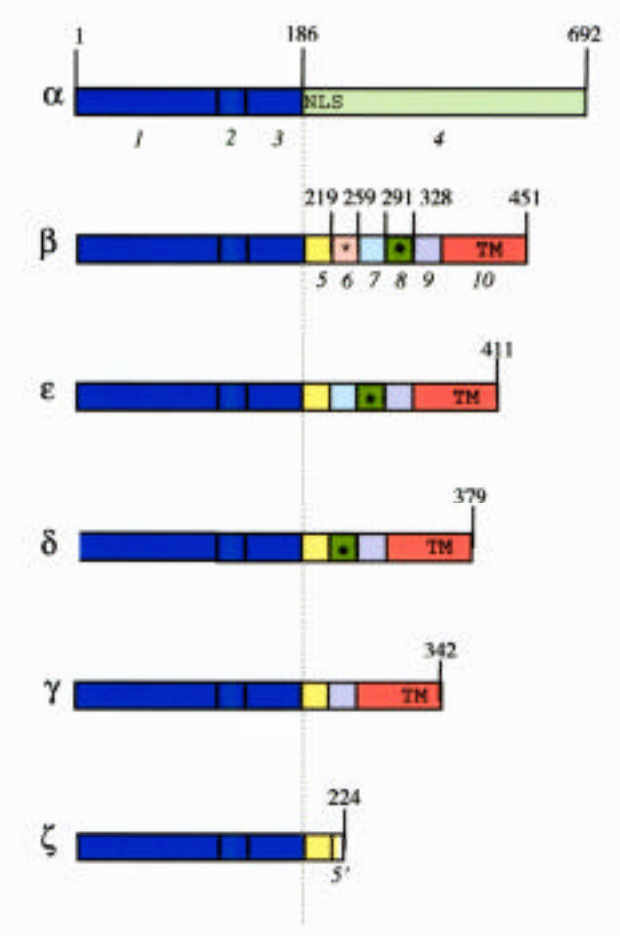

Figure 6. TMPO/LAP2 is a growing family of nuclear envelope proteins derived from a single alternatively spliced gene. The numbers above each isoform represent the corresponding amino acid. The numbers below the α and β isoforms represent the corresponding exons in the TMPO gene. The exons that are common to all TMPOs are colored in blue and the alternatively spliced exons have different colors. NLS-nuclear localization signal; TM-transmembrane domain; 5'- the last 5 amino acids of the ζ isoform that are included in intron 5 of the TMPO gene. Asterisk in β, ε and δ isoforms show the location of putative sites for the cdc2-kinase.

Mouse TMPO α, β, δ, ε, γ and ζ share an identical N-terminal 186 amino acids domain (**Figure 6**). Like the human and the bovine TMPOs, amino acids 1-49 are highly homologous to the originally purified 49-amino acid bovine TP (Schlesinger and Goldstein, 1975). After Glu186, TMPOα diverges from the other TMPOs. TMPOε differs from TMPOβ only in lacking the β-specific residues 220-259. TMPOδ lacks both the β and the ε/β-specific domains, which is contained within residues 220-291 of TMPOβ. TMPOγ is missing the β, ε/β and δ/ε/β-specific domains contained within amino acids 220-328 of TMPOβ. Interestingly, two TMPOβ 3'-UTR versions were isolated in mouse and designated TMPOβ and TMPOβ'. The two clones are identical in their ORF sequence, but differ in an additional 3'- UTR sequence starting at A1715 of the β' clone, probably due to an alternative polyadenylation signal.

D. Monoclonal antibodies identify alternatively spliced forms of TMPOs

Three monoclonal antibodies (mAbs) were generated in order to characterize the expression and localization of the different TMPO isoforms (Dr. Gideon Goldstein, generous gift). The 6E10 mAb was raised against a synthetic peptide common to the N-terminus of all TMPOs. Indeed, Western blot analysis revealed that the 6E10 antibody recognized both recombinant and native TMPOs α, β, γ and ζ as well as native TMPOs ε and δ (**Figure 7a**). Although all TMPOs are present in both the hematopoietic K562 and the epithelial HeLa cell lines, TMPOβ is clearly more abundant than all

other TMPOs and the relative amounts of the different TMPOs vary between these cell lines. Thus, it would be interesting to investigate if the different levels of expression of the various TMPOs in specific cells have a role in the biological functions that these cells have to exert. The 4G10 mAb was raised against recombinant TMPOα and the 6G11 mAb was raised against a synthetic peptide, derived from TMPOβ/LAP2 specific region (amino acids 313-330). These antibodies recognized both recombinant and native TMPOα and TMPOβ , respectively (**Figure 7b** and **c**). Interestingly, the 6G11 antibody cross-reacted with two close bands in both cell lines, suggesting two forms of TMPOβ that differ in their phosphorylation pattern (**Figure 7c**).

E. TMPOα is localized both to the nucleus and centrosomes, while the TMPO/ LAP2 type isoforms are localized to the nuclear envelope.

TMPOα is the largest member of the TMPO family. It shares the first 187 amino acids of all TMPOs but has a unique long C-terminus. This region lacks a trans-membrane domain but contains a NLS, a putative tyrosine phosphorylation site and two perfect p34cdc2 kinase phosphorylation sites (Nigg, 1993). This unique region suggests a different intra-cellular localization than that of the TMPOβ/LAP2-type isoforms.

The localization of TMPOα was studied by immunofluorescence microscopy during the different cell cycle stages of HeLa cells, using the 4G10 anti TMPOα mAb. In interphase, TMPOα was diffusely distributed throughout the nucleoplasm but was excluded from the nucleoli (**Figure 8a**). In addition, it was found in the centrosomal region (the bright spots in **Figure 8a**). In early prophase, prior to its complete disintegration of the nuclear envelope, a large fraction of TMPOα was observed at the nuclear periphery (**Figure 8a**). In metaphase and early anaphase, TMPOα was disseminated throughout the cell In addition, the two centrosomes at the spindle poles were stained brightly (**Figure 8b**). In telophase a bulk of the TMPOα protein re-localized to the two newly forming nuclear envelopes. At this stage, the nucleus itself was negatively stained (**Figure 8c**). Upon completion of mitosis and reentry of the cells to G1, TMPOα regained its typical interphase intranuclear and centrosomal localization, while the nuclear envelope was not stained. This staining pattern of TMPOα throughout the cell cycle was exemplified in other cell lines such as PTK, K562 and HL-60 (not shown). The presence of TMPOα at the nuclear periphery during prophase indicates a possible association of TMPOα with NE components during this stage. The association of TMPOα with the chromatin periphery during telophase strongly suggests a role for this isoform in nuclear assembly.

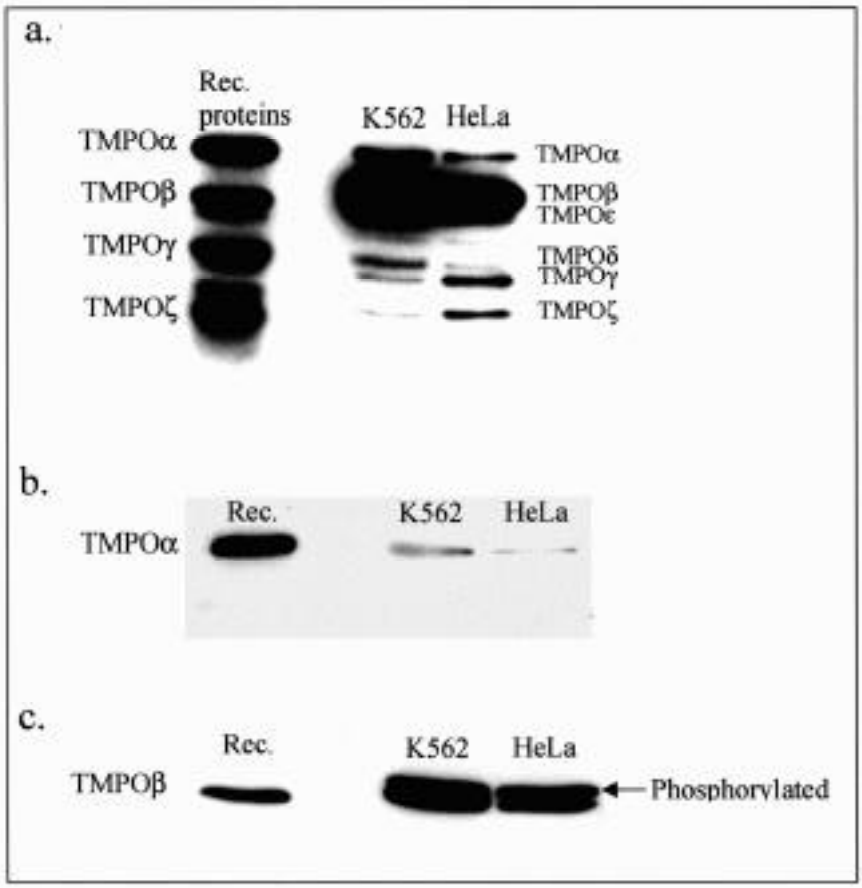

Figure 7. Different monoclonal anti-TMPO antibodies detect the various of TMPO isoforms and show variability in their relative expression in human cells. (**a**) Detection was with the 6E10 monoclonal anti-all TMPO antibody. The left lane was loaded with recombinant proteins. Although all TMPO proteins are expressed in both Hela and K562 cells, their relative abundance is different. (**b**) Detection was with 4G10 monoclonal anti-TMPOα antibody. (**c**) Detection was with 6G11 monoclonal anti-TMPOβ antibody.

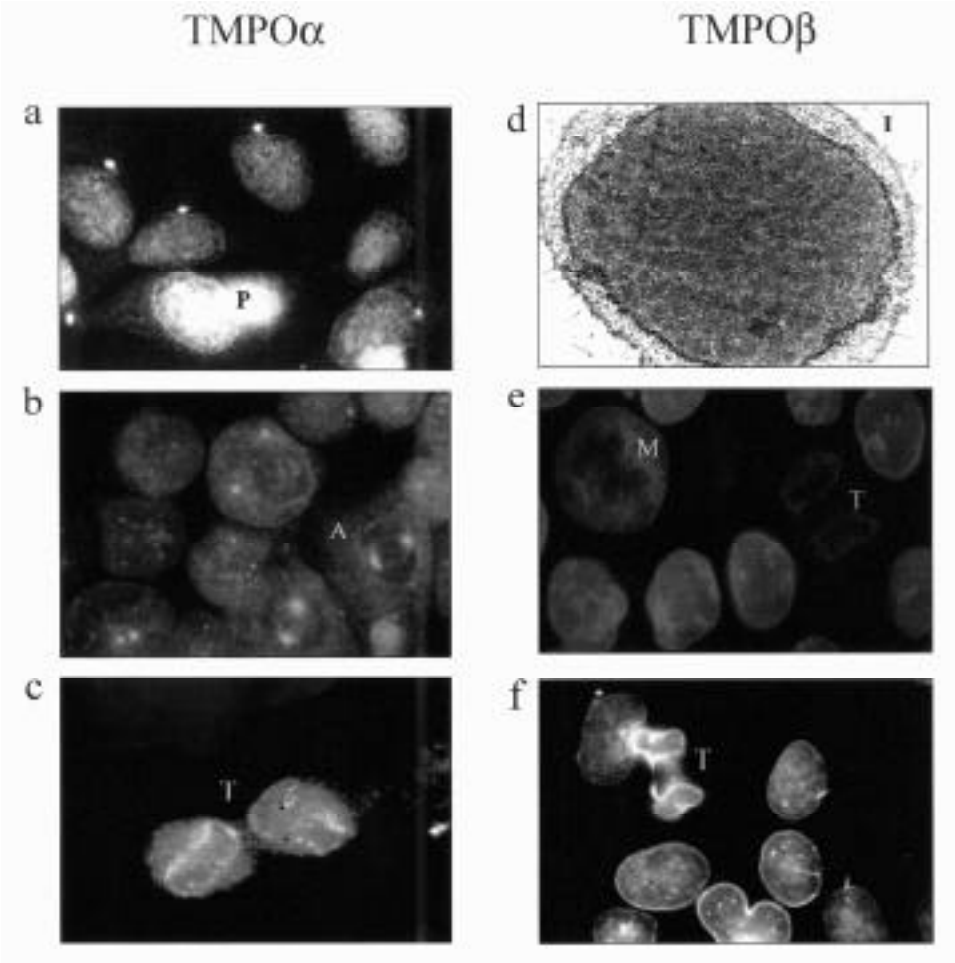

Figure 8. TMPOα and TMPOβ have different localisation in human cells. TMPOα localisation was detected with the 4G10 monoclonal anti-TMPOα antibody (**a-c**) and TMPOβ localisation was detected with the 6G11 monoclonal anti-TMPOβ antibody (**d-f**). P, a cell in prophase; M, metaphase; T, telophase. Panel d shows immnogold electron microscope detection of TMPOβ; all other panels show indirect immunofluorescence analysis.

Previous studies have shown that TMPOβ/LAP2 is an integral protein of the inner nuclear membrane (Foisner and Gerace, 1993). Its NE targeting and binding are probably mediated by its carboxy terminus-located lamin binding region and trans-membrane domain (Furukawa et al, 1995). Since TMPOs ε, δ and γ also share this carboxy-terminus and parts of the lamin binding domain, and transfected TMPOγ was localized to the NE (data not shown), we suggest that these isoforms are also nuclear membrane proteins.

The localization of TMPOβ/LAP2 to the nuclear envelope during the cell cycle was studied in Hela cells, by immunofluorescence and immuno-electron microscopy. In interphase, immuno-gold analysis demonstrated the localization of TMPOβ/LAP2 to the NE (**Figure 8d**). The immuno-fluorescence staining was confined to the NE, as well as to intra nuclear speckles (**Figure 8f**). These speckles possibly represent replication origins (Gant et al, 1999; Moir et al, 1994). In metaphase, TMPOβ was dispersed in the cytoplasm (**Figure 8e**). In telophase, TMPOβ re-localized to the two newly forming nuclear envelopes. However, the staining of TMPOβ during telophase is more continuous than that of TMPOα.

References

Anjum R and Khar A (**1997**). Caspase-mediated apoptosis in AK-5 tumor cells: a cell-free study using peptide inhibitors and antisense strategy. **Exp Cell Res** *236*, 371-377.

Antoku K, Liu Z, and Johnson DE (**1997**). Inhibition of caspase proteases by CrmA enhances the resistance of human leukemic cells to multiple chemotherapeutic agents. **Leukemia** *11*, 1665-1672.

Ashery Padan R, Ulitzur N, Arbel A, Goldberg M, Weiss AM, Maus N, Fisher PA, and Gruenbaum Y (**1997**). Localization and posttranslational modifications of otefin, a protein required for vesicle attachment to chromatin, during *Drosophila* melanogaster development. **Mol Cell Biol** *17*, 4114-4123.

Ashery Padan R, Weiss AM, Feinstein N, and Gruenbaum Y (**1997b**). Distinct regions specify the targeting of otefin to the nucleoplasmic side of the nuclear envelope. **J Biol Chem** *272*, 2493-2499.

Baricheva EA, Berrrios M, Bogachev SS, Borisevich IV, Lapik ER, Sharakhov IV, Stuurman N, and Fisher PA (**1996**). DNA from *Drosophila* _-heterochromatin binds specifically to nuclear lamins in vitro and the nuclear envelope in situ. **Gene** *171*, 171-176.

Belmont AS, Zhai Y, and Thilenius A (**1993**). Lamin B

distribution and association with peripheral chromatin revealed by optical sectioning and electron microscopy tomography. **J Cell Biol** *123*, 1671-1685.

Benavente R and Krohne G (**1986**). Involvement of nuclear lamins in postmitotic reorganization of chromatin as demonstrated by microinjection of lamin antibodies. **J Cell Biol** *103*, 1847-1854.

Benavente R, Krohne G, and Franke WW (**1985**). Cell type-specific expression of nuclear lamina proteins during development of *Xenopus* laevis. **Cell** *41*, 177-190.

Benavente RK (**1985**). Change of karyoskeleton during spermatogenesis of *Xenopus*: expression of lamin LIV, a nuclear lamina protein specific for the male germ line. **Proc Natl Acad Sci USA** *82*, 6176-6180.

Berger R, Theodor L, Shoham J, Gokkel E, Brok-Simoni F, Avraham KB, Copeland NG, Jenkins NA, Rechavi G, and Simon AJ (**1996**). The characterization and localization of the mouse thymopoietin/lamina- associated polypeptide 2 gene and its alternatively spliced products. **Genome Res** *6*, 361-370.

Bione S, Maestrini E, Rivella S, Mancini M, Regis S, Romeo G, and Toniolo D (**1994**). Identification of a novel X-linked gene responsible for Emery-Dreifuss muscular dystrophy. **Nat Genet** *8*, 323-327.

Bonne G, Di Barletta MR, Varnous S, Becane HM, Hammouda EH, Merlini L, Muntoni F, Greenberg CR, Gary F, Urtizberea JA, Duboc D, Fardeau M, Toniolo D, and Schwartz K (**1999**). Mutations in the gene encoding lamin A/C cause autosomal dominant Emery- Dreifuss muscular dystrophy. **Nat Genet** *21*, 285-288.

Bossie CA and Sanders MM (**1993**). A cDNA from *Drosophila* melanogaster encodes a lamin C-like intermediate filament protein. **J Cell Sci** *104*, 1263-1272.

Bridger JM, Kill IR, O'Farrell M, and Hutchison CJ (**1993**). Internal lamin structures within G1 nuclei of human dermal fibroblasts. **J Cell Sci** *104*, 297-306

Burke B (**1990a**). The nuclear envelope and nuclear transport. **Curr Opin Cell Biol** *2*, 514-20.

Burke B (**1990b**). On the cell-free association of lamins A and C with metaphase chromosomes. **Exp Cell Res** *186*, 169-176.

Burke B and Gerace L (**1986**). A cell free system to study reassembly of the nuclear envelope at the end of mitosis. **Cell** *44*, 639-652.

Christova R, Yaneva J, and Galcheva-Gargova Z (**1989**). Some features of DNA fragments associated in vivo with the nuclear lamina. **Biosci Rep** *9*, 587-592.

Chu A, Rassadi R, and Stochaj U (**1998**). Velcro in the nuclear envelope: LBR and LAPs. **FEBS Lett** *441*, 165-169.

Collas P and Alestrom P (1997). Nuclear localization signals: a driving force for nuclear transport of plasmid DNA in zebrafish. **Biochem Cell Biol** *75*, 633-640.

Collas P, Courvalin JC, and Poccia D (**1996**). Targeting of membranes to sea urchin sperm chromatin is mediated by a lamin B receptor-like integral membrane protein. **J Cell Biol** *135*, 1715-1725.

Corbett AH and Silver PA (**1997**). Nucleocytoplasmic transport of macromolecules. **Microbiol Mol Biol Rev** *61*, 193-211.

Courvalin JC, Segil N, Blobel G, and Worman HJ (**1992**). The lamin B receptor of the inner nuclear membrane undergoes mitosis-specific phosphorylation and is a substrate for p34cdc2-type protein kinase. **J Biol Chem** *267*, 19035-19038.

Dabauvalle MC, Loos K, Merkert H, and Scheer U (**1991**). Spontaneous assembly of pore complex-containing membranes ("annulate lamellae") in *Xenopus* egg extract in the absence of chromatin. **J Cell Biol** *112*, 1073-1082.

Davis, L. I. (**1995**). The nuclear pore complex. **Annu Rev Biochem** *64*, 865-896.

Dechat T, Gotzmann J, Stockinger A, Harris CA, Talle MA, Siekierka JJ, and Foisner R (**1998**). Detergent-salt resistance of LAP2alpha in interphase nuclei and phosphorylation-dependent association with chromosomes early in nuclear assembly implies functions in nuclear structure dynamics. **EMBO J** *17*, 4887-4902.

Dessev G, Iovcheva DC, Bischoff JR, Beach D, and Goldman R (**1991**). A complex containing p34cdc2 and cyclin B phosphorylates the nuclear lamin and disassembles nuclei of clam oocytes in vitro. **J Cell Biol** *112*, 523-533.

Eggert M, Radomski N, Linder D, Tripier D, Traub P, and Jost E (**1993**). Identification of novel phosphorylation sites in murine A-type lamins. **Eur J Biochem** *213*, 659-666.

Eggert M, Radomski N, Tripier D, Traub P, and Jost E (**1991**). Identification of phosphorylation sites on murine nuclear lamin C by RP-HPLC and microsequencing. **FEBS Lett** *292*, 205-209.

Ellis DJ, Jenkins H, Whitfield WG, and Hutchison CJ (**1997**). GST-lamin fusion proteins act as dominant negative mutants in *Xenopus* egg extract and reveal the function of the lamina in DNA replication. **J Cell Sci** *110*, 2507-2518.

Fabre E and Hurt E (**1997**). Yeast genetics to dissect the nuclear pore complex and nucleocytoplasmic trafficking. **Annu Rev Genet** *31*, 277-313.

Favreau C, Worman HJ, Wozniak RW, Frappier T, and Courvalin JC (**1996**). Cell Cycle Dependent Phosphorylation Of Nucleoporins and Nuclear Pore Membrane Protein Gp210. **Biochemistry** *35*, 8035-8044.

Fields AP, Pettit GR, and May WS (**1988**). Phosphorylation of lamin B at the nuclear membrane by activated protein kinase C. **J Biol Chem** *263*, 8253-8260.

Firmbach-Kraft I and Stick R (**1995**). Analysis of nuclear lamin isoprenylation in *Xenopus* oocytes: isoprenylation of lamin B3 precedes its uptake into the nucleus. **J Cell Biol** *129*, 17-24.

Firmbach-Kraft I and Stick R (**1993**). The role of CaaX-dependent modifications in membrane association of *Xenopus* nuclear lamin B3 during meiosis and the fate of B3 in transfected mitotic cells. **J Cell Biol** *123*, 1661-1670.

Fisher DZ, Chaudhary N, and Blobe, G (**1986**). cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins. **Proc Natl Acad Sci USA** *83*, 6450-6454.

Foisner R and Gerace L (**1993**). Integral membrane proteins of the nuclear envelope interact with lamins and chromosomes, and binding is modulated by mitotic phosphorylation. **Cell** *73*, 1267-1279.

Fraser AG, McCarthy NJ, and Evan GI (**1997**). drICE is an essential caspase required for apoptotic activity in *Drosophila* cells. **EMBO J** *16*, 6192-6199.

Furukawa K, Fritze CE, and Gerace L (**1998**). The major nuclear envelope targeting domain of LAP2 coincides with its lamin binding region but is distinct from its chromatin interaction domain. **J Biol Chem** *273,* 4213-4219.

Furukawa K and Hotta Y (**1993**). cDNA cloning of a germ cell specific lamin B3 from mouse spermatocytes and analysis of its function by ectopic expression in somatic cells. **EMBO J** *12,* 97-106.

Furukawa K, Inagaki H, and Hotta Y (**1994**). Identification and cloning of an mRNA coding for a germ cell-specific A-type lamin in mice. **Exp Cell Res** *212,* 426-430.

Furukawa K, and Kondo T (**1998**). Identification of the lamina-associated-polypeptide-2-binding domain of B-type lamin. **Eur J Biochem** *251,* 729-733.

Furukawa K, Pante N, Aebi U, and Gerace L (**1995**). Cloning of a cDNA for lamina-associated polypeptide 2 (LAP2) and identification of regions that specify targeting to the nuclear envelope. **EMBO J** *14,* 1626-1636.

Galcheva-Gargova Z and Dessev GN (**1987**). Crosslinking of DNA to nuclear lamina proteins by UV irradiation in vivo. **J Cell Biochem** *34,* 163-168.

Gant TM, Harris CA, and Wilson KL (**1999**). Roles of LAP2 Proteins in Nuclear Assembly and DNA Replication: Truncated LAP2beta Proteins Alter Lamina Assembly, Envelope Formation, Nuclear Size, and DNA Replication Efficiency in *Xenopus* laevis Extracts. **J Cell Biol** *144,* 1083-1096.

Gant TM and Wilson KL (**1997**). Nuclear assembly. **Ann Rev Cell Dev Biol** *13,* 669-695.

Gerace L and Foisner R (**1994**). Integral membrane proteins and dynamic organization of the nuclear envelope. **Trends Cell Biol** *4,* 127-131.

Glass CA, Glass JR, Taniura H, Hasel KW, Blevitt JM, and Gerace L (**1993**). The alpha-helical rod domain of human lamins A and C contains a chromatin binding site. **EMBO J** *12,* 4413-4424.

Glass JR and Gerace L (**1990**). Lamins A and C bind and assemble at the surface of mitotic chromosomes. **J Cell Biol** *111,* 1047-1057.

Goldberg M, Harel A, Brandeis M, Rechsteiner T, Richmond TJ, Weiss AM, and Gruenbaum Y (**1999**). The tail domain of lamin Dm0 binds histones H2A and H2B. **Proc Natl Acad Sci USA** *96,* 2852-2857.

Goldberg M, Jenkins H, Allen T, Whitfield WG, and Hutchison CJ (**1995**). *Xenopus* lamin B3 has a direct role in the assembly of a replication competent nucleus: evidence from cell-free egg extracts. **J Cell Sci** *108,* 3451-3461.

Goldberg M, Lu H, Stuurman N, Ashery Padan R, Weiss AM, Yu J, Bhattacharyya D, Fisher PA, Gruenbaum Y, and Wolfner MF (**1998**). Interactions among *Drosophila* nuclear envelope proteins lamin, otefin, and YA. **Mol Cell Biol** *18,* 4315-4323.

Goldman AE, Moir RD, Montag LM, Stewart M, and Goldman RD (**1992**). Pathway of incorporation of microinjected lamin A into the nuclear envelope. **J Cell Biol** *119,* 725-735.

Gorlich D and Mattaj IW (**1996**). Nucleocytoplasmic transport. Science. *271,* 1513-1518.

Goss VL, Hocevar BA, Thompson LJ, Stratton CA, Burns DJ, and Fields AP (**1994**). Identification of nuclear beta II protein kinase C as a mitotic lamin kinase. **J Biol Chem** *269,* 19074-19080.

Gruenbaum Y, Landesman Y, Drees B, Bare JW, Saumweber H, Paddy MR, Sedat JW, Smith DE, Benton BM, and Fisher PA (**1988**). *Drosophila* nuclear lamin precursor Dm0 is translated from either of two developmentally regulated mRNA species apparently encoded by a single gene. **J Cell Biol** *106,* 585-596.

Haas M and Jost E (**1993**). Functional analysis of phosphorylation sites in human lamin A controlling lamin disassembly, nuclear transport and assembly. **Eur J Cell Biol** *62,* 237-247.

Harel A, Goldberg M, Ulitzur N, and Gruenbaum Y (**1998**). Structural organization and biological roles of the nuclear lamina. In Textbook of Gene Therapy and Molecular Biology: "*From basic mechanism to clinical applications*". Ed. T. Boulikas. *1,* 529-542.

Harel A, Zlotkin E, Nainude, ES, Feinstein N, Fishe, PA, and Gruenbaum Y (**1989**). Persistence of major nuclear envelope antigens in an envelope-like structure during mitosis in *Drosophila* melanogaster embryos. **J Cell Sci** *94,* 463-470.

Harris CA, Andryuk PJ, Cline S, Chan HK, Natarajan A, Siekierka JJ, and Goldstein G (**1994**). Three distinct human thymopoietins are derived from alternatively spliced mRNAs. **Proc Natl Acad Sci USA** *91,* 6283-6287.

Harris CA, Andryuk PJ, Cline SW, Mathew S, Siekierka JJ, and Goldstein G (**1995**). Structure and mapping of the human thymopoietin (TMPO) gene and relationship of human TMPO beta to rat lamin-associated polypeptide 2. **Genomics** *28,* 198-205.

Heald R and McKeon F (**1990**). Mutations of phosphorylation sites in lamin A that prevent nuclear lamina disassembly in mitosis. **Cell** *61,* 579-589.

Hennekes H, Peter M, Weber K, and Nigg EA (**1993**). Phosphorylation on protein kinase C sites inhibits nuclear import of lamin B2. **J Cell Biol** *120,* 1293-1304.

Hocevar BA, Burns DJ, and Fields AP (**1993**). Identification of protein kinase C (PKC) phosphorylation sites on human lamin B. Potential role of PKC in nuclear lamina structural dynamics. **J Biol Chem** *268,* 7545-7552.

Hocevar BA and Fields AP (**1991**). Selective translocation of beta II-protein kinase C to the nucleus of human promyelocytic (HL60) leukemia cells. **J Biol Chem** *266,* 28-33.

Hoger TH, Krohne G, and Kleinschmidt JA (**1991**). Interaction of *Xenopus* lamins A and LII with chromatin in vitro mediated by a sequence element in the carboxyterminal domain. **Exp Cell Res.***197,* 280-289.

Hozak P, Sasseville AM, Raymond Y, and Cook PR (**1995**). Lamin proteins form an internal nucleoskeleton as well as a peripheral lamina in human cells. **J Cell Sci** *108,* 635-644.

Hutchison CJ, Bridger JM, Cox LS, and Kill IR (**1994**). Weaving a pattern from disparate threads: lamin function in nuclear assembly and DNA replication. **J Cell Sci** *107,* 3259-3269.

Ishijima Y, Toda T, Matsushita H, Yoshida M, and Kimura N (**1996**). Expression of thymopoietin beta/lamina-associated polypeptide 2 (TP beta/LAP2) and its family proteins as revealed by specific antibody induced against recombinant human thymopoietin. **Biochem Biophys Res Commun** *226,* 431-438.

Jenkins H, Holman T, Lyon C, Lane B, Stick R, and Hutchison C

(**1993**). Nuclei that lack a lamina accumulate karyophilic proteins and assemble a nuclear matrix. **J Cell Sci** *106*, 275-285

Kalderon D, Roberts BL, Richardson WD, and Smith AE (**1984**). A short amino acid sequence able to specify nuclear location. **Cell** *39*, 499-509.

Kaufmann SH (**1989**). Induction of endonucleolytic DNA cleavage in human acute myelogenous leukemia cells by etoposide, camptothecin, and other cytotoxic anticancer drugs: a cautionary note. **Cancer Res** *49*, 5870-5878.

Kawahara A, Enari M, Talanian RV, Wong WW, and Nagata S (**1998**). Fas-induced DNA fragmentation and proteolysis of nuclear proteins. **Genes Cells** *3*, 297-306.

Kluck RM, Martin SJ, Hoffman BM, Zhou JS, Green DR, and Newmeyer DD (**1997**). Cytochrome c activation of CPP32-like proteolysis plays a critical role in a *Xenopus* cell-free apoptosis system. **EMBO J** *16*, 4639-4649.

Krohne G. Lamin assembly in vivo. Vol. 31. **Subcellular Biochemistry** ed. Herrmann and Harris. New York: Plenum Press, **1998**.

Krohne G, Waizenegger I, and Hoger TH (**1989**). The conserved carboxy-terminal cysteine of nuclear lamins is essential for lamin association with the nuclear envelope. **J Cell Biol** *109*, 2003-2011.

Lazebnik YA, Cole S, Cooke CA, Nelson WG, and Earnshaw WC (**1993**). Nuclear events of apoptosis in vitro in cell-free mitotic extracts: a model system for analysis of the active phase of apoptosis. **J Cell Biol** *123*, 7-22.

Lenz-Bohme B, Wismar J, Fuchs S, Reifegerste R, Buchner E, Betz H, and Schmitt B (**1997**). Insertional mutation of the *Drosophila* nuclear lamin dm(0) gene results in defective nuclear envelopes, clustering of nuclear pore complexes, and accumulation of annulate lamellae. **J Cell Biol** *137*, 1001-1016.

Lin H, Song K, Hutcheson GA, Goutte CE, and Wolfner MF (**1991**). A maternally encoded nuclear envelope protein required for embryonic mitosis in *Drosophila*. **Cold Spring Harb, Symp Quant Biol** *56*, 719-727.

Lin HF and Wolfner MF (**1991**). The *Drosophila* maternal-effect gene fs(1)Ya encodes a cell cycle-dependent nuclear envelope component required for embryonic mitosis. **Cell** *64*, 49-62.

Liu J, Lopez JM, and Wolfner MF (**1997**). Developmental modulation of the nuclear envelope. **Curr Top Dev Biol** *35*, 47-70.

Liu J, Song K, and Wolfner MF (**1995**). Mutational analyses of fs(1)Ya, an essential, developmentally regulated, nuclear envelope protein in *Drosophila*. **Genetics** *141*, 1473-1481.

Liu J and Wolfner MF (**1998**). Functional dissection of YA, an essential, developmentally regulated nuclear lamina protein in *Drosophila* melanogaster. **Mol Cell Biol** *18*, 188-197.

Lopez JM, Son, K, Hirshfeld AB, Lin H, and Wolfner MF (**1994**). The *Drosophila* fs(1)Ya protein, which is needed for the first mitotic division, is in the nuclear lamina and in the envelopes of cleavage nuclei, pronuclei, and nonmitotic nuclei. **Dev Biol** *163*, 202-211.

Lopez JM and Wolfner MF (**1997**). The developmentally regulated *Drosophila* embryonic nuclear lamina protein young arrest (fs(1)ya) is capable of associating with chromatin. **J Cell Sci** *5*, 643-651.

Luderus ME, de Graaf A, Mattia E, den BJ, Grande MA, de Jong L, and van Driel R (**1992**). Binding of matrix attachment regions to lamin B1. **Cell** *70*, 949-959.

Luderus ME, den Blaauwen J, de Smit O, Compton DA, and van Driel R (**1994**). Binding of matrix attachment regions to lamin polymers involves single-stranded regions and the minor groove. **Mol Cell Biol** *14*, 6297-6305.

Lutz RJ, Trujillo MA, Denham KS, Wenger L, and Sinensky M (**1992**). Nucleoplasmic localization of prelamin A: implications for prenylation-dependent lamin A assembly into the nuclear. **Proc Natl Acad Sci USA** *89*, 3000-3004.

Macaulay C, Meier E, and Forbes DJ (**1995**). Differential mitotic phosphorylation of proteins of the nuclear pore complex. **J Biol Chem** *270*, 254-262.

Machiels BM, Zorenc AH, Endert JM, Kuijpers HJ, van Eys GJ, Ramaekers FC, and Broers JL (**1996**). An alternative splicing product of the lamin A/C gene lacks exon 10. **J Biol Chem** *271*, 9249-9253.

Maison C, Pyrpasopoulos A, Theodoropoulos PA, and Georgatos SD (**1997**). The inner nuclear membrane protein LAP1 forms a native complex with B-type lamins and partitions with spindle-associated mitotic vesicles. **EMBO J** *16*, 4839-4850.

Mandal M, Maggirwa, SB, Sharma N, Kaufmann SH, Sun SC, and Kumar R (**1996**). Bcl-2 prevents CD95 (Fas/APO-1)-induced degradation of lamin B and poly(ADP-ribose) polymerase and restores the NF-kappaB signaling pathway. **J Biol Chem** *271*, 30354-30359.

Manilal S, Nguyen TM, and Morris GE (**1998**). Colocalization of emerin and lamins in interphase nuclei and changes during mitosis. **Biochem Biophys Res Commun** *249*, 643-647.

Manilal S, Nguyen TM, Sewry CA, and Morris GE (**1996**). The Emery-Dreifuss muscular dystrophy protein, emerin, is a nuclear membrane protein. **Hum Mol Genet** *5*, 801-808.

Marshall ICB. and Wilson KL (**1997**). Nuclear envelope assembly after mitosis. **Trends Cell Biol** *7*, 69-74.

Martin L, Crimaud, C, and Gerace L (**1995**). cDNA cloning and characterization of lamina-associated polypeptide 1C (LAP1C), an integral protein of the inner nuclear membrane. **J Biol Chem** *270*, 8822-8828.

McKeon FD, Kirschner MW, and Caput D (**1986**). Homologies in both primary and secondary structure between nuclear envelope and intermediate filament proteins. **Nature** *319*, 463-468.

Meier J, Campbell KH, Ford CC, Stick R, and Hutchison CJ (**1991**). The role of lamin LIII in nuclear assembly and DNA replication, in cell-free extracts of *Xenopus* eggs. **J Cell Sci** *98*, 271-279.

Mical TI and Monteiro MJ (**1998**). The role of sequences unique to nuclear intermediate filaments in the targeting and assembly of human lamin B: evidence for lack of interaction of lamin B with its putative receptor. **J Cell Sci** *111*, 3471-3485.

Moir RD, Montag LM, and Goldman RD (**1994**). Dynamic properties of nuclear lamins: lamin B is associated with sites of DNA replication. **J Cell Biol** *125*, 1201-1212.

Moir RD, Spann TP, and Goldman RD (**1995**). The dynamic properties and possible functions of nuclear lamins. **Int Rev Cytol** *162b*, 141-182.

Nagano A, Koga R, Ogaw, M, Kurano Y, Kawada J, Okada R,

Hayashi YK, Tsukahara T, and Arahata K (**1996**). Emerin deficiency at the nuclear membrane in patients with Emery-Dreifuss muscular dystrophy. **Nat Genet** *12*, 254-259.

Neamati N, Fernandez A, Wright S, Kiefer J, and McConkey DJ (**1995**). Degradation of lamin B1 precedes oligonucleosomal DNA fragmentation in apoptotic thymocytes and isolated thymocyte nuclei. J. Immunol. *154*, 3788-3795.

Newport, J. W., Wilson, K. L., and Dunphy, W. G. (1990). A lamin-independent pathway for nuclear envelope assembly. **J Cell Biol** *111*, 2247-2259.

Nigg EA (**1993**). Targets of cyclin-dependent protein kinases. **Curr Opin Cell Biol** *5*, 187-193.

Nikolakaki E, Meier J, Simos G, Georgatos SD, and Giannakouros T (**1997**). Mitotic phosphorylation of the lamin B receptor by a serine/arginine kinase and p34(cdc2). **J Biol Chem** *272*, 6208-6213.

Nikolakaki E, Simos G, Georgatos SD, and Giannakouros T (**1996**). A nuclear envelope-associated kinase phosphorylates arginine-serine motifs and modulates interactions between the lamin B receptor and other nuclear proteins. **J Biol Chem** *271*, 8365-8372.

Oberhammer FA, Hochegger K, Froschl G, Tiefenbacher R, and Pavelka M (**1994**). Chromatin condensation during apoptosis is accompanied by degradation of lamin A+B, without enhanced activation of cdc2 kinase. **J Cell Biol** *126*, 827-837.

Orth K, Chinnaiyan AM, Garg M, Froelich CJ, and Dixit VM (**1996**). The CED-3/ICE-like protease Mch2 is activated during apoptosis and cleaves the death substrate lamin A. **J Biol Chem** *271*, 16443-16446.

Ottaviano Y and Gerace L (**1985**). Phosphorylation of the nuclear lamins during interphase and mitosis. **J Biol Chem** *260*, 624-632.

Padan R, Nainudel ES, Goitein R, Fainsod A, and Gruenbaum Y (**1990**). Isolation and characterization of the *Drosophila* nuclear envelope otefin cDNA. **J Biol Chem** *265*, 7808-7813.

Papoutsopoulou S, Nikolakaki E, and Giannakouros T (**1999**). SRPK1 and LBR protein kinases show identical substrate specificities. **Biochem Biophys Res Commun** *255*, 602-607.

Patschinsky T, Hunter T, Esch FS, Cooper JA, and Sefton BM (**1982**). Analysis of the sequence of amino acids surrounding sites of tyrosine phosphorylation. **Proc Natl Acad Sci USA** *79*, 973-977.

Paulin Levasseur M, Blake DL, Julien M, and Rouleau L (**1996**). The MAN antigens are non-lamin constituents of the nuclear lamina in vertebrate cells. **Chromosoma** *104*, 367-379.

Peter M, Heitlinger E, Haner M, Aebi U, and Nigg EA (**1991**). Disassembly of in vitro formed lamin head-to-tail polymers by CDC2 kinase. **EMBO J** *10*, 1535-1544.

Peter M, Kitten GT, Lehner CF, Vorburger K, Bailer SM, Maridor G, and Nigg EA (**3 1989**). Cloning and sequencing of cDNA clones encoding chicken lamins A and B1 and comparison of the primary structures of vertebrate A- and B-type lamins. **J Mol Biol** *208*, 393-404.

Peter M, Nakagawa J, Doree M, Labbe JC, and Nigg EA (**1990**). In vitro disassembly of the nuclear lamina and M phase-specific phosphorylation of lamins by cdc2 kinase. **Cell** *61*, 591-602.

Peter M, Sanghera JS, Pelech SL, and Nigg EA (**1992**). Mitogen-activated protein kinases phosphorylate nuclear lamins and display sequence specificity overlapping that of mitotic protein kinase p34cdc2. **Eur J Biochem** *205*, 287-294.

Pyrpasopoulou A, Meier J, Maison C, Simos G, and Georgatos SD (**1996**). The lamin B receptor (LBR) provides essential chromatin docking sites at the nuclear envelope. **EMBO J** *15*, 7108-7119.

Qian Y, Barton R, and Worman HJ Nuclear lamin-binding proteins. Vol. 31. **Subcellular Biochemistry** ed. Herrmann and Harris. New York: Plenum Press, **1998**.

Rao L, Modha D, and White E (**1997**). The E1B 19K protein associates with lamins in vivo and its proper localization is required for inhibition of apoptosis. **Oncogene** *15*, 1587-1597.

Rao L, Perez D, and White E (**1996**). Lamin proteolysis facilitates nuclear events during apoptosis. **J Cell Biol** *135*, 1441-1455.

Riemer D, Dodemont H, and Weber K (**1993**). A nuclear lamin of the nematode Caenorhabditis elegans with unusual structural features; cDNA cloning and gene organization. **Eur J Cell Biol** *62*, 214-223.

Rzepecki R, Bogachev SS, Kokoza E, Stuurman N, and Fisher PA (**1998**). In vivo association of lamins with nucleic acids in *Drosophila* melanogaster. **J Cell Sci** *111*, 121-129.

Sasseville AM and Langelier Y (**1998**). In vitro interaction of the carboxy-terminal domain of lamin A with actin. **FEBS Lett** *425*, 485-489.

Sasseville AM and Raymond Y (**1995**). Lamin A precursor is localized to intranuclear foci. **J Cell Sci** *108*, 273-285.

Schlesinger DH and Goldstein G (**1975**). The amino acid sequence of thymopoietin II. **Cell** *5*, 361-365.

Shimanuki M, Goebl M, Yanagida M, and Toda T (**1992**). Fission yeast sts1+ gene encodes a protein similar to the chicken lamin B receptor and is implicated in pleiotropic drug-sensitivity, divalent cation-sensitivity, and osmoregulation. **Mol Biol Cell** *3*, 263-273.

Shimizu, T, Cao CX, Shao RG, and Pommier Y (**1998**). Lamin B phosphorylation by protein kinase calpha and proteolysis during apoptosis in human leukemia HL60 cells. **J Biol Chem** *273*, 8669-8674.

Shimizu T and Pommier Y (**1997**). Camptothecin-induced apoptosis in p53-null human leukemia HL60 cells and their isolated nuclei: effects of the protease inhibitors Z-VAD-fmk and dichloroisocoumarin suggest an involvement of both caspases and serine proteases. **Leukemia** *11*, 1238-1244.

Shoeman RL and Traub P (**1990**). The in vitro DNA-binding properties of purified nuclear lamin proteins and vimentin. **J Biol Chem** *265*, 9055-9061.

Silve S, Dupuy PH, Ferrara P, and Loison G (**1998**). Human lamin B receptor exhibits sterol C14-reductase activity in Saccharomyces cerevisiae. **Biochim Biophys Acta** *1392*, 233-244.

Simos G and Georgatos SD (**1992**). The inner nuclear membrane protein p58 associates in vivo with a p58 kinase and the nuclear lamins. **EMBO J** *11*, 4027-4036.

Simos, G. and Georgatos, S. D. (2-3 1994). The lamin B receptor-associated protein p34 shares sequence homology and antigenic determinants with the splicing factor 2-associated protein p32. **FEBS Lett** *346*, 225-228.

Simos G, Maison C, and Georgato, SD (**1996**). Characterization of p18, a component of the lamin B receptor complex and a new integral membrane protein of the avian erythrocyte nuclear envelope. **J Biol Chem** *271,* 12617-12625.

Spann TP, Moir RD, Goldman AE, Stick R, and Goldman RD (**1997**). Disruption of nuclear lamin organization alters the distribution of replication factors and inhibits DNA synthesis. **J Cell Biol** *136,* 1201-1212.

Stick R (**1992**). The gene structure of *Xenopus* nuclear lamin A: a model for the evolution of A-type from B-type lamins by exon shuffling. **Chromosoma** *101,* 566-574.

Stick R (**5 1994**). The gene structure of B-type nuclear lamins of *Xenopus* laevis: implications for the evolution of the vertebrate lamin family. **Chromosome Res** *2,* 376-382.

Stuurman N, Heins S, and Aebi U (**1998**). Nuclear lamins: their structure, assembly, and interactions. **J Struct Biol** *122,* 42-66.

Takahashi A, Alnemri ES, Lazebnik YA, Fernandes Alnemri T, Litwack G, Moir RD, Goldman RD, Poirier GG, Kaufmann SH, and Earnshaw WC (**1996a**). Cleavage of lamin A by Mch2 alpha but not CPP32: multiple interleukin 1 beta-converting enzyme-related proteases with distinct substrate recognition properties are active in apoptosis. **Proc Natl Acad Sci USA** *93,* 8395-8400.

Takahashi A, Musy PY, Martins LM, Poirier GG, Moyer RW, and Earnshaw WC (**1996b**). CrmA/SPI-2 inhibition of an endogenous ICE-related protease responsible for lamin A cleavage and apoptotic nuclear fragmentation. **J Biol Chem** *271,* 32487-32490.

Taniura H, Glass C, and Gerace L (**1995**). A chromatin binding site in the tail domain of nuclear lamins that interacts with core histones. **J Cell Biol** *131,* 33-44.

Theodor L, Shoham J, Berger R, Gokkel E, Trachtenbrot L, Simon AJ, Brok Simon F, Nir U, Ilan E, Zevin Sonkin D, Friedman E, and Rechavi G (**1997**). Ubiquitous expression of a cloned murine thymopoietin cDNA. **Acta Haematol** *97,* 153-163.

Ucker D S, Obermiller PS, Eckhart W, Apgar JR, Berger NA, and Meyers J (**1992**). Genome digestion is a dispensable consequence of physiological cell death mediated by cytotoxic T lymphocytes. **Mol Cell Biol** *12,* 3060-3069.

Ulitzur N, Harel A, Feinstein N, and Gruenbaum Y (**1992**). Lamin activity is essential for nuclear envelope assembly in a *Drosophila* embryo cell-free extract. **J Cell Biol** *119,* 17-25.

Ulitzur N, Harel A, Goldberg M, Feinstein N, and Gruenbaum Y (**1997**). Nuclear Membrane Vesicle Targeting to Chromatin In a *Drosophila* Embryo Cell Free System. **Mol Biol Cell** *8,* 1439-1448.

Vorburger K, Lehner CF, Kitten GT, Eppenberger HM, and Nigg EA (**1989**). A second higher vertebrate B-type lamin. cDNA sequence determination and in vitro processing of chicken lamin B2. **J Mol Biol** *208,* 405-415.

Ward GE and Kirschner MW (**1990**). Identification of cell cycle-regulated phosphorylation sites on nuclear lamin C. **Cell** *61,* 561-577.

Wedrychowski A, Bhorjee JS, and Briggs RC (**1989**). In vivo crosslinking of nuclear proteins to DNA by cis-diamminedichloroplatinum (II) in differentiating rat myoblasts. **Exp Cell Res.***183,* 376-387.

Wilson KL and Newport J (**1988**). A trypsin-sensitive receptor on membrane vesicles is required for nuclear envelope formation in vitro. **J Cell Biol** *107,* 57-68.

Wilson KL and Wiese C (**1996**). Reconstituting the Nuclear Envelope and Endoplasmic Reticulum In Vitro. **Seminars In Cell and Developmental Biology** *7,* 487-496.

Worman HJ, Evans CD, and Blobel G (**1990**). The lamin B receptor of the nuclear envelope inner membrane: a polytopic protein with eight potential transmembrane domains. **J Cell Biol** *111,* 1535-1542.

Worman HJ, Yuan J, Blobel G, and Georgatos SD (**1988**). A lamin B receptor in the nuclear envelope. **Proc Natl Acad Sci USA** *85,* 8531-8534.

Yang L, Guan T, and Gerace L (**1997**). Lamin-binding fragment of LAP2 inhibits increase in nuclear volume during the cell cycle and progression into S phase. **J Cell Biol** *139,* 1077-1087.

Ye Q and Worman HJ (**1994**). Primary structure analysis and lamin B and DNA binding of human LBR, an integral protein of the nuclear envelope inner membrane. **J Biol Chem** *269,* 11306-11311.

Ye Q and Worman HJ (**1996**). Interaction between an integral protein of the nuclear envelope inner membrane and human chromodomain proteins homologous to *Drosophila* Hp1. **J Biol Chem** *271,* 14653-14656.

Ye QA, Callebaut I, Pezhman A, Courvalin JC, and Worman HJ (**1997**). Domain specific interactions of human hp1 type chromodomain proteins and inner nuclear membrane protein LBR. **J Biol Chem** *272,* 14983-14989.

Yuan J, Simos G, Blobel G, and Georgatos SD (**1991**). Binding of lamin A to polynucleosomes. **J Biol Chem** *266,* 9211-9215.

Zevin-Sonkin D, Ilan E, Guthmann D, Riss J, Theodor L, Shoham J, Berger R, Gokkel E, Brok-Simoni F, Avraham KB, Copeland NG, Jenkins NA, Rechavi G, and Simon AJ (**1992**). Molecular cloning of the bovine thymopoietin gene and its expression in different calf tissues: evidence for a predominant expression in thymocytes. **Immunol Lett** *31,* 301-309.

Zhao K, Harel A, Stuurman N, Guedalia D, and Gruenbaum Y (**1996**). Binding of matrix attachment regions to nuclear lamin is mediated by the rod domain and depends on the lamin polymerization state. **FEBS Lett** *380,* 161-164.

Zhivotovsky B, Gahm A, and Orrenius S (**1997**). Two different proteases are involved in the proteolysis of lamin during apoptosis. **Biochem Biophys Res Communi** *233,* 96-101.