1 INTRODUCTION

1.1 FUNCTIONAL ORGANIZATION OF THE CELL NUCLEUS

The identification of the DNA sequence of every human gene is of limited value without a description of the function and regulation of the gene products. The question how the same 35000-50000 genes contained in every metazoan cell can give rise to the vast diversity of cell specialization, is one of the fundamental, still largely unanswered questions in biology. Sure is, that it needs tight control and regulation of the gene expression.

Gene expression involves chromatin remodelling, transcription, RNA processing, RNA export and translation in the cytoplasm. Each of these steps is carried out by complex machineries consisting of hundreds of components. Efficient assembly and functioning of these machineries is greatly faciliated by the compartmentalization of the different processes (reviewed by Misteli, 2001, Carmo-Fonesca, 2002, Fackelmeyer, 2000).

Transcription and translation of genes encoded by genomic DNA, are physically separated within a cell by the nuclear envelope (NE), which surrounds the cell nucleus and thus shields it from the cytoplasm. The NE consists of outer and inner membranes joined by the pore membrane (ONM, INM and PM respectively). The INM is underlined with the lamina, an intermediate filament network which gives the NE its shape and stability. The transport of molecules to and from the nucleus pass through the nuclear pore complexes (NPC, reviewed by Stewart and Clarkson, 1996). (The NE is discussed in detail in chapter 1.2). Because of its physical space limitation and the amount of molecules contained in it, the nucleus is a very crowded place. To assure a correct course of the genetic processes, a high level of organization within the nucleus is needed.

The biggest subcompartments in the nucleus are the chromosome territories, which are defined areas occupied by the chromatin of individual chromosomes (Lichter et al., 1988). The size and position of individual chromosome territories is dependent on the amount of DNA and number of active genes. Chromosome 18 for example, which is less transcriptionally active than chromosome 19, is positioned near the NE, whereas chromosome 19 is found deep inside the nucleus (Croft et al., 1999). Specific DNA sequences within each chromosome are organized as either euchromatin or heterochromatin (reviewed in Cremer and Cremer, 2001). Heterochromatin appears as a dense structure under the microscope. It is

defined as the gene poor chromatin, packaged into a structure that is relatively inaccessible to DNA-modifying factors, as opposed to euchromatin, which is transcriptionally active (Hennig, 1999, Quemsiyeh, 1999). Euchromatin can be converted into heterochromatin to switch off specific genes, a reversible process known as 'gene silencing' (reviewed in Carmo-Fonesca, 2002). It is an open question though, whether genes are translocated to the heterochromatin compartments to become silenced, or whether already transcriptionally inactivated genes are targeted to this compartment. Heterochromatin has been found to interact with proteins of the inner nuclear membrane (Ye and Worman, 1996, Ye et al., 1997), but contacts of the inner nuclear membrane with euchromatin have also been shown (Mansharamani et al., 2001).

The position of the chromosome territories in daughter cells after mitosis is mutualy preserved when compared to the position in the mother cell (Jan Ellenberg, personal communication).

The chromosome territories are separated from each other through DNA-free inter-chromosomal-domain compartments (ICD). Proteins and ribonucleoprotein complexes involved in mRNA metabolism are found there, whereas components of the ribosome biogenesis are located to the nucleolus, which is another functional nuclear compartment (Carmo-Fonesca et al., 2000).

Other functional nuclear compartments localized in the ICD include nuclear speckles containing spliceosomal components (Misteli et al., 1997) and a number of nuclear bodies, the function of which is largely unknown. Cajal bodies are involved in snRNP biogenesis (Gall, 2000), PML nuclear bodies (nuclear domains that are specifically disrupted in human acute promyeolytic leukemia cells,) are enriched in transcriptional regulators (Zhong et al., 2000), while a variety of nuclear inclusions are often associated with degenerative diseases (Zoghbi and Orr, 2000).

The described nuclear subcompartments are not physically separated by membranes. Nevertheless, they contain specific sets of proteins, have different morphology and some can even be isolated biochemically. They form and maintain their function through specific interactions of proteins. The position of the nuclear bodies is defined by the interaction with specific gene loci. Overall, the nuclear bodies are highly dynamic structures, with their components being in perpetual flux as shown by FRAP (fluorescence recovery after photobleaching) experiments (reviewed by Misteli 2001).

The functional organization of the nucleus is schematically represented in fig.1-1.

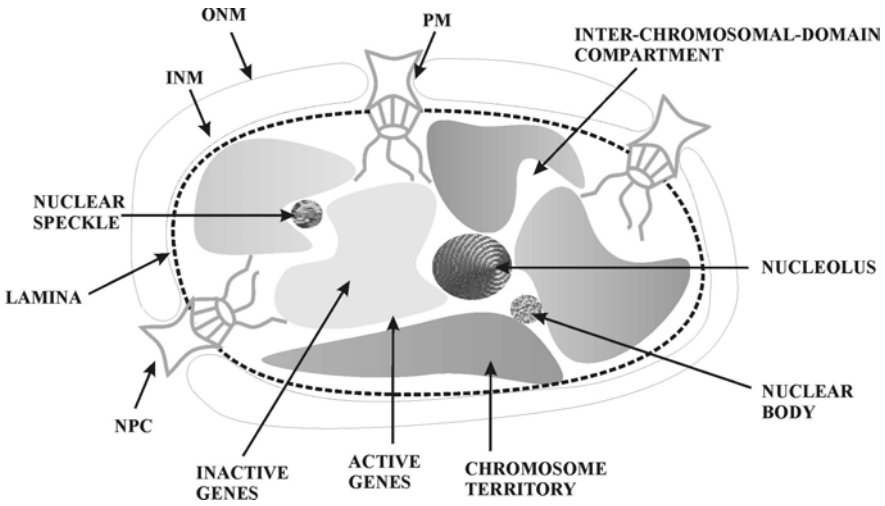

Fig.1-1 Functional organization of the nucleus. The nucleus is shielded from the cytoplasm through the nuclear envelope, which consists of outer and inner membranes joining at the pore membrane (ONM, INM and PM respectively) and is underlined with an intermediate filament network – the lamina. The transport to and from the nucleus pass through the nuclear pore complexes (NPC). The chromatin occupies defined areas – the chromosome territories (shaded structures) separated from each other through inter-chromosomal-domain compartments. Active genes are found at the outskirts of the chromosome territories, the inactive inside. Nuclear bodies and speckles are functionally defined compartments where the gene expression, RNA proliferation or genome replication takes place.

An additional level of organization in the nucleus may bring the nuclear matrix structure, existence of which is highly controversial. It is postulated, that even though nuclear compartments are highly dynamic structures, a spatial separation of these is necessary and achieved through binding to a nuclear marix/nuclear scaffold (reviewed by Fackelmeyer, 2000, Orphanides and Reinberg, 2002). The nuclear matrix supposedly consists of a 20-50nm thick network of RNA and proteins, with major building blocks being the hnRNA. Chromatin is organized in 20,000-80,000 bp long loops and specific DNA sequences on these loops interact with the nuclear matrix. That way a spatial separation of the chromatin loops is achieved, and the loops become distinct functional chromatin domains. The specific DNA sequences interacting with the nuclear matrix are called MAR (matrix associated regions). A number of MAR interacting proteins has been identified, including laminB, topoisomerase II and histone H1.

1.2 THE NUCLEAR ENVELOPE

The nuclear envelope surrounding the cell nucleus consists of three distinct but interconnected membranes: inner and outer (INM and ONM, respectively) and the pore membrane (PM) (reviewed by Gant and Wilson, 1997). The ONM is continuous with the endoplasmic reticulum (ER) membrane and shares properties with the ER. Analogously, the perinuclear space is continuous with the ER lumen and shares its properties. A specialized domain of the nuclear envelope is the PM, which connects the ONM with INM and forms the 'holes' in the nuclear membrane where the nuclear pore complexes (NPC) are inserted. The proteins pom121 and gp210, the only known to be specifically located to the pore membrane, function as anchors for the NPCs. NPCs are multiprotein complexes of ca 120MDa forming channels, through which the transport to and from the nucleus takes place (Davis, 1995). The INM contains a specific set of integral membrane proteins (see 1.2.1), providing attachment sites for chromatin and lamina (see 1.2.2). The structural features of the nuclear envelope are schematically represented in fig.1-2.

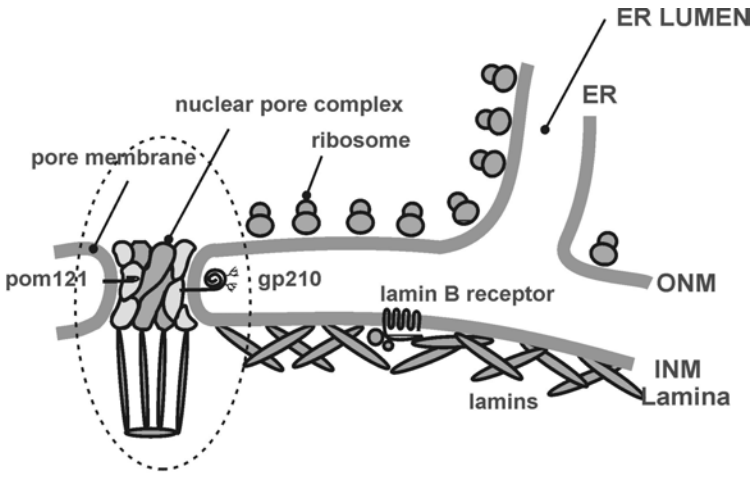

Fig.1-2 Structure of the nuclear envelope. The nuclear envelope consists of outer and inner nuclear membranes (ONM, INM) joined by the pore membrane, where the nuclear pore complexes are anchored. The ONM is continuous with the endoplasmic reticulum (ER) and the perinuclear space with the ER lumen. The pore membrane and the INM contain specific sets of integral membrane proteins. (The image was kindly provided by Henning Otto.)

The nuclear envelope is a highly dynamic structure, which completely dis- and reassembles during mitosis in higher eukaryotes. Mitosis begins at the prophase with the condensation of

the chromosomes and formation of the mitotic spindle. During prometaphase, the spindle microtubuli attach to the centromeric regions of the chromatids and the nuclear envelope breaks down. The spindle aligns the chromosomes during the metaphase and separates the daughter chromatids during anaphase. Reformation of the nucleus starts at the telophase, when the spindle disassembles, the chromatids decondensate and the nuclear envelope reassembles. During cytokinesis the cytoplasm is equally partitioned and the formation of two daughter cells is completed.

Nuclear envelope breakdown

The nuclear envelope breakdown involves depolymerization of the lamina, the deattachment of the chromatin from the nuclear membranes and the disassembly of the NPCs. All three events are facilitated by mitotic phosphorylation and, according to a novel model of nuclear envelope breakdown, through mechanic force (Beaudouin et al., 2002, Salina et al., 2002).
A-type lamins are phosphorylated by $p34^{cdc2}$ (Peter et al., 1990, Enoch et al., 1991), B-type lamins by $p34^{cdc2}$ and PKC (Collas, 1999). Also the NPCs are phosphorylated, which causes them to disassemble into different subcomplexes (McCaulay and Forbes, 1996, Matsuoka et al., 1999, Favreau et al., 1996). Likewise, a variety of integral or associated proteins of the inner nuclear membrane are phosphorylated, which causes the loss of their interactions with the lamina and the chromatin (Courvalin et al., 1992, Foisner and Gerace, 1993, Collas 1999). Phosphorylated proteins of the inner nuclear membrane are no longer retained in the nucleus and redistribute into the ER by lateral diffusion. Thus, the specific nuclear domains of the ER loose their identity upon mitosis, but the nuclear envelope and ER remain a permanent system throughout the cell cycle (Collas and Courvalin, 2000, Ellenberg et al., 1997).
The mitotic spindle gains access to the interior of the nucleus by literally tearing the nuclear envelope apart using mechanical force generated by the molecular motor dynein (Beaudouin et al., 2002, Salina et al., 2002). At the beginning of the prophase, the nuclear envelope specifically develops a pair of deep invaginations where the centrosomes are located. The invaginations also contain lamins, inner nuclear membrane markers and nucleoporins. The nuclear envelope break down begins then with formation of 1-3 holes spanning both membranes, leading to flooding of the nucleus with the cytoplasmic components. At the end of the prophase, about 30% of the nuclear envelope is drawn into the membrane folds surrounding the centrosomes, resulting in a huge hole located distal to the centrosomes. The hole formation is coincident with lamina and NPC phosphorylation events and with an

acceleration of chromatin condensation. The force required to tear down the NE is generated by dynein, a minus-end-directed, microtubule-based motor, which is specifically recruited to the nuclear envelope during late G2 or early prophase (Busson et al., 1998). Dynein attaches the spindle microtubuli emanating in both directions to the nuclear envelope and begins to pull the nuclear envelope towards the centrosomes. The increasing tension eventually tears the nuclear envelope and lamina.

Nuclear envelope reassembly

The NE is reassembled around the chromosomes during the telophase, re-establishing the architecture of the nucleus for the next interphase (reviewed by Gant and Wilson, 1997, Buendia et al., 2001). It is a stepwise process and involves targeting of INM proteins, like lamin B receptor (LBR), lamina associated polypeptide 2β and emerin (see 1.2.1) to chromosomes (Ellenberg et al., 1997, Furukawa et al., 1997, Haraguchi et al., 2000). These markers are targeted to specific chromosome surface areas that are defined by their location relative to the mitotic apparatus. LBR and LAP 2β are associated with the lateral margins of chromosomes (Buendia and Courvalin, 1997, Chaudhary and Courvalin, 1993, Haraguchi et al., 2000), emerin with the central region of the assembling nuclear rim (the 'core' region), near the spindle attachment sites (Dabauvalle et al., 1999, Haraguchi et al., 2001), whereas Nup153 decorates the entire chromosome surface (Bodoor et al., 1999). The targeting of the INM proteins to the chromosomes is mediated through their binding to DNA-interacting proteins: LBR binds to HP1 (Ye and Worman, 1996), LAP 2β and emerin bind to BAF (Furukawa, 1999, Lee et al., 2001). Mutations in BAF abolish the recruitment of emerin to the core region, which leads to its improper interphase localization. Also LAP 2β and A-type lamins are mislocalized as a consequence of mutations in BAF, whereas lamin B remains unaffected, suggesting a distinct mechanism for its assembly (Haraguchi et al., 2001).
The reassembling NE seals during late telophase and has at that point fully functional NPCs (Bodoor et al., 1999). The reactivation of the nucleocytoplasmic transport correlates with rapid chromatin decondensation and nucleolar assembly. Lamins are imported into the nucleus and assembled into the lamina during cytokinesis (Broers et al., 1999). This is a crucial step for nuclear growth and chromatin decondensation (Benavente and Krohne, 1986).

1.2.1 The integral proteins of the inner nuclear membrane

There is only a limited number of proteins known to specifically reside in the inner nuclear membrane and the functions of most of them remain to be discovered. For a long time, these proteins were considered being merely structural proteins involved in attachment of the lamina to the nuclear envelope. With the discovery of novel integral proteins and a closer examination of the functions of the known proteins, a new exciting picture of the functions of the INM emerges. Proteins of the INM are involved in and regulate events as distinct as gene expression, dis- and reassembly of the NE during mitosis, signal transduction during interphase, nuclear anchoring and migration and are implicated in disease. The following chapter summarizes all what is known up to date about the INM proteins and their functions.

LAP 1

The lamina-associated polypeptide 1 (LAP 1) has three isoforms, all arising through alternative splicing of the mRNA transcipt (Foisner and Gerace, 1993, Martin et al., 1995). The isoforms are differentially expressed during development, with 1A and 1B isoforms being synthesized in differentiated cells. Only LAP 1C has been biochemically characterized so far (Maison et al., 1997). It interacts with lamin B and is phosphorylated by an unknown kinase. After the breakdown of the nuclear envelope and with a preparation of the membranes under experimental conditions which cause vesiculation (for a discussion on exsistance or not of mitotic vesicles see Buendia et al., 2001), LAP 1 is found in mitotic vesicles void of LAP 2. Based on this observation, it was suggested that the inner nuclear membrane contains discrete territories, which accommodate specific integral membrane proteins and are differentially disassembled during mitosis.

LAP 2

LAP 2 proteins were identified using monoclonal antibodies generated against isolated nuclear envelopes (Senior and Gerace, 1988). The gene encodes six different, ubiquitously expressed proteins, that are produced by alternative RNA splicing (Harris et al., 1995). The best characterized isoforms of LAP 2 are the integral membrane protein LAP 2β and the soluble LAP 2α. All LAP 2 isoforms have a common N-terminal region of 187 residues, which contains the chromatin binding region - the LEM box (Lin et al., 2000). The LEM box is a 40 amino acid homologous sequence motif also found in emerin and MAN1 (hence its name: LAP 2/emerin/MAN1). The binding to chromatin is mediated through BAF, a 10 kDa

DNA bridging protein (Furukawa, 1999, Zheng et al., 2000). The affinity to BAF:DNA complexes is three fold higher than to BAF alone (Shumaker et al., 2001). LAP 2β is also found in complexes with LBR (see below) and HA95. HA95 is a chromatin- and lamina-associated protein homologous to the nuclear A-kinase anchoring protein AKAP95 (Martins et al., 2000, Eide et al., 1998, Örstavik et al., 2000). Disrupting the interaction between HA95 and its interactors inhibits chromatin condensation and disassembly of nuclear membranes, but does not affect lamin depolymerization. HA95`s function is probably the recruitment of the kinases required for regulation of chromatin attachment to the INM to the chromatin attachment 'points' – the integral membrane proteins like LAP 2β.

Another argument for LAP 2β`s role in chromatin structure and organization is the observation that a recombinant polypeptide that contains the chromatin-binding region of LAP 2β, enhances the efficiency of DNA replication in *Xenopus* extracts (Gant et al., 1999).

LAP 2β may also have an important function in the regulation of gene expression. It was recently reported to mediate transcriptional repression either alone or with its binding partner GCL (Nili et al., 2001). GCL (germ cell less) belongs to the BTB/POZ domain containing proteins. Nearly all mammalian proteins that contain this protein-protein interacting domain are zinc-finger proteins involved in transcriptional regulation (Albagli et al., 1995). GCL interacts with DP, which heterodimerizes with the transcription factor E2F. This interaction causes the E2F-DP complex to translocalize to the NE and by doing that, reduces its transcriptional activity (La Luna et al., 1999).

The activity of LAP 2β is probably regulated, since it is differentially phosphorylated during interphase (Dreger et al., 1999) and mitosis (Foisner and Gerace, 1993).

LAP 2β binds lamin B in a phosphorylation dependent manner (Foisner and Gerace, 1993). The injection of recombinant lamin binding region of LAP 2β inhibited nuclear volume increase and progression into the S phase (Yang et al., 1997). The addition of recombinant LAP 2β truncation mutants to cell-free nuclear assembly reactions severly affected nuclear envelope structure and the nuclear assembly (Gant et al., 1999). Taken together, these observations indicate that LAP 2β also plays a role in lamin dynamics, which are crucial for NE assembly and nuclear growth.

The soluble LAP 2 isoform LAP 2α is found throughout the nuclear matrix during interphase (Dechat et al., 1998). LAP 2α binds also to A-type lamins (Vlcek et al., 1999).

LBR

The lamin B receptor (LBR) was the first integral membrane protein of the INM to be identified (Worman et al., 1988, Worman et al., 1990). It contains eight putative transmembrane domains with both termini facing the nucleoplasma (Worman et al., 1990, Schuler et al., 1994, Ye and Worman, 1994). The first 60 amino acids of the LBR seem to be critical for binding to lamin B (Lin et al., 1996), although the interaction may not be direct (Mical et al., 1998). The N-terminal domain also binds to DNA. The binding characteristics are similar to that of histone H1 and non-histone protein HMG1/2, which both bind preferentially to linker DNA (Ye and Worman, 1994, Duband-Goulet and Courvalin, 2000). Apart of binding nucleosomal DNA, LBR also binds heterochromatin through its interaction with the chromoshadow domain of human chromodomain protein $HP1^{Hs\alpha}$ and $HP1^{Hs\gamma}$ (Ye and Worman, 1996, Ye et al., 1997). This interaction may not be direct, but mediated by histone H3 (Polioudaki et al., 2001).

LBR contains a RS-domain (arginin/serin rich domain) also found in splicing factors (Mistelli and Spector, 1997). RS-domains are targets of RS-specific kinases (RSPKs), which functionally regulate splicing factors through phosphorylation (Mistelli et al., 1997). Such a kinase, LBR kinase, was found associated with LBR (Simos und Georgatos, 1992; Nikolakaki et al., 1996; Nikolakaki et al., 1997) and could be shown to have a substrate specificity identical to that of RSPKs (Papoutsopoulou et al., 1999).

The carboxyterminal part containing the transmembrane domains of LBR is highly homologous to yeast and plant sterol reductases (Holmer et al., 1998). In a complementation study different LBR constructs were able to rescue strains of *Saccharomyces cerevisiae* deficient in sterol reductase (Silve et al., 1998). This could implicate a role for LBR in the gene regulation by nuclear receptors.

Emerin

Positional cloning of a gene responsible for X-linked Emery-Dreifuss muscular dystrophy led to the discovery of emerin. Emerin contains one transmembrane domain, a LEM–box in its N-terminal part and localizes specifically to the inner nuclear membrane (Bione et al., 1994, Nagano et al., 1996, Manilal et al., 1996). The exact mechanism of how mutations in emerin cause the disease are unclear, but all pathological mutations in emerin lead to its mislocalization, either caused by aberrant phosphorylation during the intephase (Ellis et al., 1998) or because of deletion of the C-terminus and hence loosing its transmembrane domain

(Nigro et al., 1995). Emerin interacts with BAF:DNA complexes through the LEM-box and with lamin A through a functional domain in the central region (Lee et al., 2001). The binding to BAF is required for emerin assembly into the reforming nuclear envelope (Haraguchi et al., 2001). The strongest evidence for an interaction between emerin and lamin A comes from the study of a lamin A knockout mouse. The autosomal dominant form of Emery-Dreifuss muscular dystrophy is caused by mutations in the lamin A gene and a knockout of lamin A leads to mislocalization of emerin and an aberrant nuclear shape (Bonne et al., 1999, Sullivan et al., 1999).

MAN1

MAN1 was identified as one of the antigens of the autoantibodies from a patient with an ill-defined collagen vascular disease (Lin et al., 2000). The autoantibodies recognize three polypeptides called 'MAN antigenes', which are localized exclusively to the nuclear envelope and cofractionate biochemically with the nuclear lamins (Paulin-Levasseur et al., 1996). MAN1 contains two transmembrane domains and a N-terminal LEM-box, which makes MAN1 a putative binding partner of BAF:DNA complexes.

Otefin

Otefin is an either peripheral or integral nuclear envelope protein found in *Drosophila* (Harel et al., 1989, Padan et al., 1990). It interacts with lamin Dm0 (a dominating lamin form in Drosophila) and a protein called YA (young arrest) (Goldberg et al., 1998). Otefin does not have any apparent sequence similarity to other known proteins, except of the LEM-domain (Shumaker et al., 2001), which makes it a potential candidate for interaction with BAF:DNA complexes. Otefin is required for vesicle attachment to chromatin (Padan et al., 1997).

Nesprins

The mouse proteins Syne 1 and its homolog Syne-2 were identified in a yeast two-hybrid screen aiming at identification of novel neuromuscular junction components (Apel et al., 2000). MuSK, a receptor tyrosine kinase that is highly concentrated at the postynaptic membrane (Valenzuela et al., 1995), was used as a bait. Both Syne-1 and –2 contain multiple spectrin repeats similar to those found in dystrophin and utrophin, and a domain homologous to the C-terminus of Klarsicht. Klarsicht is a protein associated with nuclei and is required for a subset of nuclear migrations in Drosophila (Welte et al., 1998 and Mosley-Bishop et al., 1999). Syne-1 is associated with nuclear envelopes in skeletal, cardiac and smooth muscle

cells only. Additionally, its expression is highest in the postsynaptic nuclei of myotubes. Syne-2 is ubiquitously expressed. Alternative splicing gives rise to the isoforms 1A and 1B.
The rat orthologs of Syne-1 and –2 were recently described by Zhang et al., 2001 under the name 'nesprin'. A whole family of nesprins was identified. Nesprins are ubiquitously expressed type II integral membrane proteins of the inner nuclear membrane encoded by two genes: nesprin-1 and nesprin-2. Tissue-specific alternative mRNA initiation and splicing generate at least two major isoforms of each protein. Nesprins contain multiple spectrin repeats, a bipartite nuclear localization signal, a single conserved C-terminal transmembrane domain and colocalize with heterochromatin, as revealed by electron microscopy (Zhang et al., 2001). The C-terminus and the transmembrane domain are required for the inner nuclear membrane localization of the nesprins, which indicates that their N-terminus is oriented towards the ER lumen.
The attachment of dynein to the NE preceeding the NE breakdown is mediated by the dynactin complex (Salina et al., 2002) and the dynactin-interacting component on other target membranes in the cell is spectrin (Karcher et al., 2002). This observation and the presence of multiple spectrin repeats makes nesprins good candidates for dynein attachment sites at the NE.

RFBP

RFBP is an acronym for RING finger binding protein and was discovered in a screen for RING motif interacting proteins (Mansharamani et al., 2001). RING finger is a protein motif that binds two zinc ions to form an intergrated structural unit that mediates protein-protein interactions (Saurin et al., 1996, Borden and Freemont, 1996, Mackay and Crossley, 1998, Borden, 2000). It is apparently necessary for the formation of speckled nuclear bodies (Le et al., 1996). RUSH proteins are hormonally regulated SWI/SNF-related transcription factors with RING finger signatures near their COOH termini (Hayward-Lester et al., 1996). When the RING motif of the RUSH gene product was used to clone the RFBP, a new Type IV P-type ATPase was identified in the inner nuclear membrane. RFBP is ubiquitously expressed and the level of expression is subject to hormonal regulation depending on the level of expression of the RUSH protein. RFBP spans the membrane nine times and its flexible loop (a structure element found in all P-Type ATPases) extends into the nucleoplasm where it interacts with euchromatin. RFBP may be a part of a mechanism that targets transcription factors to specific domains within the nucleus.

Nurim

Nurim was identified as a INM protein in a visual screen of GFP fusion proteins in tissue culture cells (Rolls et al., 1999). This 29kDa protein consists of five transmembrane domains with short amino acid loops between them. There are no homologues or homology domains. Biochemically, nurim behaves as a lamin-binding protein.

UNC-84, UNC-83

Both UNC-84 and UNC-83 were identified in a genetic screen in C.elegans (Malone et al., 1999 and Starr et al., 2001 respectively). Mutations in the genes encoding these proteins have an effect on nuclear migration during the development of C.elegans. UNC-84A contains a C-terminal SUN domain also found in Sad1p, a yeast protein localizing to the nuclear envelope and the spindle pole body. Sad1p was shown to play a role in spindle body architecture (Hagan and Yanmagida, 1995). Therefore, UNC-84 was suggested to function in centrosome attachment to nuclei. UNC-84A interacts with UNC-83 through its SUN domain and this interaction is supposed to recruit UNC-83 to the nuclear membrane. UNC-84 colocalizes with the lamina and thus represents an integral membrane protein of the inner nuclear membrane-Like UNC-84, UNC-83 is essential for a correct nuclear migration in C.elegans, but is not necessary for the positioning of centrosomes. It is suggested that UNC-83 is important for the attachment of microtubuli to the nuclear envelope.

PROTEIN	ISOFORMS	MW (kDa)	NO OF TM	HOMOLOGY DOMAINS	ASSIGNED FUNCTION/ INTERACTION PARTNERS
LAP 1	LAP 1A	75	1	-	Interaction with A and B type lamins
	LAP 1B	68	1		Interaction with A and B type lamins
	LAP 1C	55	1		Interaction with lamin B
LAP 2	LAP 2α	76	-	LEM domain	Interaction with BAF, lamin A/C
	LAP 2β	53	1		Interaction with lamin B, BAF, DNA, HA95, GCL; regulation of gene expression, lamin dynamics and nuclear architecture
	LAP 2δ	42	1		?
	LAP 2ε	45	1		
	LAP 2γ	38	1		
	LAP 2ζ	24	-		
LBR		58	8	RS-domain sterol reductase	Interaction with HP1, histone H3, lamin B Anchoring of the heterochromatin to the NE
Emerin		34	1	LEM domain	Mutations cause Emery-Dreifuss Syndrom; Interaction with BAF
MAN1		82	2	LEM domain	Putative interaction with BAF
Otefin		45	1?	LEM domain	Interaction with lamin Dm and YA; putative interaction with BAF; required for vesicle atachment to chromatin
Nesprin-1	Nesprin-1α	112	1	spectrin repeats bipartite NLS	Muscle differentiation
	Nesprin-1α2 (= Syne-1A)	112	1	spectrin repeats bipartite NLS	Anchoring of nuclei at the postsynapse Muscle differentiation Nuclear migration
	Nesprin-1β (= Syne-1B)	380	1	spectrin repeats bipartite NLS protein-DNA binding motif	
Nesprin-2	Nesprin-2α	61	1	spectrin repeats	?
	Nesprin-2β	87	1	spectrin repeats	
	Nesprin-2γ	377	1	spectrin repeats bipartite NLS leucine zipper	
RFBP		126	9	Atypical P-type ATPase	Putative phospholipid pump; binds RING-motif proteins; regulation of transcription?
Nurim	-	29	5	-	?
UNC-84	UNC-84A	122	8	SUN domain	anchoring of nuclei and nuclear migration in C.elegans
	UNC-84B	97	8	-	
UNC-83	UNC-83A	115	1	coiled-coil structure	nuclear migration in C.elegans
	UNC-83B	107	1		

Tab. 1-1 Known proteins of the inner nuclear membrane.

1.2.2 The lamina

The nuclear lamina lines the inner nuclear membrane. Its major building blocks are intermediate filament-type proteins – the nuclear lamins (reviewed by Nigg, 1992). The nuclear lamins possess a central α-helical coiled-coil "rod" domain flanked by non-helical N-terminal "head" and C-terminal "tail" domains (Nigg, 1992). Cdc2 phosphorylation sites are found on both sides of the rod domain, a nuclear localization signal in the tail domain and a CaaX box for post-translational isoprenylation and carboxymethylation at the carboxy terminus. The two major types of lamins are: lamin A and lamin B, each of which names a group of isoforms differently expressed in different species (reviewed by Georgatos et al., 1994 and Moir and Spann, 2001). Lamin B is constitutively expressed at all developmental stages, whereas lamin A is absent from embroynic cells. Both lamin types are subject to farnesylation at the cysteine of the CaaX box, subsequent cleavage of the three carboxy terminal amino acids and carboxymethylation of the farnesylated cysteine. Once translated and modified in the cytoplasm, the lamin monomers are imported into the nucleus through the nuclear pore complex (Aebi et al., 1986). The monomers dimerize through formation of a coiled coil structure with their rod domains. The dimers then polymerize head to tail to form a two-dimensional, near-tetragonal, 10nm meshwork of lamina filaments (Aebi et al., 1986, Zhang et al., 1996). The lamins are not distributed homogenously along the nuclear membrane (Paddy et al., 1990). The areas with high lamin concentration colocalize with local high concentration of chromosomes (Belmont et al., 1993), whereas a low concentration of lamins are found at sites where the nuclear pore complexes are inserted (Belmont et al., 1993).

Besides being part of the protein network lining the inner nuclear membrane, lamins are also found in the nuclear interior as intranuclear spots. The lamin foci may represent sites where the intermediate assembly of the lamin protein takes place before its stable incorporation into the peripheral lamina. Lamin B foci were shown to colocalize with sites of DNA replication (reviewed by Stuurman et al., 1998, Moir and Spann, 2001).

The most prominent role of the nuclear lamina is to support the nuclear membrane and to determine the shape and the mechanical strength of the nucleus. Another function is to provide anchorage sites for chromatin and inner nuclear membrane proteins (Glass and Gerace, 1990, reviewed by Gant and Wilson, 1997, Chu et al., 1998). The lamina also affects the DNA replication. In a cell-free nuclear assembly system in *Xenopus laevis*, nuclear

envelopes form after depletion of lamins but the developing nuclei are not replication competent (Jenkins et al, 1993). In addition, the disturbance of the lamina structure by a dominant negative lamin A-mutant in the same experimental system causes an abnormal distribution of replication factors (Spann et al, 1997). The nuclear lamina may also be involved in the regulation of gene activity (Bell et al., 2001). Mutations in lamin A lead to the autosomal dominant form of Emery Dreifuss muscular dystrophy, limb girdle muscular dystrophy, dilated cardiomyopathy and lipodystrophy (Bonne et al., 1999). Emery Dreifuss muscular dystrophy is also developed as a consequence of mutations in emerin, a lamin A interaction partner of the inner nuclear membrane (Manilal et al, 1996, Clements et al., 2000).

1.3 QUESTIONS AND APPROACHES

The inner nuclear membrane remains a poorly understood structure, mostly because of the limited knowledge about the proteins residing in it. There is only a small number of proteins known to specifically reside in the inner nuclear membrane and the functions of most of them remain to be discovered. The presented work focusses on two questions which are fundamental for the nuclear envelope research. First, we wanted to know if there are still any unidentified protein components residing in the inner nuclear membrane. Second, we wanted to functionally characterize LAP 2β, one of the few known proteins of the inner nuclear membrane.

The first part of the presented work deals with the detection and characterization of novel integral membrane proteins of the inner nuclear membrane. The proteome of the nuclear envelope was examined by a combination of subcellular fractionation and MALDI MS based protein identification. The approach is called 'subcellular proteomics' and was conducted by Mathias Dreger. It resulted in identification of two novel integral membrane proteins of the inner nuclear membrane, localization of which was to be confirmed by an independent method. The tools of molecular and cell biology, along with the bioinformatics were further utilized for characterization of the novel proteins. The public available data bases were searched for information on the novel proteins. The retrieved information was used for cloning of the proteins and deletion mutant construction. The subcellular localization of the proteins and the changes that are imposed on the cells when the proteins are overexpressed or downregulated, were examined by immunofluorescence on transfected cells.

In the second part of the presented work, the LAP 2β containing protein complexes were isolated and characterized using the tools of protein chemistry. The native complexes were isolated from solubilized nuclear envelopes prepared from N2a cells and analyzed with help of the size-exclusion chromatography, the glycerol-gradient centrifugation and the blue native gel electrophoresis.