3 SUMMARY

The nuclear envelope (NE) is one of the least characterized structures in the eukaryotic cell. Although the dynamics of its alterations during the cell cycle are well described, the detailed study of its functional roles is hampered by the small number of known proteins specifically located to it. This work focuses on the identification of novel components of the inner nuclear membrane (INM) and their characterization. Besides that, interaction partners of LAP 2β, a protein known to reside in the INM, were searched for.

Through combining subcellular fractionation methods and proteomic tools, a proteomic screen of the NE was performed. Based on the experimental evidence that INM proteins resist extraction with TritonX-100, two novel integral membrane proteins were classified as INM proteins. These proteins are KIAA0810 and LUMA. Their localization to the INM was independently confirmed by indirect immunofluorescence of transiently transfected cells overexpressing these proteins in combination with confocal laser scanning microscopy. Public available data bases were searched for information on the novel proteins regarding diverse physical parameters, the presence of homology domains or the existence of homologous proteins. The retrieved information was used to design further experiments (especially for LUMA) and to speculate on possible functions of these proteins.
KIAA0810 (apparent mass of 100 kDa) was found to be related to C. elegans UNC-84A, an integral nuclear envelope protein involved in the interaction between centrosome and the nucleus. KIAA0810 has two membrane spanning segments, a DNA binding motif (BRLZ domain) and a C-terminal SUN domain. KIAA0810's similarity to UNC-84A and also to some extent to structural proteins like myosin, vimentin or kinesin suggests that it is a probable candidate for anchoring the centrosome during mitosis in vertebrates.

KIAA0668, a close homolog of KIAA0810, was found through a data base search. Indirect immunofluorescence of transiently transfected cells overexpressing this protein in combination with confocal laser scanning microscopy revealed a rim-like staining around the nucleus. In addition, the biochemical characterization of KIAA0668 showed that this protein resists extraction by TX-100. Since both criteria for INM localization were met, KIAA0668 was classified as the third novel INM protein discovered in this study. KIAA0668 has a similar domain structure as KIAA0810, spans the membrane two times, contains a DNA

binding motif and a C-terminal SUN domain. No homologues other than KIAA0810 were found.

The second novel protein of INM membrane identified in the proteomic screen was a 45 kDa protein which we named LUMA. LUMA displays no similarity to any known protein and contains no homology domains. The hydropathy profile for LUMA suggests four membrane spanning segment with a large loop between the first two. By overexpressing deletion mutants in COS-7 cells, the topology of LUMA could be determined and the INM targeting domain identified. Both, N- and C-terminus of LUMA point towards the ER lumen, the large loop is oriented to the nucleoplasmic side and the smallest part of LUMA which was sufficient for INM localization were the amino acids 201-345, comprising the C-terminal part of the nucleoplasmic loop and the second transmembrane segment.

Overexpression of full length LUMA induces a pronounced phenotype in the cells – the NE shows a swollen appearance and has vesicle like structures attached to it. Additionally, the nuclear chromatin appears less dense. A knock-down of LUMA leads to the formation of vesicle–like structures containing chromatin outside the nucleus and an altered chromatin structure inside. A chromosome binding assay showed that LUMA binds chromosomes and causes their decondensation. These observations indicate a role for LUMA in the regulation of gene expression and/or the cell cycle. They also show that LUMA is crucial for the integrity of the NE structure.

To understand the functions of NE, a detailed understanding of the interplay between its proteins is necessary. To investigate that, native complexes of LAP 2β were purified and characterized. Applying different techniques, after solubilization of the membranes with n-dodecyl-β-maltoside, high molecular weight complexes containing LAP 2β could be isolated. Besides LAP 2β, LAP 2ε was present in these complexes, but no lamins. Further experiments are needed for closer characterization of the isolated complexes.