THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Comparative proteomic analyses of the nuclear envelope and pore complex suggests a wide range of heretofore unexpected functions

OPEN ACCESS

Comparative Proteomic Analyses of the Nuclear Envelope and Pore Complex Suggests a Wide Range of Heretofore Unexpected Functions

Dzmitry G. Batrakou, Alastair R. W. Kerr, and Eric C. Schirmer

Wellcome Trust Centre for Cell Biology and Institute of Cell Biology, University of Edinburgh, Edinburgh, EH9 3JR, UK

Corresponding author:

Eric Schirmer (e.schirmer@ed.ac.uk)

Wellcome Trust Centre for Cell Biology

University of Edinburgh, Kings Building Campus

Swann 5.22, Mayfield Road

Edinburgh EH9 3JR

UK

Tel: +44 131 650 7075

Fax: +44 131 650 7360

Abstract

Since the discovery of several inherited diseases linked to the nuclear envelope the number of functions ascribed to this subcellular organelle has skyrocketed. However the molecular pathways underlying these functions are not clear in most cases, perhaps because of missing components. Several recent proteomic analyses of the nuclear envelope and nuclear pore complex proteomes have yielded not only enough missing components to potentially elucidate these pathways, but suggest an exponentially greater number of functions at the nuclear periphery than ever imagined. Many of these functions appear to derive from recapitulation of pathways utilized at the plasma membrane and from other membrane systems. Additionally, many proteins identified in the comparative nuclear envelope studies have sequence characteristics suggesting that they might also contribute to nuclear pore complex functions. In particular, the striking enrichment for proteins in the nuclear envelope fractions that carry phenylalanine-glycine (FG) repeats may be significant for the mechanism of nuclear transport. In retrospect, these findings are only surprising in context of the notion held for many years that the nuclear envelope was only a barrier protecting the genome. In fact, it is arguably the most complex membrane organelle in the cell.

1. Introduction

The structure of the nuclear envelope (NE) is complex (for reviews see [1-4]). It is a double membrane system continuous with the endoplasmic reticulum (ER) that consists of three connected but distinguishable membrane domains: the outer, inner and pore membranes (Fig. 1A). The outer nuclear membrane (ONM) is studded with ribosomes [5,6] and contains many ER proteins in addition to having a set of unique proteins, some of which appear to be involved in tethering the nucleus to cytoplasmic filament systems [7-12]. Some of these proteins in turn connect across the lumen of the nuclear envelope to the inner nuclear membrane (INM), which contains its own unique set of proteins (Fig. 1B; reviewed in [3,13]). Many of these have been shown to bind both to the intermediate filament lamin polymer [14-19] and chromatin (reviewed in [20]). Nuclear pore complexes (NPCs) are inserted at the pore membrane (PoM) that connects the ONM and INM (Fig. 1A; reviewed in [4]). The NPCs regulate directional transport of proteins and mRNA between the nucleus and cytoplasm that exceed the measured maximum free diffusion limit of ~40-60 kDa [21] and can accommodate very large molecules or complexes that have been experimentally tested up to 39 nm diameter [22] (to put this in perspective the longest dimension of an assembled ribosome is ~25 nm). To accommodate such large substrates, the NPCs are necessarily large, on the order of 44 to 125 MDa. These large complexes are tethered to the membrane by at least three unique transmembrane proteins [23-28] that also contribute to a ring component of the NPC in the NE lumen [29-31]. The lumen of the nuclear envelope is largely unexplored territory, but is likely to have its own unique functions.

NE proteins have now been shown to influence a wide range of functions, although it is unclear whether their effects are direct or indirect. These functions include nuclear morphology and stability [32-36], nuclear anchoring/ migration within the cell [8-10], signaling cascades [37-40], and support of DNA replication [41-43], transcription [44-46], and RNA splicing [47]. Consistent with the notion that the nuclear lamina carries out or facilitates a diverse range of basic cellular functions, over a dozen inherited diseases and syndromes are linked to lamins and certain associated NE proteins. NPC proteins have also been linked to disease. These nuclear envelope diseases include muscular

dystrophies, lipodystrophies, neuropathy, cardiomyopathies, dermopathy, bone disorders, and premature aging syndromes (reviewed in [48-51]). The proteins thus far linked to disease are lamins A/C, B1, and B2, emerin, LBR, LAP2, MAN1, Syne/Nesprin-1, FACE-1/ZMPSTE24, torsin A, and the NPC proteins Aladin, and Nup62. The favored hypotheses to explain how these proteins can yield so many different diseases are: 1) reduced resistance to mechanical stress, 2) disruption of gene regulation, 3) alterations in cell cycle and signaling pathways. However, none of these hypotheses can fully explain by themselves how mutations in the same widely expressed proteins can yield different diseases that each have distinctive tissue pathologies. Correspondingly, it is hard to imagine the many functions ascribed to lamins and associated proteins being due to diverse enzymatic activities encoded within the same proteins. The resolution in both cases likely involves additional partner proteins that provide these functions and have yet to be identified, hence the need for proteomic analysis.

The combination of its inclusion of cytoskeletal elements, the lamin polymer, integral membrane proteins, NPCs, a membrane and its lumenal content that is continuous with the ER together with the many associations with chromatin proteins, transport receptors and cargos, and indirectly attached peripheral components of the cytoskeleton give the NE a wide range of biochemical properties. This has the consequence that any biochemical fractionation methodology will necessarily remove some true NE components and bring along some contaminants. As the ONM is continuous with the ER and connected to cytoplasmic filament systems [9,52] these structures are difficult to separate. INM proteins bind chromatin (reviewed in [20]) and in yeast the NPCs are connected to nucleoplasmic filaments [53,54] that in turn connect to telomeres [55,56]. All of these connections on both cytoplasmic and nucleoplasmic faces of the NE and NPC further compound difficulties in their purification.

2. The Nuclear Pore Proteome

2.1 Pre-proteomics studies

As one of the largest macromolecular complexes in biology it is not surprising that most of the original characterization of the NPC was through the electron microscope. This work determined that the Xenopus NPC had a diameter of ~120 nm with eight-fold radial symmetry perpendicular to the membrane and a predicted mass of roughly 125 MDa [29,31,57] while the yeast NPC was somewhat smaller at 55-72 MDa [58,59]. Thus the NPC could be made up of a very large number of distinct proteins. Determining its composition was therefore not trivial even in the eras of genomics and proteomics. As the average mammalian nucleus has 2-3,000 NPCs [60] identification of NPC component proteins by biochemical fractionation was initially attempted and was successful in some cases. For example the first individual NPC component identified was gp210 that was isolated from a rat liver NE fraction and used to make antibodies that labeled the NPCs by immunogold electron microscopy ([61]; note this was originally called gp190 and renamed after the gene was cloned 7 years later [28]). Many other NPC proteins were soon identified using similar approaches and the proteins were called nucleoporins or NUPs [62-64]. Many of these antibodies cross-reacted among mammals, Xenopus and yeast [65-67] thus facilitating cloning of NUPs. Xenopus oocytes were the best system for biochemical purification while yeast was the most genetically tractable, so that many of the first NUP sequences were from yeast (*e.g.* NUP1 [66], NSP1 [68]). Once the first NUPs were identified it was discovered that they tend to form subcomplexes with 3-5 proteins and this rapidly facilitated further NUP identifications. For example p62 was among the first NUPs cloned [69] due to its abundance and strong antigenicity and it was subsequently found to be part of a complex with p54 and p58 in mammalian cells [70,71]. In yeast Nup170p was found to be in a complex with Nup53p and Nup59p [72] and Nic96p was identified through its interaction with Nsp1p [73]. Many individual studies over nearly two decades had identified 26 core NPC components in yeast (Table 1) by the time that the first major proteomic studies were done on the NPC. However, absent knowing how many copies of each protein were in each NPC it was impossible to gauge how many more components still needed to be identified and it was generally thought that between 50 and 100 proteins would be required to account for the estimated 55 to 125 MDa mass predicted by electron microscopy.

2.2 The yeast NPC proteome

The first comprehensive determination of NPC composition was the product of collaboration between the Rout, Aitchison and Chait laboratories [74]. Critical to a successful proteomic study is the choice of experimental system and the purity of the fractions analyzed. This study used yeast (*Saccharomyces cerevisiae*) because it had the highest NPC:nuclear volume ratio of any organism tested [75]. A haploid yeast nucleus has 65-182 NPCs depending on the cell cycle stage [76]. Unlike mammalian cells yeast do not have a lamina, which has the advantage of less contamination from connections to such a promiscuous structure and the disadvantage of losing the added stability to NEs from the lamina during purification. Yeast cells were spheroplasted, lysed and nuclei were isolated on sucrose gradients. The chromatin was enzymatically digested and extracted with heparin to isolate NEs. NPCs were released from NEs with mild detergent followed by isolation through their partitioning on a continuous sucrose velocity gradient [58]. Three separate rounds of separation by HPLC and SDS-PAGE yeilded 465 protein bands that were digested and analyzed by matix-associated laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry and an additional 177 protein bands were analyzed by MALDI-ion trap tandem mass spectrometry. A total of 174 protein identifications were made [74]. Among these were all previously characterized NUPs, many proteins with known functions at the NPC such as transport factors and chaperones, many other proteins with known functions elsewhere that were considered to be contaminants, and 34 previously uncharacterized ORFs.

All previously uncharacterized ORFs and several known NUPs were genomically tagged with a protein A epitope and tested in yeast for their localization at the NE by immunofluorescence and specifically at the NPC by immuno-electron microscopy. Those that fulfilled these criteria and also were significantly enriched in a NE fraction by Western blot were classified as core NPC components, a total of 30 proteins (two of which are products of the same gene). Thus the yeast proteomic analysis identified only 4 additional core NPC components on top of those previously identified (Table 1). An additional ten NPC-associated proteins that did not fulfill their stringent criteria for inclusion as core

components were classified among those identified, leaving 134 of the 174 proteins identified

classified as contaminants by the authors.

2.3 The mammalian NPC proteome

      Subsequently the Matunis laboratory determined the composition of the mammalian NPC

[77]. They used rat liver because previous work had developed procedures for isolating extremely

clean fractions of nuclear envelopes from this material [61,78-80]. These procedures take advantage

of the relative softness of liver as a tissue to lyse cells without breaking their nuclei and also benefit

from the stability conferred to the NE by the lamin polymer to allow several steps of douncing,

floating contaminating membranes on sucrose cushions, chromatin digestion and salt washes without

fragmenting NEs. For fear that some NPC components might be also removed by high salt treatments,

they however replaced the salt with heparin and spermidine so that extraction would favor removal of

just histones. They next took advantage of the biochemical characteristics of the lamin polymer and

its binding partners that are highly resistant to extraction by high salt and detergent treatments and

tested a variety of mild detergents for those that would solubilize NPC components while leaving

lamina components insoluble. They found that all well-characterized NPC components could be

extracted while leaving most of the lamina components in the pellet using a hypotonic solution

containing a low concentration (0.3%) of the detergent Empigen BB. Simlarly to the yeast study, this

material was separated by HPLC prior to SDS-PAGE and analyzed using a combination of single step

and tandem mass spectrometry. Previously uncharacterized proteins were tagged with GFP and tested

for NPC colocalization using an antibody (mAb414) that recognizes O-linked glycosylation on several

nucleoporins [62]. This study identified 23 proteins that were classified as core NUPs and 18 proteins

classified as NPC-associated proteins (Table 1).

2.4 The revised NPC

      The big surprise from both the yeast and mammalian studies was the relatively small number

of proteins identified. Original estimates of protein content based on electron microscopy studies had

predicted between 50 and 100 distinct protein components for the NPC [29,31,57]. The total number

of only ~30 can in part be explained by the observations that several components have well above

average molecular weights and that many components are duplicated many times per NPC. Based on

the eight-fold symmetry of the NPC core NUPs would be expected to be duplicated eight times, but

the abundance of many components suggested that they are represented 16 and 32 times within a

single NPC [74,77]. Furthermore, the yeast study excluded the Mlp (Tpr in mammals) proteins that

make up much of a "nuclear basket" extending into the nucleus from the NPC that was observed in

electron microscopy studies. Nonetheless, even taking into account these considerations, the total

mass of the core NPC calculated from summing up the components identified in the proteomic

analyses (44 MDa in yeast [74] and ~60 MDa in mammals [77]) was suprisingly lower than the mass

predicted from electron microscopy studies (55-72 MDa in yeast [58,59] and 125 MDa in mammals

[29,57].

A potential explanation for this discrepency may lie in the additional proteins that both studies

identified, but excluded because they did not fulfill their very conservative definition for core NPC

components. There are a great many NPC proteins that have only transient associations during the

transport process. For example the Ran GTPase is a very abundant protein that is involved in release

of transport substrates from NPC proteins in the nucleus (Fig. 2A). Importins and exportins are

transport receptors that bind to the transport cargo and interact with "core" NPC components to

facilitate transport of the cargo through the central channel of the NPC (Fig. 2B and C). These

interactions presumably occur through repeat motifs containing phenylalanine and glycine (FG

repeats) that appear on both NUPs and many transport receptors. Because their associations are

transient neither Ran proteins nor transport receptors were considered as core NPC components, yet

due to their high abundance these proteins were also identified in these studies and could moreover

account for a significant portion of the mass difference between the calculations from the "core" NPC

components and those measured from electron microscopy studies. Even with these, however, the

mass difference of almost 2-fold for the mammalian NPC would likely indicate the existence of

additional proteins not yet identified (either core components or transient NPC proteins) that were

8

extracted during the preparation of the core NPC fraction. Another possibility is that some of the proteins that were discounted because of other previously characterized cellular functions and localizations actually have separate functions at the NPC. For example the Sec13 proteins are known to function in vesicle formation in the ER [81], yet also were shown to function in nucleo-cytoplasmic transport [82]. Thus, additional NPC components might be identified if these studies were repeated using multiple and varied purification methodologies and if proteins identified with distinct known cellular functions were also tested for NPC function.

It is also worthy of note that the original 125 MDa mass estimate for the NPC of higher eukaryotes was actually principally determined from Xenopus, which was much more accessible to analysis than mammalian nuclei. Thus it is possible that the Xenopus NPC, not yet analyzed by proteomic approaches, is larger than either yeast or mammalian NPCs. Comparison of the yeast and mammalian NPCs would suggest that if this is the case the mass difference likely is accounted for mostly in more peripherally associated proteins because the differences between the yeast and mammalian proteins identified as core structural NPC components is smaller than it appears (Table 1). While several proteins did not have homologues, they nevertheless had analogues: although no clear sequence homology links yeast Pom152 to the mammalian Pom121, both are transmembrane proteins with similar functions. Likewise yeast Ndc1 had no clear mammalian homologue identifiable by genome mining and was not found in the mammalian NPC study, but the mammalian Ndc1 analogue was subsequently identified in a NE proteomic study ([26,83]; see below) giving direct support to the notion that differences in purification methodologies will also contribute to differences in identifications. The mammalian Nup50 appears to be an analogue of Nup2p in yeast, which was not considered in the yeast proteomic study to be part of the core because its association is too dynamic. Although we cannot tell if completely novel or as yet unidentified core components are part of the Xenopus NPC until it has been also analyzed by proteomics, nonetheless, blast searching yeast and mammalian nucleoporins against the *Xenopus laevis* and *Xenopus tropicalis* genomes indicates only

Nup133, PoM152, Gle1, Nup60, and PoM34 among the proteins listed in Table 1 do not have homologs in frogs.

Phylogenetic analysis of the sequences of these core NPC proteins brought another striking observation. The yeast NPC proteins could be separated into pairs that clearly diverged after a gene duplication event [84]. This is consistent with predictions based on the yeast genome sequence of a general genome duplication that was followed by asynchronous differentiation of the duplicated genes [85]. The gene duplications expanded the range of phenylalanine-glycine (FG) repeat motif NUPs and of beta barrel/ alpha solenoid fold NUPs. These latter are related to coatamer proteins and have been suggested to facilitate bending of the pore membrane during NE/ NPC assembly [84]. Thus, though at the sequence level not all NPC proteins are conserved, the central evolutionary mechanism underlying their development argues that they will be essentially conserved at the core.

3. The Nuclear Envelope Proteome

3.1 Approaches prior to proteomics

The abundance (~3 million copies per mammalian nucleus [60]) and biochemical properties of the intermediate filament lamins enabled their being the first NE proteins to be identified [78,86]. Following on this the first NE transmembrane proteins (NETs) identified were INM proteins that bound to lamins and so were resilient to extraction by procedures for isolating NEs that were based largely on the resistance of the supporting lamin polymer to high salt and detergents. The lamin B receptor (LBR) was so named because of its identification through its binding to lamin B1 [19] while LAP1 and LAP2 that also bind lamins were identified as major proteins in a NE/ lamina fraction that was used to generate monoclonal antibodies [15,87]. The methods of discovering the next round of NETs were surprisingly varied ranging from autoimmune antibodies that stained the NE in the case of MAN1 [88] to a 2-hybrid screen for partners of a kinase of the postsynaptic membrane in muscle that identified the Syne/Nesprin-1 proteins [7].

As different groups were using different organisms to identify NETs and the timing correlated with the beginning of large-scale genome sequencing projects, once the first several proteins had been identified, attempts were made to identify related proteins through sequence similarity. This type of analysis found the Syne/Nesprin-2 NE protein family from its sequence similarity to Syne/Nesprin-1 [7], but it did not identify the Nesprin-3 family that was subsequently identified by proteomics [12,83]. Moreover, the two first identified *Drosophila* NETs do not have mammalian homologs (Otefin [89] and YA [90]). Thus the only way to determine the components of the NE would be directly through proteomic analyses.

3.2 Strategies for NE proteome determination

The continuity between the ONM and the peripheral ER on one side and many connections to chromatin on the other require creative approaches to the identification of NE proteins by proteomics, especially after the high number of proteins defined as contaminants in the NPC proteomes (provided they are truly contaminants). Two studies that were relatively similar in purifications yet different in strategies yielded strikingly different results [83,91]. Both studies used equivalent procedures for isolating mammalian NEs as were used in the mammalian NPC study; however, the NEs were either extracted to enrich for proteins associated with the intermediate filament lamin polymer or to enrich for proteins embedded in the membranes.

The first study from the Otto laboratory generated three separate NE fractions: a chaotrope-insoluble fraction, a non-ionic detergent-insoluble fraction and a salt-insoluble fraction [91]. Extraction with chaotropes (4 M Urea, 200 mM sodium carbonate) solubilizes the lamin polymer and most protein-protein interactions, but has no effect on membranes so that proteins embedded in the membrane are protected and maintained in the membrane fraction. The extraction with detergent (1% Triton X-100) should remove all membranes and so only proteins tightly bound to the lamin polymer should remain. Finally extraction with high salt (1 M NaCl) should also leave the lamina intact, but wash away soluble proteins that are weakly associated with it. Each fraction was separated on 2-D gels, and the protein spots were excised and analyzed by MALDI mass spectrometry. Proteins that

were found in both the chaotrope and detergent-resistant fractions were considered as candidate NETs. In this way they could remove contamination from the ER and ONM proteins: as the chaotrope fraction contains both NE and ER transmembrane proteins, it alone is insufficient to distinguish INM proteins. This analysis identified most, but not all, previously characterized INM NETs, as well as mammalian Unc84A/SUN1 and a novel protein with no predicted functions that was named LUMA. Both novel mammalian NETs were shown to target to the NE by exogenous expression of the proteins fused to GFP [91].

Where the first study used a "comparative" approach to exclude peripheral ER proteins that also were present in the NE fraction, the second study from the Gerace and Yates laboratories used a "subtractive" approach [83].   In this case a microsomal membrane (MM) fraction was used to identify peripheral ER proteins. The MM fraction was analyzed separately from the NE fraction and all proteins appearing in both fractions were subtracted from the NE fraction. As there are no membranes in the nucleus besides the NE membrane and contaminating membranes of the NE fraction should in theory all also occur in the MM fraction, those transmembrane proteins in just the NE fractions were considered to be true NETs. Multiple NE fractions were also analyzed, but instead of using chaotropes an alkali extraction (0.1 N NaOH) was used to enrich for transmembrane proteins and salt and detergent (400 mM NaCl, 1% Triton X-100) were combined in one extraction to generate a cleaner lamina fraction. Combining both fractions resulted in a more comprehensive analysis of proteins with different biochemical characteristics as compared to the other study in which the different fractions were used to increase the stringency of inclusion. Fractions were analyzed using Multi-Dimensional Protein Identification Technology (MudPIT) [92,93], which couples tandem mass spectrometry with multiple liquid chromatography steps to analyze the complex mixture of peptides generated by direct digestion of isolated membranes. This avoids loss of membrane proteins that are poorly resolved on 2-D gels [94]. Details of the method are given in [95]. The subtractive approach was validated by the identification of all expected previously characterized NETs in the NE fraction and their absence from the MM fraction. Moreover, the numbers of peptides recovered suggests that

they are the most abundant NETs and this is why they were first identified by other means. In addition, 67 previously uncharacterized putative NETs were identified in the NE fraction, which were absent from the MM fraction [83]. All of the original eight tested targeted to the NE in the original study, suggesting that all would prove to be valid identifications; however of the 2/3 now tested only ~70% are valid NETs that are integral to the membrane and target to the NE (P. Malik, N. Korfali, N. Zuleger, V. Lazou, D. M. Kavanagh, G. S. Wilkie, D. G. Batrakou, and E. C. Schirmer, in preparation). Thus this method, like the NPC studies, still brings considerable contaminants. It appears, nonetheless, that some of this failure rate is not due to mis-identification or contamination, but rather to mis-prediction of transmembrane helices.

3.3 Comparison of benefits and disadvantages between the two approaches

The "subtractive" and "comparative" approaches used to identify NE-specific proteins both had limitations. The comparative approach disregarded NE-specific proteins that were not associated with the salt and nonionic detergent-insoluble lamina fraction, because no other basis was provided for distinguishing between the NE and ER transmembrane proteins that were present in the membrane-enriched fraction. The more conservative requirement of appearing in both membrane-enriched and lamina-enriched fractions also served to limit the number of identifications.

The subtractive approach had the disadvantage of disregarding proteins that have functions in both the ER and the NE. For example, a known ER protein, torsinA, appeared in both NE and MM fractions. However, torsinA is now known to move between the ER and INM where it interacts with multiple NETs [96].

While the subtraction limited the identifications, the combining all proteins in membrane-enriched and lamina-enriched datasets allowed for a more comprehensive analysis that covered a wider range of biochemical characteristics. This approach is not unreasonable considering the wide range of biochemical characteristics observed just for splice variants of one of the first identified NETs, LAP1 [87]. There were three variants of LAP1 recognized by a monoclonal antibody: the smallest was extracted with less than 200 mM salt, the intermediate sized variant was only about 50%

extracted at this salt concentration while the largest remained fully associated with the lamin polymer [15]. Non-equivalence has also been observed for different transmembrane proteins of the NE with regard to their extraction to chaotropes and different detergents. Just as empigen BB favored extraction of NPC components over other integral membane proteins and lamins, partitioning of NETs in the first NE proteomic study indicated that emerin is more extractible by detergents than many other NETs while LBR is more extractible by chaotropes [91]. Thus the two studies highlight the persistent struggle between comprehensive identifications and contaminants.

Another reason for discrepancies between datasets is the randomness of getting single peptides into the mass spec and in obtaining good fragmentation to make identifications. In the present instance this was unlikely to be a primary contributor to variation as 92.7% of the proteins identified in the comparative study were also found in the subtractive study. Moreover 32.9% of the proteins identified in the NPC study were found in the comparative study (59.2% of core NPC proteins) and 89.3% in the subtractive study (if accepting variants or related proteins then this number jumps to 98.9%). This argues that the subtractive study was the most comprehensive, but the caveat as discussed above is that it likely also has the highest number of contaminants.

4. Recapitulation of other organellar functions in the nuclear envelope

4.1 NPC and NE proteins with dual roles

There are many previously characterized examples of proteins that have dual functions in the NE and other cellular structures or organelles. The first was Sec13p that functions in both ER vesicle formation [81] and NPC transport [82]. Additionally the DEAD-box helicases An3 and Dbp5 that bind RNA were also found to play an important role in RNA export [97-99]. Many core or peripheral NPC components have now been found to play separate roles on mitotic chromosomes when the NPCs are disassembled in mitosis. Ran, importinß and the Nup107-160 complex have been found to function in mitotic spindle assembly and on kinetochores [100-102].

NE proteins outside the NPC have also been found to have dual localizations and/ or functions. Lamins were originally thought to reside only under the inner nuclear membrane and provide structural support [60]; however in the past several years it has become apparent that lamins assemble and function also in the nucleoplasm [103] and on mitotic spindles [104]. Emerin is clearly an INM protein, binding to lamin A [14,18], the INM protein MAN1 [17], the chromatin binding protein BAF [105], the splicing factor YT521-B [106] and the transcriptional repressors germ cell-less, Btf, and Lmo7 [107-109], but emerin has recently been shown to function also in the outer nuclear membrane and ER [110]. Nesprins were named for their NE association, but are now recognized as a complex family containing many splice variants located throughout the cell [111]. Conversely Torsin A, like Sec13p, was originally characterized as an ER protein but is now known to normally sample the INM and accumulate there with certain point mutants associated with disease [112,113]. Torsin A has also been found to bind LAP1 in the INM and another protein that the authors renamed LULL1 and claimed resides in the ER [96]. However LULL1 was originally identified as NET9 [83] and has been confirmed in the INM [114]. Presumably NET9/LULL1, like Torsin A, can sample both compartments.

4.2 Newly identified NE proteins with apparent dual roles

Only a small percentage of the total proteins identified in the liver NE datasets were novel proteins with no predicted functions. Even among the 67 novel putative NETs identified, nearly a third had predicted functions based on sequence homology. An analysis of both predicted and characterized functional regions indicates a wide range of functions represented in both the NE and MM datasets (Fig. 2A; [115,116]). These functions range from transport and signaling functions to specialized functions of differentiated cells. Some of the proteins associated with cell signaling might converge on or add additional pathways to those recently identified at the NE [37-40]. A clear enrichment in proteins associated with DNA and RNA functions was noticeable in the NE compared to the MM. Lamins have been shown to have roles in DNA replication [41,42], transcription [45,46], and RNA splicing [47], but it is unclear whether lamin effects are direct or reflect their serving as

15

recruitment sites for specific enzymes. It has already been shown that transcriptional regulators pRb and germ cell-less respectively interact with lamins [46] and several NETs [17,44,109]. These proteins are among those identified in the liver NE dataset and so other proteins in this dataset that function in DNA and RNA processes might also mediate such lamin effects. Looking in more detail at the types of DNA functions represented in the NE dataset shows a striking enrichment of proteins involved in chromatin organization and remodeling compared to the total proteins in the human genome that function on DNA (Fig. 3A). For proteins functioning on RNA there is both an abundance and enrichment for those involved in RNA splicing and pre-mRNA processing (Fig. 3B). Though less abundant, there is also enrichment for those involved in mRNA end processing and stability, RNA localization and rRNA metabolism. This indicates that there are many potential mechanisms that could direct disease pathology from the NE involving disruption of gene expression beyond those currently being investigated.

Within the transporter category there were many pathways at play in addition to the expected NPC transport-associated proteins. This is not surprising in retrospect as regulated transport of smaller molecules such as ions should be important in the nucleus and potentially for disease, yet there has been very little focus in this area within the now chromatin-oriented NE field. Inositol(1,4,5)P_3 receptors were long ago reported at the NE [117,118], and specific ones were also found in the liver NE proteomic study that have been since directly tested [119]. Other regulators of Ca^{2+} transport and signaling were also found such as the ryanodine receptor, which has since been shown to function in the NE from several studies [120-122]. Indeed Ca^{2+} oscillations have been shown to affect gene expression [123] and so regulation of ion transport could also affect disease pathology through gene expression. Zn^{2+} transporters, Na^{2+}/H^+ exchangers and many other ion transporters were also found in the NE proteomic datasets, many of which have since been directly shown at the NE [124,125], and there was a striking enrichment for electron transporters.

Detailed analysis of the enrichment in signaling proteins indicates that lipid signaling mechanisms in particular could be important in transducing signals to the nucleus in a separate or

16

backup mechanism to those that depend on transport of molecules through the NPC. In support of this NET39 that was identified in the liver NE proteomic study and confirmed at the NE [83] is a lipid phosphatase/lipid phosphotransferase of the candidate sphingomyelin synthase class and thus also now called CCS2ß [126]. This is the least studied subclass of the lipid phosphatase/ transferases, but others have been shown to play roles in regulation of cell growth and survival. There appear to be many proteins in the NE involved in these processes as NET13, also identified in the liver NE study, has now been shown to be a sphingomyelin synthase [127].

The various biochemical functions observed for these proteins and our confirmation of their partial representation in the NE has significant implications for many aspects of NE biology. Some studies have argued that nuclear membrane growth during interphase requires vesicle fusion or that ER membranes perfuse around the NPCs, but the identification of several enzymes involved in lipid generation in both NE datasets argues for de novo generation of lipid during interphase when the NE grows 3- to 4-fold. The recently reported function of NET8 (LPGAT1) as a phosphatidylglycerol acyltransferase [128] could serve to direct lipid content in the nuclear membrane, which has been reported to be one of the subcellular membranes containing phosphatidylglycerol [129]. The membrane trafficking function of NET24 (ERGIC-32; [130]) could have relevance for NE disassembly as Rab5 (which also appeared in the NE proteomics datasets) functions in NE disassembly in addition to its normal vesicle transport role [131]. Similarly, the signaling roles of emerin, MAN1 and AKAP149 [37-40] could be facilitated by functions of NETs 45 and 55 that respectively have been reported to directly function in signaling and to be a homolog of a protein involved in signaling [132-134]. Thus these NETs could be involved in signal transduction pathways similar to those of the well-characterized NET MAN1 in Smad/ BMP/ TGFß signaling [135].

5. Future Directions

5.1 Tissue variation in the NE proteome

17

There is precedent for tissue differences in NE proteins from the distribution of different lamin subtypes that have been shown to vary in relative concentrations during development and in different tissues [136-138]. There are also tissue-specific splice variants of lamins such as the lamin C2 that appears during spermatogenesis [139]. Although the first identified NETs were widely expressed, comparison of the novel NETs identified in the liver proteomic study to a transcriptome database [140] indicated that a significant percentage of the proteins recovered were preferentially expressed in liver and/ or had restricted tissue expression [13,141]. Moderate tissue variation has been reported recently in proteomes from mitochondria and other organelles [142,143]. A current study analyzing the NE proteome from several different tissues confirms that there is not only considerable tissue variation in the NE proteome but further suggests that there is more variation in the NE than these other subcellular organelles (N. Korfali, G.S Wilkie, E.A.L. Fairley, S.K. Swanson, D.G. Batrakou, P. Malik, A.R.W. Kerr, L. Florens, and E.C. Schirmer, in preparation).

The finding of tissue variation in the NE proteome together with observations that different epitopes on NE proteins are occupied in different tissues [144] argue that tissue-preferential binding partners of the NE proteins mutated in disease might mediate the tissue-preferential phenotypes of the wide range of NE-related diseases [13,145]. Different diseases preferentially affect muscle, neurons, bone, skin, heart, fat, and immune cells. Some diseases have partial overlap between a subset of these tissues. Thus a particular point mutant might disrupt a functional complex in one tissue where a specific partner within the complex is expressed, but have only a minimal effect on a different functional complex in another tissue where a different partner that has slightly different binding characteristics occupies the same general binding site. If this is the case, then determining the protein composition of the NE in different tissues may be critical to understanding these diseases. As variation will likely mostly occur in terms of relative amounts of protein as opposed to all or none effects, it will also be important for such future analyses to be performed using recently developed quantitative approaches such as SILAC, iTRAQ, and protein correlation profiling.

18

This also applies for the NPC as a study on the integral NPC component gp210 revealed that it has restricted tissue expression in mammals [146]. It is nonetheless essential for viability in *C. elegans* by RNAi [147]. Gp210 was also reported to be essential in HeLa cells [147] but other studies found that it could be deleted and that, rather, another integral NPC component, NDC1/NET3, is essential in HeLa cells [26,148,149]. Interestingly, they also found that NDC1 was not essential in *C. elegans* [148]. It is possible thus that different combinations of NPC components are expressed in different tissues, thus rendering them essential depending on the tissue being sampled. Accordingly it will be important to directly compare knockdown of several different NUPs over a panel of tissues. This, however, also means that aspects of nuclear pore structure and function may differ between different tissues and indicates a need to compare the NPC proteome in different tissues using identical extraction methodologies and quantitative approaches so that differences in extractability due to different detergents or salt concentrations used are not misread as tissue variation.

5.2 A further use of proteomics to gain structural insights into the NPC

The two NPC proteomic studies were considered at the time to be the definitive conclusion to NPC proteomics, but a recent yeast study indicates that mass spectrometry can contribute much more to understanding the NPC even without counting potential tissue variation. This study performed rapid pulldowns with each component of the NPC followed by quantitative mass spectrometry to identify binding partners and their ratios [30,150]. Stringent conditions were used so that by comparing the ratios of NUPs in different fractions those most likely to be in direct contact with one another could be ascertained. These data were combined with position measurements from immuno-electron microscopy for epitopes on NUPs and other available data to generate what is by far the highest resolution structure of the NPC to date [30]. This structure gives a more detailed view of the internal structure of the core NPC and the organization of the many core NUPs that is diagrammed schematically in figure 1C.

5.3 Additional NPC proteins in the NE proteome datasets?

It is noteworthy that the integral NPC component NDC1/NET3 was not identified in the mammalian NPC proteomic study [77], apparently due to its resilience to the extraction with empigen BB that was used to isolate the NPC proteins as their method relied on extracting NPC components from the membrane and lamina. NDC1/NET3 was, however, identified in the less conservative study of the NE that enriched for transmembrane proteins [83] and was later shown to be the third integral component of the NPC in mammalian cells [26,27,148] (it had previously been identified in yeast, but was too divergent for mammalian homologs to be identified by genome searching [23]), and this suggests that other proteins may have been missed in the NPC studies that appear in the NE proteomic datasets. If this is the case, it might account in part for the large differences between the masses predicted by electron microscopy studies and the final masses calculated from the proteins identified in the proteomic studies.

Searching the liver NE datasets for characteristics of NPC proteins such as the appearance of FG repeats indicates that proteins containing this motif are significantly enriched in the NE compared to the whole genome. FG repeats were observed in some of the first NUPs identified and it soon became apparent that they represented a common motif among most of the NUPs when three NUPs were identified using an antibody that recognized regions containing a GLFG motif: NUP49, NUP100 and NUP116 were found to have respectively 13, 29 and 33 GLFG repeats [151]. It is noteworthy, however, that only 10 of the 30 core NUPs have six or more FG repeats and 13 have one to five FGs (Table 1). Thus very large numbers may not be required for some FG functions. The FG repeat regions of NUPs are thought to be disordered [152] and tend to have >30% of polar residues between them. It has been suggested that the FGs within unstructured regions interact within the central channel of the NPC to provide an entropic barrier to passive diffusion [153], though the exact nature of that barrier is the subject of active debate [154,155]. One possible mechanism for transport through the entropic barrier would be exchange of FG interactions in the barrier with FG motifs on the surface of proteins associated with transport cargos [156]. The transport receptor importinß has 3 FG repeats so that coating the surface of the substrate cargo with importinß could potentially provide these FG

repeats on the outer surface to facilitate navigation through the central channel (Fig. 2B). If this hypothesis is correct, then other proteins that have FG repeats could similarly coat cargoes to facilitate their transport. A general search of all proteins in the human genome database reveals that only 1.86% have 5 or more FG repeats with 30% or more polar residues between at least 4 of them. In contrast, in the liver NE-enriched dataset of proteins 5.3% of proteins fulfill these criteria. It is particularly interesting that some DEAD box helicases have been shown to be important for nuclear export of mRNAs (including DEAD box protein 5 that has 5 FGs; [98,99]) and among the components of the TREX complex that is important for RNA export [157] is Sub2p, a DEAD box ATPase containing 2 FGs [158]. Strikingly, several of the liver NE proteins carrying multiple FGs are also RNA binding proteins including DEAD box proteins (Table 2). Thus if these stay bound to the mRNA during the process of transport they might facilitate the act of transport by coating the surface of the RNA with FGs (Fig. 2D).

These observations indicate that the NE has a great many more functions and diversity than ever considered. To gain clarity of these functions it will be necessary to sample further methods of enrichment to reduce the number of contaminants and also to sample from different tissues, in both cases using new quantitative approaches. Even in the absence of further sampling, mining of the existing NE proteome datasets should yield considerable treasure for many years to come.

Figure Legends and Tables

Figure 1.
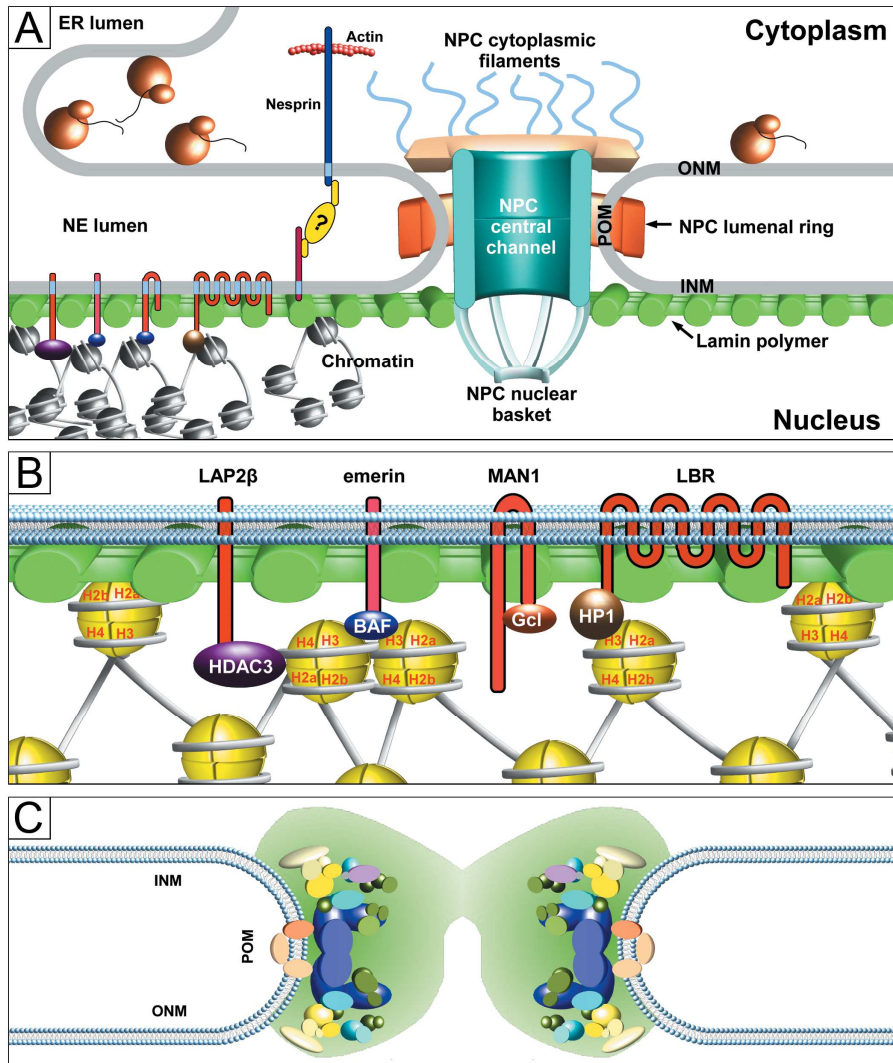
Schematic diagram of the NE with details of the NPC. (A) The nuclear envelope consists of outer and

inner nuclear membranes connected at the "pore membrane". The outer nuclear membrane (ONM) is

continuous with the rough and smooth endoplasmic reticulum (ER). The inner nuclear membrane

(INM) contains many unique integral proteins, which commonly are associated with the intermediate

filament lamin polymer. The pore membrane apposed to the nuclear pore complexes (NPCs) contains

specific integral proteins involved in membrane tethering of NPCs. Depending on their topology and

membrane subdomain, NE transmembrane proteins could have functions in the cytoplasm, nucleoplasm, or the perinuclear lumenal space. Some ONM-specific proteins have been identified such as the Syne/Nesprin protein families, but most proteins identified in this region such as ribosomal proteins share functions with the ER. Only a few characterized proteins have both been shown to contain most of their mass in the lumen, though more are likely based on proteomic results. (B) Many transmembrane proteins of the INM directly interact with the lamin polymer and/ or chromatin proteins, though only a small number of the first identified proteins have been tested for such characteristics. Among these, LAP2ß interacts with the chromatin remodeling protein histone deacetylase 3 (HDAC3). LAP2ß, emerin, and MAN1 all interact with the chromatin crosslinking protein BAF and transcriptional repressors such as germ cell-less (Gcl). LBR binds to both the heterochromatin protein HP1 and histone H3, and even lamins can directly bind to the core histones H2A and H2B. (C) The NPC proteomic studies identified many proteins that recently have been mapped into a high resolution structure (compared to electron microscope images; [30]). A depiction of this structure shows the rough positions of many nucleoporins and a density gradient of unstructured phenylalanine-glycine (FG) repeat containing regions (represented as a haze around the NPC core components) that are thought to play a significant role in the transport process.

Figure 2.
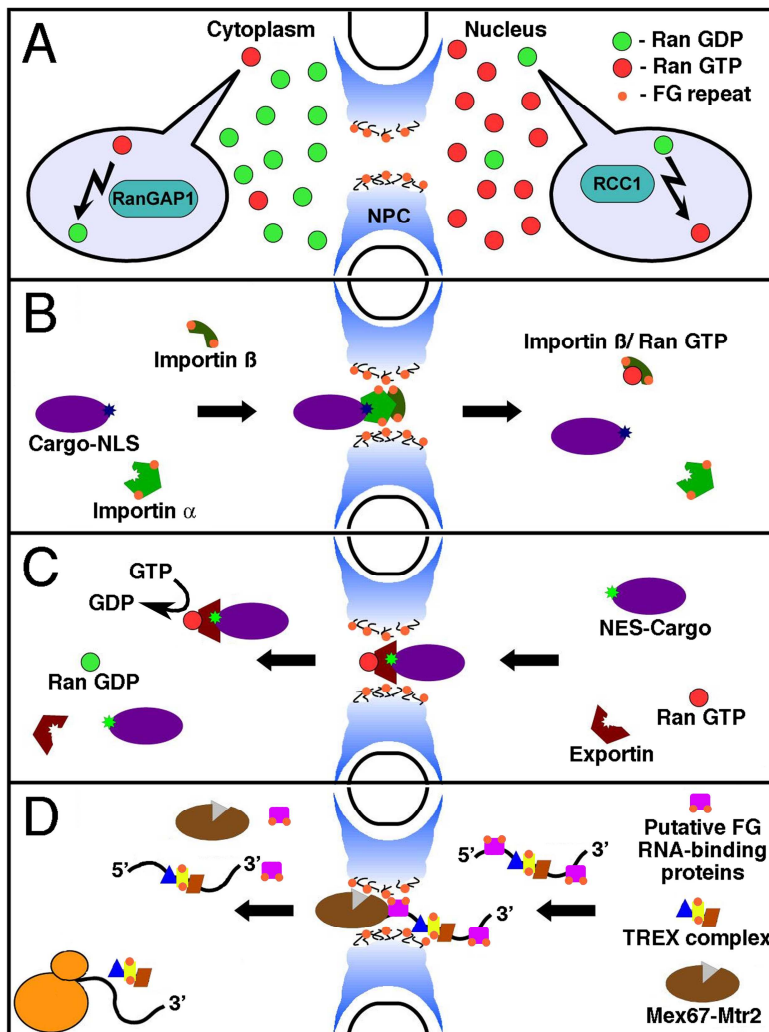
Transport through the NPC. (A) Transport through the central channel of the NPC is facilitated by the action of the small GTPase Ran. RanGTP is concentrated in the nucleus and RanGDP in the cytoplasm. RanGAPs (Ran GTPase Activating Proteins) facilitate conversion of the GTP to the GDP form in the cytoplasm while RanGEFs (Guanine nucleotide Exchange Factors) such as RCC1 mediate replenishment of RanGTP in the nucleus. Ran is thus a very abundant protein that will be associated with NPC proteins even though it is not part of the core NPC structure. (B) In nuclear import a transport cargo that has a nuclear localization signal (NLS) (depicted as "*") is recognized by the transport receptor importin α. Another transport receptor, importin ß, then recognizes the importin α-

receptor cargo and facilitates its transport through the central channel of the NPC. This is thought to be mediated by interactions between the FG repeats on core NPC proteins and FG repeats on the transport receptors that now coat the cargo. Once the receptor-cargo complex has passed through the NPC RanGTP in the nucleus binds to importin ß to facilitate dissociation of the receptor-cargo complex and thus release of the free cargo into the nucleoplasm. (C) In nuclear export a different kind of transport receptor called an exportin recognizes a nuclear export signal (NES) (depicted as "*") on the cargo and binds together with RanGTP. The complex then transports through the central channel of the NPC and GTP hydrolysis initiated by the RanGAPs releases both the Ran and the exportin. (D) For RNA export several transport receptors have been identified such as the Mex67-Mtr2 heterodimer. Several additional proteins, some of which contain FGs, have also been found to be important that form a complex called the TREX complex in yeast. We propose that the many RNA-binding proteins that harbor FGs identified in NE proteomic datasets might be part of an even greater complex of RNA-binding proteins that coat the surface of the RNAs and expose FGs to interact with the FGs on the core NPC proteins and facilitate transport. The proteins shown in Table 2 are good candidates for this task.

Figure 3.
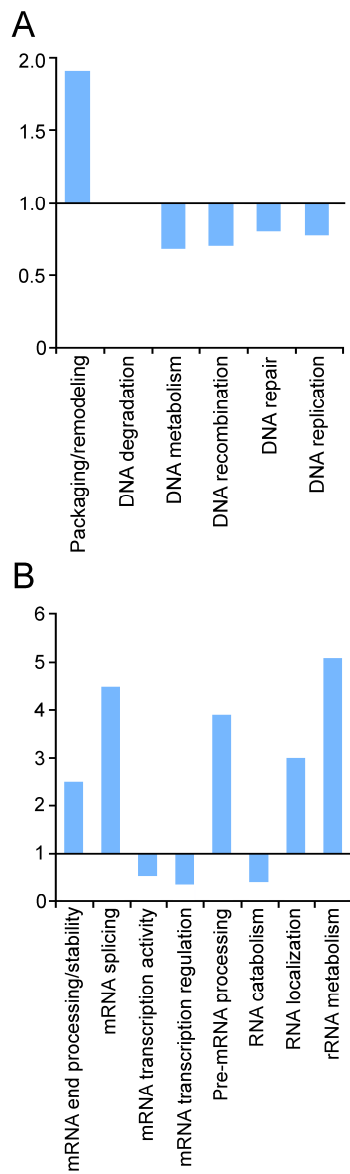
Functional distribution of proteins identified in the liver NE and MM proteomic study. (A) As subtraction of all proteins identified in the MM datasets would potentially miss interesting proteins that are nonetheless enriched in the NE, NE- and MM-enriched datasets were generated by comparing the number of spectra recovered per run for each dataset. If a NE protein had at least 5x more spectra than were recovered in the MM or vice-versa they were included in these datasets. The Panther (Protein ANalysis THrough Evolutionary Relationships) Classification System [115,116] was then

used to organize proteins in each dataset according to functional annotations. A clear enrichment for

proteins involved in DNA and RNA functions (DNA/RNA) was observed in the NE compared to the

MM. As only 2 proteins (<1%) recovered in the liver MM had anything to do with DNA function and

4 (1.7%) with RNA function, the % distribution of all DNA or RNA functions in the human genome

was compared with that for the proteins recovered in the NE-enriched dataset. The ratio of the percent

of a function in the NE to its percent represented in the total human proteome is presented. (A) DNA

functions represented 65/854 proteins in the NE-enriched dataset compared to 977/25431 proteins in

the total human dataset (or 7.6% vs 3.8%). Within the DNA set ther was a strong enrichment at the

NE for proteins involved in chromatin structure and remodeling. All other categories showed a

relative decrease in functions at the NE. (B) RNA functions represented 330 of the 854 proteins in

the NE-enriched dataset compared to 4295 of the 25431 proteins in the total human dataset (or 38.6%

vs 16.8%). Within the RNA dataset functions in RNA splicing and pre-mRNA processing were both

abundant and enriched for. Additionally functions in mRNA end processing and stability, RNA

localization and rRNA metabolism were enriched for, though much less abundant.

Table 1. NPC core proteins [74,77] with number of phenyalanine-glycine (FG) repeats.

Yeast	FG	Year (PubMed ID)	Mammalian	FG	Year (PubMed ID)
Nup1	17	**1990** (2190694)	Nup153	30	**1993** (8422679)
Nsp1	33	**1990** (2112428)	Nup62	6	**1990** (2295087)
Nic96	1	**1993** (7688296)	Nup93	3	**1997** (9348540)
Nup145N	14	**1994** (8044840)	Nup98	40	**1995** (7878057)
Nup145C	1	**1994** (8044840)	Nup96	1	**1999** (10087256)
Nup133	0	**1994** (7813444)	Nup133	2	**2001** (11564755)
Pom152	1	**1994** (8138573)	–	-	-
Nup42	29	**1995** (7634338)	NLP1/hCG1 (45)	14	**1999** (10358091)
Nup49	18	**1995** (1385442)	Nup58	11	**1991** (2050741)
Nup57	16	**1995** (7828598)	Nup54	8	**1991** (2050741)
Nup82	2	**1995** (7559750)	Nup88	2	**1997** (9049309)
Nup100	45	**1995** (1385442)	Nup98	40	**1995** (7878057)
Nup116	47	**1995** (1385442)	Nup98	40	**1995** (7878057)
Nup120	2	**1995** (8557736)	Nup160	3	**2001** (11684705)
Nup157	2	**1995** (8522578)	Nup155	0	**1993** (8458861)
Nup170	0	**1995** (8522578)	Nup155	0	**1993** (8458861)
Nup159	28	**1996** (8898365)	Nup214/CAN	45	**1994** (8108440)
Nup188	5	**1996** (8682854)	Nup188	3	**2000** (11029043)

Gle1(62)	1	**1996** (8848052)	hGle1(85)	0	**1998** (9618489)
Gle2(41)	0	**1996** (8970155)	Rae1/Gle2b (41)	3	**1997** (9370289)
Nup85	1	**1996** (8816998)	Nup75/Nup85	2	**1996** (8816998)
Nup84	0	**1997** (9166401)	Nup107	3	**1994** (8021268)
Ndc1 (74)	3	**1998** (9864355)	–	-	-
Nup53	4	**1998** (9864357)	Nup35	3	2002 (12196509)
Nup59	6	**1998** (9864357)	Nup35	3	2002 (12196509)
Nup192	4	**1999** (10428845)	Nup205	4	**2000** (11029043)
Seh1 (39)	0	2000 (10684247)	Sec13-like	0	2002 (12196509)
Nup60	0	2000 (10684247)	-	-	-
CDC31	0	2000 (10684247)	-	-	-
Pom34	1	2000 (10684247)	-	-	-
-	-	-	Pom121	24	**1993** (8335683)
-	-	-	Gp210	5	**1990** (2184032)
-	-	-	Nup358/RanBP2	20	**1995** (7775481)
-	-	-	ALADIN (60)	1	2004 (15666842)
-	-	-	Nup37	0	2002 (12196509)
-	-	-	Nup43	1	2002 (12196509)
-	-	-	Tpr (266)	0	**1994** (7798308)
-	-	-	Nup50	5	**1997** (9073512)

Table 2. RNA-binding proteins with FG repeats separated by polar residues found in the liver NE datasets.

FG Repeats	Protein Name	Accession
9	hnRNP core protein A1	NP_058944.1
9	hnRNP A2/B1 isoform 1	NP_058086.2
8	RNA binding motif protein 19	XP_222200.4
6	hnRNP M	NP_446328.1
6	hnRNP A0	XP_001001311.1
6	U5 snRNP-specific protein, 116 kDa	XP_001081526.1
6	DEAD box polypeptide 42	XP_001081592.1

Criteria used to search for FG repeat containing proteins were 5 or more FG repeats, at least 4 of which have >= 30% polar residues between them (provided >3 residues separate the FGs).

References

[1] M.W. Hetzer, T.C. Walther and I.W. Mattaj. Pushing the envelope: structure, function, and dynamics of the nuclear periphery. Annu Rev Cell Dev Biol 21 (2005) 347-380.

[2] A.J. Prunuske and K.S. Ullman. The nuclear envelope: form and reformation. Curr Opin Cell Biol 18 (2006) 108-116.

[3] E.C. Schirmer and R. Foisner. Proteins that associate with lamins: many faces, many functions. Exp Cell Res 313 (2007) 2167-2179.

[4] M. Suntharalingam and S.R. Wente. Peering through the pore: nuclear pore complex structure, assembly, and function. Dev Cell 4 (2003) 775-789.

[5] H.G. Callan and S.G. Tomlin. Experimental studies on amphibian oocyte nuclei. I. Investigation of the structure of the nuclear membrane by means of the electron microscope. Proc R Soc of Lond [Biol] 137 (1950) 367-378.

[6] D.J. Fry The nuclear envelope in mammalian cells, in: G.A. Jameson and D.M. Robinson (Eds.), Mammalian Cell Membranes, Butterworth, Woburn, Mass, 1976, pp. 197-265.

[7] E.D. Apel, R.M. Lewis, R.M. Grady and J.R. Sanes. Syne-1, a dystrophin- and Klarsicht-related protein associated with synaptic nuclei at the neuromuscular junction. J Biol Chem 275 (2000) 31986-31995.

[8] C.J. Malone, W.D. Fixsen, H.R. Horvitz and M. Han. UNC-84 localizes to the nuclear envelope and is required for nuclear migration and anchoring during C. elegans development. Development 126 (1999) 3171-3181.

[9] D.A. Starr and M. Han. Role of ANC-1 in tethering nuclei to the actin cytoskeleton. Science 298 (2002) 406-409.

[10] D.A. Starr and M. Han. A genetic approach to study the role of nuclear envelope components in nuclear positioning. Novartis Found Symp 264 (2005) 208-219; discussion 219-230.

[11] Q. Zhang, J.N. Skepper, F. Yang, J.D. Davies, L. Hegyi, R.G. Roberts, P.L. Weissberg, J.A. Ellis and C.M. Shanahan. Nesprins: a novel family of spectrin-repeat-containing proteins that localize to the nuclear membrane in multiple tissues. J Cell Sci 114 (2001) 4485-4498.

[12] K. Wilhelmsen, S.H. Litjens, I. Kuikman, N. Tshimbalanga, H. Janssen, I. van den Bout, K. Raymond and A. Sonnenberg. Nesprin-3, a novel outer nuclear membrane protein, associates with the cytoskeletal linker protein plectin. J Cell Biol 171 (2005) 799-810.

[13] E.C. Schirmer and L. Gerace. The nuclear membrane proteome: extending the envelope. Trends Biochem Sci 30 (2005) 551-558.

[14] L. Clements, S. Manilal, D.R. Love and G.E. Morris. Direct interaction between emerin and lamin A. Biochem Biophys Res Commun 267 (2000) 709-714.

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

[16] D.M. Hodzic, D.B. Yeater, L. Bengtsson, H. Otto and P.D. Stahl. Sun2 is a novel mammalian inner nuclear membrane protein. J Biol Chem 279 (2004) 25805-25812.

[17] M. Mansharamani and K.L. Wilson. Direct binding of nuclear membrane protein MAN1 to emerin in vitro and two modes of binding to barrier-to-autointegration factor. J Biol Chem 280 (2005) 13863-13870.

[18] M. Sakaki, H. Koike, N. Takahashi, N. Sasagawa, S. Tomioka, K. Arahata and S. Ishiura. Interaction between emerin and nuclear lamins. J Biochem (Tokyo) 129 (2001) 321-327.

[19] H.J. Worman, J. Yuan, G. Blobel and S.D. Georgatos. A lamin B receptor in the nuclear envelope. Proc Natl Acad Sci U S A 85 (1988) 8531-8534.

[20] A. Mattout-Drubezki and Y. Gruenbaum. Dynamic interactions of nuclear lamina proteins with chromatin and transcriptional machinery. Cell Mol Life Sci 60 (2003) 2053-2063.

[21] P.L. Paine, L.C. Moore and S.B. Horowitz. Nuclear envelope permeability. Nature 254 (1975) 109-114.

[22] N. Pante and M. Kann. Nuclear pore complex is able to transport macromolecules with diameters of about 39 nm. Mol Biol Cell 13 (2002) 425-434.

[23] H.J. Chial, M.P. Rout, T.H. Giddings and M. Winey. Saccharomyces cerevisiae Ndc1p is a shared component of nuclear pore complexes and spindle pole bodies. J Cell Biol 143 (1998) 1789-1800.

[24] U.F. Greber, A. Senior and L. Gerace. A major glycoprotein of the nuclear pore complex is a membrane-spanning polypeptide with a large lumenal domain and a small cytoplasmic tail. EMBO J 9 (1990) 1495-1502.

[25] E. Hallberg, R.W. Wozniak and G. Blobel. An integral membrane protein of the pore membrane domain of the nuclear envelope contains a nucleoporin-like region. J Cell Biol 122 (1993) 513-521.

[26] J. Mansfeld, S. Guttinger, L.A. Hawryluk-Gara, N. Pante, M. Mall, V. Galy, U. Haselmann, P. Muhlhausser, R.W. Wozniak, I.W. Mattaj, U. Kutay and W. Antonin. The conserved transmembrane nucleoporin NDC1 is required for nuclear pore complex assembly in vertebrate cells. Mol Cell 22 (2006) 93-103.

[27] C.K. Lau, V.A. Delmar and D.J. Forbes. Topology of yeast Ndc1p: predictions for the human NDC1/NET3 homologue. Anat Rec A Discov Mol Cell Evol Biol 288 (2006) 681-694.

[28] R.W. Wozniak, E. Bartnik and G. Blobel. Primary structure analysis of an integral membrane glycoprotein of the nuclear pore. J Cell Biol 108 (1989) 2083-2092.

[29] C.W. Akey and M. Radermacher. Architecture of the Xenopus nuclear pore complex revealed by three-dimensional cryo-electron microscopy. J Cell Biol 122 (1993) 1-19.

[30] F. Alber, S. Dokudovskaya, L.M. Veenhoff, W. Zhang, J. Kipper, D. Devos, A. Suprapto, O. Karni-Schmidt, R. Williams, B.T. Chait, A. Sali and M.P. Rout. The molecular architecture of the nuclear pore complex. Nature 450 (2007) 695-701.

[31] J.E. Hinshaw, B.O. Carragher and R.A. Milligan. Architecture and design of the nuclear pore complex. Cell 69 (1992) 1133-1141.

[32] J. Lammerding, P. Schulze, T. Takahashi, S. Kozlov, T. Sullivan, R. Kamm, C. Stewart and R. Lee. Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. J Clin Invest 113 (2004) 370-378.

[33] J. Liu, T. Ben-Shahar, D. Riemer, M. Treinin, P. Spann, K. Weber, A. Fire and Y. Gruenbaum. Essential roles for Caenorhabditis elegans lamin gene in nuclear organization, cell cycle progression, and spatial organization of nuclear pore complexes. Mol Biol Cell 11 (2000) 3937-3947.

[34] A.C. Rowat, J. Lammerding and J.H. Ipsen. Mechanical properties of the cell nucleus and the effect of emerin deficiency. Biophys J 91 (2006) 4649-4664.

[35] E.C. Schirmer and L. Gerace. The stability of the nuclear lamina polymer changes with the composition of lamin subtypes according to their individual binding strengths. J Biol Chem 279 (2004) 42811-42817.

[36] E.C. Schirmer, T. Guan and L. Gerace. Involvement of the lamin rod domain in heterotypic lamin interactions important for nuclear organization. J Cell Biol 153 (2001) 479-489.

[37] S. Osada, S.Y. Ohmori and M. Taira. XMAN1, an inner nuclear membrane protein, antagonizes BMP signaling by interacting with Smad1 in Xenopus embryos. Development 130 (2003) 1783-1794.

[38] D. Pan, L.D. Estevez-Salmeron, S.L. Stroschein, X. Zhu, J. He, S. Zhou and K. Luo. The integral inner nuclear membrane protein MAN1 physically interacts with the R-Smad proteins to repress signaling by the transforming growth factor-{beta} superfamily of cytokines. J Biol Chem 280 (2005) 15992-16001.

[39] R.L. Steen, S.B. Martins, K. Tasken and P. Collas. Recruitment of protein phosphatase 1 to the nuclear envelope by A-kinase anchoring protein AKAP149 is a prerequisite for nuclear lamina assembly. J Cell Biol 150 (2000) 1251-1262.

[40] E. Markiewicz, K. Tilgner, N. Barker, M. van de Wetering, H. Clevers, M. Dorobek, I. Hausmanowa-Petrusewicz, F.C. Ramaekers, J.L. Broers, W.M. Blankesteijn, G. Salpingidou, R.G. Wilson, J.A. Ellis and C.J. Hutchison. The inner nuclear membrane protein emerin regulates beta-catenin activity by restricting its accumulation in the nucleus. EMBO J 25 (2006) 3275-3285.

[41] D.J. Ellis, H. Jenkins, W.G. Whitfield and C.J. Hutchison. 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 (1997) 2507-2518.

[42] T.P. Spann, R.D. Moir, A.E. Goldman, R. Stick and R.D. Goldman. Disruption of nuclear lamin organization alters the distribution of replication factors and inhibits DNA synthesis. J Cell Biol 136 (1997) 1201-1212.

[43] S. Martins, S. Eikvar, K. Furukawa and P. Collas. HA95 and LAP2 beta mediate a novel chromatin-nuclear envelope interaction implicated in initiation of DNA replication. J Cell Biol 160 (2003) 177-188.

[44] E. Nili, G.S. Cojocaru, Y. Kalma, D. Ginsberg, N.G. Copeland, D.J. Gilbert, N.A. Jenkins, R. Berger, S. Shaklai, N. Amariglio, F. Brok-Simoni, A.J. Simon and G. Rechavi. Nuclear membrane protein LAP2beta mediates transcriptional repression alone and together with its binding partner GCL (germ-cell-less). J Cell Sci 114 (2001) 3297-3307.

[45] B.R. Johnson, R.T. Nitta, R.L. Frock, L. Mounkes, D.A. Barbie, C.L. Stewart, E. Harlow and B.K. Kennedy. A-type lamins regulate retinoblastoma protein function by promoting subnuclear localization and preventing proteasomal degradation. Proc Natl Acad Sci U S A 101 (2004) 9677-9682.

[46]   T. Ozaki, M. Saijo, K. Murakami, H. Enomoto, Y. Taya and S. Sakiyama. Complex formation between lamin A and the retinoblastoma gene product: identification of the domain on lamin A required for its interaction. Oncogene 9 (1994) 2649-2653.

[47]   R.I. Kumaran, B. Muralikrishna and V.K. Parnaik. Lamin A/C speckles mediate spatial organization of splicing factor compartments and RNA polymerase II transcription. J Cell Biol 159 (2002) 783-793.

[48]   B.C. Capell and F.S. Collins. Human laminopathies: nuclei gone genetically awry. Nat Rev Genet 7 (2006) 940-952.

[49]   R. Foisner, U. Aebi, G. Bonne, Y. Gruenbaum and G. Novelli. 141st ENMC International Workshop inaugural meeting of the EURO-Laminopathies project "Nuclear Envelope-linked Rare Human Diseases: From Molecular Pathophysiology towards Clinical Applications", 10-12 March 2006, Naarden, The Netherlands. Neuromuscul Disord 17 (2007) 655-660.

[50]   H.J. Worman and G. Bonne. "Laminopathies": a wide spectrum of human diseases. Exp Cell Res 313 (2007) 2121-2133.

[51]   S. Shaklai, N. Amariglio, G. Rechavi and A.J. Simon. Gene silencing at the nuclear periphery. FEBS J 274 (2007) 1383-1392.

[52]   M. Crisp, Q. Liu, K. Roux, J.B. Rattner, C. Shanahan, B. Burke, P.D. Stahl and D. Hodzic. Coupling of the nucleus and cytoplasm: role of the LINC complex. J Cell Biol 172 (2006) 41-53.

[53]   B. Kosova, N. Pante, C. Rollenhagen, A. Podtelejnikov, M. Mann, U. Aebi and E. Hurt. Mlp2p, a component of nuclear pore attached intranuclear filaments, associates with nic96p. J Biol Chem 275 (2000) 343-350.

[54]   C. Strambio-de-Castillia, G. Blobel and M.P. Rout. Proteins connecting the nuclear pore complex with the nuclear interior. J Cell Biol 144 (1999) 839-855.

[55]   V. Galy, J.C. Olivo-Marin, H. Scherthan, V. Doye, N. Rascalou and U. Nehrbass. Nuclear pore complexes in the organization of silent telomeric chromatin. Nature 403 (2000) 108-112.

[56]   H. Scherthan, M. Jerratsch, B. Li, S. Smith, M. Hulten, T. Lock and T. de Lange. Mammalian meiotic telomeres: protein composition and redistribution in relation to nuclear pores. Mol Biol Cell 11 (2000) 4189-4203.

[57]   R. Reichelt, A. Holzenburg, E.L. Buhle, Jr., M. Jarnik, A. Engel and U. Aebi. Correlation between structure and mass distribution of the nuclear pore complex and of distinct pore complex components. J Cell Biol 110 (1990) 883-894.

[58]   M.P. Rout and G. Blobel. Isolation of the yeast nuclear pore complex. J Cell Biol 123 (1993) 771-783.

[59]   Q. Yang, M.P. Rout and C.W. Akey. Three-dimensional architecture of the isolated yeast nuclear pore complex: functional and evolutionary implications. Mol Cell 1 (1998) 223-234.

[60]   L. Gerace and B. Burke. Functional organization of the nuclear envelope. Annu Rev Cell Biol 4 (1988) 335-374.

[61]   L. Gerace, Y. Ottaviano and C. Kondor-Koch. Identification of a major polypeptide of the nuclear pore complex. J Cell Biol 95 (1982) 826-837.

[62]   L.I. Davis and G. Blobel. Identification and characterization of a nuclear pore complex protein. Cell 45 (1986) 699-709.

[63]   M.K. Park, M. D'Onofrio, M.C. Willingham and J.A. Hanover. A monoclonal antibody against a family of nuclear pore proteins (nucleoporins): O-linked N-acetylglucosamine is part of the immunodeterminant. Proc Natl Acad Sci U S A 84 (1987) 6462-6466.

[64]   C.M. Snow, A. Senior and L. Gerace. Monoclonal antibodies identify a group of nuclear pore complex glycoproteins. J Cell Biol 104 (1987) 1143-1156.

[65]   J.P. Aris and G. Blobel. Yeast nuclear envelope proteins cross react with an antibody against mammalian pore complex proteins. J Cell Biol 108 (1989) 2059-2067.

[66]   L.I. Davis and G.R. Fink. The NUP1 gene encodes an essential component of the yeast nuclear pore complex. Cell 61 (1990) 965-978.

[67]   M. Carmo-Fonseca, H. Kern and E.C. Hurt. Human nucleoporin p62 and the essential yeast nuclear pore protein NSP1 show sequence homology and a similar domain organization. Eur J Cell Biol 55 (1991) 17-30.

[68]   U. Nehrbass, H. Kern, A. Mutvei, H. Horstmann, B. Marshallsay and E.C. Hurt. NSP1: a yeast nuclear envelope protein localized at the nuclear pores exerts its essential function by its carboxy-terminal domain. Cell 61 (1990) 979-989.

[69]   C.M. Starr, M. D'Onofrio, M.K. Park and J.A. Hanover. Primary sequence and heterologous expression of nuclear pore glycoprotein p62. J Cell Biol 110 (1990) 1861-1871.

[70]   T. Hu, T. Guan and L. Gerace. Molecular and functional characterization of the p62 complex, an assembly of nuclear pore complex glycoproteins. J Cell Biol 134 (1996) 589-601.

[71]   K. Kita, S. Omata and T. Horigome. Purification and characterization of a nuclear pore glycoprotein complex containing p62. J Biochem 113 (1993) 377-382.

[72]   M. Marelli, J.D. Aitchison and R.W. Wozniak. Specific binding of the karyopherin Kap121p to a subunit of the nuclear pore complex containing Nup53p, Nup59p, and Nup170p. J Cell Biol 143 (1998) 1813-1830.

[73]   P. Grandi, V. Doye and E.C. Hurt. Purification of NSP1 reveals complex formation with 'GLFG' nucleoporins and a novel nuclear pore protein NIC96. EMBO J 12 (1993) 3061-3071.

[74]   M.P. Rout, J.D. Aitchison, A. Suprapto, K. Hjertaas, Y. Zhao and B.T. Chait. The yeast nuclear pore complex: composition, architecture, and transport mechanism. J Cell Biol 148 (2000) 635-651.

[75]   G.G. Maul and L. Deaven. Quantitative determination of nuclear pore complexes in cycling cells with differing DNA content. J Cell Biol 73 (1977) 748-760.

[76]   M. Winey, D. Yarar, T.H. Giddings, Jr. and D.N. Mastronarde. Nuclear pore complex number and distribution throughout the Saccharomyces cerevisiae cell cycle by three-dimensional reconstruction from electron micrographs of nuclear envelopes. Mol Biol Cell 8 (1997) 2119-2132.

[77]   J. Cronshaw, A. Krutchinsky, W. Zhang, B. Chait and M. Matunis. Proteomic analysis of the mammalian nuclear pore complex. J Cell Biol 158 (2002) 915-927.

[78]   R.P. Aaronson and G. Blobel. Isolation of nuclear pore complexes in association with a lamina. Proc Natl Acad Sci U S A 72 (1975) 1007-1011.

[79]   G. Blobel and V.R. Potter. Nuclei from rat liver: isolation method that combines purity with high yield. Science 154 (1966) 1662-1665.

[80]   N. Dwyer and G. Blobel. A modified procedure for the isolation of a pore complex-lamina fraction from rat liver nuclei. J Cell Biol 70 (1976) 581-591.

[81]   N.K. Pryer, N.R. Salama, R. Schekman and C.A. Kaiser. Cytosolic Sec13p complex is required for vesicle formation from the endoplasmic reticulum in vitro. J Cell Biol 120 (1993) 865-875.

[82]   S. Siniossoglou, C. Wimmer, M. Rieger, V. Doye, H. Tekotte, C. Weise, S. Emig, A. Segref and E.C. Hurt. A novel complex of nucleoporins, which includes Sec13p and a Sec13p homolog, is essential for normal nuclear pores. Cell 84 (1996) 265-275.

[83]   E.C. Schirmer, L. Florens, T. Guan, J.R.r. Yates and L. Gerace. Nuclear membrane proteins with potential disease links found by subtractive proteomics. Science 301 (2003) 1380-1382.

[84]   D. Devos, S. Dokudovskaya, R. Williams, F. Alber, N. Eswar, B.T. Chait, M.P. Rout and A. Sali. Simple fold composition and modular architecture of the nuclear pore complex. Proc Natl Acad Sci U S A 103 (2006) 2172-2177.

[85]   R.B. Langkjaer, P.F. Cliften, M. Johnston and J. Piskur. Yeast genome duplication was followed by asynchronous differentiation of duplicated genes. Nature 421 (2003) 848-852.

[86]   L. Gerace, A. Blum and G. Blobel. Immunocytochemical localization of the major polypeptides of the nuclear pore complex-lamina fraction. Interphase and mitotic distribution. J Cell Biol 79 (1978) 546-566.

[87]   A. Senior and L. Gerace. Integral membrane proteins specific to the inner nuclear membrane and associated with the nuclear lamina. J Cell Biol 107 (1988) 2029-2036.

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

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

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

[91]   M. Dreger, L. Bengtsson, T. Schoneberg, H. Otto and F. Hucho. Nuclear envelope proteomics: novel integral membrane proteins of the inner nuclear membrane. Proc Natl Acad Sci U S A 98 (2001) 11943-11948.

[92]   M.P. Washburn, D. Wolters and J.R.r. Yates. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. Nat Biotechnol 19 (2001) 242-247.

[93]   D.A. Wolters, M.P. Washburn and J.R.r. Yates. An automated multidimensional protein identification technology for shotgun proteomics. Anal Chem 73 (2001) 5683-5690.

[94]   V. Santoni, M. Molloy and T. Rabilloud. Membrane proteins and proteomics: un amour impossible? Electrophoresis 21 (2000) 1054-1070.

[95]   L. Florens, N. Korfali and E.C. Schirmer. Subcellular fractionation and proteomics of nuclear envelopes. Methods Mol Biol 432 (2008) 117-137.

[96] R.E. Goodchild and W.T. Dauer. The AAA+ protein torsinA interacts with a conserved domain present in LAP1 and a novel ER protein. J Cell Biol 168 (2005) 855-862.

[97] P. Askjaer, A. Bachi, M. Wilm, F.R. Bischoff, D.L. Weeks, V. Ogniewski, M. Ohno, C. Niehrs, J. Kjems, I.W. Mattaj and M. Fornerod. RanGTP-regulated interactions of CRM1 with nucleoporins and a shuttling DEAD-box helicase. Mol Cell Biol 19 (1999) 6276-6285.

[98] S.S. Tseng, P.L. Weaver, Y. Liu, M. Hitomi, A.M. Tartakoff and T.H. Chang. Dbp5p, a cytosolic RNA helicase, is required for poly(A)+ RNA export. EMBO J 17 (1998) 2651-2662.

[99] J. Zhao, S.B. Jin, B. Bjorkroth, L. Wieslander and B. Daneholt. The mRNA export factor Dbp5 is associated with Balbiani ring mRNP from gene to cytoplasm. EMBO J 21 (2002) 1177-1187.

[100] T. Ohba, M. Nakamura, H. Nishitani and T. Nishimoto. Self-organization of microtubule asters induced in Xenopus egg extracts by GTP-bound Ran. Science 284 (1999) 1356-1358.

[101] C. Wiese, A. Wilde, M.S. Moore, S.A. Adam, A. Merdes and Y. Zheng. Role of importin-beta in coupling Ran to downstream targets in microtubule assembly. Science 291 (2001) 653-656.

[102] N. Belgareh, G. Rabut, S.W. Bai, M. van Overbeek, J. Beaudouin, N. Daigle, O.V. Zatsepina, F. Pasteau, V. Labas, M. Fromont-Racine, J. Ellenberg and V. Doye. An evolutionarily conserved NPC subcomplex, which redistributes in part to kinetochores in mammalian cells. J Cell Biol 154 (2001) 1147-1160.

[103] R.D. Moir, T.P. Spann and R.D. Goldman. The dynamic properties and possible functions of nuclear lamins. Int Rev Cytol (1995) 141-182.

[104] M.Y. Tsai, S. Wang, J.M. Heidinger, D.K. Shumaker, S.A. Adam, R.D. Goldman and Y. Zheng. A mitotic lamin B matrix induced by RanGTP required for spindle assembly. Science 311 (2006) 1887-1893.

[105] K.K. Lee, T. Haraguchi, R.S. Lee, T. Koujin, Y. Hiraoka and K.L. Wilson. Distinct functional domains in emerin bind lamin A and DNA-bridging protein BAF. J Cell Sci 114 (2001) 4567-4573.

[106] F.L. Wilkinson, J.M. Holaska, Z. Zhang, A. Sharma, S. Manilal, I. Holt, S. Stamm, K.L. Wilson and G.E. Morris. Emerin interacts in vitro with the splicing-associated factor, YT521-B. Eur J Biochem 270 (2003) 2459-2466.

[107] J.M. Holaska, S. Rais-Bahrami and K.L. Wilson. Lmo7 is an emerin-binding protein that regulates the transcription of emerin and many other muscle-relevant genes. Hum Mol Gen 15 (2006) 3459-3472.

[108] L. Bengtsson and K.L. Wilson. Multiple and surprising new functions for emerin, a nuclear membrane protein. Curr Opin Cell Biol 16 (2004) 73-79.

[109] J.M. Holaska, K. Lee, A.K. Kowalski and K.L. Wilson. Transcriptional repressor germ cell-less (GCL) and barrier to autointegration factor (BAF) compete for binding to emerin in vitro. J Biol Chem 278 (2003) 6969-6975.

[110] G. Salpingidou, A. Smertenko, I. Hausmanowa-Petrucewicz, P.J. Hussey and C.J. Hutchison. A novel role for the nuclear membrane protein emerin in association of the centrosome to the outer nuclear membrane. J Cell Biol 178 (2007) 897-904.

[111] Q. Zhang, C.D. Ragnauth, J.N. Skepper, N.F. Worth, D.T. Warren, R.G. Roberts, P.L. Weissberg, J.A. Ellis and C.M. Shanahan. Nesprin-2 is a multi-isomeric protein that binds lamin and emerin at the nuclear envelope and forms a subcellular network in skeletal muscle. J Cell Sci 118 (2005) 673-687.

[112] R.E. Goodchild and W.T. Dauer. Mislocalization to the nuclear envelope: an effect of the dystonia-causing torsinA mutation. Proc Natl Acad Sci U S A 101 (2004) 847-852.

[113] T.V. Naismith, J.E. Heuser, X.O. Breakefield and P.I. Hanson. TorsinA in the nuclear envelope. Proc Natl Acad Sci U S A 101 (2004) 7612-7617.

[114] I.H. Chen, M. Huber, T. Guan, A. Bubeck and L. Gerace. Nuclear envelope transmembrane proteins (NETs) that are up-regulated during myogenesis. BMC Cell Biology 7 (2006) 38.

[115] H. Mi, N. Guo, A. Kejariwal and P.D. Thomas. PANTHER version 6: protein sequence and function evolution data with expanded representation of biological pathways. Nucleic Acids Res 35 (2007) D247-252.

[116] P.D. Thomas, M.J. Campbell, A. Kejariwal, H. Mi, B. Karlak, R. Daverman, K. Diemer, A. Muruganujan and A. Narechania. PANTHER: a library of protein families and subfamilies indexed by function. Genome Res 13 (2003) 2129-2141.

[117] J.P. Humbert, N. Matter, J.C. Artault, P. Koppler and A.N. Malviya. Inositol 1,4,5-trisphosphate receptor is located to the inner nuclear membrane vindicating regulation of nuclear calcium signaling by inositol 1,4,5-trisphosphate. Discrete distribution of inositol phosphate receptors to inner and outer nuclear membranes. J Biol Chem 271 (1996) 478-485.

[118] L. Lanini, O. Bachs and E. Carafoli. The calcium pump of the liver nuclear membrane is identical to that of endoplasmic reticulum. J Biol Chem 267 (1992) 11548-11552.

[119] C. Cruttwell, J. Bernard, M. Hilly, V. Nicolas, R.E. Tunwell and J.P. Mauger. Dynamics of the inositol 1,4,5-trisphosphate receptor during polarisation of MDCK cells. Biol Cell (2005).

[120] J.V. Gerasimenko, Y. Maruyama, K. Yano, N.J. Dolman, A.V. Tepikin, O.H. Petersen and O.V. Gerasimenko. NAADP mobilizes Ca2+ from a thapsigargin-sensitive store in the nuclear envelope by activating ryanodine receptors. J Cell Biol 163 (2003) 271-282.

[121] E.S. Erickson, O.L. Mooren, D. Moore-Nichols and R.C. Dunn. Activation of ryanodine receptors in the nuclear envelope alters the conformation of the nuclear pore complex. Biophys Chem 112 (2004) 1-7.

[122] G.C. Pare, J.L. Easlick, J.M. Mislow, E.M. McNally and M.S. Kapiloff. Nesprin-1alpha contributes to the targeting of mAKAP to the cardiac myocyte nuclear envelope. Exp Cell Res 303 (2005) 388-399.

[123] R.E. Dolmetsch, K. Xu and R.S. Lewis. Calcium oscillations increase the efficiency and specificity of gene expression. Nature 392 (1998) 933-936.

[124] M.F. Arteaga, R. Gutierrez, J. Avila, A. Mobasheri, L. Diaz-Flores and P. Martin-Vasallo. Regeneration influences expression of the Na+, K+-atpase subunit isoforms in the rat peripheral nervous system. Neuroscience 129 (2004) 691-702.

[125] P.T. Ronaldson, M. Bendayan, D. Gingras, M. Piquette-Miller and R. Bendayan. Cellular localization and functional expression of P-glycoprotein in rat astrocyte cultures. J Neurochem 89 (2004) 788-800.

[126] Y.J. Sigal, M.I. McDermott and A.J. Morris. Integral membrane lipid phosphatases/phosphotransferases: common structure and diverse functions. Biochem J 387 (2005) 281-293.

[127] O. Krut, K. Wiegmann, H. Kashkar, B. Yazdanpanah and M. Kronke. Novel tumor necrosis factor-responsive mammalian neutral sphingomyelinase-3 is a C-tail-anchored protein. J Biol Chem 281 (2006) 13784-13793.

[128] Y. Yang, J. Cao and Y. Shi. Identification and characterization of a gene encoding human LPGAT1, an endoplasmic reticulum-associated lysophosphatidylglycerol acyltransferase. J Biol Chem 279 (2004) 55866-55874.

[129] M. Hallman and L. Gluck. Phosphatidylglycerol in lung surfactant. II. Subcellular distribution and mechanism of biosynthesis in vitro. Biochim Biophys Acta 409 (1975) 172-191.

[130] L. Breuza, R. Halbeisen, P. Jeno, S. Otte, C. Barlowe, W. Hong and H.P. Hauri. Proteomics of endoplasmic reticulum-Golgi intermediate compartment (ERGIC) membranes from brefeldin A-treated HepG2 cells identifies ERGIC-32, a new cycling protein that interacts with human Erv46. J Biol Chem 279 (2004) 47242-47253.

[131] A. Audhya, A. Desai and K. Oegema. A role for Rab5 in structuring the endoplasmic reticulum. J Cell Biol 178 (2007) 43-56.

[132] J.A. Jacobsson, T. Haitina, J. Lindblom and R. Fredriksson. Identification of six putative human transporters with structural similarity to the drug transporter SLC22 family. Genomics 90 (2007) 595-609.

[133] M.W. Coolen, K.M. Van Loo, N.N. Van Bakel, D.J. Pulford, L. Serneels, B. De Strooper, B.A. Ellenbroek, A.R. Cools and G.J. Martens. Gene dosage effect on gamma-secretase component Aph-1b in a rat model for neurodevelopmental disorders. Neuron 45 (2005) 497-503.

[134] F. Diao, S. Li, Y. Tian, M. Zhang, L.G. Xu, Y. Zhang, R.P. Wang, D. Chen, Z. Zhai, B. Zhong, P. Tien and H.B. Shu. Negative regulation of MDA5- but not RIG-I-mediated innate antiviral signaling by the dihydroxyacetone kinase. Proc Natl Acad Sci U S A 104 (2007) 11706-11711.

[135] L. Bengtsson. What MAN1 does to the Smads TGFß/BMP signaling and the nuclear envelope. FEBS J 274 (2007) 1374-1382.

[136] J.L. Broers, B.M. Machiels, H.J. Kuijpers, F. Smedts, R. van den Kieboom, Y. Raymond and F.C. Ramaekers. A- and B-type lamins are differentially expressed in normal human tissues. Histochem Cell Biol 107 (1997) 505-517.

[137] M. Jansen, B. Machiels, A. Hopman, J. Broers, F. Bot, J. Arends, F. Ramaekers and H. Schouten. Comparison of A and B-type lamin expression in reactive lymph nodes and nodular sclerosing Hodgkin's disease. Histopathology 31 (1997) 304-312.

[138] R.A. Rober, K. Weber and M. Osborn. Differential timing of nuclear lamin A/C expression in the various organs of the mouse embryo and the young animal: a developmental study. Development 105 (1989) 365-378.

[139] M. Alsheimer and R. Benavente. Change of karyoskeleton during mammalian spermatogenesis: expression pattern of nuclear lamin C2 and its regulation. Exp Cell Res 228 (1996) 181-188.

[140] A.I. Su, M.P. Cooke, K.A. Ching, Y. Hakak, J.R. Walker, T. Wiltshire, A.P. Orth, R.G. Vega, L.M. Sapinoso, A. Moqrich, A. Patapoutian, G.M. Hampton, P.G. Schultz

and J.B. Hogenesch. Large-scale analysis of the human and mouse transcriptomes. Proc Natl Acad Sci U S A 99 (2002) 4465-4470.

[141] D.M. Kavanagh, W.E. Powell, P. Malik, V. Lazou and E.C. Schirmer. Organelle proteome variation among different cell types: lessons from nuclear membrane proteins. Subcell Biochem 43 (2007) 51-76.

[142] T. Kislinger, B. Cox, A. Kannan, C. Chung, P. Hu, A. Ignatchenko, M.S. Scott, A.O. Gramolini, Q. Morris, M.T. Hallett, J. Rossant, T.R. Hughes, B. Frey and A. Emili. Global survey of organ and organelle protein expression in mouse: combined proteomic and transcriptomic profiling. Cell 125 (2006) 173-186.

[143] V.K. Mootha, J. Bunkenborg, J.V. Olsen, M. Hjerrild, J.R. Wisniewski, E. Stahl, M.S. Bolouri, H.N. Ray, S. Sihag, M. Kamal, N. Patterson, E.S. Lander and M. Mann. Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. Cell 115 (2003) 629-640.

[144] D. Tunnah, C.A. Sewry, D. Vaux, E.C. Schirmer and G.E. Morris. The apparent absence of lamin B1 and emerin in many tissue nuclei is due to epitope masking. J Mol Histol 36 (2005) 337-344.

[145] G.S. Wilkie and E.C. Schirmer. Guilt by association: the nuclear envelope proteome and disease. Mol Cell Proteomics 5 (2006) 1865-1875.

[146] M. Olsson, S. Scheele and P. Ekblom. Limited expression of nuclear pore membrane glycoprotein 210 in cell lines and tissues suggests cell-type specific nuclear pores in metazoans. Exp Cell Res 292 (2004) 359-370.

[147] M. Cohen, N. Feinstein, K.L. Wilson and Y. Gruenbaum. Nuclear pore protein gp210 is essential for viability in HeLa cells and Caenorhabditis elegans. Mol Biol Cell 14 (2003) 4230-4237.

[148] F. Stavru, B.B. Hulsmann, A. Spang, E. Hartmann, V.C. Cordes and D. Gorlich. NDC1: a crucial membrane-integral nucleoporin of metazoan nuclear pore complexes. J Cell Biol 173 (2006) 509-519.

[149] F. Stavru, G. Nautrup-Pedersen, V.C. Cordes and D. Gorlich. Nuclear pore complex assembly and maintenance in POM121- and gp210-deficient cells. J Cell Biol 173 (2006) 477-483.

[150] F. Alber, S. Dokudovskaya, L.M. Veenhoff, W. Zhang, J. Kipper, D. Devos, A. Suprapto, O. Karni-Schmidt, R. Williams, B.T. Chait, M.P. Rout and A. Sali. Determining the architectures of macromolecular assemblies. Nature 450 (2007) 683-694.

[151] S.R. Wente, M.P. Rout and G. Blobel. A new family of yeast nuclear pore complex proteins. J Cell Biol 119 (1992) 705-723.

[152] D.P. Denning, S.S. Patel, V. Uversky, A.L. Fink and M. Rexach. Disorder in the nuclear pore complex: the FG repeat regions of nucleoporins are natively unfolded. Proc Natl Acad Sci U S A 100 (2003) 2450-2455.

[153] R.Y. Lim, N.P. Huang, J. Koser, J. Deng, K.H. Lau, K. Schwarz-Herion, B. Fahrenkrog and U. Aebi. Flexible phenylalanine-glycine nucleoporins as entropic barriers to nucleocytoplasmic transport. Proc Natl Acad Sci U S A 103 (2006) 9512-9517.

[154] R.Y. Lim, B. Fahrenkrog, J. Koser, K. Schwarz-Herion, J. Deng and U. Aebi. Nanomechanical basis of selective gating by the nuclear pore complex. Science 318 (2007) 640-643.

[155] S. Frey, R.P. Richter and D. Gorlich. FG-rich repeats of nuclear pore proteins form a three-dimensional meshwork with hydrogel-like properties. Science 314 (2006) 815-817.

[156] M. Rexach and G. Blobel. Protein import into nuclei: association and dissociation reactions involving transport substrate, transport factors, and nucleoporins. Cell 83 (1995) 683-692.

[157] K. Strasser, S. Masuda, P. Mason, J. Pfannstiel, M. Oppizzi, S. Rodriguez-Navarro, A.G. Rondon, A. Aguilera, K. Struhl, R. Reed and E. Hurt. TREX is a conserved complex coupling transcription with messenger RNA export. Nature 417 (2002) 304-308.

[158] K. Strasser and E. Hurt. Splicing factor Sub2p is required for nuclear mRNA export through its interaction with Yra1p. Nature 413 (2001) 648-652.