Background

The nuclear envelope (NE)

The nucleus is enclosed by two concentric lipid bilayers, the outer nuclear membrane (ONM) which, is continuous with the rough endoplasmic reticulum (ER), and the inner nuclear membrane (INM)(Stewart et al., 2007; Doucet and Hetzer, 2010). The two membranes are separated by the perinuclear space (PNS). Together these membranes create a barrier between the nucleus and the cytoplasm, called the nuclear envelope (NE). The NE is perforated by nuclear pores, points of fusion between the membranes, occupied by the nuclear pore complexes (NPC), that account for regulated nucleo-cytoplasmic transport(Rout and Aitchison, 2001). The ONM is continuous with the ER and is covered with ribosomes, yet it contains a distinct set of proteins. One group of ONM proteins is the KASH, (Klarsicht, ANC-1 and Syne homology) domain proteins. These proteins are called Nesprins and are involved in an array of functions such as centrosome positioning, nuclear migration and attachment of the nucleus to the microtubule and actin cytoskeleton (Starr and Fischer, 2005). These functions are carried out in complex with Sun domain proteins of the INM, which binds to KASH-domains in the perinuclear space (Starr and Fischer, 2005). The ONM provides a connective platform for cytoplasmic components docking or associating with the nucleus, important for i.e. cell signaling and gene regulation (Markiewicz et al., 2006). See INM proteins section page 5.

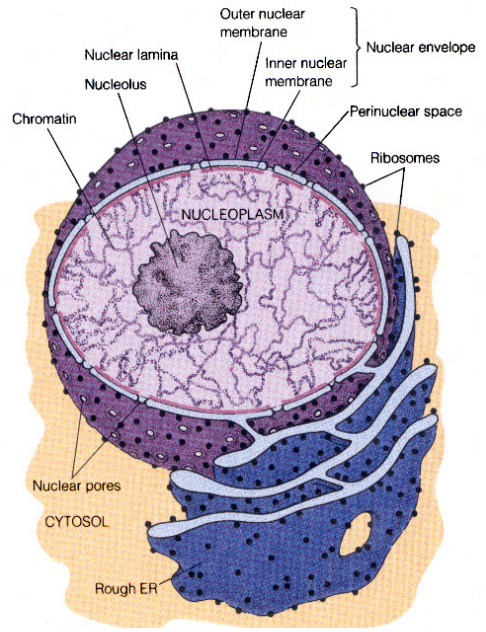

Fig 1: Adapted from

www.salem.k12.va.us/staff/jwright/vocabulary/nuclear_envelope.html

The inner nuclear membrane (INM)

The INM constitutes a highly specialized membrane domain with a specific subset of membrane proteins, many of which associates with the nuclear lamina (Holaska et al., 2002; Gruenbaum et al., 2003). The nuclear lamina is built up of type V intermediate filaments and lamina binding proteins. The nuclear lamina gives structure and rigidity to the nucleus (Dahl et al., 2004), and serves as a docking site for heterochromatin as well as for NPCs (Lammerding et al., 2004). There are two major types of lamina groups, A-type lamins consisting of Lamin A and C and B-type lamins (Stuurman et al., 1998). The nuclear lamins are transiently disassembled during mitosis, the A-type lamins are completely solubilized, whereas the B type lamins remain associated with the membranes (Gerace and Blobel, 1980).

Traditionally the NE has primarily been looked upon as a passive compartment barrier but since the early eighties, research groups have generated hypotheses that subscribes it a more active role i.e. in chromatin organization (Blobel, 1985). Some of these hypotheses have been experimentally tested and it has been shown that the INM is indeed involved in nuclear architecture and chromatin organizations. As an example it was shown that alterations in the INM affects both transcription and proliferation (Van de Vosse et al., 2011). The nuclear periphery is generally considered as docking site for heterochromatin, mainly containing transcriptionally inactive genes. However with new elegant methods such as DAM-ID and chromatin immunoprecipitation (ChIP) research groups have now established that there are islets of euchromatin even in the peripheral nucleus i.e. in the vicinity of the NPC (Casolari et al., 2004; Taddei et al., 2004).

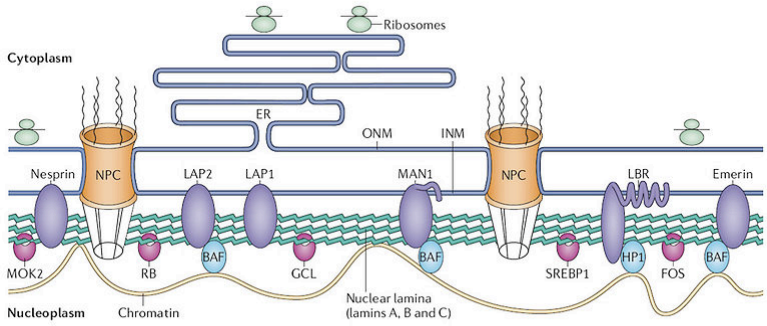

Fig 2 Adapted from Coutinho et al 2009 (Coutinho et al., 2009)

INM proteins

INM proteins are synthesized as transmembrane proteins on the RER. Then they are thought to travel to their resident location in the inner nuclear membrane via the nuclear pore membrane (Smith and Blobel, 1993; Soullam and Worman, 1993). It has long been debated whether this requires a recognition signal (Zuleger et al., 2012). The INM proteins have long been an elusive area mainly due to difficulties working with low levels of hard-to-solubilize proteins. Therefore only a handful of INM proteins have been characterized. However proteomic advances in the field has given rise to the finding of hundreds of new INM proteins (Schirmer et al., 2003), of which only a few have been investigated further. One of the first INM protein to be characterized was the Lamin B receptor LBR (Worman et al., 1988). LEM [LAP2 (lamina-associated polypeptide 2)/Emerin/MAN1] domain proteins, Emerin, Man1 and Lem2 belong to the more studied proteins of the INM. They share a common structural motif (LEM domain motif) known to interact with BAF (barrier to auto integration factor), a well-conserved chromatin associated protein that in this manner connects the LEM domain proteins to chromatin (Shimi et al., 2004). Several studies have shown redundancy between LEM domain proteins, where depletion of one of the members only has mild effects but combined depletion give severe phenotypes in mitosis and differentiation(Liu et al., 2003; Huber et al., 2009). LEM domain proteins are also known to be involved in signaling pathways (Holaska and Wilson, 2007). Furthermore, Emerin gene deletions or mutations is linked to the muscular dystrophy, X-linked Emery Driefuss Muscular Dystrophy (Tifft et al., 2009). Emerin is found both in the ONM, INM and the ER has been shown to bind to both A- and B-type lamins as well as BAF and nesprins on the ONM and Sun domain proteins in the INM (Wilson et al., 2005).

The LINC complex

As mentioned earlier Sun domain proteins of the INM and nesprin proteins of the ONM build up transcisternal bridges that provide a mechanical communication route between the nucleus and cytoplasm, bypassing the NPC. These bridges are referred to as LINC (LInker of nucleoskeleton and cytoskeleton) complexes and serve as connectors between the cytoskeleton and nucleoskeleton (Östlund et al., 2009). The Sun domain proteins interconnects with Lamin A and other components of the INM, and nesprins of the OMN and thereby connects indirectly with microtubule and actin filaments in the cytoplasm (Stewart et al., 2007).

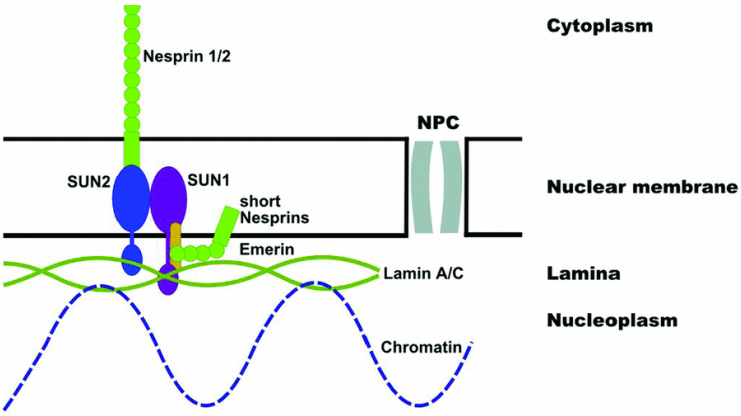

Fig 3: Adapted from Meinke et al 2011 (Meinke et al., 2011)

The nuclear pore complex (NPC)

The nuclear pore complex is a multi protein complex of approximately 125MDa (Panté and Aebi, 1993). The complex is situated at the fusion pores of the NE (Dingwall and Laskey, 1992). The structure of the NPC has an eight fold rotational symmetry. The general organization of the pore complex is based on a central ring structure with surrounding spokes and a basket structure on the nucleoplasmic side and cytoplasmic fibrils facing the cytoplasm (Goldberg and Allen, 1993). There are approximately 30

different nucleoporins (nups) that build up the NPC, some of which are present in several copies per subunit. Based on their positioning, the nups can be divided into different sub classes: Membrane layer, Scaffold layer and Barrier layer (Onischenko and Weis, 2011). Three individual proteins, gp210 (Wozniak et al 1989) POM 121, (Hallberg et al., 1993) and NDC1 (Mansfeld et al., 2006) are the pore membrane proteins. NDC1 is conserved down to yeast whereas POM121 only exists in vertebrates and gp210 is detected in both animals and plants suggesting that its function has been conserved during evolution (Cohen et al., 2003). Gp210 has a large luminal domain in the N-terminus and only a short C-terminal tail facing the cytoplasm. Pom121 has approximately the opposite structure with a large domain facing the cytoplasm and small domain exposed to the lumen. The two most stable subunits of the NPC are the Nup107-160 complex (Belgareh et al., 2001; Vasu et al., 2001) and the Nup93 complex (Grandi et al., 1997). The main activity of the NPC is to selectively mediate the nucleo-cytoplasmic transport of macromolecules during interphase. The NPC makes up a selective permeability barrier, created by FG (phenylalanine-glycine) repeat containing nups. These FG repeats creates a meshwork that does not allow passage of inert molecules larger than 40kDa, but has a high capacity for mediating transport receptor mediated passage. These transport receptors and other non-nup components involved in the transport machinery are presented in the Ran section, page 10. In addition to transport, in recent years, the NPC has been assigned function in gene regulation (Capelson et al., 2010)

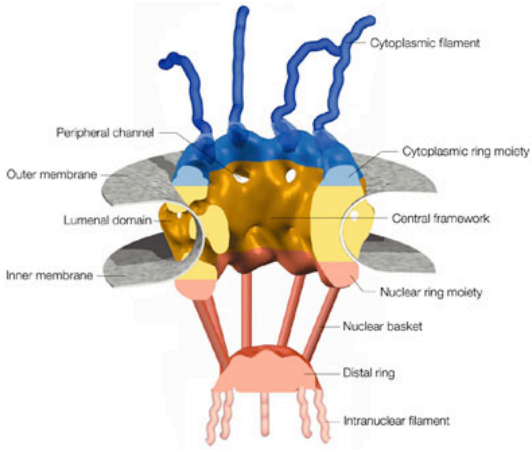

Fig 4: Adapted from Fahrenkrog and Abei 2003(Fahrenkrog and Aebi, 2003)

During the mitotic disassembly of the NPC individual nups are dispersed into the cytoplasm or ER. However it has now been established that a subset of nups are recruited to mitotic chromosomes i.e. Nup358, Nup98 and the Nup107/130 which have small populations found at kinetochores (Joseph et al., 2004; Loïodice et al., 2004), here, these proteins have roles in the spindle checkpoint machinery (Salina et al., 2003; Jeganathan et al., 2005; Zuccolo et al., 2007)

The Ran GTPase system

Ran is a small GTPase protein belonging to the Ras family. Ran is found in a GTP bound state and GDP bound state. RanGTP versus RanGDP is distributed along a concentration gradient which is predicted by the localization of its guanine nucleotide exchange factor (GEF) RCC1 (regulator of chromosome condensation 1) that is bound to chromatin (Bischoff and Ponstingl, 1991) and RanGAP (GTPase-activating protein and RanBP1 (binding protein 1) both localized to the cytoplasmic side of the NPC (Moore and Blobel, 1992). Due to this gradient RanGTP is predicted to localize to the nucleus during interphase and on mitotic chromosomes during mitosis. In its GTP bound state Ran binds to importin β, a nuclear transport receptor. Binding of RanGTP alters the

conformation of importin β, which thereby releases its bound cargo (Bischoff and Görlich, 1997). The importin β/RanGTP complex is then recycled back to the cytoplasm where the Ran bound GTP is hydrolyzed to GDP and Ran loses its affinity for importin β. This machinery is referred to as the RanGTPase system and gives the directionality to the nucleocytoplasmic transport. The last decade the RanGTPase system has been shown to regulate several assembly events in mitosis by the same mechanism as during nucleocytoplasmic transport in interphase i.e. the spindle assembly, where RanGTP releases and hence activates spindle assembly factors bound to importin β (Nachury et al., 2001). It has also been shown that the RanGTPase system regulates post mitotic nuclear reassembly mechanisms such as reassembly process of the NE and the NPC (Zhang et al., 2002).

Mitosis-NE disassembly and mitotic phosphorylation

The cell division cycle is divided into four phases: **G1**-the cell has received division signal and starts growing, **S phase**- the cell replicate its DNA, the amount of NPCs are doubled and centrosome duplication occurs, **G2**- the cell continue to grow and prepare for division, **Mitosis**- the genetic material and the cell divide. These phases are characterized by a strictly regulated set of events that is controlled by extensive signaling networks. The different Cyclin dependent kinases (CDKs) are the major regulators of these cell cycle phases. Their co regulators, Cyclins, are timely regulated by expression and degradation (Murray, 1987). The process of mitosis is complex, with morphologically identifiable stages: **Prophase-** condensation of chromatin, centrosome separation, **Prometaphase-,** NEBD, **Metaphase-** alignment of chromosomes, **Anaphase**-separation of chromosomes, **Telophase-**formation of new daughter nuclei. All these steps are regulated by phosphorylation /dephosphorylation involving multiple

kinases, and phosphatases. However the main player is CDK1/Cyclin B. This kinase complex phosphorylates an array of proteins during mitosis (Dephoure et al., 2008). The disassembly of the NPC is also believed to be governed by mitotic phosphorylation. The recent study of Laurell and colleagues shows that the phosphorylation of Nup98 is important event as it breaks the NPC permeability barrier, which is a crucial step of NPC disassembly (Laurell et al., 2011), compare conclusion in paper I. During the nuclear envelope breakdown (NEBD) in prophase CDK/Cyclin B phosphorylates lamins, microtubule proteins, nups, histones and INM proteins, this ensures the dismantling of the NE and its rupture (Salina et al., 2003). The NE is pulled apart by a cross connection network of microtubule motor protein and NE components. The transmembrane proteins of the NE are redistributed into the mitotic ER (Ellenberg et al., 1997; Lu et al., 2011) whereas the soluble components disperse into to the mixed nucleo-cytoplasm. However, recently transmembrane proteins of the INM have been shown to associate to the microtubules of the mitotic spindle (Buch et al., 2009; Lu et al., 2009; Wilkie et al., 2011). In a study from 2008 Galy and colleges show that antibodies against the transmembrane protein gp210 delay the NEBD process, of in vitro assembled nuclei, hence signing gp210 a partial role of in NEBD (Galy et al., 2008).

Reassembly of the NPC

Pore complex assembly occurs in two different ways by mechanisms that differ significantly. At the end of mitosis of mammalian cells, the reformation of the NE begins already at anaphase when the ER membrane is recruited to the separating chromosomes. This reforming NE includes Emerin, LBR and Sun2, characteristic proteins for the NE, but not the ER membrane protein Ret4a (Lu et al., 2011). Slightly

later in anaphase several nups associate with the chromosomes and the reforming nuclear membrane and together start forming the NPC. Mel28, Nup98, Pom121, 107-160 complex and Nup153 are all recruited to the reforming NE in anaphase (Rabut et al., 2004; Dultz et al., 2008). Subsequently in telophase the Nup93 complex, Nup 214, Nup352 and Nup62 are recruited. Last of the nups are gp210 and Tpr that are recruited in early G1 (Burke and Ellenberg, 2002). During S phase the second assembly of NPCs occurs, as the cell needs to double the amount of NPCs to ensure an equal distribution in the daughter cells. In this process the pore membrane protein POM 121 is believed to be recruited to Lamin B rich regions of the inner nuclear membrane, where POM121 recruits further NPC components to the assembling pore (Imamoto and Funakoshi, 2012).

Aim

The overall aim of my research has been to investigate protein-protein interactions of nuclear envelope proteins.

One specific aim has been to study the effect of mitotic phosphorylation on binding of the pore membrane protein gp210 to the nuclear pore complex. The second specific aim has been to identify binding partners to the INM protein Samp1.

Methodological considerations

Live cell imaging

The ability to visualize proteins in the live cell, with high spatial and temporal resolution has revolutionized the field of cell biology. It has been made possible by the creation of protein chimeras consisting of the protein of interest and the green fluorescent protein (GFP) or any of its variants (Tsien, 1998). GFP can be exited with light of certain wavelength causing GFP to emit this energy as light of a higher wavelength in the visible range. Hence it is possible to follow the chimerical protein with non-invasive methods (Lippincott-Schwartz, 2003). When using high power illumination of live cells one has to be aware that this can cause cytotoxicity. Therefore the selection of illumination intensity, fluorophores and optical system are crucial. The ideal is being able to run experiments on cells expressing low to medium levels of the fluorophore fusion protein.

Confocal microscopy

Confocal microscopy is an optical technique that allows us to collect images with higher optical depth resolution compared to conventional microscopy. This is achieved by excluding light from out of focus regions. This exclusion of light is obtained by introducing a pinhole, the smaller the pinhole, the thinner the focal plane, from which we collect light. This increase in resolution is limited by the physical properties of light to approximately 200x100x800 nm.

FRAP

Fluorecsence Recovery After Photobleaching (FRAP) is a non-invasive live cell imaging method that enables studies of protein dynamics. In FRAP the dynamics of a fluorophore tagged protein is studied by irreversible bleaching out the fluorescence of a selected area of the cell and then follow the redistribution of the unbleached pool of the fusion protein. By measuring the mobile fraction and diffusion constant of the fusion protein one can determine its mobility properties. FRAP has been extensively used to study the mobility, and hence complex association of membrane proteins in the nuclear membrane (Ellenberg et al., 1997; Ostlund et al., 1999). In specific, FRAP experiments was used to study the dynamic properties of the nups in their association to the NPC (Daigle et al., 2001). This study revealed that the nups has extensively different turnover at the NPC and contribute to their biological functions (Rabut et al., 2004).

In vivo crosslinking

The DSP-crosslinker has an 8-carbon spacer arm with an amine-reactive *N*-hydroxysuccinimide (NHS) ester at each end. NHS esters react with primary amines at pH 7-9 and form stable amide bond. Proteins generally have several primary amines in the side chain of lysine (K) residues and the N-terminus of each polypeptide and these are available as substrates for NHS-ester crosslinking reagents. We added 1μM DSP to cells in culture to enable cross-linking of proteins, thereby introducing a stable amide bond between interacting proteins. This bond will persists the 7M urea treatment needed to solubilize the INM protein Samp1.

Immunoprecipitation

The solubilized, DSP crosslinked cell lysates from either HeLa cells transiently expressing YFP-Samp1 or U2OS cells stably expressing YFP-Samp1, were incubated with antibodies against GFP and then subjected to immunoprecipitation. One drawback with performing IP and eluting the beads under reducing conditions, is that the light chain of the antibody give rise to background signals in the 20-30kDA region obscuring detection of proteins in this region of the western blot. To avoid this, we took advantage of camel anti-GFP antibodies covalently bound to agarose beads (GFP Trap, ChromeTek) that only consists of a heavy chain.

Nuclear preparation

We preformed the immunoprecipitation using either stably or transiently expressed Samp1 fused to YFP. As over expression of inner nuclear membrane proteins generate an excess pool that resides in the ER we needed a tool that allowed us to primarily focus on Samp1 interactions taking place at the nuclear envelope. Therefore we performed subcellular fractionation to obtain a nuclear enriched fraction. We performed it as in (Kihlmark et al., 2004)

Cell lines

NIH 3T3 cells are mouse fibroblasts, which naturally lack gp210, therefore they are an ideal model system to study dynamics of mutant variants of gp210, as there will be no interference with the wild type protein.

Results and Discussion

Paper I) "Phosphomimetic mutation of the mitotically phosphorylated serine 1880 compromises the interaction of the transmembrane nucleoporin gp210 with the nuclear pore complex."

The role of mitotic phosphorylation has not been clearly demonstrated but one idea is that the mitotic phosphorylation cascade, mainly carried out by CDK1, alters the charge of many NE proteins and therefore break up their interaction with neighboring proteins leading to disassembly. Lamins were the first NE components shown to be phosphorylated and depolymerized in mitosis (Gerace and Blobel, 1980). CDK1 was also shown to phosphorylate several nups during mitosis in vitro (Favreau et al., 1996). In this study Favreau et al showed that pore membrane protein gp210 becomes specifically phosphorylated in mitosis at a single site, serine 1880. Gp210 is a bitopic type I integral membrane protein, it has a large lumenal domain in the N-terminal and a smaller C-terminal domain exposed to the cytoplasm. The serine 1880 is positioned 6 amino acids from the C-terminus and is exposed to the cytoplasm/nucleoplasm, in the region of gp210 that is available for interaction with other nups.
Our laboratory (Onischenko et al., 2005) showed that CDK1 activity is required to break up the NPC during mitosis in Drosophila syncytial embryos. In the same study, inhibition of phosphatases by oakadaic acid inhibited post mitotic reassembly of the NPC. These results suggest that mitotic phosphorylation alone is responsible for the disassembly of the NPC. Here, we wanted to investigate the functionality of mitotic phosphorylation of nups and how it affects their binding to the NPC. As the NPC is disassembled during mitosis and its components are dispersed out in the cell, the

functionality of mitotic phosphorylation on binding to the interphase NPC is hard to study. The NPC and mitotically phophorylated nups do simply not coexist in the same temporal context. To circumvent this we used a phosphomimetic substitution approach where a serine (S) is substituted to a glutamate (E) which, with its negative charge mimics the phosphoserine. This approach has been widely used in phosphoprotein studies previously (Casolari et al., 2004; Pitula et al., 2004; Taddei et al., 2004; Yang et al., 2007).

In our study we took advantage of the transmembrane nucleoporin gp210, carrying only a single mitotic phosphorylation site (Favreau et al., 1996), and the NIH 3T3 cell line since it is known that they naturally lack endogenous gp210 (Eriksson et al., 2004; Olsson et al., 2004). Thereby we could, by transient transfection, study the functional binding of gp210 to the NPC without the ambiguity of endogenous gp210 expression.

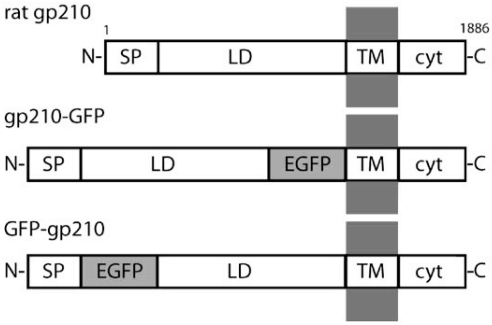

Fig 5 Gp210-GFP fusion constructs(Onischenko et al., 2007).

Gp210(S1880E) mutant shows decreased incorporation to the NPC

When performing co-expression of wt gp210 with the phosphomimetic mutant, gp210(S1880E) we observed a significantly lower accumulation of the phosphomimetic mutant at the NE. The gp210(S1880E) did also show an increased mobility at the NE in

FRAP experiments compared to the wild type protein indicating that the mutant is less tightly associated to the NPC.

We also studied the degree of incorporation to the NPC by comparing the ratio between the wt gp210-YFP and gp210(S1880E) in the ER and the NE. In this manner wt gp210 serve as an internal control for differences in expression levels accumulated in the ER compared to the NE due to natural variation. We named this Relative NE Accumulation Factor RNAF. The results present strong evidence that gp210(S1880E) has decreased ability to incorporate to the NPC. As this phosphorylation of gp210 has been shown to specifically occur in mitosis (Favreau et al., 1996) one can speculate that phosphorylation contributes to gp210 disassociation from the NPC during NPC disassembly in mitosis.

Dephosphorylation of gp210 is required for efficient post mitotic recruitment to the NPC

Additionally we wanted to investigate if post mitotic recruitment of gp210 was affected by phosphorylation of serine 1880. In post-mitotic reassembly of the NPC, gp210 is one of the last nups to be recruited to the reassembled pore, and is not fully recruited until G1. We showed that in post mitotic cells the gp210(S1880E) incorporated less efficiently than the wt gp210. This result suggests that dephosphorylation is required for post mitotic gp210 reassembly to the NPC. All together these results provide experimental evidence that are consistent with the idea that mitotic phosphorylation of nups plays a direct role in disassembly of the NPC. We have in his study been able to investigate how phosphomimetic substitution mutation affects NPC binding using non-invasive techniques.

Paper II) "In vivo interaction between the transmembrane inner nuclear membrane protein Samp1 and LINC complex proteins."

Manuscript

Recent advances in the field has suggested novel and unexpected roles of the INM proteins in nuclear organization and chromatin organization. These are central regulatory mechanisms that are disrupted in many diseases (Lammerding et al., 2004). Investigation of new interaction partners of INM proteins like Samp1 is essential to understand the functions of INM proteins in interphase and in mitosis. We have previously shown that Samp1 is an integral membrane protein locating to the INM (Buch et al., 2009). In the same study Samp1 was also shown to have a specific localization to the mitotic spindle in mitosis. Samp1 is a polytopic type II membrane protein and has a relatively large (approximately half of the polypeptide length) N-terminal domain exposed in the nucleoplasm. This domain contains four conserved CXXC motifs (C=Cys ; X= any amino acid), potentially giving rise to two zinc fingers. These are followed by four transmembrane segments and the protein is ending with a small C-terminal tail facing the nucleoplasm (Gudise et al., 2011). During our previous studies we found that Samp1 partially co-localized with the LINC complex component Sun1. In addition to this, Samp1 was shown to co-precipitate with Emerin in vitro in a Zinc-dependent manner (Gudise et al., 2011). Due to difficulties with solubilization of transmembrane proteins this interaction has so far only been demonstrated in vitro. Given the peculiar mitotic localization of Samp1 and its interaction with the LINC complex proposed in Gudise et al 2011 we wanted to find a robust and efficient way to investigate the binding partners of Samp1 in vivo. Immunoprecipitation of integral

membrane proteins is difficult to perform as the harsh conditions often needed for solubilization of these proteins disrupt their interaction with other proteins. Therefore interaction studies made on transmembrane INM protein has earlier mostly been restricted to pull down experiments with the recombinantly expressed soluble domain of the INM proteins (Holaska and Wilson, 2007; Haque et al., 2010).

Samp1 interacts with Emerin at the INM in vivo

To improve our chances to identify interaction partners of Samp1 in vivo we took advantage of the cell permeable reversible crosslinker DSP (Dithiobis(succinimidyl propionate)) which is an amine reactive succinidyl ester that crosslinks primary amines within a 12Å distance (Smith et al., 2011), thereby substituting the protein-protein interaction with a covalent S-S bond. The covalent link resists 7M urea and 1% Triton X-100 necessary to solubilize INM proteins. This enables immunoprecipitation with in vivo interacting proteins. We estimated Samp1 to pull down approximately 15-20% of total Emerin. This interaction was further confirmed performing the reverse immunoprecipitation using cells expressing YFP-Emerin. These results show that Samp1 interacts with Emerin at the INM in live cells.

Samp1 interacts with Sun1 at the INM in vivo

The fact that Samp1 was shown to partially co-localize with Sun1 (Gudise et al., 2011) tempted us to investigate if Sun1 would precipitate with YFP-Samp1 using our in vivo crosslinking immunoprecipitation approach. Indeed Sun1 co-precipitates with YFP-Samp1. Using the same approach we showed that Sun1 also co-precipitated with YFP-Emerin. This is consistent with earlier in vitro experiments published by Östlund and

collegues in 2009 (Östlund et al., 2009) and studies from Haque et al 2010 (Haque et al., 2010). This result suggests that Sun1 can interact with both Samp1 and Emerin at the INM. As both Sun1 and Emerin have been suggested to be components of the LINC complex it is tempting to speculate that also Samp1 can be a part of Sun1 containing LINC complexes. LINC complexes are heterogeneous transcisternal complexes that exist in different sub variants involving different isoforms and connecting to different classes of the cytoskeleton proteins (Roux et al., 2009). Our results show that Samp1 interacts with Sun1 and Emerin in vivo. This, together with the previous data from (Gudise et al., 2011) where Samp1 was shown to be essential for centrosome location close to the NE, gives the opportunity to hypothesize that Samp1 is a part of Sun1 containing LINC complexes possibly connecting to the centrosome and the microtubule cytoskeleton.

The small GTPase Ran co-precipitates with Samp1

Samp1 is predicted to interact with Ran in the Funcoup (functional coupling) database (www.***funcoup****.sbc.su.se),* an interactome prediction database set up from networks of functional coupling that have been constructed by Bayesian integration of diverse high-throughput data.

We wanted to investigate if the predicted interaction between Samp1 and Ran could be confirmed using in vivo crosslinking immunoprecipitation. Indeed Ran co-precipitated with YFP-Samp1 in vivo. RanGTP together with importinβ is a master regulator of various protein complexes and hereby regulates several spatio-temporal cellular functions such as nucleo-cytoplasmic transport (Moore and Blobel, 1992), microtubule assembly (Nachury et al., 2001) and post mitotic nuclear assembly (Walther et al., 2003; Harel and Forbes, 2004). Therefor interaction between Samp1 and Ran awakes the

intriguing hypothesis that Ran might regulate Samp1 associated protein complexes, possibly the LINC complex. In summary we have presented a new method that overcome difficulties with solubilizing INM proteins without disrupting their interactions that could become an invaluable tool to reveal interactomes of INM proteins in live cells. With this method we have been able to reveal interactions occurring between INM proteins Sun1, Samp1 and Emerin and also between Samp1 and Ran in live cells.

Conclusions

Paper I)

- Phosphomimetic substitution of Ser1880 to Glu of gp210 compromises its interaction with the NPC.
- Phosphomimetic substitution of Ser1880 to Glu of gp210 hinders its recruitment to the reforming NPC.
- The results support a model where phosphorylation plays a direct role in the disassembly the NPC during mitosis.

Paper II)

- We have developed a cross-link immunoprecipitation protocol for transmembrane proteins in vivo.
- Using crosslink immunoprecipitation we show that Samp1 interacts with the INM protein Emerin in vivo.
- Using crosslink immunoprecipitation we show that Sun1 and Samp1 interact in vivo.
- Using crosslink immunoprecipitation we show an in vivo interaction between Ran and Samp1 at the INM.

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