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THE FUNCTIONAL ORGANIZATION OF NUCLEAR ENVELOPE PROTEINS

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THE FUNCTIONAL ORGANIZATION OF NUCLEAR ENVELOPE PROTEINS

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To my family

Madhuri & Anvi

ABSTRACT

In eukaryotic cells, the nucleus is enclosed by a double lipid membrane, termed the nuclear envelope (NE). The NE consists of the outer nuclear membrane (ONM), the inner nuclear membrane (INM), the nuclear pore complexes (NPCs) and the nuclear lamina. Recently it has been realized that the NE proteins not only serve structural functions but are also involved in a diverse group of genetic diseases collectively termed laminopathies or envelopathies. So far, only a few NE proteins have been characterized in detail. Here, we have identified and investigated a novel transmembrane protein from the NE, which is highly conserved in evolution. We termed the protein, Spindle associated membrane protein 1 (Samp1). During mitosis, a subpopulation of Samp1 is concentrated in the mitotic spindle. Samp1 has four transmembrane domains and is specifically localized to the INM. The N-terminal half of Samp1 contains a Zinc finger domain and is exposed in the nucleoplasm. Over expression of Zinc finger mutants of Samp1 gave an abnormal phenotype characterized by disruption of the localization of endogenous Samp1 and a specific set of NE proteins, suggesting that Samp1 is functionally associated with LINC complex and A-type lamina network proteins. After posttranscriptional silencing of Samp1 expression we showed that Samp1 is required for correct localization of Emerin to the NE. We also showed that Samp1 interacts with Emerin in live cells and that this interaction can occur by direct binding. The fact that the interaction between Emerin and Samp1 depended on Zinc, supports the idea that Samp1 has functional Zinc finger(s).

Posttranscriptional silencing of Samp1 gave rise to an increase in the distance between the centrosome and the NE, suggesting that Samp1 is functionally associated with the microtubule cytoskeleton, most likely mediated via the LINC complexes. Using high-resolution fluorescence microscopy we showed that Samp1 is distributed in a distinct pattern in the NE and partially colocalized with the LINC complex protein, Sun1. We also showed that the Samp1 can interact with Sun1 in live cells.

We developed a novel method, Membrane protein Cross-Link ImmunoPrecipitation (MCLIP) that enables detection of specific interactions of NE proteins in live cells. Using MCLIP we identified specific interaction partners of Samp1 in U2OS cells.

Human induced pluripotent stem cells (hiPSCs) displayed increased expression of Samp1 during differentiation. Over expression of YFP-Samp1 induced a rapid differentiation of hiPSCs into neurons. The medium from the Samp1 over expressing hiPSCs was sufficient to differentiate untransfected hiPSCs.

LIST OF SCIENTIFIC PAPERS

I.   An integral protein of the inner nuclear membrane localizes to the mitotic spindle in mammalian cells.
Buch, C.*, Lindberg, R.*, Figueroa, R., **Gudise, S.**, Onischenko, E., and Hallberg, E.
Journal of Cell Science (2009) 122, 2100-7.

II.   Samp1 is functionally associated with the LINC complex and A-type lamina networks.
Gudise, S.*, Figueroa, RA.*, Lindberg, R., Larsson, V., and Hallberg, E.
Journal of Cell Science (2011) 124, 2077-85.

III.   MCLIP, an effective method to detect interactions of transmembrane proteins of the nuclear envelope in live cells.
Jafferali, MH., Vijayaraghavan, B., Figueroa, RA., Crafoord, E., **Gudise, S.**, Larsson, VJ., and Hallberg , E.
Biochim Biophys Acta (2014) 1838, 2399-2403.

IV.   The inner nuclear membrane protein, Samp1 induces differentiation of human induced pluripotent stem cells into neurons.
Gudise, S., Markus, R., Bergqvist, C., and Hallberg, E., *Manuscript.*

* These authors contributed equally to this work.

ADDITIONAL PUBLICATIONS

I. A transmembrane inner nuclear membrane protein in the mitotic spindle.

Figueroa, R., **Gudise, S.**, Larsson, V., and Hallberg, E.

Nucleus (2010) 1, 249-253.

II. Microtubule-associated nuclear envelope proteins in interphase and mitosis.

Figueroa, RA., **Gudise, S.**, and Hallberg, E.

Biochemical Society Transactions (2011) 39, 1786-9.

CONTENTS

LIST OF ABBREVIATIONS

AD-EDMD	Autosomal dominant EDMD
BAF	Barrier-to-autointegration factor
Co-IP	Co-immunoprecipitation
CSLM	Confocal laser scanning microscopy
Ct	Chaetomium thermophilum
DamID	DNA adenine methyltransferase identification
DNA	Deoxy ribonucleic acid
DSP	Dithiobis (succinimidyl propionate)
EDMD	Emery-Dreifuss Muscular Dystrophy
ER	Endoplasmic reticulum
ESCs	Embryonic stem cells
FG	Phenylalanine-Glycine
GFP	Green fluorescent protein
HGPS	Hutchinson-Gilford progeria syndrome
hiPSCs	Human induced pluripotent stem cells
HP1	Heterochromatin protein 1
ICM	Inner cell mass
Ima1	Inner MTOC attachment site protein 1
INM	Inner nuclear membrane
IP	Immunoprecipitation
KASH	Klarsicht/Anc-1/Syne Homology
LADs	Lamina associated domains
LBR	Lamin B receptor
LEM	Lap2, Emerin and Man1
LINC	Linker of nucleoskeleton and cytoskeleton
MCLIP	Membrane protein Cross-Link ImmunoPrecipitation
mRNA	messenger RNA
MTOC	Microtubule-organizing center
NE	Nuclear envelope
NEBD	Nuclear envelope breakdown
NPC	Nuclear pore complex
Nups	nucleoporins
ONM	Outer nuclear membrane
PNS	Perinuclear space
POMs	Pore membrane proteins
PSCs	Pluripotent stem cells
RER	Rough endoplasmic reticulum
RNA	Ribonucleic acid
RNAi	RNA interference
ROS	Reactive oxygen species
Samp1	Spindle associated membrane protein 1
siRNA	Short interference RNA
SPB	Spindle pole body
Sun	Sad1/UNC-84 homology
TF	Transcription factor
X-EDMD	X-linked EDMD
YFP	Yellow fluorescent protein

1 INTRODUCTION

1.1 The Nucleus

In eukaryotic cells (Fig 1), the nucleus encloses most of the cell's genetic material and controls several functions, for example gene regulation and chromatin organization. The nucleus is the principal site for the synthesis of DNA and RNA (Alberts et al., 2008). Transcription factors (TFs) in the nucleus regulate the gene expression according to the cellular requirement. Recent findings suggest that the nuclear periphery also may have important roles in the gene expression, but exact mechanism remains to be elucidated. The nucleus is enclosed by two lipid bilayer membranes termed the nuclear envelope (NE) (Stewart et al., 2007). During cell division, the disassembly of the nucleus allows to form the mitotic spindle and connect to centrosomes in order to separate the duplicated chromosomes into daughter cells.
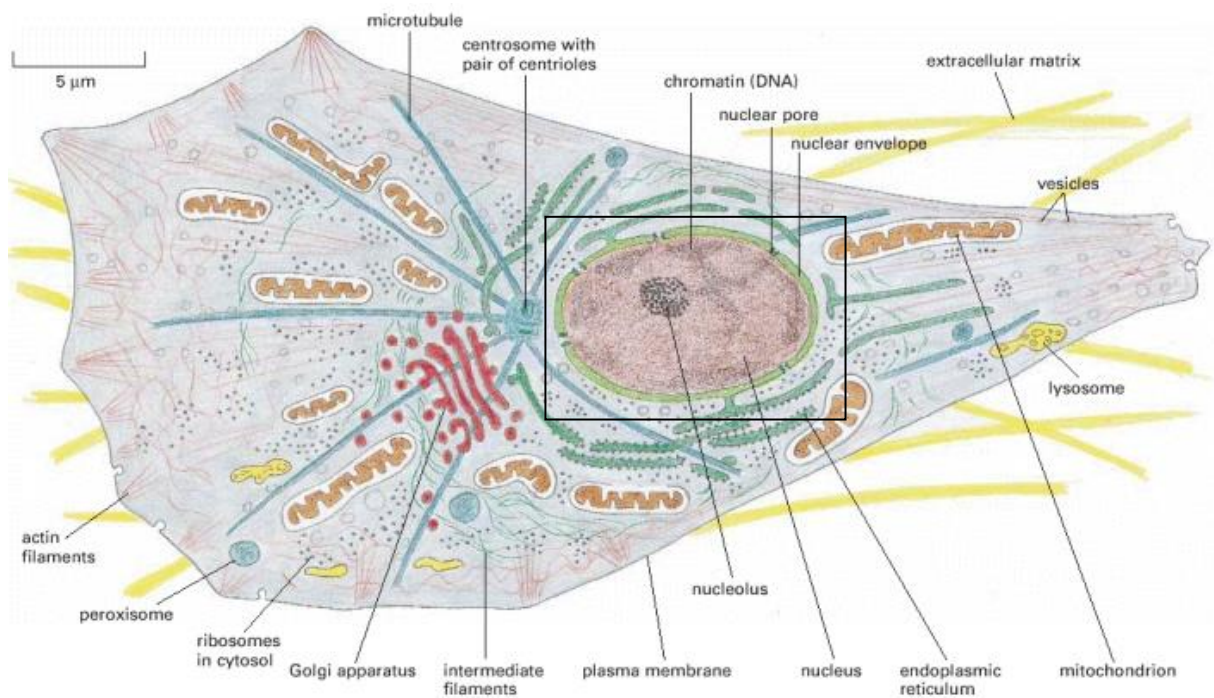

Fig 1. *Schematic view of a Eukaryotic cell. Modified from (Alberts et al., 2008)*

1.2 Nuclear envelope organization

The NE is made up of different components, the nuclear membranes, the nuclear pore complexes (NPCs) and the nuclear lamina (Stewart et al., 2007) (Fig 2). The inner nuclear membrane (INM) and the outer nuclear membrane (ONM) are separated by perinuclear space (PNS), which is continuous with the lumen of the ER (Gerace and Burke, 1988; Hetzer, 2010). The ONM is continuous with the rough endoplasmic reticulum (RER) and contains ribosomes. The traditional view of the function of the NE was to maintain the shape of the nucleus and act as a selective barrier between the nucleoplasm and the cytoplasm. Already more than three decades ago it was proposed that the NE has functions in regulation and organization of the genome (Blobel, 1980). Since last decade the NE has taken more attention of the researchers because of the NE proteins were found to be involved in several genetic diseases, commonly termed laminopathies or envelopathies. The ONM and the INM contain unique sets of proteins and the repertoire varies between different cell types and tissues (Korfali et al., 2012). The differential expression of NE proteins in different tissues suggests that the NE might have a role in differentiation.
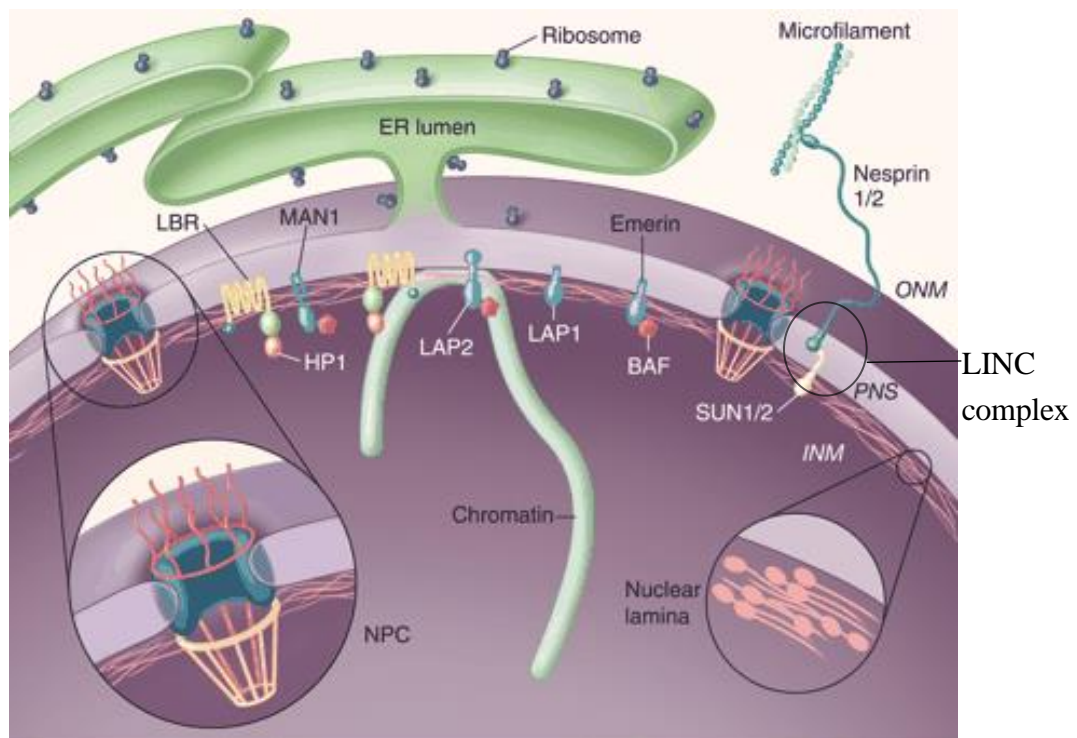

Fig 2. *Overview of the nuclear envelope organization. Modified from (Stewart et al., 2007)*

1.3 Nuclear envelope proteins

Previously subtractive proteomic studies have shown that the INM contains approximately 80 unique transmembrane proteins (Schirmer et al., 2003, 2005; Schirmer and Gerace, 2005). In a recent study, Korfali and colleagues reported that the INM may contain hundreds of proteins and they are differentially expressed in different tissues (Korfali et al., 2012). Still, only a few of the INM proteins have been characterized in detail. The transmembrane INM proteins are synthesized on the RER and transported to their correct localization by a diffusion retention mechanism. According to this model, the transmembrane proteins laterally diffuse in the ER/NE membrane to their correct localization and then retained in the INM by binding to other INM proteins, or nuclear lamina (Schirmer and Foisner, 2007), or chromatin (Dorner et al., 2007; Ellenberg et al., 1997; Soullam and Worman, 1995).

1.3.1 Samp1

Samp1 is a transmembrane protein that specifically localized to the INM in human cells (Buch et al., 2009) (see paper-I & II results in the thesis). It is also referred Net5 or TMEM201 (Schirmer et al., 2003). Borrego-Pinto and colleagues showed that Samp1 is involved in nuclear migration and cell polarization in wound healing assay in NIH-3T3 cells (Borrego-Pinto et al., 2012). The Samp1 homologue in *S. pombe*, Ima1 is involved in the connection of MTOC (Microtubule-organizing center)/SPB (Spindle pole body) to the nuclear interior (King et al., 2008; Steglich et al., 2012).

1.3.2 Emerin

Emerin is a serine rich INM protein of 294 aminoacids, which is encoded by *EMD* gene located on the X-chromosome. Emerin is a type II integral membrane protein, which exposes its N-terminal domain in the nucleoplasm and C-terminus in the PNS. Emerin is very dynamic and laterally diffuses between the INM, the ONM and the ER (Ostlund et al., 1999; Salpingidou et al., 2007). Emerin is one of the LEM domain containing proteins (Lap2, Emerin and Man1). The LEM domain interact with a DNA binding protein, BAF (Barrier-to-autointegration factor), thereby tethering chromatin to the NE (Brachner and Foisner, 2011). Emerin also interacts with several other proteins including the nuclear lamina (Holaska and Wilson, 2007; Libotte et al., 2005; Sullivan et al., 1999), and cytoskeletal components, tubulin (Salpingidou et al., 2007) and actin (Holaska et al., 2004; Lattanzi et al., 2003).

1.3.3 BAF (Barrier-to-autointegration factor)

BAF is a 10 KDa protein, which is localized in the nucleoplasm and enriched at the NE (Furukawa, 1999; Haraguchi et al., 2001; Margalit et al., 2007; Margalit et al., 2005; Shimi et al., 2004). BAF directly binds double stranded DNA (dsDNA), several transcription factors, the LEM domain proteins Lap2, Emerin and Man1, and indirectly interact with A-type lamins, reviewed in (Margalit et al., 2007). BAF regulates several cellular functions in the cell, which includes cell cycle progression, developmental processes (Furukawa et al., 2003; Margalit et al., 2005) and gene expression (Holaska et al., 2003; Wang et al., 2002). BAF is also involved in the heterochromatin formation by its DNA-bridging activity and ability to bind to the core histone H3 to repress the gene activity (Montes de Oca et al., 2005). However, BAF mediated gene repression is not known in detail. Down regulation of BAF by RNAi in *C. elegans* and deletion of the *baf* gene in Drosophila flies leads to embryonic/larval lethality, respectively (Furukawa et al., 2003; Margalit et al., 2005).

1.3.4 The LINC complex

The LINC (Linker of Nucleoskeleton and Cytoskeleton) complexes are trans-cisternal bridges across the two concentric lipid membranes of the NE (Burke and Stewart, 2002; D'Angelo and Hetzer, 2006). The LINC complex is formed by the interaction in the PNS between the ONM proteins, Nesprins and Sun proteins from the INM (shown in Fig 2) (Wilhelmsen et al., 2006). The LINC complexes are conserved from yeast to humans. The LINC complexes has been found to play a major role in different cellular functions including cell division, centrosome association to the NE, cell polarization, nuclear migration, nuclear anchorage and mechanotransduction, recently reviewed in (Burke and Roux, 2009; Lei et al., 2009; Méjat and Misteli, 2010; Starr, 2009; Wilson and Berk, 2010; Wilson and Foisner, 2010; Zhang et al., 2009).

1.3.5 Sun domain proteins

Sun proteins have a C-terminal coiled coil Sun domain located in the PNS. Sun proteins expose their N-terminal domains in the nucleoplasm, which interacts with the nuclear lamina. However, this interaction is not required for their localization to INM (Crisp et al., 2006). Sun proteins associate with each other to form homo or hetero oligomers (Lu et al., 2008; Wang et al., 2006). However, recently the structure of KASH-Sun1 complex was resolved by X-ray

crystallography showing three KASH peptide domains of Nesprins interacting with homo trimeric Sun1 (Sosa et al., 2013; Sosa et al., 2012).

1.3.6 Nesprins

Most Nesprins are ONM proteins, which have a KASH (Klarischt, Anc-1, Syne homology) domain in their C-terminus, located in the PNS of the NE. KASH proteins have different sizes of their N-terminal domains projecting into the nucleo/cytoplasm. The N-terminal domains of KASH proteins can interact with the cytoskeletal elements actin, plectin or tubulin, reviewed in (Méjat and Misteli, 2010). However, shorter KASH protein isoforms when situate in the INM interact with the nucleoskeleton.

1.3.7 The nuclear pore complex

The nuclear pore complexes (NPCs) are highly conserved multi protein assemblies of approximately 125 MDa molecular mass, which are harbored by the nuclear pores (Fig 3) (D'Angelo and Hetzer, 2008; Hetzer, 2010; Tran and Wente, 2006). The NPCs are responsible for nucleocytoplasmic transport of proteins, RNA and ribonucleoprotein complexes (Beck et al., 2004; Terry et al., 2007). The NPCs allows passage of small molecules by passive diffusion. However, proteins with a molecular mass larger than 40 KDa require specific signals and are transported by an active mechanism involving nuclear transport receptors and the Ran GTPase (Rout and Aitchison, 2000). The NPCs consists of multiple copies of ~30 different proteins termed nucleoporins (Nups), arranged in an eight-fold symmetry. Many Nups are conserved from humans to yeast (Akey, 1995; Asakawa et al., 2014; Cronshaw et al., 2002; Rout and Aitchison, 2000; Strambio-De-Castillia et al., 2010). The Nups can be sub-divided into four groups. The membrane associated proteins, termed the pore membrane proteins (POMs) (Hallberg et al., 1993), anchor the NPC to the membrane. The core scaffold proteins form the outer and inner rings. The phenylalanine-glycine (FG) Nups, line the surface of the central channel that connects the nuclear and cytoplasmic face. The adaptor Nups, connects the core scaffold proteins and FG Nups. (Fig 3) (Asakawa et al., 2014; Cronshaw et al., 2002; Rout and Aitchison, 2000; Strambio-De-Castillia et al., 2010). The NPCs are nonrandomly distributed in the NE and is dependent on the nuclear lamina (Aaronson and Blobel, 1975). In one study, The LINC complex protein, Sun1 was associated with the NPCs and depletion of Sun1 or over expression of dominant-negative forms of Sun1 lead to nuclear pore clustering (Liu et al., 2007).
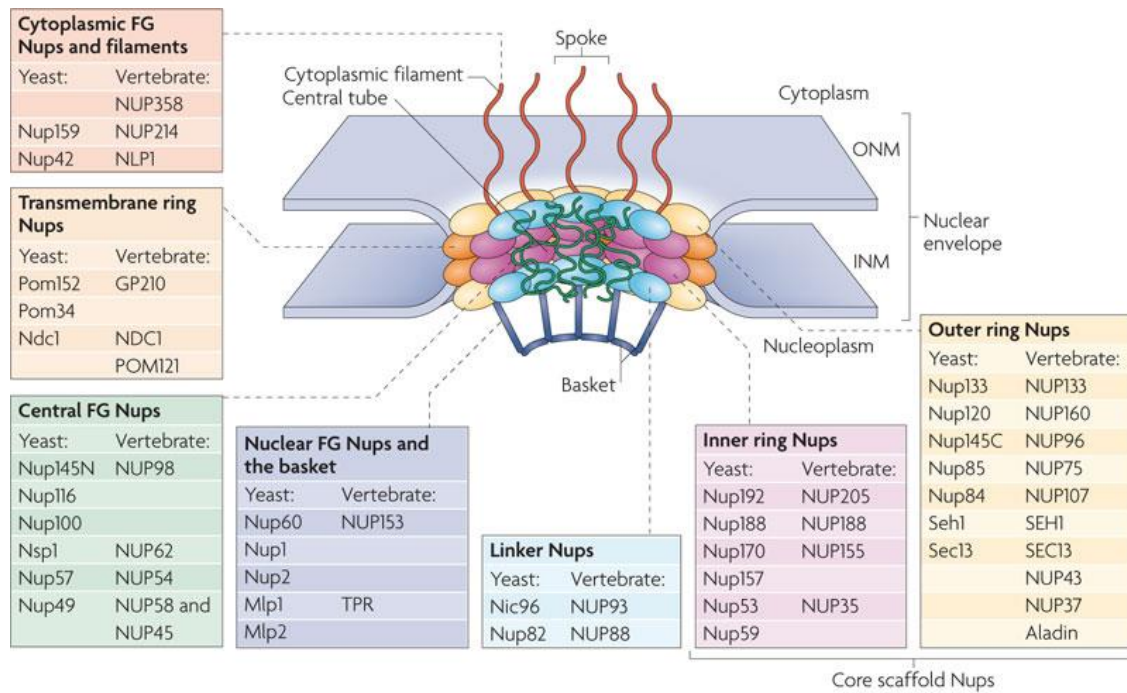
Fig 3. *Schematic view of the nuclear pore complex and comparison of Nups between vertebrates and yeast. From (Strambio-De-Castillia et al., 2010)*

1.3.8 The nuclear lamina

The nuclear lamina is a thin mesh like network of type-V intermediate filament proteins, which underlies the INM (Stuurman et al., 1998). The nuclear lamina is known to associate with proteins of both the INM and chromatin (Aebi et al., 1986; Gerace et al., 1978; Wilson and Foisner, 2010). The nuclear lamina consists of two different types of mutually exclusive networks, termed A- and B-type lamin networks (Gruenbaum et al., 2000; Shimi et al., 2008). Lamin A and C are two splice variants encoded by a single gene, *LMNA*. Lamin B1 and B2 are B-type lamins, encoded by separate genes *LMNB1* and *LMNB2*, respectively (Dittmer and Misteli, 2011). Lamin proteins have three domains termed, N-terminal "head domain", a central coiled-coil "rod domain" and C-terminal tail including an "Ig-fold domain" (Dechat et al., 2008; Dittmer and Misteli, 2011). Previous reports showed that purified lamins *in vitro* first dimerize by their rod domain, and then the dimers associate in a head-to-tail manner to form linear polymers. Finally, linearized polymers associate laterally in a staggered antiparallel manner to form a lamina network (Simon and Wilson, 2013). However, the *in vivo* lamina network arrangement needs to be further investigated. Lamin proteins are synthesized on free polysomes and post-translationally modified prior to filament assembly. Lamin proteins maturation undergoes the following steps. Prelamin A is first farnesylated at the cysteine residue of the C-terminal -CaaX motif, followed by proteolytic cleavage by

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either Rce1 or Zmpste24 to release aminoacids -aaX from the C-terminus (Fig 4). After proteolytic cleavage, the farnesylated cysteine is carboxymethylated by carboxyl methyltransferase. Finally farnesylated, carboxymethylated prelamin A undergoes further proteolytic cleavage by Zmpste24 to form mature Lamin A devoid of its farnesylated C-terminal tail (Fig 4) (Broers et al., 2006; Dechat et al., 2010; Dechat et al., 2008; Simon and Wilson, 2013). However, B-type lamins keep their farnesyl group, which facilitates association with the NE.
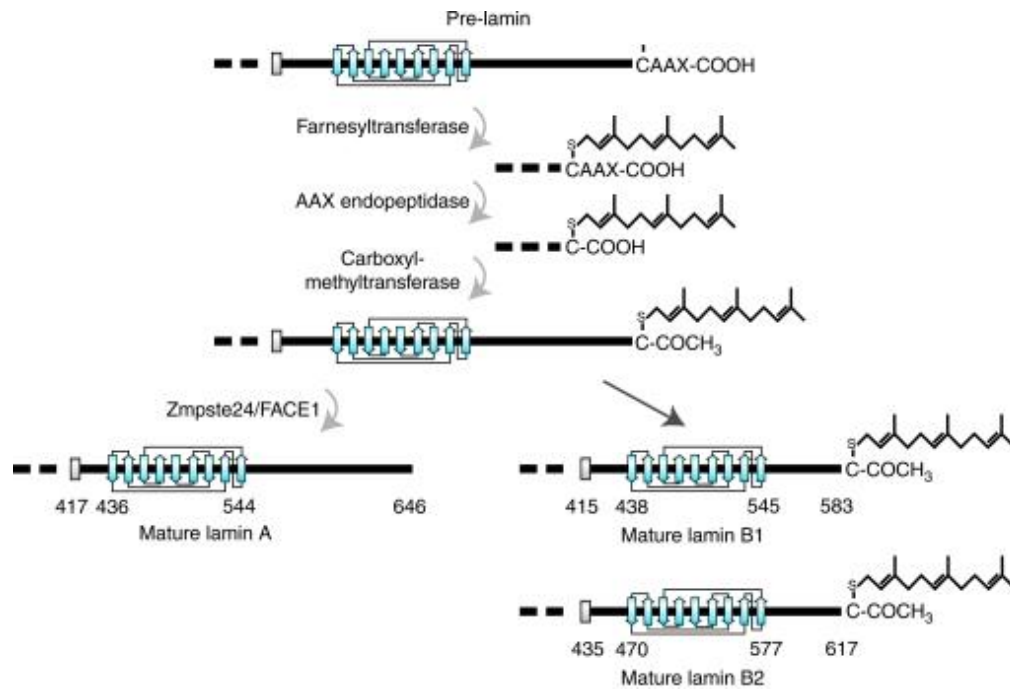

Fig 4. *Post-translational modifications of lamins. From (Dechat et al., 2010)*

The nuclear lamina has been shown to be important for the nuclear stability, especially in tissues involved in mechanical force generation as muscle fibers (Cohen et al., 2008). Also, it has been suggested that the nuclear lamina is involved in chromatin organization, differentiation, DNA replication and DNA repair processes. For example, using the DamID (DNA adenine methyltranferase Identification) technique it has recently been shown that the nuclear periphery associates with specific regions of the chromatin termed Lamina Associated Domains (LAD's) which associate with the down regulation of specific genes (Kind et al., 2013; Kind and van Steensel, 2010; Luperchio et al., 2014; Peric-Hupkes and van Steensel, 2010). B-type lamins are expressed and localized to the NE in most cell types in both embryos and adult animals (Lehner et al., 1987; Lourim and Lin, 1989; Stuurman et al., 1998). Posttranscriptional silencing of B-type lamins in somatic cells induced apoptosis

(Harborth et al., 2001), suggesting that B-type lamins are essential for survival of cells. However, by creating embryonic stem cells (ESCs) null for Lamin B1 and B2 it was demonstrated that B-type lamins are not essential for survival of ESCs (Hutchison, 2014; Kim et al., 2011; Kim et al., 2013), suggesting that in contrast to somatic cells the ESCs may not require any type of lamins for their survival. In contrast, A-type lamins are highly expressed in differentiated cells but not in undifferentiated cells (Constantinescu et al., 2006; Eckersley-Maslin et al., 2013), suggesting that A-type lamins are not an essential element for cell survival. However, in several studies Lamin A/C was used as differentiation marker.

1.4 Laminopathies

Laminopathies or envelopathies are a diverse group of genetic diseases, which are caused due to mutations in genes, encoding or affecting NE proteins. For example mutations or deletions in *EMD* or few mutations in *LMNA* genes give rise to Emery-Dreifuss Muscular Dystrophy (EDMD) (Broers et al., 2006). The laminopathies usually set on in adults and are characterized by different phenotypes including nuclear morphology aberrations, NE protein aggregation and/or detachment of heterochromatin from the NE, reviewed in (Davidson and Lammerding, 2014; Wilson and Foisner, 2010). The laminopathies can be further divided into different categories based on the affected tissue. In muscular dystrophy diseases mainly weakening/wasting of muscles occurs (Bione et al., 1994) without affecting other tissues. In accelerated aging syndrome, or premature aging, several tissues are affected (Azibani et al., 2014; Hutchinson, 1886).

The major cause of several diverse laminopathies including muscular dystrophies, cardiomyopathies and lipodystrophies is due to mutations or loss of the *LMNA* gene that encodes A-type lamins (Broers et al., 2006; Gruenbaum et al., 2005; Mattout et al., 2006; Mounkes et al., 2003; Muchir et al., 2004; Scaffidi and Misteli, 2006; Worman, 2012). Bonne and colleagues identified mutations in the *LMNA* gene that cause Autosomal-Dominant EDMD (AD-EDMD) (Bonne et al., 1999). So far, a couple of hundred disease causing mutations have been identified in the *LMNA* gene. However, the list of mutations is expanding (Azibani et al., 2014; Butin-Israeli et al., 2012). Several labs created *LMNA-/-* and disease mutation models. Most of the model animals were normal at birth but rapidly displayed defects in their growth, especially in muscles.

So far, very few studies have been reported about diseases with mutations in B-type lamins. Duplication of the *LMNB1* gene gave rise to leukodystrophy (Padiath et al., 2006) and

mutations in the *LMNB2* gene gave rise to partial lipodystrophy (Hegele et al., 2006). Mice with B-type lamin deficiency displayed abnormal nuclear morphology in neuronal cells, which led to defects in the neuronal development (Coffinier et al., 2010; Coffinier et al., 2011).

Bione and colleagues identified mutations in the *EMD* gene that leads to a genetic disorder termed X-linked EDMD (Bione et al., 1994). In different cases of X-EDMD, Emerin is functionally inactive. Patient cells carrying mutations in the *EMD* gene display an interesting phenotype, an increased distance between the centrosome and the NE (Salpingidou et al., 2007). In several cases of AD-EDMD patient cells displayed similar phenotypes of X-EDMD, for example centrosome detachment from the NE.

In other studies it has been shown that mutations in genes encoding LINC complex proteins also give rise to EDMD phenotypes. For example dominant mutations in *SYNE-1* and *-2* genes which encodes for Nesprin-1 and -2 proteins, respectively give rise to detachment of centrosome from the NE (Puckelwartz et al., 2009; Puckelwartz et al., 2010; Zhang et al., 2007). Recently, Li and colleagues discovered that at least one X-EDMD patient cells displayed additional mutations in the LINC complex protein, Sun1 (Li et al., 2014). Suggesting, there are more unknown players which may have involved in EDMD disease mechanism.

In different cases of laminopathies, including HGPS (Hutchinson-Gilford progeria syndrome) (Viteri et al., 2010) and Dunningan-type familial partial lipodystrophy (Verstraeten et al., 2009), patient cells displayed a significant increase in the ROS (reactive oxygen species) levels and accumulation of oxidized proteins. The above results suggests that the increased levels and accumulation of ROS in the laminopathic patient cells may led to DNA damage, reviewed in (Hutchison, 2011).

1.5 Chromatin organization

The chromatin is made up of dsDNA and histone proteins. The DNA is wrapped around the nucleosomes, which are composed of histones. The chromatin is classified into two different types based on their condensation. Several studies showed that the chromatin is highly condensed in the nuclear and nucleolar periphery, this chromatin is transcriptionally inactive or silent and termed heterochromatin, reviewed in (Padeken and Heun, 2014; Stancheva and Schirmer, 2014). In the interior of the nucleus, the chromatin termed euchromatin tends to be more loosely organized to give access to DNA and RNA polymerases for their activity in

transcription. The chromatin organization at the nuclear periphery involve the nuclear lamina and associated proteins (Bickmore, 2013; Kind et al., 2013; Kind and van Steensel, 2010; Luperchio et al., 2014; Peric-Hupkes and van Steensel, 2010; Zuleger et al., 2011). The Tudor domain of the INM protein lamin B receptor (LBR) interacts with heterochromatin protein 1 (HP1), which is important for heterochromatin formation. The LEM domain proteins from the INM interact with BAF, which is able to bind directly to DNA (See the BAF section) (Foisner and Gerace, 1993; Segura-Totten and Wilson, 2004; Tifft et al., 2006; Umland et al., 2000). During differentiation of pluripotent cells the chromatin becomes less dynamic by formation of heterochromatin in the nuclear periphery, which is a hallmark of the differentiated cell (Bhattacharya et al., 2009; Meshorer and Misteli, 2006). So far, only a few studies have elaborated on the mechanism of how NE proteins participate in chromatin organization. However, further studies of the NE organization in detail may give more information about the chromatin organization and also how NE proteins are involved in several genetic diseases (See laminopathies section).

1.6 Differentiation

In multi cellular organisms, the pluripotent cells are able to differentiate into three germ layers termed ectoderm, endoderm and mesoderm (Gieseck et al., 2014). The germ layers can further differentiate into several different cell lineages, for example muscular or neuronal cells. The differentiated cells are the building blocks of an organism. Cell lineage specific transcription regulators are expressed to differentiate pluripotent cells into specialized cell types, although the mechanism is not clearly known. Also, the chromatin is reorganized during the differentiation process (See the chromatin organization section) and chromatin modifications might influence the differentiation process (Bernstein et al., 2006). So far, very few studies have reported NE proteins to be involved in the differentiation process (D'Angelo et al., 2012; Smith et al., 2011). However, the role of the NE in differentiation is a challenging task for the future.

1.7 Mitosis

Mitosis is the event of nuclear division, where the genetic material is equally divided into daughter nuclei (Fig 5). Metazoans undergo open mitosis, where the NE disassembles to give access for microtubules to form the mitotic spindle. In contrast, yeast undergoes closed mitosis, where the NE remains intact and MTOC/SPB is inserted into the NE (Smoyer and Jaspersen, 2014). *Aspergillus nudulans* and *Saccharomyces japonicus* undergoes semi-open

mitosis, where the NE remains intact but the NPCs disassemble, this allows the diffusion of proteins involved in the mitotic machinery. Mitosis is an accurately controlled event with several checkpoints (Nezi and Musacchio, 2009; Zhou et al., 2002). Missegregation of chromosomes results in aneuploidy, which is the major cause of development of chromosome instability and cancer (Weaver and Cleveland, 2006; Weaver et al., 2006). Prior to mitosis in S-phase, the centrosome/MTOC and the genome are duplicated. During prophase, the microtubules assemble into a mitotic spindle, initiated by the nuclear envelope breakdown (NEBD). This is crucial for the chromosome alignment and separation, and at the same time the duplicated genetic material is condensed to form visible chromosomes. In prometaphase, the duplicated centrosomes start to move to opposite poles of the NE and also microtubules form a mitotic spindle with attached chromosomes. In metaphase, the chromosomes are arranged in a flat metaphase plate connected to the bipolar spindle. In anaphase, the spindle poles move further apart with the forces generated by primarily overlapping and astral microtubules to segregate the chromosomes. The NE reassembles on the separated chromosomes in late anaphase/telophase forming two daughter nuclei (Beaudouin et al., 2002; Zhou et al., 2002).
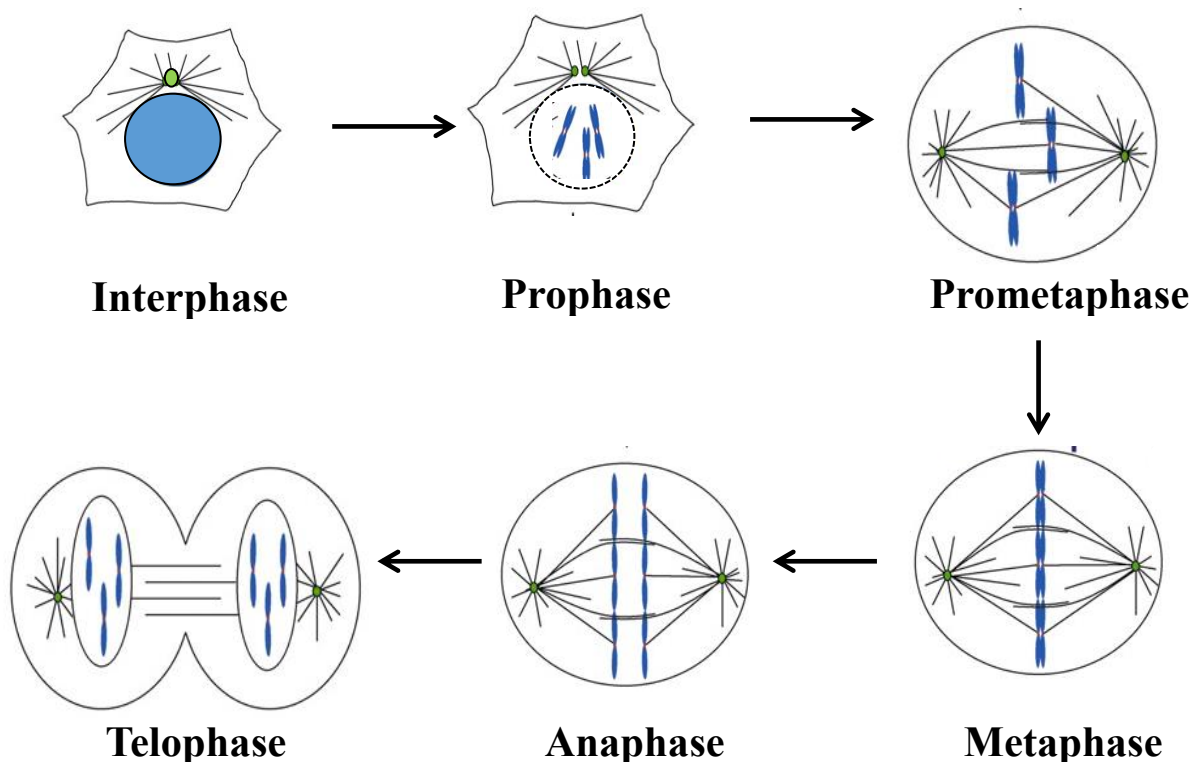

Interphase   **Prophase**   **Prometaphase**

Telophase   **Anaphase**   **Metaphase**

Fig 5. *Schematic representation of Mitosis. Modified from (Zhou et al., 2002)*

After nuclear division, the newly formed nuclei and the cytoplasm are divided into two daughter cells by the contraction and abscission of the plasma membrane. This is termed cytokinesis.

1.7.1 Nuclear envelope breakdown (NEBD)

In open mitosis the NEBD is one of the crucial step, marking the end of prophase, reviewed in (Burke and Stewart, 2002; Hetzer et al., 2005; Smoyer and Jaspersen, 2014). The NEBD is initiated through disassembly of NPCs and nuclear lamina. The nuclear lamins and INM proteins are phosphorylated, which counteract protein-protein interactions. As soon as the nuclear lamina disassembles, the microtubule cytoskeleton physically tears the membrane to complete the NEBD process.

Previously, it was assumed that the nuclear lamina solubilizes and transmembrane NE proteins disperses into the ER during mitosis (Fig 6a) (Ellenberg et al., 1997). However, in 2009 we reported the existence of a specific membrane domain of the mitotic spindle (Buch et al., 2009), an observation confirmed by other labs (Lu et al., 2009; Wilkie et al., 2011) (Fig 6b). However, the function of membranes and membrane proteins in the mitosis has to be elucidated in detail.
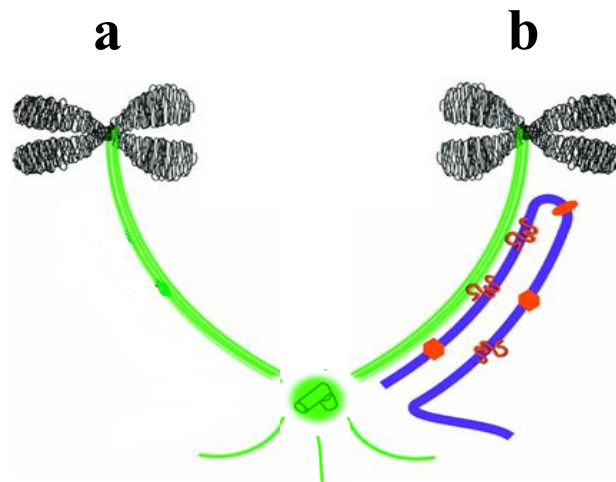

Fig 6. *Schematic sketch of spindle endomembranes (SE). Modified from (Figueroa et al., 2011)*

1.8 Embryonic stem (ES) cells and human induced pluripotent stem (hiPS) cells

Stem cells are the pluripotent cells, they can differentiate into specialized cells of the organism. In 1998, for the first time the ES/PS cells (Fig 7) were isolated from the mouse

embryos (Mountford et al., 1998). In mice, the zygote is formed after fertilization and undergoes several cell divisions to give rise to a mass of cells, termed the morula that consists of 16 cells. As development proceeds, the cells in the morula further divide and form the blastocyst. In the blastocyst, the cells are arranged in two different layers; the outer layer or trophoectoderm and the inner layer or inner cell mass (ICM). The ICM is the source of the pluripotent stem cells in embryos (Gieseck et al., 2014; Sánchez Alvarado and Yamanaka, 2014).
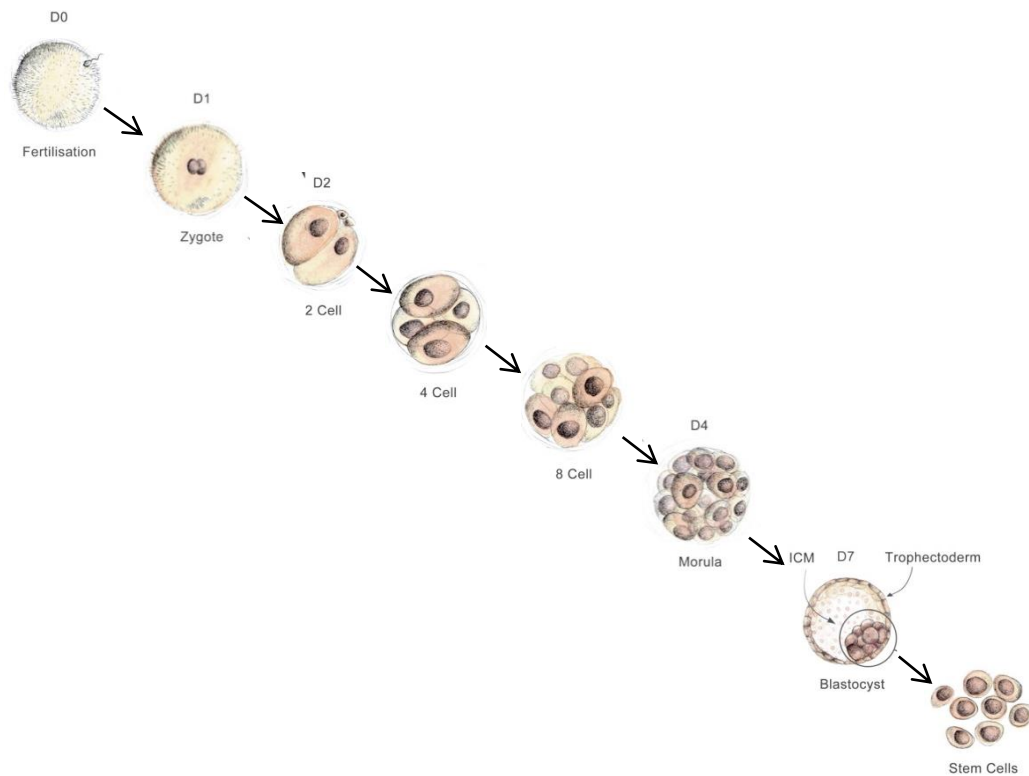

Fig 7. *Schematic view of embryonic stem cells development. Modified from (Gieseck et al., 2014)*

Human induced pluripotent stem cells (Fig 8) were first generated in the year 2006 (Takahashi and Yamanaka, 2006; Yamanaka and Takahashi, 2006) and represents a novel discovery in regenerative medicine. hiPSCs were derived from fibroblasts after ectopic over expression of only four specific transcription factors namely, Sox2, Oct4, c-myc and Klf4 (Nakagawa et al., 2008; Takahashi et al., 2007; Yamanaka, 2008a, b). Development of hiPSCs from patients has advantages and several applications because of their immune specificity. hiPSCs can be used in cell replacement therapies, disease models and also in development of new drugs. The major disadvantage of hiPSCs over ESCs is they are produced by using viral transduction and associated risks. hiPSCs are pluripotent and can differentiate into several other cell types. For example, over expression of specific cell

lineage regulators/transcription factors (TFs) differentiate hiPSCs into neurons, under conditions promoting differentiation, reviewed in (de Peppo and Marolt, 2012; Zhang et al., 2013). It has been reported that Silencing of lamin A enhances the generation of hiPSCs (Zuo et al., 2012) suggesting that the nuclear periphery might play a role in the production of hiPSCs. However, the mechanism is not known.
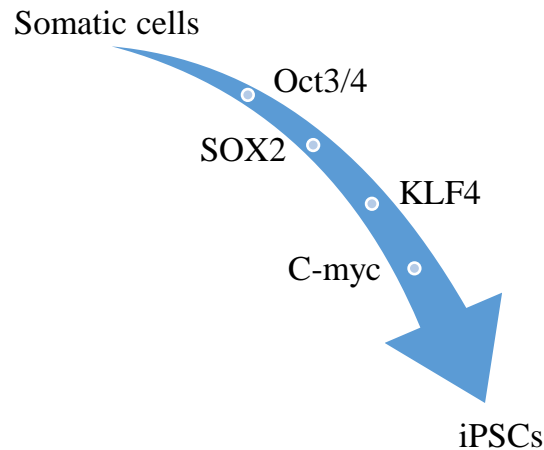

Fig 8. *Flow chart of induced pluripotent stem cells generation.*

Recent studies showed that expression of specific NE proteins might have a role in the generation of specialized differentiated cells from the pluripotent stem cells (Constantinescu et al., 2006; D'Angelo et al., 2012; Eckersley-Maslin et al., 2013; Smith et al., 2011; Zhang et al., 2013). In the present thesis (Paper-IV), we discuss how the INM transmembrane protein, Samp1 enhances the differentiation of hiPSCs into neurons.

ES and iPS cells have similar important properties; they are self-renewable, pluripotent, express pluripotent markers and they grow as multi layered colonies with boundaries. Self-renewal means that they can divide indefinitely and increase their population without any genomic abnormalities. Pluripotency means that they can differentiate into several specialized cells of an organism. However, ES and iPS cells have dissimilarities in the epigenetic landscape, protein expression and posttranslational modifications (Bernstein et al., 2006; Brumbaugh et al., 2011).

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2 METHODOLOGY

2.1 Microscopy

Microscopy in combination with computational image analysis is a powerful tool in scientific research. In our studies very often we used confocal laser scanning microscopy (CSLM). The major advantage of CSLM is elimination of out-of-focus light from the illuminated sample. A pinhole aperture is placed in front of the detector, which gives crisp optical sections at different focal depths of the sample. The size of the pinhole can be adjusted, by this adjustment only light emitted from the focal plane of the sample will be detected. Series of optical sections can be projected to give a 3D-image of the sample.
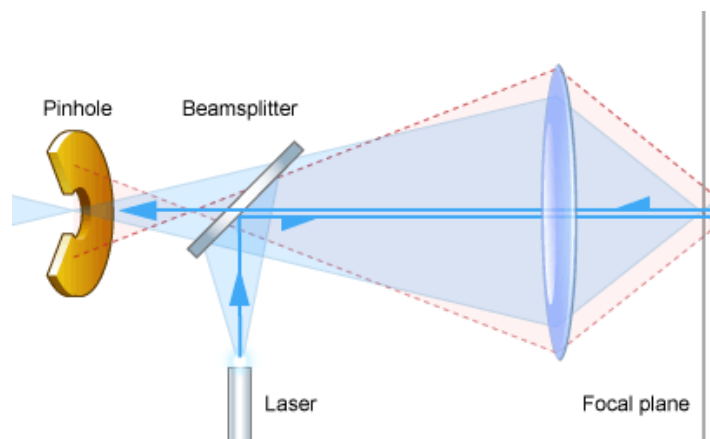

Fig 9*. Schematic representation of confocal laser scanning microscopy. From public domain.*

2.2 Culture and plating of human induced pluripotent stem (hiPS) cells.

Human induced pluripotent stem cells (hiPSCs) were grown in serum free and sterile pluripotent stem cell medium on a cell matrix basement membrane gel. ROCK inhibitor was used to prevent dissociation derived differentiation. To prevent spontaneous differentiation of hiPSCs, we needed to change the medium every 24 hrs and passage colonies after every 4 or 5 days. We developed specific techniques to plate hiPSC colonies onto glass bottom dishes coated with cell matrix basement membrane gel in order to keep the hiPSC colonies in the middle of the dish, which was very tricky. To overcome this we used a phase contrast microscope in combination with micromanipulator to cut out selected monolayer areas of colonies in the cultures and plate them in the middle of a 35mm glass bottom dish. In this way we could preserve pluripotency for up to 30 passages.

2.3 MCLIP (Membrane protein Cross-Link ImmunoPrecipitation)

Investigating interactions of transmembrane NE proteins is very difficult because extracting them from the membranes requires very harsh conditions, such as urea, which denatures the proteins and disrupts interactions. Therefore, we developed a novel method to study the interactions of hard-to-extract proteins. In this method we used a cell permeable *in vivo* crosslinker Dithiobis [succinimidyl propionate] (DSP), which has a spacer arm length of 12 Å. DSP contains a disulphide bond in the middle allowing reversal of the crosslink. Live cells were incubated with DSP for 15 min to crosslink associated neighboring proteins. After incubation, we quenched the reaction using Tris-HCl (pH-7.4). Solubilization of crosslinked proteins was performed using 7M urea and 1% Triton X-100. After solubilization of protein complexes, the urea concentration was reduced to 0.8M, which is tolerated by antibodies to perform specific co-immunoprecipitation of binding partners.

3 AIM OF THE THESIS

The overall aim of this thesis is to elucidate the functional organization of nuclear envelope proteins.

3.1 Individual aims of papers:

Paper-I

To characterize a novel transmembrane protein from the nuclear envelope.

Paper-II

To elucidate the functional domains and interactions of the nuclear envelope protein, Samp1.

Paper-III

To investigate protein-protein interactions of nuclear envelope proteins.

Paper-IV

To investigate the role of the nuclear envelope proteins in differentiation.

4   RESULTS AND DISCUSSION

4.1   Samp1 is a novel transmembrane protein specifically located in the inner nuclear membrane and functionally associated with the microtubule cytoskeleton (paper-I).

Subtractive proteomic studies for NE proteins in rat liver (Schirmer et al., 2003), leukocytes (Korfali et al., 2010) and muscles (Wilkie et al., 2011) has resulted in a long list of putative novel NE protein candidates. In this paper we focused on one of these putative transmembrane NE proteins identified in rat liver, Net5 (Schirmer et al., 2003). We found this protein especially interesting because it was conserved from humans to *S. pombe* and silencing of its homologue in a genome-wide siRNA screen in *C. elegans*, gave rise to phenotypes associated with mitotic defects (Sönnichsen et al., 2005). We termed this protein Samp1, because of reasons that will be discussed later (Page 19-20).

To characterize Samp1, we raised antibodies against a peptide located in the C-terminus and purified them using affinity chromatography. The purified antibodies gave rise to a single band of expected size in Western blotting of total cell lysates. The antibodies specifically stained nuclear rims in immunofluorescence microscopy. Both the 43 KDa band in Western blot and nuclear rim labeling disappeared after posttranscriptional silencing of Samp1 using siRNA. Hence, we concluded that the antipeptide antibodies were specific for Samp1 and that Samp1 was specifically localized in the NE. In humans Samp1 has three splice isoforms. We focused on the shortest isoform, which has a unique C-terminus recognized by our antibodies. Furthermore, Samp1 resisted 7M urea extraction, which proves that Samp1 is a transmembrane protein. Using immunoelectron microscopy and epitope accessibility assay in semi permeabilized cells we showed that Samp1 was specifically located in the INM. Taken together our data show that Samp1 is a novel transmembrane protein that specifically localizes to the INM.

We also investigated phenotypic changes after siRNA mediated silencing of Samp1 expression. Surprisingly, we noticed a significant increase in the distance between the NE and the centrosomes of Samp1 depleted cells (Fig 10), showing that Samp1 is functionally associated with the centrosome and microtubule cytoskeleton. Detachment of centrosomes from the NE has also been observed, in cells from Emery-Dreifuss muscular dystrophy patients carrying mutations in *LMNA* or *EMD* (Hale et al., 2008; Salpingidou et al., 2007), in

embryonic fibroblasts from *LMNA*-/- mice and fibroblasts over expressing A-type lamin mutants (Hale et al., 2008). Centrosome detachment was also observed in cells post-transcriptionally silenced with siRNA against Emerin (Salpingidou et al., 2007), as well as in cells carrying mutations in Nesprins (Malone et al., 2003). This suggests that A-type lamins, the LINC complex proteins, Emerin and Samp1, function together in a cellular process which is important for the specific disease mechanism behind muscular dystrophy.
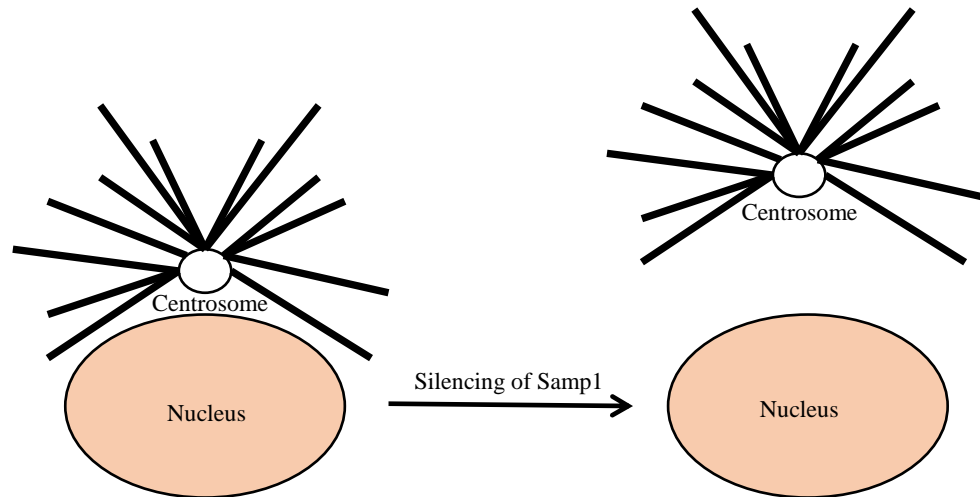

Fig 10. Centrosome detachment from the NE after post-transcriptional silencing of Samp1.

4.2   Samp1 is concentrated in the mitotic spindle during mitosis (paper-I).

To study the distribution of Samp1 during the cell cycle, we performed immunofluorescence microscopy using antipeptide antibodies specific for Samp1 and live cell imaging in cells expressing YFP-Samp1. During mitosis, INM proteins are known to disperse into the ER (Ellenberg et al., 1997). Surprisingly, we noticed that a significant fraction of Samp1 was concentrated in the mitotic spindle during metaphase in both live (Fig 11A, arrow) and fixed cells (Fig 11B, arrow). Hence, we termed this protein Samp1 (Spindle associated membrane protein 1). The localization of Samp1 to the mitotic spindle was not specific for HeLa cells, but also occurred in neuroblastoma (SH-SY5Y) and MDCK cells. The enrichment of Samp1 in the mitotic spindle even after post-transcriptional silencing, suggests that Samp1 might have an important role in the mitotic machinery. Our study was the first to demonstrate the existence of a specific membrane domain in the mitotic spindle. After our study, Lu and colleagues (Lu et al., 2009) showed that several ER proteins tagged with GFP also located in the mitotic spindle. Although the ER proteins were not enriched in the spindle, the study supports the idea of the presence of membranes in the spindle (Lu et al., 2009). More recently, Wilkie and colleagues showed that two novel proteins from the INM, WFS1 and

Tmem214 (Wilkie et al., 2011) were also concentrated in the mitotic spindle. The nucleoplasmically exposed N-terminal domain of Samp1 is sufficient to localize Samp1 to the mitotic spindle. Intersetingly, genome wide RNAi screens in HeLa cells (Neumann et al., 2010) and in *C. elegans* (Sönnichsen et al., 2005) siRNA mediated silencing of Samp1 expression resulted in mitosis defective phenotypes.
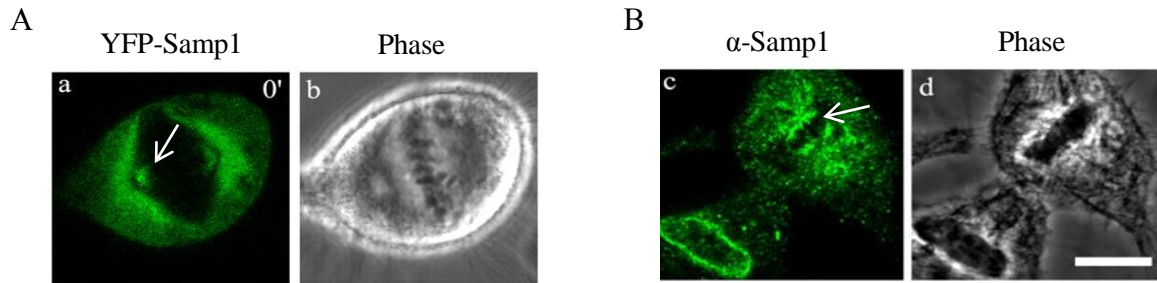

Fig 11. *A sub fraction of Samp1 localized to the mitotic spindle in live (A) and fixed (B) cells. Modified from (Buch et al., 2009)*

4.3 Samp1 distributes in specific micro-domains partially overlapping with LINC complex protein, Sun1 (paper-II).

With the development of better microscopes more and more labs have showed that many NE proteins distribution in specific micro-domains (Liu et al., 2007; Lu et al., 2008; Maeshima et al., 2006; Shimi et al., 2008). For example Lamin A/C and Lamin B distributes in separate networks with mutual exclusion (Shimi et al., 2008). To investigate the distribution of Samp1 in the INM we used high resolution fluorescence microscopy. We applied deconvolution on immunofluorescence microscopy image stacks along the Z-axis acquired by CLSM. Immunostaining of Samp1 was not uniformly distributed in the INM but instead gave rise to a distinct dotty pattern, which showed a partial colocalization with the LINC complex protein, Sun1. This is interesting in light of the functional association between Samp1 and the microtubule cytoskeleton, *c.f.* 4.1.

4.4 Membrane topology of Samp1 (paper-II).

Samp1 was suggested to have five transmembrane domains by membrane topology prediction programs. To investigate the functional domains of Samp1, we created deletion mutants from the C-terminus of YFP-Samp1. Surprisingly, deletion mutants lacking the four predicted transmembrane domains in the C-terminal half of Samp1 distributed in the nucleoplasm instead of nuclear rim. We concluded that the first hydrophobic domain is not a

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true transmembrane domain and that both the N- and C-terminal ends are exposed in the nucleoplasm (Fig 12). This is in agreement with the topology of homologues of Samp1 in fission yeast (King et al., 2008) and *Chaetomium thermophilum (Ct)* (Jafferali et al., 2014)
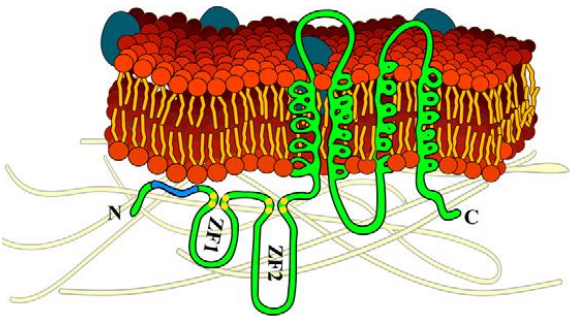

Fig 12. *Membrane topology of Samp1. Adapted from (Gudise et al., 2011)*

4.5 The Zinc finger domain of Samp1 is responsible for its localization to the NE and interaction with Emerin (paper-II).

The N-terminal tail of Samp1 contains eight conserved cysteine residues organized in four -CXXC- motifs, which might be able to form two Zinc fingers. Furthermore, silencing of Samp1 expression showed that Emerin localization to the INM was dependent on the presence of Samp1. To investigate a possible interaction between Samp1 and Emerin we performed co-immunoprecipitation using GFP-antibodies in cells over expressing YFP-Samp1. The experiment showed that Samp1 interacts with Emerin and that the interaction only occurred in the presence of Zinc ions (Fig 13), supporting the existence of functional Zinc finger(s) in Samp1.
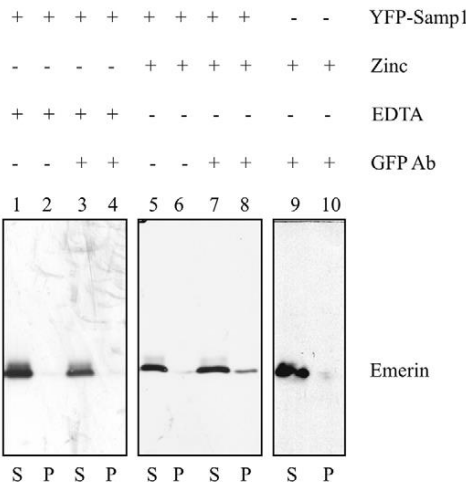

Fig 13. *Samp1 interacts with Emerin in the presence of Zinc ions. Adapted from (Gudise et al., 2011)*

4.6 Zinc finger mutants of Samp1 disrupts NE and chromatin organization (Paper-II)

We constructed cysteine to alanine substitution mutations in YFP-Samp1 to disrupt Zinc finger formation. These mutants showed mislocalization to cytoplasmic membranes, suggesting that Zinc finger formation might be responsible for the proper localization of Samp1 to the INM.

After over expression of Zinc finger mutants, we noticed that not only Samp1 was mislocalized but also that the nuclei acquired a lobulated shape and loss of peripheral chromatin. The effect was very strong and appeared similar to morphologies of nuclei in cells expressing laminopathic disease mutations or down regulation of lamins (Dechat et al., 2008; Gruenbaum et al., 2005; Sullivan et al., 1999). Interestingly, over expression of Zinc finger mutants also showed effects on the localization to the INM of a specific set of INM proteins including Emerin, Lamin A/C and Sun1, but not Sun2, nuclear pores and Lamin B. The effect on the INM organization suggests that Samp1 functionally associates with the A-type lamina network proteins. Samp1 association/interaction with the A-type lamina network proteins may be essential for Samp1 anchorage to the NE.

4.7 Samp1 interacts with Sun1, Emerin, Lamin B1 and Ran in live cells (paper-III).

It has traditionally been very difficult to study protein-protein interactions of membrane proteins of the NE because they are difficult to extract from the membranes in their native form. To overcome this problem we developed an efficient method, to identify and study interactions of NE proteins in live cells that we termed MCLIP (Membrane protein Cross-Link ImmunoPrecipitation).

To elucidate the interaction network of Samp1, we used MCLIP (See methodology section) to crosslink interacting proteins using a cell permeable crosslinker followed by complete solubilization in Triton X-100 and 7M urea. After dilution of the urea, the samples were subjected to immunoprecipitation using α-GFP antibodies. In U2OS cells expressing YFP-Samp1 or Samp1-YFP MCLIP was able to specifically coprecipitate Emerin, Sun1, Ran and Lamin B1 but not Lamin A/C, Sun2, p62, Nup214, POM 121 or Nup210/gp210, demonstrating that MCLIP detects specific interactions. The previously reported interaction between Samp1 and Emerin using Co-IP *in vitro* (Gudise et al., 2011), was reproduced *in vivo*. Also, the interaction between Samp1 and Sun1 that was suggested in paper-II was

reproduced and could explain the centrosome detachment observed in Paper-I. Furthermore, using recombinantly expressed proteins in *E. coli* we showed that N-terminal domains of *Ct. Samp1* and human Emerin can bind to each other directly.

4.8   Samp1 in differentiation (paper-IV).

It is known that the nuclear periphery plays important roles in organization of chromatin and may influence the differentiation of cells. However, there is very little known about the role of individual NE proteins during differentiation. Yet, it is known that Lamin A/C expression correlates with differentiation of pluripotent cells (Constantinescu et al., 2006; Eckersley-Maslin et al., 2013). Samp1 is differently expressed in different tissues (Figueroa et al., 2010; Korfali et al., 2012). In order to investigate the Samp1 expression during differentiation we employed human induced pluripotent stem cells (hiPSCs) as model system in paper-IV.

4.9   Samp1 is localized to the NE in the spontaneously differentiated pluripotent cells but not in undifferentiated cells (paper-IV).

We characterized the nuclear envelope organization in differentiating hiPSCs by immunofluorescence using antibodies specific for different NE proteins. The distribution and expression of the LINC complex proteins, Sun1 and Sun2 were similar in both undifferentiated and differentiated cells. We observed low levels of Lamin A/C in undifferentiated cells and higher levels in early stages of differentiation, consistent with previous studies on ES cells showing increased Lamin A/C staining after spontaneous differentiation (Constantinescu et al., 2006; Eckersley-Maslin et al., 2013). We found that Samp1 displayed a similar expression pattern as Lamin A/C. This is consistent with the expression pattern in differentiating hES cells (unpublished). Emerin was expressed in both undifferentiated and differentiated cells but the enrichment in the NE was higher in differentiated cells correlating with the appearance of its binding partners, Samp1 (Gudise et al., 2011) and Lamin A/C (Libotte et al., 2005; Sullivan et al., 1999). Previously, Emerin was shown to be localized to the NE after 2-5 days of differentiation of ES cells (Butler et al., 2009) and redistributed during adipogenic differentiation (Verstraeten et al., 2011). These results call for further investigation of the relation between the differential expression of NE proteins and the differentiation process.

4.10 Over expression of Samp1 induces rapid differentiation in hiPSCs into neurons (paper-IV).

To our surprise we discovered that after transient over expression of YFP-Samp1 in undifferentiated hiPSC colonies, the morphology of the colonies were drastically changed even under conditions promoting pluripotency. The effect was massive, affecting all cells in the colony and occurred very rapidly (within 24 hrs). The effect was specific for Samp1 because there are no changes in the morphology of hiPSC colonies transfected with cDNA encoding YFP or YFP-Emerin.

Interestingly, an increased level of Lamin A/C and βIII-tubulin were observed in cells transfected with YFP-Samp1 (Fig 14, arrow) but not in untransfected cells or, cells transfected with YFP alone. Our study is, to our knowledge, the first to show that transient over expression of an INM protein induces rapid (6 days) differentiation of hiPSCs into neurons. Previously, several different labs reported that the differentiation of pluripotent stem cells into neurons under conditions promoting differentiation required longer times varying between 1 to 6 weeks, reviewed in (Zhang et al., 2013). In another report, over expression of a LINC complex protein, Nesprin-1, enhanced differentiation of ES cells most likely by modifying the architecture and chromatin binding property of the NE (Smith et al., 2011). Also, a recent study showed that Nup210/gp210 promoted differentiation by increasing the activity of specific set of genes in ES and myoblasts (D'Angelo et al., 2012). However, the mechanism has to be studied in detail.
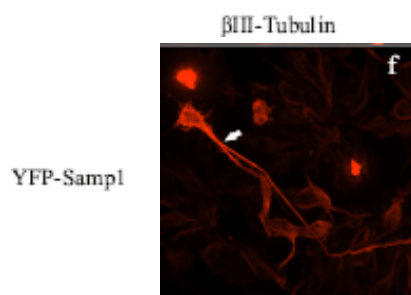

Fig 14. *Samp1 specifically induces differentiation of hiPSCs into neurons.*

Finally, I speculate that transient over expression of YFP-Samp1 may organize the NE composition and the organized NE might have an impact on the chromatin organization and gene expression to induce differentiation of hiPSCs into neurons. In regard to a role of Samp1 in chromatin organization, I would like to mention that Samp1 has been reported to affect intranuclear positioning of specific chromosomes in human cells (Zuleger et al., 2013) and

that its homologue in fission yeast, Ima1, was found to preferentially colocalize with heterochromatin (Steglich et al., 2012) and interact with centromeric heterochromatin (King et al., 2008).

4.11 The medium from hiPSC colonies transfected with YFP-Samp1 cDNA is sufficient to differentiate undifferentiated hiPSC colonies (paper-IV).

We noticed that during YFP-Samp1 induced differentiation, only a few cells were actually transfected. Nevertheless the effect was global in hiPSC colonies. We hypothesized that the transfected cells, when differentiating may secrete soluble factor(s), which could induce differentiation of other cells in the colony. Medium supplementation experiment supported our hypothesis but the exact mechanism remains to be studied in detail.

The major impact from our study may be the unusually rapid generation of a specific cell type from pluripotent stem cells. This might contribute to improve regenerative medicine in the future and gives faster help to patients suffering from cell degeneration diseases.

4.12 CONCLUSIONS

In this thesis, we focused on a novel transmembrane protein from the INM that we termed Samp1 (Paper-I). Samp1 is the first transmembrane protein shown to colocalize with microtubules of the mitotic spindle.

➢ Samp1 is specifically located in the INM, and it is also essential for the correct localization of another INM protein, Emerin (Paper II). The interaction between Samp1 and Emerin occurs in live cells (Paper-III) and requires Zinc ions (Paper-II) and can occur by direct binding (Paper-III).

➢ Samp1 is important for the attachment of the centrosome to the NE, a phenomenon most likely mediated via the LINC complexes (Paper-I). Samp1 is distributed in distinct microdomains in the NE displaying partial colocalization with the LINC complex protein, Sun1 (paper-II). Samp1 also interacts with Sun1 in live U2OS cells (Paper-III).

➢ Samp1 has four well conserved –CXXC– motifs in the N-terminal nucleoplasmicdomain, which can form two putative Zinc fingers (Paper-I). The Zinc finger domain of Samp1 is important for its correct localization to INM, chromatin and nuclear envelope organization (Paper-II) and interacting with Emerin (Paper-II). Taken together, the results suggest that the Samp1 might have one or two functional Zinc fingers.

➢ Samp1 expression is induced upon differentiation of hiPSCs and Samp1 over expression induces rapid differentiation of hiPSCs into neurons (Paper-IV). Medium from the hiPSCs over expressed with Samp1 is sufficient to differentiate untransfected hiPSCs.

4.13 FUTURE DIRECTIONS

In the past years of my Ph.D study we have found different interesting aspects of Samp1, which remains to be elucidated in detail. Here, I would like to take a chance to mention some of the possible future directions.

Structural organization of Zinc fingers in Samp1

In paper-II we showed that Samp1 has four conserved –CXXC- motifs in the N-terminal nucleoplasmic domain, which is likely to form two Zinc fingers. However, the organization and number of Zinc fingers has to be investigated in detail. To study the structural organization of the Zinc finger domain, we tried to produce crystals using recombinantly expressed and purified His-tagged N-terminal tail of human Samp1. Unfortunately, we have not succeeded to get crystals. At present, we turned to produce N-terminal tail of Samp1 homologue in *Chaetomium thermophilum*, which has higher possibilities to form crystals. As soon as we get crystals of Samp1 we perform X-ray crystallography to reveal how Zinc finger(s) are arranged. Furthermore, we will perform co-crystallization along with the interaction partners such as the nucleoplasmic N-terminal domain of Emerin to explain how Samp1 and Emerin interaction takes place in the presence of Zinc ions using 3D-structure.

Proteome analysis of Samp1 interaction partners

In paper-III, We developed a novel method termed MCLIP to investigate the interaction partners of Samp1 in live cells. Using MCLIP we showed specific interactions of Samp1 in live U2OS cells. To find out unknown interaction partners of Samp1 we will perform mass spectrometric analysis after MCLIP. We will also perform reciprocal immunoprecipitation to confirm the interactions. We will continue with posttranscriptional silencing of interesting interacting partners to find out the importance of the interaction in cells. Furthermore, we can extend MCLIP in different stages of cell cycle. For example, investigating interaction partners of Samp1 in the mitotic spindle may give clues about the function of Samp1 and membranes in the mitotic spindle.

Samp1 in differentiation

In paper-IV, we tried to find out the role of Samp1 during differentiation of hiPSCs. After over expression of YFP-tagged Samp1 in hiPSCs, we noticed a rapid differentiation into neurons. However, the role of Samp1 in differentiation has to be studied in detail. To find out whether the neurons produced after differentiation are functional or not we will perform

electrophysiological experiments in collaboration with Prof. Gilad Silberberg at Karolinska Institutet. To study the mechanism in neuronal differentiation we will investigate which neural genes are up or down regulated by using microarray technique or deep sequencing. Also, we can extend the study by finding the role of other NE proteins in the differentiation process. For example, Lamin proteins.

5 ACKNOWLEDGMENTS

6 REFERENCES

Aaronson, R. P., and G. Blobel, 1975, Isolation of nuclear pore complexes in association with a lamina: Proc Natl Acad Sci U S A, v. 72, p. 1007-11.

Aebi, U., J. Cohn, L. Buhle, and L. Gerace, 1986, The nuclear lamina is a meshwork of intermediate-type filaments: Nature, v. 323, p. 560-4.

Akey, C. W., 1995, Structural plasticity of the nuclear pore complex: J Mol Biol, v. 248, p. 273-93.

Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter, 2008, Molecular biology of the cell, Garland Science.

Asakawa, H., H. J. Yang, T. G. Yamamoto, C. Ohtsuki, Y. Chikashige, K. Sakata-Sogawa, M. Tokunaga, M. Iwamoto, Y. Hiraoka, and T. Haraguchi, 2014, Characterization of nuclear pore complex components in fission yeast Schizosaccharomyces pombe: Nucleus, v. 5, p. 149-62.

Azibani, F., A. Muchir, N. Vignier, G. Bonne, and A. T. Bertrand, 2014, Striated muscle laminopathies: Semin Cell Dev Biol, v. 29C, p. 107-115.

Beaudouin, J., D. Gerlich, N. Daigle, R. Eils, and J. Ellenberg, 2002, Nuclear envelope breakdown proceeds by microtubule-induced tearing of the lamina: Cell, v. 108, p. 83-96.

Beck, M., F. Förster, M. Ecke, J. M. Plitzko, F. Melchior, G. Gerisch, W. Baumeister, and O. Medalia, 2004, Nuclear pore complex structure and dynamics revealed by cryoelectron tomography: Science, v. 306, p. 1387-90.

Bernstein, B. E., T. S. Mikkelsen, X. Xie, M. Kamal, D. J. Huebert, J. Cuff, B. Fry, A. Meissner, M. Wernig, K. Plath, R. Jaenisch, A. Wagschal, R. Feil, S. L. Schreiber, and E. S. Lander, 2006, A bivalent chromatin structure marks key developmental genes in embryonic stem cells: Cell, v. 125, p. 315-26.

Bhattacharya, D., S. Talwar, A. Mazumder, and G. V. Shivashankar, 2009, Spatio-temporal plasticity in chromatin organization in mouse cell differentiation and during Drosophila embryogenesis: Biophys J, v. 96, p. 3832-9.

Bickmore, W. A., 2013, The spatial organization of the human genome: Annu Rev Genomics Hum Genet, v. 14, p. 67-84.

Bione, S., E. Maestrini, S. Rivella, M. Mancini, S. Regis, G. Romeo, and D. Toniolo, 1994, Identification of a novel X-linked gene responsible for Emery-Dreifuss muscular dystrophy: Nat Genet, v. 8, p. 323-7.

Blobel, G., 1980, Intracellular protein topogenesis: Proc Natl Acad Sci U S A, v. 77, p. 1496-500.

Bonne, G., M. R. Di Barletta, S. Varnous, H. M. Bécane, E. H. Hammouda, L. Merlini, F. Muntoni, C. R. Greenberg, F. Gary, J. A. Urtizberea, D. Duboc, M. Fardeau, D. Toniolo, and K. Schwartz, 1999, Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy: Nat Genet, v. 21, p. 285-8.

Borrego-Pinto, J., T. Jegou, D. S. Osorio, F. Auradé, M. Gorjánácz, B. Koch, I. W. Mattaj, and E. R. Gomes, 2012, Samp1 is a component of TAN lines and is required for nuclear movement: J Cell Sci, v. 125, p. 1099-105.

Brachner, A., and R. Foisner, 2011, Evolvement of LEM proteins as chromatin tethers at the nuclear periphery: Biochem Soc Trans, v. 39, p. 1735-41.

Broers, J. L., F. C. Ramaekers, G. Bonne, R. B. Yaou, and C. J. Hutchison, 2006, Nuclear lamins: laminopathies and their role in premature ageing: Physiol Rev, v. 86, p. 967-1008.

Brumbaugh, J., C. M. Rose, D. H. Phanstiel, J. A. Thomson, and J. J. Coon, 2011, Proteomics and pluripotency: Crit Rev Biochem Mol Biol, v. 46, p. 493-506.

Buch, C., R. Lindberg, R. Figueroa, S. Gudise, E. Onischenko, and E. Hallberg, 2009, An integral protein of the inner nuclear membrane localizes to the mitotic spindle in mammalian cells: J Cell Sci, v. 122, p. 2100-7.

Burke, B., and K. J. Roux, 2009, Nuclei take a position: managing nuclear location: Dev Cell, v. 17, p. 587-97.

Burke, B., and C. L. Stewart, 2002, Life at the edge: the nuclear envelope and human disease: Nat Rev Mol Cell Biol, v. 3, p. 575-85.

Butin-Israeli, V., S. A. Adam, A. E. Goldman, and R. D. Goldman, 2012, Nuclear lamin functions and disease: Trends Genet, v. 28, p. 464-71.

Butler, J. T., L. L. Hall, K. P. Smith, and J. B. Lawrence, 2009, Changing nuclear landscape and unique PML structures during early epigenetic transitions of human embryonic stem cells: J Cell Biochem, v. 107, p. 609-21.

Coffinier, C., S. Y. Chang, C. Nobumori, Y. Tu, E. A. Farber, J. I. Toth, L. G. Fong, and S. G. Young, 2010, Abnormal development of the cerebral cortex and cerebellum in the setting of lamin B2 deficiency: Proc Natl Acad Sci U S A, v. 107, p. 5076-81.

Coffinier, C., H. J. Jung, C. Nobumori, S. Chang, Y. Tu, R. H. Barnes, Y. Yoshinaga, P. J. de Jong, L. Vergnes, K. Reue, L. G. Fong, and S. G. Young, 2011, Deficiencies in lamin B1 and lamin B2 cause neurodevelopmental defects and distinct nuclear shape abnormalities in neurons: Mol Biol Cell, v. 22, p. 4683-93.

Cohen, T. V., L. Hernandez, and C. L. Stewart, 2008, Functions of the nuclear envelope and lamina in development and disease: Biochem Soc Trans, v. 36, p. 1329-34.

Constantinescu, D., H. L. Gray, P. J. Sammak, G. P. Schatten, and A. B. Csoka, 2006, Lamin A/C expression is a marker of mouse and human embryonic stem cell differentiation: Stem Cells, v. 24, p. 177-85.

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

Cronshaw, J. M., A. N. Krutchinsky, W. Zhang, B. T. Chait, and M. J. Matunis, 2002, Proteomic analysis of the mammalian nuclear pore complex: J Cell Biol, v. 158, p. 915-27.

D'Angelo, M. A., J. S. Gomez-Cavazos, A. Mei, D. H. Lackner, and M. W. Hetzer, 2012, A change in nuclear pore complex composition regulates cell differentiation: Dev Cell, v. 22, p. 446-58.

D'Angelo, M. A., and M. W. Hetzer, 2006, The role of the nuclear envelope in cellular organization: Cell Mol Life Sci, v. 63, p. 316-32.

D'Angelo, M. A., and M. W. Hetzer, 2008, Structure, dynamics and function of nuclear pore complexes: Trends Cell Biol, v. 18, p. 456-66.

Davidson, P. M., and J. Lammerding, 2014, Broken nuclei--lamins, nuclear mechanics, and disease: Trends Cell Biol, v. 24, p. 247-56.

de Peppo, G. M., and D. Marolt, 2012, State of the art in stem cell research: human embryonic stem cells, induced pluripotent stem cells, and transdifferentiation: J Blood Transfus, v. 2012, p. 317632.

Dechat, T., S. A. Adam, P. Taimen, T. Shimi, and R. D. Goldman, 2010, Nuclear lamins: Cold Spring Harb Perspect Biol, v. 2, p. a000547.

Dechat, T., K. Pfleghaar, K. Sengupta, T. Shimi, D. K. Shumaker, L. Solimando, and R. D. Goldman, 2008, Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin: Genes Dev, v. 22, p. 832-53.

Dittmer, T. A., and T. Misteli, 2011, The lamin protein family: Genome Biol, v. 12, p. 222.

Dorner, D., J. Gotzmann, and R. Foisner, 2007, Nucleoplasmic lamins and their interaction partners, LAP2alpha, Rb, and BAF, in transcriptional regulation: FEBS J, v. 274, p. 1362-73.

Eckersley-Maslin, M. A., J. H. Bergmann, Z. Lazar, and D. L. Spector, 2013, Lamin A/C is expressed in pluripotent mouse embryonic stem cells: Nucleus, v. 4, p. 53-60.

Ellenberg, J., E. D. Siggia, J. E. Moreira, C. L. Smith, J. F. Presley, H. J. Worman, and J. Lippincott-Schwartz, 1997, Nuclear membrane dynamics and reassembly in living cells: targeting of an inner nuclear membrane protein in interphase and mitosis: J Cell Biol, v. 138, p. 1193-206.

Figueroa, R., S. Gudise, V. Larsson, and E. Hallberg, 2010, A transmembrane inner nuclear membrane protein in the mitotic spindle: Nucleus, v. 1, p. 249-53.

Figueroa, R. A., S. Gudise, and E. Hallberg, 2011, Microtubule-associated nuclear envelope proteins in interphase and mitosis: Biochem Soc Trans, v. 39, p. 1786-9.

Foisner, R., and L. Gerace, 1993, Integral membrane proteins of the nuclear envelope interact with lamins and chromosomes, and binding is modulated by mitotic phosphorylation: Cell, v. 73, p. 1267-79.

Furukawa, K., 1999, LAP2 binding protein 1 (L2BP1/BAF) is a candidate mediator of LAP2-chromatin interaction: J Cell Sci, v. 112 (Pt 15), p. 2485-92.

Furukawa, K., S. Sugiyama, S. Osouda, H. Goto, M. Inagaki, T. Horigome, S. Omata, M. McConnell, P. A. Fisher, and Y. Nishida, 2003, Barrier-to-autointegration factor plays crucial roles in cell cycle progression and nuclear organization in Drosophila: J Cell Sci, v. 116, p. 3811-23.

Gerace, L., A. Blum, and G. Blobel, 1978, Immunocytochemical localization of the major polypeptides of the nuclear pore complex-lamina fraction. Interphase and mitotic distribution: The Journal of Cell Biology, v. 79, p. 546-566.

Gerace, L., and B. Burke, 1988, Functional organization of the nuclear envelope: Annu Rev Cell Biol, v. 4, p. 335-74.

Gieseck, R. L., J. Colquhoun, and N. R. Hannan, 2014, Disease modeling using human induced pluripotent stem cells: Lessons from the liver: Biochim Biophys Acta.

Gruenbaum, Y., A. Margalit, R. D. Goldman, D. K. Shumaker, and K. L. Wilson, 2005, The nuclear lamina comes of age: Nat Rev Mol Cell Biol, v. 6, p. 21-31.

Gruenbaum, Y., K. L. Wilson, A. Harel, M. Goldberg, and M. Cohen, 2000, Review: nuclear lamins--structural proteins with fundamental functions: J Struct Biol, v. 129, p. 313-23.

Gudise, S., R. A. Figueroa, R. Lindberg, V. Larsson, and E. Hallberg, 2011, Samp1 is functionally associated with the LINC complex and A-type lamina networks: J Cell Sci, v. 124, p. 2077-85.

Hale, C. M., A. L. Shrestha, S. B. Khatau, P. J. Stewart-Hutchinson, L. Hernandez, C. L. Stewart, D. Hodzic, and D. Wirtz, 2008, Dysfunctional connections between the nucleus and the actin and microtubule networks in laminopathic models: Biophys J, v. 95, p. 5462-75.

Hallberg, E., R. W. Wozniak, and G. Blobel, 1993, An integral membrane protein of the pore membrane domain of the nuclear envelope contains a nucleoporin-like region: J Cell Biol, v. 122, p. 513-21.

Haraguchi, T., T. Koujin, M. Segura-Totten, K. K. Lee, Y. Matsuoka, Y. Yoneda, K. L. Wilson, and Y. Hiraoka, 2001, BAF is required for emerin assembly into the reforming nuclear envelope: J Cell Sci, v. 114, p. 4575-85.

Harborth, J., S. M. Elbashir, K. Bechert, T. Tuschl, and K. Weber, 2001, Identification of essential genes in cultured mammalian cells using small interfering RNAs: J Cell Sci, v. 114, p. 4557-65.

Hegele, R. A., H. Cao, D. M. Liu, G. A. Costain, V. Charlton-Menys, N. W. Rodger, and P. N. Durrington, 2006, Sequencing of the reannotated LMNB2 gene reveals novel mutations in patients with acquired partial lipodystrophy: Am J Hum Genet, v. 79, p. 383-9.

Hetzer, M. W., 2010, The Nuclear Envelope: Cold Spring Harbor Perspectives in Biology, v. 2.

Hetzer, M. W., T. C. Walther, and I. W. Mattaj, 2005, Pushing the envelope: structure, function, and dynamics of the nuclear periphery: Annu Rev Cell Dev Biol, v. 21, p. 347-80.

Holaska, J. M., A. K. Kowalski, and K. L. Wilson, 2004, Emerin caps the pointed end of actin filaments: evidence for an actin cortical network at the nuclear inner membrane: PLoS Biol, v. 2, p. E231.

Holaska, J. M., K. K. Lee, A. K. Kowalski, and K. L. Wilson, 2003, Transcriptional repressor germ cell-less (GCL) and barrier to autointegration factor (BAF) compete for binding to emerin in vitro: J Biol Chem, v. 278, p. 6969-75.

Holaska, J. M., and K. L. Wilson, 2007, An emerin "proteome": purification of distinct emerin-containing complexes from HeLa cells suggests molecular basis for diverse roles including gene regulation, mRNA splicing, signaling, mechanosensing, and nuclear architecture: Biochemistry, v. 46, p. 8897-908.

Hutchinson, J., 1886, Case of congenital absence of hair, with atropic condition of the skin and its appendages, in a boy whose mother had been almost wholly bald from alopecia areata from the age of six.: Med Chir Trans., p. 473-477.

Hutchison, C. J., 2011, The role of DNA damage in laminopathy progeroid syndromes: Biochem Soc Trans, v. 39, p. 1715-8.

Hutchison, C. J., 2014, B-type lamins in health and disease: Semin Cell Dev Biol, v. 29C, p. 158-163.

Jafferali, M. H., B. Vijayaraghavan, R. A. Figueroa, E. Crafoord, S. Gudise, V. J. Larsson, and E. Hallberg, 2014, MCLIP, an effective method to detect interactions of transmembrane proteins of the nuclear envelope in live cells: Biochim Biophys Acta.

Kim, Y., A. A. Sharov, K. McDole, M. Cheng, H. Hao, C. M. Fan, N. Gaiano, M. S. Ko, and Y. Zheng, 2011, Mouse B-type lamins are required for proper organogenesis but not by embryonic stem cells: Science, v. 334, p. 1706-10.

Kim, Y., X. Zheng, and Y. Zheng, 2013, Proliferation and differentiation of mouse embryonic stem cells lacking all lamins: Cell Res, v. 23, p. 1420-3.

Kind, J., L. Pagie, H. Ortabozkoyun, S. Boyle, S. S. de Vries, H. Janssen, M. Amendola, L. D. Nolen, W. A. Bickmore, and B. van Steensel, 2013, Single-cell dynamics of genome-nuclear lamina interactions: Cell, v. 153, p. 178-92.

Kind, J., and B. van Steensel, 2010, Genome-nuclear lamina interactions and gene regulation: Curr Opin Cell Biol, v. 22, p. 320-5.

King, M. C., T. G. Drivas, and G. Blobel, 2008, A network of nuclear envelope membrane proteins linking centromeres to microtubules: Cell, v. 134, p. 427-38.

Korfali, N., G. S. Wilkie, S. K. Swanson, V. Srsen, D. G. Batrakou, E. A. Fairley, P. Malik, N. Zuleger, A. Goncharevich, J. de Las Heras, D. A. Kelly, A. R. Kerr, L. Florens, and E. C. Schirmer, 2010, The leukocyte nuclear envelope proteome varies with cell activation and contains novel transmembrane proteins that affect genome architecture: Mol Cell Proteomics, v. 9, p. 2571-85.

Korfali, N., G. S. Wilkie, S. K. Swanson, V. Srsen, J. I. de las Heras, D. G. Batrakou, P. Malik, N. Zuleger, A. R. W. Kerr, L. Florens, and E. C. Schirmer, 2012, The nuclear envelope proteome differs notably between tissues: Nucleus, v. 3, p. 552-564.

Lattanzi, G., V. Cenni, S. Marmiroli, C. Capanni, E. Mattioli, L. Merlini, S. Squarzoni, and N. M. Maraldi, 2003, Association of emerin with nuclear and cytoplasmic actin is regulated in differentiating myoblasts: Biochem Biophys Res Commun, v. 303, p. 764-70.

Lehner, C. F., R. Stick, H. M. Eppenberger, and E. A. Nigg, 1987, Differential expression of nuclear lamin proteins during chicken development: J Cell Biol, v. 105, p. 577-87.

Lei, K., X. Zhang, X. Ding, X. Guo, M. Chen, B. Zhu, T. Xu, Y. Zhuang, R. Xu, and M. Han, 2009, SUN1 and SUN2 play critical but partially redundant roles in anchoring nuclei in skeletal muscle cells in mice: Proc Natl Acad Sci U S A, v. 106, p. 10207-12.

Li, P., P. Meinke, l. T. Huong, M. Wehnert, and A. A. Noegel, 2014, Contribution of SUN1 mutations to the pathomechanism in muscular dystrophies: Hum Mutat, v. 35, p. 452-61.

Libotte, T., H. Zaim, S. Abraham, V. C. Padmakumar, M. Schneider, W. Lu, M. Munck, C. Hutchison, M. Wehnert, B. Fahrenkrog, U. Sauder, U. Aebi, A. A. Noegel, and I. Karakesisoglou, 2005, Lamin A/C-dependent localization of Nesprin-2, a giant scaffolder at the nuclear envelope: Mol Biol Cell, v. 16, p. 3411-24.

Liu, Q., N. Pante, T. Misteli, M. Elsagga, M. Crisp, D. Hodzic, B. Burke, and K. J. Roux, 2007, Functional association of Sun1 with nuclear pore complexes: J Cell Biol, v. 178, p. 785-98.

Lourim, D., and J. J. Lin, 1989, Expression of nuclear lamin A and muscle-specific proteins in differentiating muscle cells in ovo and in vitro: J Cell Biol, v. 109, p. 495-504.

Lu, L., M. S. Ladinsky, and T. Kirchhausen, 2009, Cisternal organization of the endoplasmic reticulum during mitosis: Mol Biol Cell, v. 20, p. 3471-80.

Lu, W., J. Gotzmann, L. Sironi, V. M. Jaeger, M. Schneider, Y. Lüke, M. Uhlén, C. A. Szigyarto, A. Brachner, J. Ellenberg, R. Foisner, A. A. Noegel, and I. Karakesisoglou, 2008, Sun1 forms immobile macromolecular assemblies at the nuclear envelope: Biochim Biophys Acta, v. 1783, p. 2415-26.

Luperchio, T. R., X. Wong, and K. L. Reddy, 2014, Genome regulation at the peripheral zone: lamina associated domains in development and disease: Curr Opin Genet Dev, v. 25, p. 50-61.

Maeshima, K., K. Yahata, Y. Sasaki, R. Nakatomi, T. Tachibana, T. Hashikawa, F. Imamoto, and N. Imamoto, 2006, Cell-cycle-dependent dynamics of nuclear pores: pore-free islands and lamins: J Cell Sci, v. 119, p. 4442-51.

Malone, C. J., L. Misner, N. Le Bot, M. C. Tsai, J. M. Campbell, J. Ahringer, and J. G. White, 2003, The C. elegans hook protein, ZYG-12, mediates the essential attachment between the centrosome and nucleus: Cell, v. 115, p. 825-36.

Margalit, A., A. Brachner, J. Gotzmann, R. Foisner, and Y. Gruenbaum, 2007, Barrier-to-autointegration factor--a BAFfling little protein: Trends Cell Biol, v. 17, p. 202-8.

Margalit, A., M. Segura-Totten, Y. Gruenbaum, and K. L. Wilson, 2005, Barrier-to-autointegration factor is required to segregate and enclose chromosomes within the nuclear envelope and assemble the nuclear lamina: Proc Natl Acad Sci U S A, v. 102, p. 3290-5.

Mattout, A., T. Dechat, S. A. Adam, R. D. Goldman, and Y. Gruenbaum, 2006, Nuclear lamins, diseases and aging: Curr Opin Cell Biol, v. 18, p. 335-41.

Meshorer, E., and T. Misteli, 2006, Chromatin in pluripotent embryonic stem cells and differentiation: Nat Rev Mol Cell Biol, v. 7, p. 540-6.

Montes de Oca, R., K. K. Lee, and K. L. Wilson, 2005, Binding of barrier to autointegration factor (BAF) to histone H3 and selected linker histones including H1.1: J Biol Chem, v. 280, p. 42252-62.

Mounkes, L. C., S. Kozlov, L. Hernandez, T. Sullivan, and C. L. Stewart, 2003, A progeroid syndrome in mice is caused by defects in A-type lamins: Nature, v. 423, p. 298-301.

Mountford, P., J. Nichols, B. Zevnik, C. O'Brien, and A. Smith, 1998, Maintenance of pluripotential embryonic stem cells by stem cell selection: Reprod Fertil Dev, v. 10, p. 527-33.

Muchir, A., J. Medioni, M. Laluc, C. Massart, T. Arimura, A. J. van der Kooi, I. Desguerre, M. Mayer, X. Ferrer, S. Briault, M. Hirano, H. J. Worman, A. Mallet, M. Wehnert, K. Schwartz, and G. Bonne, 2004, Nuclear envelope alterations in fibroblasts from patients with muscular dystrophy, cardiomyopathy, and partial lipodystrophy carrying lamin A/C gene mutations: Muscle Nerve, v. 30, p. 444-50.

Méjat, A., and T. Misteli, 2010, LINC complexes in health and disease: Nucleus, v. 1, p. 40-52.

Nakagawa, M., M. Koyanagi, K. Tanabe, K. Takahashi, T. Ichisaka, T. Aoi, K. Okita, Y. Mochiduki, N. Takizawa, and S. Yamanaka, 2008, Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts: Nat Biotechnol, v. 26, p. 101-6.

Neumann, B., T. Walter, J. K. Hériché, J. Bulkescher, H. Erfle, C. Conrad, P. Rogers, I. Poser, M. Held, U. Liebel, C. Cetin, F. Sieckmann, G. Pau, R. Kabbe, A. Wünsche, V. Satagopam, M. H. Schmitz, C. Chapuis, D. W. Gerlich, R. Schneider, R. Eils, W. Huber, J. M. Peters, A. A. Hyman, R. Durbin, R. Pepperkok, and J. Ellenberg, 2010, Phenotypic profiling of the human genome by time-lapse microscopy reveals cell division genes: Nature, v. 464, p. 721-7.

Nezi, L., and A. Musacchio, 2009, Sister chromatid tension and the spindle assembly checkpoint: Curr Opin Cell Biol, v. 21, p. 785-95.

Ostlund, C., J. Ellenberg, E. Hallberg, J. Lippincott-Schwartz, and H. J. Worman, 1999, Intracellular trafficking of emerin, the Emery-Dreifuss muscular dystrophy protein: J Cell Sci, v. 112 (Pt 11), p. 1709-19.

Padeken, J., and P. Heun, 2014, Nucleolus and nuclear periphery: Velcro for heterochromatin: Curr Opin Cell Biol, v. 28C, p. 54-60.

Padiath, Q. S., K. Saigoh, R. Schiffmann, H. Asahara, T. Yamada, A. Koeppen, K. Hogan, L. J. Ptácek, and Y. H. Fu, 2006, Lamin B1 duplications cause autosomal dominant leukodystrophy: Nat Genet, v. 38, p. 1114-23.

Peric-Hupkes, D., and B. van Steensel, 2010, Role of the nuclear lamina in genome organization and gene expression: Cold Spring Harb Symp Quant Biol, v. 75, p. 517-24.

Puckelwartz, M. J., E. Kessler, Y. Zhang, D. Hodzic, K. N. Randles, G. Morris, J. U. Earley, M. Hadhazy, J. M. Holaska, S. K. Mewborn, P. Pytel, and E. M. McNally, 2009, Disruption of nesprin-1 produces an Emery Dreifuss muscular dystrophy-like phenotype in mice: Hum Mol Genet, v. 18, p. 607-20.

Puckelwartz, M. J., E. J. Kessler, G. Kim, M. M. Dewitt, Y. Zhang, J. U. Earley, F. F. Depreux, J. Holaska, S. K. Mewborn, P. Pytel, and E. M. McNally, 2010, Nesprin-1 mutations in human and murine cardiomyopathy: J Mol Cell Cardiol, v. 48, p. 600-8.

Rout, M. P., and J. D. Aitchison, 2000, Pore relations: nuclear pore complexes and nucleocytoplasmic exchange: Essays Biochem, v. 36, p. 75-88.

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

Scaffidi, P., and T. Misteli, 2006, Lamin A-dependent nuclear defects in human aging: Science, v. 312, p. 1059-63.

Schirmer, E. C., L. Florens, T. Guan, J. R. Yates, and L. Gerace, 2003, Nuclear membrane proteins with potential disease links found by subtractive proteomics: Science, v. 301, p. 1380-2.

Schirmer, E. C., L. Florens, T. Guan, J. R. Yates, and L. Gerace, 2005, Identification of novel integral membrane proteins of the nuclear envelope with potential disease links using subtractive proteomics: Novartis Found Symp, v. 264, p. 63-76; discussion 76-80, 227-30.

Schirmer, E. C., and R. Foisner, 2007, Proteins that associate with lamins: many faces, many functions: Exp Cell Res, v. 313, p. 2167-79.

Schirmer, E. C., and L. Gerace, 2005, The nuclear membrane proteome: extending the envelope: Trends Biochem Sci, v. 30, p. 551-8.

Segura-Totten, M., and K. L. Wilson, 2004, BAF: roles in chromatin, nuclear structure and retrovirus integration: Trends Cell Biol, v. 14, p. 261-6.

Shimi, T., T. Koujin, M. Segura-Totten, K. L. Wilson, T. Haraguchi, and Y. Hiraoka, 2004, Dynamic interaction between BAF and emerin revealed by FRAP, FLIP, and FRET analyses in living HeLa cells: J Struct Biol, v. 147, p. 31-41.

Shimi, T., K. Pfleghaar, S. Kojima, C. G. Pack, I. Solovei, A. E. Goldman, S. A. Adam, D. K. Shumaker, M. Kinjo, T. Cremer, and R. D. Goldman, 2008, The A- and B-type nuclear lamin networks: microdomains involved in chromatin organization and transcription: Genes Dev, v. 22, p. 3409-21.

Simon, D. N., and K. L. Wilson, 2013, Partners and post-translational modifications of nuclear lamins: Chromosoma, v. 122, p. 13-31.

Smith, E. R., X. Y. Zhang, C. D. Capo-Chichi, X. Chen, and X. X. Xu, 2011, Increased expression of Syne1/nesprin-1 facilitates nuclear envelope structure changes in embryonic stem cell differentiation: Dev Dyn, v. 240, p. 2245-55.

Smoyer, C. J., and S. L. Jaspersen, 2014, Breaking down the wall: the nuclear envelope during mitosis: Curr Opin Cell Biol, v. 26, p. 1-9.

Sosa, B. A., U. Kutay, and T. U. Schwartz, 2013, Structural insights into LINC complexes: Current Opinion in Structural Biology, v. 23, p. 285-291.

Sosa, Brian A., A. Rothballer, U. Kutay, and Thomas U. Schwartz, 2012, LINC Complexes Form by Binding of Three KASH Peptides to Domain Interfaces of Trimeric SUN Proteins: Cell, v. 149, p. 1035-1047.

Soullam, B., and H. J. Worman, 1995, Signals and structural features involved in integral membrane protein targeting to the inner nuclear membrane: J Cell Biol, v. 130, p. 15-27.

Stancheva, I., and E. C. Schirmer, 2014, Nuclear envelope: connecting structural genome organization to regulation of gene expression: Adv Exp Med Biol, v. 773, p. 209-44.

Starr, D. A., 2009, A nuclear-envelope bridge positions nuclei and moves chromosomes: J Cell Sci, v. 122, p. 577-86.

Steglich, B., G. J. Filion, B. van Steensel, and K. Ekwall, 2012, The inner nuclear membrane proteins Man1 and Ima1 link to two different types of chromatin at the nuclear periphery in S. pombe: Nucleus, v. 3, p. 77-87.

Stewart, C. L., K. J. Roux, and B. Burke, 2007, Blurring the boundary: the nuclear envelope extends its reach: Science, v. 318, p. 1408-12.

Strambio-De-Castillia, C., M. Niepel, and M. P. Rout, 2010, The nuclear pore complex: bridging nuclear transport and gene regulation: Nat Rev Mol Cell Biol, v. 11, p. 490-501.

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

Sullivan, T., D. Escalante-Alcalde, H. Bhatt, M. Anver, N. Bhat, K. Nagashima, C. L. Stewart, and B. Burke, 1999, Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy: J Cell Biol, v. 147, p. 913-20.

Sánchez Alvarado, A., and S. Yamanaka, 2014, Rethinking differentiation: stem cells, regeneration, and plasticity: Cell, v. 157, p. 110-9.

Sönnichsen, B., L. B. Koski, A. Walsh, P. Marschall, B. Neumann, M. Brehm, A. M. Alleaume, J. Artelt, P. Bettencourt, E. Cassin, M. Hewitson, C. Holz, M. Khan, S. Lazik, C. Martin, B. Nitzsche, M. Ruer, J. Stamford, M. Winzi, R. Heinkel, M. Röder, J. Finell, H. Häntsch, S. J. Jones, M. Jones, F. Piano, K. C. Gunsalus, K. Oegema, P. Gönczy, A. Coulson, A. A. Hyman, and C. J. Echeverri, 2005, Full-genome RNAi profiling of early embryogenesis in Caenorhabditis elegans: Nature, v. 434, p. 462-9.

Takahashi, K., K. Okita, M. Nakagawa, and S. Yamanaka, 2007, Induction of pluripotent stem cells from fibroblast cultures: Nat Protoc, v. 2, p. 3081-9.

Takahashi, K., and S. Yamanaka, 2006, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors: Cell, v. 126, p. 663-76.

Terry, L. J., E. B. Shows, and S. R. Wente, 2007, Crossing the nuclear envelope: hierarchical regulation of nucleocytoplasmic transport: Science, v. 318, p. 1412-6.

Tifft, K. E., M. Segura-Totten, K. K. Lee, and K. L. Wilson, 2006, Barrier-to-autointegration factor-like (BAF-L): a proposed regulator of BAF: Exp Cell Res, v. 312, p. 478-87.

Tran, E. J., and S. R. Wente, 2006, Dynamic nuclear pore complexes: life on the edge: Cell, v. 125, p. 1041-53.

Umland, T. C., S. Q. Wei, R. Craigie, and D. R. Davies, 2000, Structural basis of DNA bridging by barrier-to-autointegration factor: Biochemistry, v. 39, p. 9130-8.

Verstraeten, V. L., S. Caputo, M. A. van Steensel, I. Duband-Goulet, S. Zinn-Justin, M. Kamps, H. J. Kuijpers, C. Ostlund, H. J. Worman, J. J. Briedé, C. Le Dour, C. L. Marcelis, M. van Geel, P. M. Steijlen, A. van den Wijngaard, F. C. Ramaekers, and J. L. Broers, 2009, The R439C mutation in LMNA causes lamin oligomerization and susceptibility to oxidative stress: J Cell Mol Med, v. 13, p. 959-71.

Verstraeten, V. L., J. Renes, F. C. Ramaekers, M. Kamps, H. J. Kuijpers, F. Verheyen, M. Wabitsch, P. M. Steijlen, M. A. van Steensel, and J. L. Broers, 2011, Reorganization of the nuclear lamina and cytoskeleton in adipogenesis: Histochem Cell Biol, v. 135, p. 251-61.

Viteri, G., Y. W. Chung, and E. R. Stadtman, 2010, Effect of progerin on the accumulation of oxidized proteins in fibroblasts from Hutchinson Gilford progeria patients: Mech Ageing Dev, v. 131, p. 2-8.

Wang, Q., X. Du, Z. Cai, and M. I. Greene, 2006, Characterization of the structures involved in localization of the SUN proteins to the nuclear envelope and the centrosome: DNA Cell Biol, v. 25, p. 554-62.

Wang, X., S. Xu, C. Rivolta, L. Y. Li, G. H. Peng, P. K. Swain, C. H. Sung, A. Swaroop, E. L. Berson, T. P. Dryja, and S. Chen, 2002, Barrier to autointegration factor interacts with the cone-rod homeobox and represses its transactivation function: J Biol Chem, v. 277, p. 43288-300.

Weaver, B. A., and D. W. Cleveland, 2006, Does aneuploidy cause cancer?: Curr Opin Cell Biol, v. 18, p. 658-67.

Weaver, B. A., A. D. Silk, and D. W. Cleveland, 2006, Cell biology: nondisjunction, aneuploidy and tetraploidy: Nature, v. 442, p. E9-10; discussion E10.

Wilhelmsen, K., M. Ketema, H. Truong, and A. Sonnenberg, 2006, KASH-domain proteins in nuclear migration, anchorage and other processes: J Cell Sci, v. 119, p. 5021-9.

Wilkie, G. S., N. Korfali, S. K. Swanson, P. Malik, V. Srsen, D. G. Batrakou, J. de las Heras, N. Zuleger, A. R. Kerr, L. Florens, and E. C. Schirmer, 2011, Several novel nuclear envelope transmembrane proteins identified in skeletal muscle have cytoskeletal associations: Mol Cell Proteomics, v. 10, p. M110.003129.

Wilson, K. L., and J. M. Berk, 2010, The nuclear envelope at a glance: J Cell Sci, v. 123, p. 1973-8.

Wilson, K. L., and R. Foisner, 2010, Lamin-binding Proteins: Cold Spring Harbor Perspectives in Biology, v. 2.

Worman, H. J., 2012, Nuclear lamins and laminopathies: J Pathol, v. 226, p. 316-25.

Yamanaka, S., 2008a, Induction of pluripotent stem cells from mouse fibroblasts by four transcription factors: Cell Prolif, v. 41 Suppl 1, p. 51-6.

Yamanaka, S., 2008b, Pluripotency and nuclear reprogramming: Philos Trans R Soc Lond B Biol Sci, v. 363, p. 2079-87.

Yamanaka, S., and K. Takahashi, 2006, [Induction of pluripotent stem cells from mouse fibroblast cultures]: Tanpakushitsu Kakusan Koso, v. 51, p. 2346-51.

Zhang, Q., C. Bethmann, N. F. Worth, J. D. Davies, C. Wasner, A. Feuer, C. D. Ragnauth, Q. Yi, J. A. Mellad, D. T. Warren, M. A. Wheeler, J. A. Ellis, J. N. Skepper, M. Vorgerd, B. Schlotter-Weigel, P. L. Weissberg, R. G. Roberts, M. Wehnert, and C. M. Shanahan, 2007, Nesprin-1 and -2 are involved in the pathogenesis of Emery Dreifuss muscular dystrophy and are critical for nuclear envelope integrity: Hum Mol Genet, v. 16, p. 2816-33.

Zhang, X., K. Lei, X. Yuan, X. Wu, Y. Zhuang, T. Xu, R. Xu, and M. Han, 2009, SUN1/2 and Syne/Nesprin-1/2 complexes connect centrosome to the nucleus during neurogenesis and neuronal migration in mice: Neuron, v. 64, p. 173-87.

Zhang, Y., C. Pak, Y. Han, H. Ahlenius, Z. Zhang, S. Chanda, S. Marro, C. Patzke, C. Acuna, J. Covy, W. Xu, N. Yang, T. Danko, L. Chen, M. Wernig, and T. C. Südhof, 2013, Rapid single-step induction of functional neurons from human pluripotent stem cells: Neuron, v. 78, p. 785-98.

Zhou, J., J. Yao, and H. C. Joshi, 2002, Attachment and tension in the spindle assembly checkpoint: J Cell Sci, v. 115, p. 3547-55.

Zuleger, N., S. Boyle, D. A. Kelly, J. I. de Las Heras, V. Lazou, N. Korfali, D. G. Batrakou, K. N. Randles, G. E. Morris, D. J. Harrison, W. A. Bickmore, and E. C. Schirmer, 2013, Specific nuclear envelope transmembrane proteins can promote the location of chromosomes to and from the nuclear periphery: Genome Biol, v. 14, p. R14.

Zuleger, N., M. I. Robson, and E. C. Schirmer, 2011, The nuclear envelope as a chromatin organizer: Nucleus, v. 2, p. 339-49.

Zuo, B., J. Yang, F. Wang, L. Wang, Y. Yin, J. Dan, N. Liu, and L. Liu, 2012, Influences of lamin A levels on induction of pluripotent stem cells: Biol Open, v. 1, p. 1118-27.