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Interaction networks and dynamics of three inner nuclear membrane LEM proteins

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Abstract

The LEM domain is a structural motif initially named after its identification in three mammalian nuclear envelope proteins: LAP2, Emerin and MAN1. Most LEM domain-containing proteins represent type II transmembrane proteins and localize specifically at the inner nuclear membrane (INM). LEM proteins interact directly via the LEM domain with the chromatin component barrier-to-autointegration factor, BAF, and are thereby involved in chromatin tethering, nuclear architecture and regulation of gene expression at the nuclear periphery. Mutations in LEM proteins have been linked to human diseases including muscular dystrophy, dilated cardiomyopathy and osteopoikilosis.

This work aims at elucidating the complex interaction network between three inner nuclear membrane LEM domain-containing proteins, Emerin, LEM2 and MAN1, based on the identification via BioID. This recently developed *in vivo* biotin-labelling technique exploits a genetically engineered, promiscuous biotin ligase which biotinylates proteins in a proximity-dependent manner. It is specifically suited to study low-affinity and transient interactions as well as protein complexes, which are difficult to isolate under physiological lysis conditions, like in the case of inner nuclear membrane proteins. Besides a number of common and specific candidate interaction partners of LEM2, MAN1 and Emerin, BioID revealed that, the structurally related proteins LEM2 and MAN1 have a larger overlap in binding partners than either LEM2 or MAN1 with Emerin, a more distantly related LEM protein. Surprisingly, within the unique LEM2 BioID partners many components of the nucleotide excision repair machinery were identified. Further investigations point to an impairment of DNA damage repair upon loss of LEM2, which describes a promising novel function for LEM2.

At the *in vivo* level, the dynamics and the functional behavior of LEM proteins was investigated by live cell imaging. We found that LEM proteins were located in nuclear envelope invaginations, which were often seen connected to nucleoli. The live cell imaging results provide strong evidence for a possible role of nuclear envelope invaginations in reassembling and relocalization of nucleoli during the cell cycle.

Zusammenfassung

Die LEM Domäne ist ein spezifisches Strukturmotiv, benannt nach den Proteinen in denen sie entdeckt wurde: LAP2, Emerin und MAN1. Die meisten LEM Proteine sind Typ II Transmembranproteine und lokalisieren spezifisch in der inneren Zellkernmembran. LEM Proteine binden direkt über ihre LEM Domäne an das DNA-bindende Protein BAF (barrier-to-autointegration factor), und sind dadurch an Chromatin-Anbindung, der Kernarchitektur, und der Regulation der Genexpression beteiligt. Mutationen in LEM Proteinen werden mit humanen Erkrankungen wie Muskeldystrophie, Kardiomyopathie und Osteopoikilose assoziiert.

Diese Arbeit befasst sich mit der Aufklärung des Interaktionsnetzwerks von drei LEM Proteinen der inneren Zellkernmembran, Emerin, LEM2 und MAN1, anhand einer kürzlich entwickelten Identifizierungsmethode namens BioID. Diese *in vivo* Biotin-Markierungs Methode basiert auf einer genetisch veränderten Biotin Ligase, welche wahllos und entfernungsabhängig biotinyliert und sich deswegen besonders für Proteininteraktionen mit besonders geringer Affinität, sowie auch für schwierig zu isolierende Proteinkomplexe wie die der inneren Zellkernmembran eignet. BioID identifizierte viele spezifische und gemeinsame Bindungspartner von LEM2, MAN1 und Emerin. Außerdem zeigten die BioID Ergebnisse, dass die strukturell-ähnlichen LEM Proteine, LEM2 und MAN1, mehr gemeinsame Bindungspartner haben als LEM2 oder MAN1 mit Emerin, einem strukturell nicht vergleichbaren LEM Protein. Überraschenderweise wurden einige Komponenten des Nukleotidexzisionsreparatur-Weges als potentielle LEM2-spezifische Bindungspartner identifiziert. Weiterführende knockdown-Experimente zeigten eine Beeinträchtigung des DNA Reparaturpotentials von humanen Zellen, in Abwesenheit von LEM2, welches möglicherweise eine neue Funktion für LEM2 beschreibt.

Des Weiteren wurde das funktionelle Verhalten und die Dynamiken von LEM Proteinen anhand von Live Cell Imaging Experimenten untersucht. Wir fanden heraus, dass LEM Proteine in Invaginationen der Kernmembran lokalisieren, welche oft an Nukleoli binden. Diese Resultate zeigen, dass die Invaginationen der inneren Zellkernmembran möglicherweise eine wichtige Bedeutung für die Relokalisierung und Reassemblierung von Nukleoli während des Zell Zyklus haben.

1 Introduction

1.1 The nucleus

1.1.1 The nuclear structure

The nucleus is a highly regulated double-membraned system and the hallmark of all eukaryotic cells (Hetzer, 2010; Laird et al., 1913). It can be compartmentalized in three major parts, the nucleolus, which constitutes the largest compartment and harbors most of the ribosome biosynthesis (Hernandez-Verdun, 2006; Raska et al., 2006b), the nucleoplasm, a viscous suspension medium for nucleoli, chromatin and a variety of nuclear protein complexes, and the nuclear envelope (NE), which is made up of the inner nuclear membrane (INM) and the outer nuclear membrane (ONM).

The nucleolus of higher eukaryotes can be divided in three different compartments: the dense fibrillar component, the granular component and the fibrillary center (Thiry and Lafontaine, 2005). In the early 1960s, the presence of RNAs and ribosomal genes in the nucleoli was confirmed for the first time (Perry, 1962; Ritossa and Spiegelman, 1965). Since then many nucleolar compartments were characterized and the ribosome biosynthesis was found to occur in nucleoli, which represent its main function. Besides the commitment to polymerase I-dependent transcription of ribosomal, genes and the assembly of pre-ribosomal particles, the nucleolus regulates protein interactions by capturing specific proteins, also known as nucleolar detention (Audas et al., 2012; Raska et al., 2006a). The nucleolus is made of RNA and proteins that disperse during mitosis. The reassembly starts at the telophase and early G_1-phase around transcriptionally active nucleolar organizing centers, which represent specific genetic loci on several chromosomes (Benavente et al., 1987; Jimenez-Garcia et al., 1994). Today the nucleolus is known for several other important functions. It assembles signal recognition particles which direct the correct traffic and synthesis of secreted proteins (Sommerville et al., 2005; Walter et al., 2000) and it senses

cellular stress and regulates p53-dependent and -independent responses (Holmberg Olausson et al., 2012), just to name a few.

In mammalian cells, nucleoli are in direct contact with "tube-like" invaginations of the NE (Figure 1). The NE can fold to produce invaginations, which increases the NE surface and may thus increase the efficiency of NE-linked cellular processes (Bootman et al., 2009; Geraud et al., 1989; Hernandez-Verdun, 2006). Although the functions are poorly understood, there is increasing evidence that NE invaginations are involved in gene expression, calcium signaling, nuclear lipid transport and metabolism (Boisvert et al., 2007; Bootman et al., 2009).

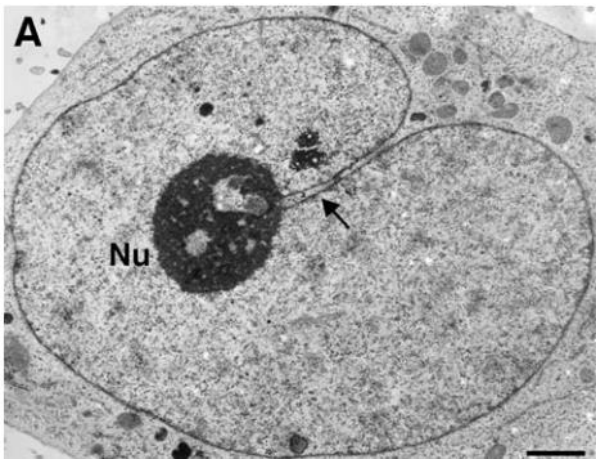


Figure 1: NE forms deep, branching invaginations
Electron microscopy shows mammalian cells where the NE is in direct contact with nucleoli (Nu) by "tube-like" invaginations of the NE. Figure adapted from (Bootman et al., 2009).

The NE (Figure 2) is the major functional hallmark of the eukaryotic cell, which physically separates the transcription and the translation machinery. It consists of two lipid bilayers, the INM and the ONM, which maintain the nucleus as an individual biochemical and structural compartment by preventing the free diffusion of molecules, and separating the nuclear contents from the cytoplasm. Controlled trafficking of molecules is ensured through nuclear pore complexes (NPC) which regularly perforate the two bilayers (Mekhail and Moazed, 2010). Inner and outer nuclear membranes are separated by the perinuclear space (Figure 2 "Lumen"), which is continuous with the lumen of the endoplasmic reticulum (ER) and contains numerous specific

proteins. The INM and the ONM are joined at sides of NPCs and the ONM connects to the ER, which is also studded with ribosomes (Burke and Ellenberg, 2002).

Integral and membrane-associated proteins of the INM provide a binding site for the chromatin and the nuclear lamina (Worman and Courvalin, 2000) which is a network of lamin polymers and lamin binding proteins, underlying the INM and providing stability and integrity for the nucleus (Gruenbaum et al., 2005).

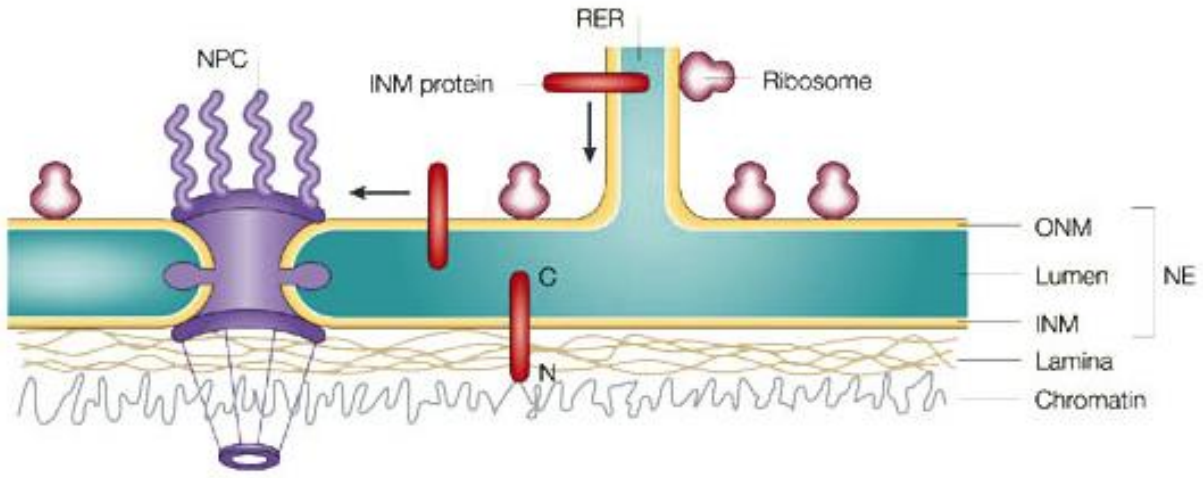


Figure 2: The structural constitution of the nuclear envelope
The ONM and the INM are continuous and linked at the NPC. The rough ER (RER) is continuous with the ONM and both are studded with ribosomes. Proteins of the INM are inserted into the RER and reach their destination by diffusing through the ONM to INM through channels, provided by NPCs (Mattaj, 2004)

1.1.2 The nuclear lamina

The nuclear lamina fulfills numerous of regulatory activities including gene expression, chromatin organization, developmental signaling and tissue maintenance (Andres and Gonzalez, 2009; Dauer and Worman, 2009; Meister et al., 2011). It represents a thin fibrous protein meshwork (15-20nm in mammals) is flanked by peripheral and integral membrane proteins of the NE and the chromatin at the nuclear periphery (Burke and Stewart, 2013; Gesson et al., 2014). Lamin type V intermediate filaments are the main constituents of the nuclear lamina, besides numerous lamin-binding proteins (Dechat et al., 2008). Lamins are further subdivided into A- and B-type lamins (Butin-Israeli et

al., 2012), both existing in several isoforms (Figure 3) . Major A type isoforms: lamin A and lamin C, which are derived from the *LMNA* gene by alternative splicing, are developmentally expressed depending on the differentiation status of the cell and expressed much later than B-type lamins. These are encoded by *LMNB1* and *LMNB2* genes, giving rise to two ubiquitously expressed proteins: lamin B1 and lamin B2 (Dechat et al., 2010). The linkage between INM and B-type lamins is much stronger than that of A-type lamins, which are also found in a more mobile and dynamic pool in the nucleoplasm. This is due to the different processing during lamin maturation. Lamin A, B1 and B2 have a C-terminal –CAAX box (C=Cysteine, AA= two aliphatic residues, X=C-terminal amino acid (aa)) which determines the site of methylation and farnesylation, and in this way the post-translational processing (Rusinol and Sinensky, 2006). B-type lamins are post-translationally processed to yield a mature protein, which is farnesylated at the C-terminus allowing the association with the INM. The maturation of A-type lamins includes in a final step, in which a C-terminal farnesylated peptide is endoproteolytically cleaved by a zinc metalloprotease, resulting in a non-farnesylated mature lamin A (Barrowman et al., 2012; Rusinol and Sinensky, 2006).

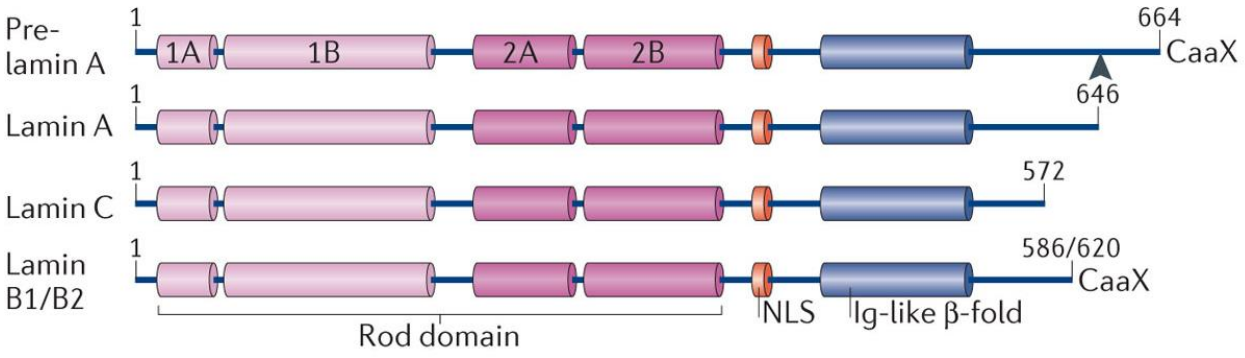


Figure 3: Structure of nuclear lamins
Schematic illustration of a pre-lamin polypeptide (at the top), and mature lamins A, C, B1 and B2. Lamin B1, B2 and Pre-lamin A contain a C-terminal –CAAX motif. The non-helical domain is organized by β-sheets of the Ig-fold (blue) followed a nuclear localization signal (NLS) (red), and the long α-helical rod domain containing four coiled-coil regions (purple) (Burke and Stewart, 2013).

Although the structures of nuclear lamins are well described, the organization and functions are complex and poorly understood. Previous data established a connection between the nuclear lamina and a plethora of different functions. Recent studies showed that nuclear lamins play a major role in providing the mechanical stability to the nuclear architecture by establishing and

maintaining the integrity of the nucleus, especially in tissue which is under high mechanical stress (Gotzmann and Foisner, 2006; Houben et al., 2007; Lee et al., 2007).

In addition, it plays an essential role in maintaining genomic stability and thus is involved in regulating senescence, DNA damage repair, differentiation, gene expression and proliferation. The gene expression regulating function is supported by dates, showing a direct interaction of lamins with transcription factors like retinoblastoma protein (pRB) or the repressor protein germ cell less (GCL). pRB regulates the cell cycle by inhibiting the transcriptional activator E2F (Frolov and Dyson, 2004), plays an essential role in muscle cell differentiation (Korenjak and Brehm, 2005) and cellular senescence (Ohtani et al., 2004). GCL provides a possibility to control the cell differentiation and proliferation through the interaction with lamin A in a pRB-independent manner (Andres and Gonzalez, 2009; Nili et al., 2001). A-type lamins also interact with other transcription factors such as c-Fos (Gonzalez et al., 2008) and suppress activator-protein-1-dependent (AP-1) transcription, or the sterol regulatory element-binding protein 1 (SREBP1) (Lloyd et al., 2002), involved in the regulation of cholesterol biosynthesis and the gene expression of fatty acid metabolism genes (Kim and Spiegelman, 1996; Yokoyama et al., 1993). Furthermore it interacts with ERK 1/2 (extracellular signal regulated kinase), which functions in a complex with lamin A and c-Fos as a molecular switch, which provides a rapid activation of AP-1 in a mitogen-dependent manner (Gonzalez et al., 2008). Lamins have the ability to interact with chromatin directly and indirectly through chromatin organizing components, such as histones and other lamin-associated proteins, like the barrier-to-autointegration factor (BAF), which interacts with histones and DNA and has the ability to “bridge” DNA (Margalit et al., 2007). In view of the variety of different functions of the nuclear lamina. It is understandable that a multitude of diseases are linked to the nuclear lamina. These rare genetic disorders, caused by mutations in lamins and lamin-binding proteins and termed laminopathies, ranging from Hutchinson–Gilford progeria syndrome (HGPS), over lipodystrophies and leukodystrophies over to muscular dystrophies (Burke and Stewart, 2006; Worman, 2012).

1.2 The LEM-domain containing protein family

The LEM (LAP2-Emerin-MAN1) domain containing proteins represent a prominent family of lamin-binding proteins, which are involved in chromatin organization and maintaining the nuclear architecture, by linking the nuclear envelope to the lamina scaffold. The predicted number of genes within the genome of mammals encoding for LEM domain-containing proteins, increased during evolution. In mammals seven individual genes encode for LEM domain containing proteins, including LAP2 (Lamina-associated polypeptide), Emerin (EMD), MAN1, LEM2, LEM3 (ANKLE1), LEM4 (ANKLE2), LEM5 (LEMD1) (Brachner and Foisner, 2014) (Figure 4) *Drosophila melanogaster* contains specific MAN1, ANKLE1, ANKLE2 and two *Drosophila melanogaster* specific proteins named Otefin (Padan et al., 1990) and Bocksbeutel α and β (Wagner et al., 2004). Most LEM proteins are INM proteins. Two LAP2 isoforms and ANKLE1 however, are located in the nucleoplasm or in the cytoplasm (Brachner et al., 2012; Dechat et al., 1998; Shaklai et al., 2008), whereas ANKLE2 has been found to be an endoplasmic reticulum resident protein (Asencio et al., 2012).

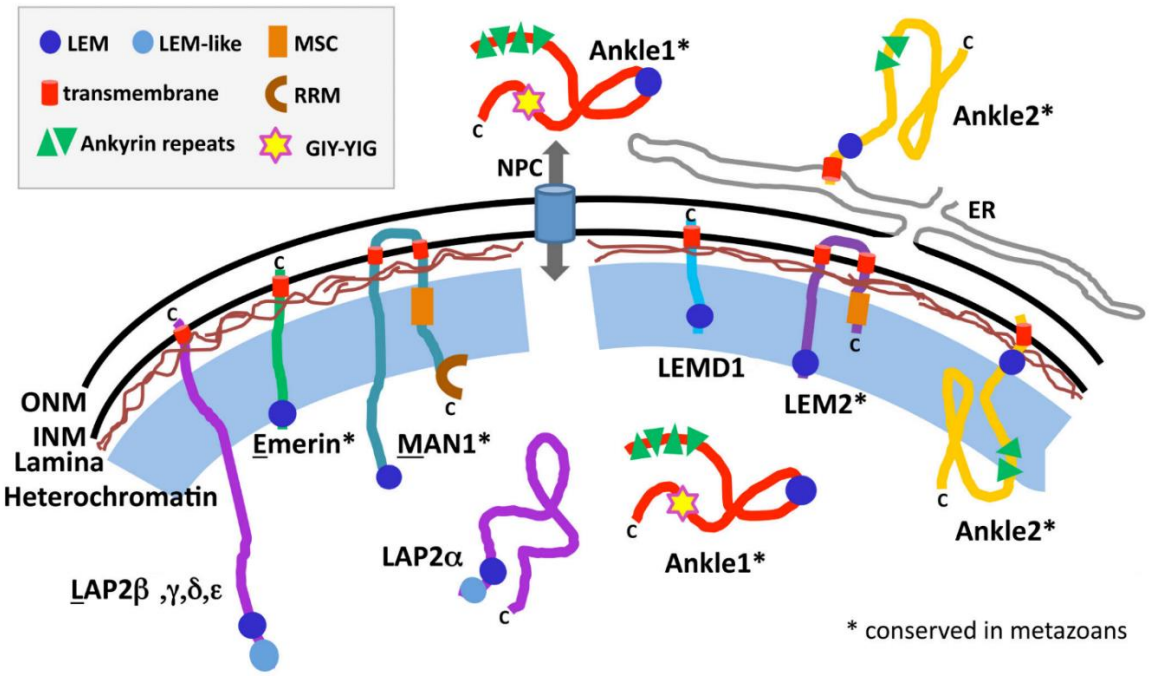


Figure 4: Schematic drawing of mammalian LEM protein family
GIY-YIG= endonuclease domain; RRM=RNA recognition motif; MSC= MAN1-SRC1p-C-terminal domain; LEM= LAP2-Emerin-MAN1domain; ; NPC=nuclear pore complex; ER=endoplasmic reticulum; ONM=outer nuclear membrane; INM=inner nuclear membrane; Figure adapted from (Brachner and Foisner, 2014).

The LEM domain-containing protein family members share a structural motif of 40 aa, termed LEM domain, which binds BAF (Laguri et al., 2001). BAF binds in a sequence independent manner to DNA as a homodimer and mediates the interaction between chromatin and the nuclear lamina (Dechat et al., 2004; Lee et al., 2001; Margalit et al., 2007; Shumaker et al., 2001). Thus a complex network of lamins, BAF and LEM proteins tether chromatin to the nuclear periphery (Wilson and Foisner, 2010). The interaction between the nuclear lamina and chromatin is essential for nuclear reassembly after mitosis and for the organization of the chromatin during interphase and knockdown of BAF leads to a deficient chromatin organization and chromosome segregation and to mislocalized lamins and LEM proteins (Furukawa et al., 2003; Margalit et al., 2005). Additionally, BAF binds histones H1.1 and H3 with high affinity (Montes de Oca et al., 2005), competes with the transcriptional repressor GCL for Emerin (Holaska et al., 2003) and represses the transcription factor cone-rod homebox (CRX) (Wang et al., 2002). Affinity purification and mass spectrometry revealed that BAF binds to core proteins of the nucleotide excision repair (NER) pathway, DDB1 and DDB2 (DNA damage repair binding protein 1 & 2), which implies a role of BAF in DNA damage repair (Montes de Oca et al., 2009).

The testis specific LEM protein LEMD1 is expressed as six alternatively spliced isoforms (Ghafouri-Fard et al., 2010; Yuki et al., 2004). It may play a role in tumorgenesis, but its function is still unknown.

LAP2 generates various isoforms (α, β, γ, δ, ε, ζ), by alternative splicing (Berger et al., 1996; Dechat et al., 2000). All LAP2 isoforms contain an additional N-terminal LEM-like motif that binds DNA directly (Cai et al., 2001). LAP2 isoforms are known to be important for several cellular processes, such as nuclear reassembly (Dechat et al., 2004; Vlcek et al., 1999), cell cycle and transcriptional regulation or tumorgenesis (Brachner and Foisner, 2011; Dorner et al., 2006).

The LEM proteins ANKLE1 and ANKLE2 carry a cluster of 3 to 4 N-terminal Ankyrin repeats, which generally function as protein interaction platforms (Gaudet, 2008; Li et al., 2006). The ANKLE1 specific GIY-YIG endonuclease domain at the C-terminal end which is highly related to a similar motif found in UvrC proteins (Dunin-Horkawicz et al., 2006), which have an essential role in the NER pathway in bacteria (Prammananan et al., 2012). Despite their similar structure, ANKLE1 and ANKLE2 differ substantially in their subcellular localization and function. Whereas ANKLE2 is implicated in the phosphorylation of BAF and located at the endoplasmic reticulum

(Asencio et al., 2012), ANKLE1 shuttles between the nucleoplasm and the cytoplasm and mediates the cleavage of DNA *in vivo* presumably as a component of DNA damage repair pathways (Brachner et al., 2012).

1.3 LEM2, MAN1 and Emerin

The INM LEM domain-containing protein LEM2 forms a small subgroup of LEM proteins with MAN1, which has a highly related structure. Both proteins contain 2 transmembrane domains, one N-terminal LEM domain and one C-terminal winged-helix fold MSC (MAN1-SRC1p-C-terminal) domain which has proposed DNA binding affinity (Caputo et al., 2006; Mans et al., 2004). MAN1 contains an additional and specific RNA recognition motif (RRM) at the very C-terminal end (Brachner et al., 2005; Pan et al., 2005; Wagner and Krohne, 2007). Emerin, a ubiquitously expressed LEM protein, which is structurally unrelated to LEM2 and MAN1, contains one transmembrane domain and one LEM domain at the N-terminus (Figure 5). All three proteins bind to the lamin- and DNA-binding protein BAF, and lamin A/C (Brachner et al., 2005; Laguri et al., 2001; Margalit et al., 2007; Sakaki et al., 2001), however their complete interactome is poorly reported.

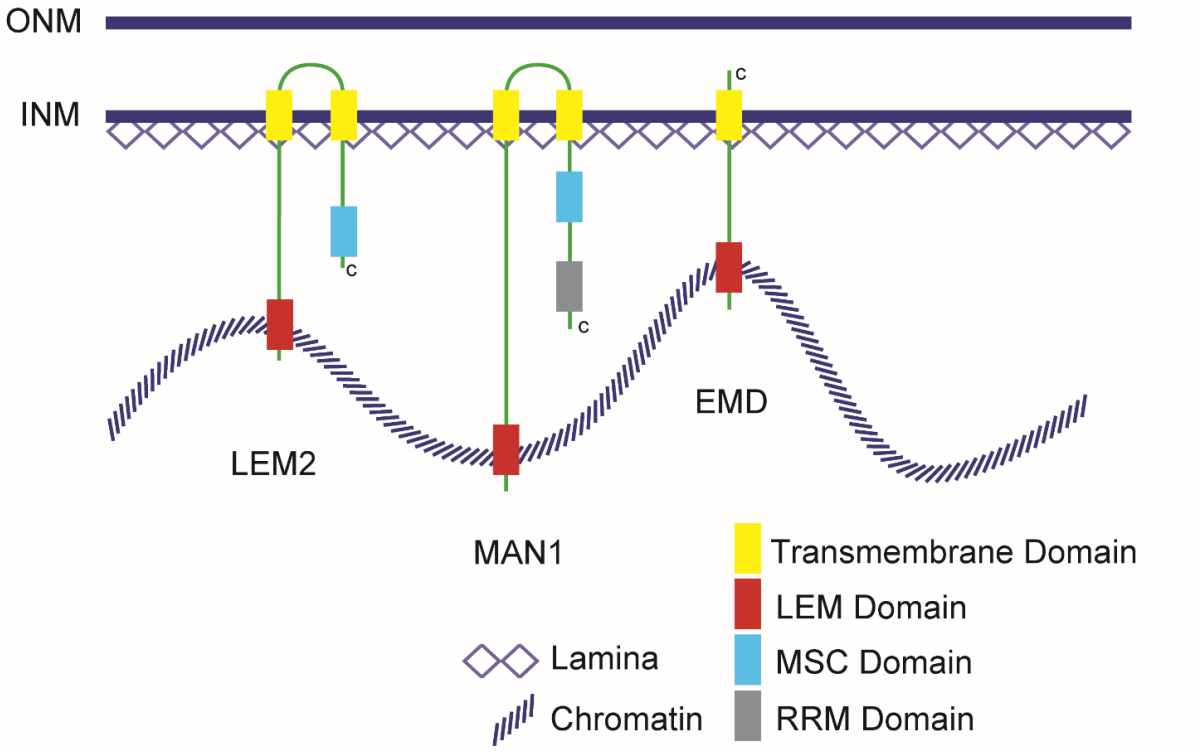


Figure 5: Schematic drawing of LEM proteins which are discussed in this thesis
LEM2 (56kDa) and MAN1 (82kDa) are composed of a nucleoplasmic LEM domain at the N-terminal end, 2 transmembrane domains and one winged-helix fold MSC (MAN1-SRC1p-C-terminal) domain at the C-terminal end, which directly binds DNA (Caputo et al., 2006; Mans et al., 2004). MAN1 contains an additional and specific RNA recognition motif (RRM) at the very C terminus (Brachner et al., 2005; Pan et al., 2005; Wagner and Krohne, 2007). Emerin (34kDa) consist of just one C-terminal transmembrane domain and nucleoplasmic LEM domain at the N-terminal end (Lee et al., 2001).

1.3.1 LEM2

The ubiquitously expressed INM LEM protein LEM2 is composed of 503 aa and has a predicted molecular mass of 56 kDa (Brachner et al., 2005). It contains a highly conserved MSC domain with proposed DNA binding affinity (Caputo et al., 2006; Mans et al., 2004) between aa 435-493, two transmembrane domains between aa 372-394 and 209-231 and one N-terminal LEM domain between aa 1-42. The N-terminal domain of LEM2, is necessary to bind the lamin A/C tail directly and is required for the proper localization of LEM2 at the INM (Brachner et al., 2005).

RNA interference mediated knockdown showed that the depletion of human LEM2 leads to an abnormally shaped nuclear structure and to a decreased proliferation or even a proliferation stop (Ulbert et al., 2006). LEM2 is strongly upregulated during myoblast differentiation (Chen et al., 2006) and its depletion causes a hyperactivation of ERK 1 and 2. (Huber et al., 2009). LEM2 is implicated in several signalling pathways as supported by recent findings. Depletion of LEM2 in mouse myoblasts (C2C12) showed an essential role in the regulation of MAP kinase and AKT/mTORC signalling and in addition in mouse development (Tapia et al., 2015). Studies on LEM2 homologues in *Caenorhabditis elegans* (ceLEM-2) and "fission yeast" *Schizosaccharomyces pombe* (HeH1 or Src1p), ascribed significant roles of these proteins in tethering chromosomes to the nuclear periphery. The helix-extension-helix motif 1 protein (HeH1) binds to small repetitive loci to transcriptionally silent rDNA and to subtelomers, causing a stabilization by repressing and limiting their exposure to deleterious recombination events (Chan et al., 2011; Mekhail et al., 2008). In *Caenorhabditis elegans,* LEM2-acossiated chromatin subdomains at chromosome ends contain increased levels of H3K9me3 and H3K27me3, which are marks of epigenetic silenced regions, whereas regions between those LEM2-acossiated domains, which loop-out into the nucleoplasm, tend to be active (Ikegami et al., 2010).

High-level overexpression of LEM2 in HeLa and C2C12 cells leads to an accumulation of the protein in functionally undetermined patchy structures. Furthermore, transmission electron microscopy revealed that this overexpression leads to numerous finger-type invaginations of the NE, which were not observed upon overexpression of the highly related protein MAN1 (Brachner et al., 2005). The identification of Emerin, BAF and lamin A to these LEM2 patches, implicates LEM2 in the spatial organization of NE components (Brachner et al., 2005). The catalytic subunit (CA or α) of the protein phosphatase 1 (PP-1), which is implicated in the regulation of a variety of

cellular processes from mitosis to biosynthesis (Wurzenberger and Gerlich, 2011), represents the only previously reported specific binding partner of LEM2. PP-1α was co-immunoprecipitated with LEM2 but not with Emerin or ANKLE2 (Asencio et al., 2012), but function of LEM2 on PP-1α as well as other LEM2 specific functions or binding partners are still unknown.

1.3.2 MAN1

Human MAN1, which is structurally related to LEM2, is composed of 911 aa (82kDa). It contains two transmembrane domains between aa 472-494 and 627-649 which are flanked by a MSC domain between aa 692-730, proposed to bind DNA (Caputo et al., 2006; Mans et al., 2004) and an N-terminal LEM domain between aa 7-50. MAN1 contains a C-terminal RRM between aa 785-684, which is absent in LEM2 proteins (Brachner et al., 2005; Wagner and Krohne, 2007) (Figure 5).

The RRM enables MAN1 to regulate several signalling pathways, by binding to receptor-regulated Smads (r-Smad (Mothers against decapentaplegic), which results in a specific repression of activin, BMP (bone morphogenetic protein) or TGF-β (transforming growth factor) signalling (Pan et al., 2005). The r-Smads, Smad2 and Smad3, are intracellular signal transducers, which are phosphorylated by activated type I receptor kinases and oligomerize with the mediator Smad4. Subsequently, this complex translocates into the nucleus, where they interact with various transcription factors and bind the promotor region of several genes (Massague et al., 2005). *In vivo* experiments showed that MAN1 binds to the r-Smads, Smad2 and Smad3 and mediates their dephosphorylation by protein phosphatase 1A (PPM1A), a direct interaction partner of MAN1, thus antagonizing their binding to Smad4 (Bourgeois et al., 2013). Recent studies have ascribed a novel role of the nuclear periphery in regulating circadian rhythmic-linked pathways, as MAN1 binds directly to the promotor of the core clock component BMAL1, resulting in its enhanced transcription (Lin et al., 2014). A role of MAN1 in development was shown by knockdown experiments in *Xenopus laevis*, which revealed that MAN1 is implicated in organogenesis and tissue homeostasis by regulating specific genes (Reil and Dabauvalle, 2013). The fission yeast *Schizosaccharomyces japonicas* breaks up its nuclear envelope during mitosis, and requires MAN1 for its equal partitioning, the reformation of nucleoli and also the correct inheritance of

nuclear pore complexes, by linking them to segregating chromatin (Yam et al., 2013). Loss of functions mutations of the MAN1-encoding mammalian gene *LEMD3*, resulted in various bone disorders including Melorheostosis, Osteopoikilosis and Buschke-Ollendorff Syndrome (Hellemans et al., 2004). MAN1 binds Emerin and both have overlapping functions binding to GCL (Holaska et al., 2003), the death promoting transcriptional repressor Bcl-2-associated transcription factor (Btf) and BAF (Mansharamani and Wilson, 2005).

1.3.3 Emerin

Emerin is a ubiquitously expressed single pass inner nuclear membrane protein, with one C-terminal transmembrane domain between aa 225-247 and one N-terminal LEM domain between aa 2-45 reaching into the nucleoplasm (Brachner et al., 2005) (Figure 5). It is, among all LEM domain-containing proteins, the best-studied protein, for which many functions, interaction partners, roles in development and disease are well documented (Koch and Holaska, 2014).

Like LEM2 and MAN1, Emerin binds directly to lamin A/C (Sakaki et al., 2001), however, Emerin binds additionally to actin, spectrin and myosin (Bengtsson and Wilson, 2004; Holaska et al., 2004) and to structural components of the LINC complex (linker of nucleoskeleton and cytoskeleton) like nesprins (nuclear envelope spectrin repeat proteins), Sun1 and Sun2 (Haque et al., 2010; Wheeler et al., 2007). In this way, it plays an essential role in stabilizing and promoting the nuclear actin cortical network, and maintaining the nuclear structure. The absence of Emerin leads to several diseases, including Emery-Dreifuss muscular dystrophy (EDMD) (Yorifuji et al., 1997), which reflects its significance in development and health. Via BAF-binding, it enriches at chromosomes, ensuring the correct assembly of the NE (Bengtsson and Wilson, 2004). Moreover, Emerin stimulates the activity of the catalytic subunit of the nuclear co-repressor complex, histone deacetylase 3 (HDAC3), and recruits it to the nuclear periphery (Demmerle et al., 2012). It binds directly to signaling transcription factors like the transcriptional repressors GCL, which competes with BAF for binding to Emerin (Holaska et al., 2003), LIM domain only 7 (Lmo7) which regulates the transcription of genes implicated in EMDM (Dedeic et al., 2011), and Btf, which is highly expressed in skeletal muscle and may be involved muscle atrophy in EDMD (Haraguchi et al., 2004). Emerin interacts with the transcription coactivator β-catenin (Markiewicz et al., 2006),

which acts as a signal transducer in the Wnt-signaling pathway (MacDonald et al., 2009), via an adenomatous polyposis coli (APC)-like domain. In this way Emerin influences the nuclear accumulation of β-catenin through a CRM1 (also known as Exportin-1)-dependent nuclear export pathway (Markiewicz et al., 2006). In Emerin deficient human fibroblasts, the centrosome is detached from the nucleus. This ascribes a novel role for Emerin, suggesting a small but significant amount of Emerin at the outer nuclear membrane and the peripheral endoplasmic reticulum, which is necessary to link centrosomes to the nucleus via a microtubule association (Salpingidou et al., 2007).

1.4 DNA damage repair

The integrity of DNA is constantly threatened by several factors such as environmental chemicals, endogenously formed metabolic products, reactive oxygen species (ROS), alkylating agents, radiation or defective cellular processes, which leads to approximately 104–105 DNA lesions per day per mammalian cell (Figure 6) (Marteijn et al., 2014; Swenberg et al., 2011) . Different types of lesions are caused by different exogenous and endogenous stress factors. Many DNA lesions cause structural abnormalities to the DNA molecule, which affect DNA replication and transcription. This may lead to DNA mutations, abnormal cell cycle regulation, chromosomal aberrations, senescence and in further consequence to cancer or cell death. This fact makes it essential for the cell to find several ways to detect and to remove these structural damages fast and effectively.

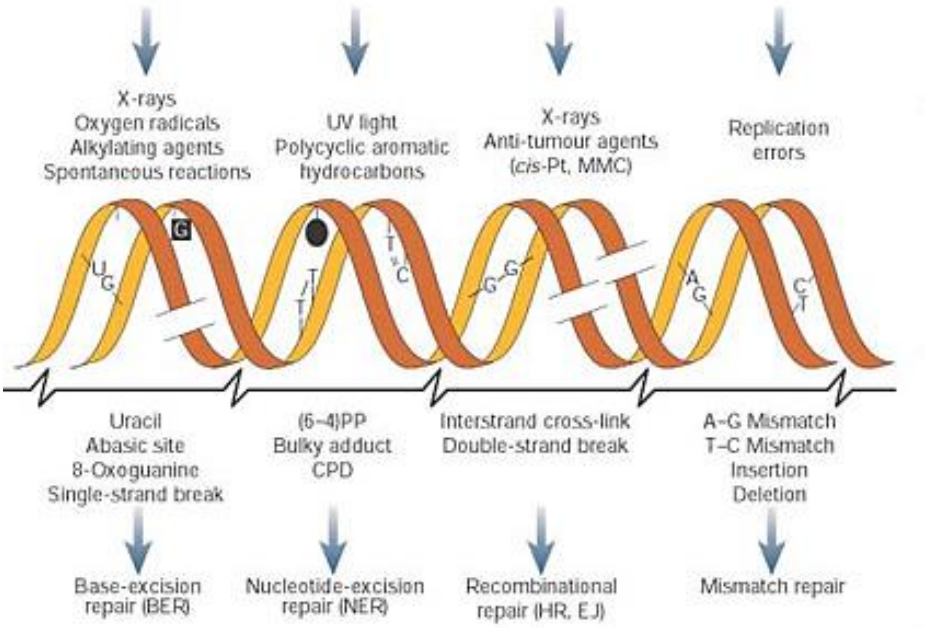


Figure 6: Cellular DNA damage response
Schematic overview of how cells recognize and response to different DNA damages. Common different DNA damage inducers (top) can result from exogenous and endogenous influences. This leads to different DNA lesions (middle), which are repaired by the cell by specific mechanisms (bottom).
cis-Pt=cisplatin; MMC=mitomycin C; (6–4)PP=6–4 photoproduct CPD= cyclobutane pyrimidine dimer; BER=base-excision repair; NER=nucleotide-excision repair; HR=homologous recombination; EJ= end joining. Figure adapted from (Hoeijmakers, 2001).

DNA damage repair is time-intensive, uses energetic, metabolic and chemical resources and requires accessibility to the lesion. In order to ensure efficient repair, cells get arrested in the S-phase, G_1/S or at the G_2/M transition, using sophisticated DNA damage checkpoints surveillance mechanisms. The checkpoint kinase 2 (Chk2) accomplishes the G_1 arrest, by inhibiting the M-phase inducer phosphatase 3 (CDC25C) and stabilizing p53, whereas checkpoint kinase 1 (Chk1) inhibits the M-phase inducer phosphatase 1 (CDC25A) and CDC25C leading to a G_2- and S- phase arrest (Finn et al., 2012; Malumbres and Barbacid, 2009).

The accessibility to detect DNA lesions is provided by chromatin relaxation which is accomplished by various remodeling complexes or histone modifications such as the phosphorylation of the histone variant H2Ax (γH2Ax), by kinases of the PI3 (phosphoinositide 3-kinase)-family (ATR (Ataxia-telangiectasia and Rad3-related gene) (Ward and Chen, 2001), ATM (Ataxia-telangiectasia mutated gene) (Burma et al., 2001) and DNA-PK (DNA-activated protein kinase) (Stiff et al., 2004), which in turn triggers various DNA damage repair pathways, formation of DNA damage foci, cell cycle arrest and/or apoptosis (Rogakou et al., 1998).

1.4.1 DNA damage repair mechanisms

The genome of cells is constantly exposed to several types of damage inducers and it is essential to start and complete DNA repair as soon and as accurate as possible. All repair mechanisms (Figure 6, bottom) follow a similar process after DNA damage has occurred: recognition of the lesion, excision of hindering regions, resynthesis and ligation of new fragments. In general, there are two main types of DNA damages, single strand DNA lesions and double strand breaks, which both activate specific repair pathways.

Double strand DNA breaks are particularly dangerous to the cell, because they can lead to genome translocations, DNA fusions, deletions and cell death. Non-homologous end joining (NHEJ) and homologous recombination (HR) are the two main repair pathways which are engaged with DNA double strand breaks (Wood et al., 2005). The decision which repair mechanism is used to repair double strand breaks is essential for the survival of cells and is tightly connected to different cell cycle phases (Chapman et al., 2012). In contrast to HR, NHEJ does not require any homologous template and promotes a direct rejoining of the two ends, when a template is not available. This

error-prone mechanism is favored in G_0/G_1 phase and frequently results in translocations, insertions, deletions and small substitutions (Chapman et al., 2012; Giglia-Mari et al., 2011; Lieber, 2010). HR is a largely error-free replication associated repair mechanism, which can only be used in S/G_2 phase, because the damaged region is copied from the sister chromatid needing large stretches for repair (Chapman et al., 2012; Giglia-Mari et al., 2011).

In NHEJ the DNA double strand break ends are held in close proximity by the heterodimer Ku70-Ku80 that activates the PI3 kinase DNA-PK. The recruitment of the MRN-complex (MRE11-RAD50-NBS1) leads to the processing of DNA ends and the DNA ligase IV complex (ligase IV-XRCC4) performs the ligation (Giglia-Mari et al., 2011; Wilson et al., 1997).
In the HR pathway, the MRN-complex is recruited to the double strand breaks and functions to hold the ends together (de Jager et al., 2001). The CtIP nuclease binds to the MRN-complex and catalyzes end resection together with exonuclease I (Limbo et al., 2007). The newly synthesized ends are bound by the replication protein A (RPA) and subsequently replaced by RAD51, which initiates strand invasion (Giglia-Mari et al., 2011).

Single strand DNA lesions are discontinuities in one DNA strand, which are caused by several different attacks of endogenous and exogenous stress factors. If these lesions are not repaired correctly, single strand breaks can interrupt the replication machinery, cause mutations or lethal DNA double strand breaks. According to the type of lesions, most single strand damages are repaired by nucleotide excision repair machinery, which excises a small DNA fragment of about 24-30 nucleotides (Huang et al., 1992; Sugasawa et al., 2001) or base excision repair (BER) excising a short strand or a single nucleotide (Sharma and Dianov, 2007). DNA damages can also be repaired without removing damaged nucleotides by a direct chemical reversing, which is very specific to the type of damage and does not need any template.

In BER, small lesions are recognized and replaced by specific DNA glycosylases, which results in basic sites, which are cleaved by an abasic site-specific endonuclease. Afterwards the BER-specific DNA polymerase β fills the gap and is ligated by the XRCC1/Ligase III complex. The Poly-ADP-Ribose Polymerase (PARP) is recruited to the BER machinery, when single strand breaks occur, which, in further consequence, causes auto-poly-ADP-ribosylation and the recruitment of the XRCC1/ligase III and the end-processing protein aprataxin (Gueven et al., 2004) and tyrosyl-DNA-phosphodiesterase (TDP1) (Caldecott, 2007; Giglia-Mari et al., 2011).

The direct reversion of DNA lesions is another possibility to remove single strand lesions. The O-6-methylguanine-DNA methyltransferase (MGMT) reverses alkylations by transferring the methyl group of the DNA to its internal cysteine residue, thereby destroying its own enzymatic activity (Sharma and Dianov, 2007). In bacteria, animals and fungi, pyrimidine dimers resulting from UV irradiation, can be directly reversed by photolyases (Lucas-Lledo and Lynch, 2009; Sancar, 2003). Mammals do not have any photolyses, because they rely on a much more complex mechanism to remove DNA damages, which are introduced through UV irradiation: the NER.

1.4.1.1 Nucleotide excision repair

The nucleotide excision repair pathway is one of the most versatile mechanism in all organisms. In mammals, among all DNA repair pathways, it has the ability to remove the widest range of unrelated abnormal DNA structures including bulky chemical adducts, lesions which are induced by ultraviolet radiation (6-4 pyrimidine-pyrimidone photoproducts (6-4PPs) and cyclobutane-pyrimidine dimers CPD)), lesions which are induced by therapeutic drugs (cisplatin) or ROS-generated oxidative DNA lesions (8,5'-cyclopurines-2'-deoxynucleosides (cPu)) (Marteijn et al., 2014). In contrast to the prokaryotic NER pathway which get along with 4 different proteins (UvrA, UvrB, UvrC and UvrD) (Truglio et al., 2006), the eukaryotic pathway uses around 30 proteins (Li et al., 2011a). The NER pathway is subdivided in global genome nucleotide excision repair (GG-NER) and transcription coupled nucleotide excision repair (TC-NER), which follow the same procedure for incision, repair and lesion however differ in their type of sensing DNA damages. GG-NER repairs lesion in non-transcribed DNA and TC-NER in transcriptionally active regions (Hanawalt, 2002).

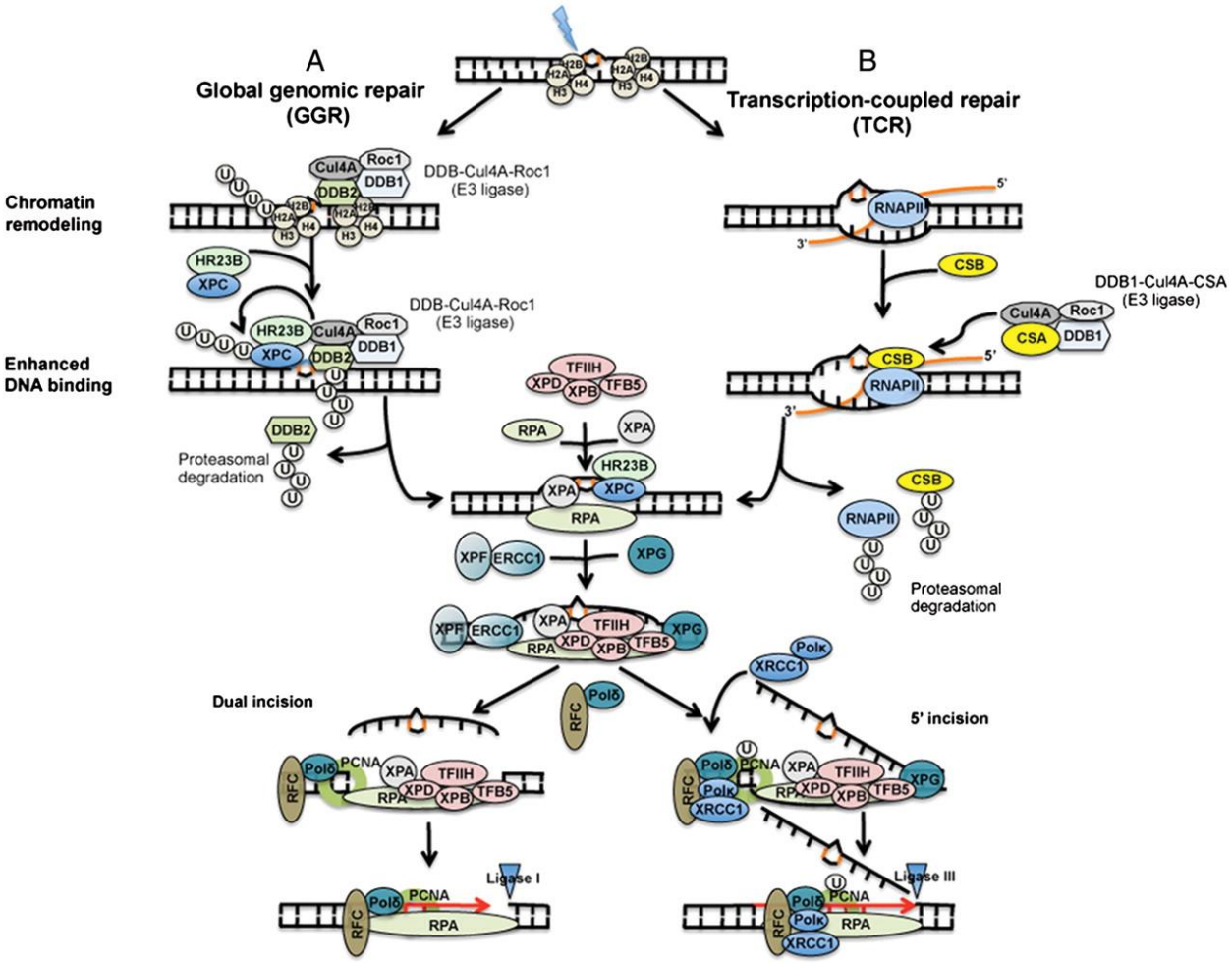


Figure 7: The nucleotide excision repair pathway
This model shows the multi-step process including recognition, excision, resynthesis and ligation of the nucleotide excision repair machinery, which needs (in mammals) a concerted action of about 30 proteins to remove DNA single strand lesions correctly. Figure from (Li et al., 2011a).

In all organisms, NER starts with lesion recognition. The GG-NER senses DNA lesions using XPC/hHR23B, which is necessary for recruiting downstream factors and UV-DDB consisting of DDB1 and DDB2/XPE, which in turn recruits XPA (xeroderma pigmentosum A) to the damage site (Hanawalt, 2002; Sugasawa, 2006). The UV-DDB complex, the ubiquitin ligase Cullin4A and Roc1 facilitates access of other repair proteins by poly-ubiquitinatination of histone H2A, and DDB2 leads to a higher DNA binding efficiency.

TC-NER recognizes DNA damages via a complex consisting of the stalled RNA polymerase II, CSA and CSB (Cockayne syndrome factors A and B) loading XPA to the damaged DNA which initiates the repair process (Fousteri and Mullenders, 2008; Fousteri et al., 2006). The E3-ligase

consisting of DDB1, Cullin4A and CSA poly-ubiquitinates RNA polymerase II and CSB leading to degradation of both proteins. After lesion recognition, TC-NER and GG-NER converge into a common mechanism.

The unwinding of approximately 30 nucleotides at damage sites is carried out by two helicases, XPB and XPD, which are part of the basal transcription factor IIH complex (TFIIH) (Coin et al., 2007; Drapkin et al., 1994). THIIH is a general transcription factor consisting of 10 proteins, which make up the RNA polymerase II preinitiation complex. Its core complex (p34, p44, p52, p62, XPB, XPD) is coupled via XPD to the cyclin activating kinase-sub-complex (cyclin H, MAT1, and CDK7) (Jawhari et al., 2002). XPA and the RPA stabilizes the unwound DNA and place the structure specific endonuclease ERCC1-XPF complex and XPG (Giglia-Mari et al., 2011; McNeil and Melton, 2012), which lead to the incisions of 3' and 5' sites and to a gap which results in the removement of 25-30 nucleotides (Sijbers et al., 1996; Volker et al., 2001). Normal replication proteins are needed to resynthesize the new DNA strand. The DNA polymerases δ and ε uses the complementary strand as a template and recruits proliferating cell nuclear antigen (PCNA), replication factor C (RFC), XRCC1 and the polymerase κ to the DNA damage site (Coverley et al., 1992; Giglia-Mari et al., 2011; Ogi et al., 2010; Shivji et al., 1992). To complete the repair, DNA ligase I or III ligate the newly synthesized DNA strand (Moser et al., 2007).

The DNA damage response and consequently the survival of cells depends crucially on the balance between GG-NER and TC-NER. Slightest of changes can lead to dysfunctions of the NER machinery, the accumulation of DNA damages, and subsequently to cell death. Accordingly, defects in GG-NER and TC-NER can cause specific dysfunctional molecular mechanisms and a variety of diseases, like premature aging (TC-NER) and cancer (GG-NER), to name a few (Marteijn et al., 2014).

1.5 Aim of the thesis

1) BioID

The aim of this thesis was to elucidate the complex interaction network of the inner nuclear membrane LEM proteins LEM2, MAN1 and Emerin with a special focus on LEM2 which represents, compared to MAN1 and Emerin, a poorly studied LEM protein concerning its specific functions and interaction partners.

Using the *in vivo* biotin labelling method BioID and comparison of the interactomes of LEM2, MAN1 and Emerin, we intended to identify potential LEM2 specific and overlapping functions.

BioID exploits a genetically engineered, promiscuous biotin ligase which biotinylates proteins in a proximity-dependent manner, and is specifically suited to study low-affinity and transient interactions as well as protein complexes, which are difficult to isolate under physiological lysis conditions, like in this case of lamina and inner nuclear membrane proteins.

2) NE invaginations

Using a live cell imaging approach we investigated the dynamics of LEM2 focusing on LEM protein containing NE invaginations, which were often seen in close association with nucleoli.

2 Materials and Methods

2.1 Cell culture

U2OS cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) (GE Healthcare) supplemented with 10% fetal calf serum (Invitrogen), nonessential amino acids, 100 U/ml Penicillin and Streptomycin and 2 mM L-Glutamine in an atmosphere of 37°C and 8,5% CO_2. Gene knock down (KD) in cells was done by RNA interference using 5µM siRNA pool (Dharmacon/GE) and DharmaFECT (Thermo Scientific) as transfection reagent according to the manufacturers protocol. All U2OS cells were washed once with 1xDPBS (Gibco) before they were detached with trypsin (HyperClone). For Live cell imaging, cells were seeded in a 35mm imaging dish with a glass bottom (Ibidi) and grown in Dulbecco's Modified Eagle Medium (described above) lacking phenol red.

2.2 Immunofluorescence and antibodies

For immunofluorescence microscopy, U2OS cells were grown on glass coverslips rinsed once with 1xPBS and fixed in 4% paraformaldehyde/PBS for 10 minutes at room temperature. The fixed cells were washed once with 1xPBS and permeabilized for 5 minutes in 1xPBS containing 0.5% Triton X-100. Non-specific antibody binding was blocked by incubation of the coverslips in IF blocking buffer (PBS containing 0.5% gelatine (Sigma)) for 15 minutes. All primary (Table 1) and secondary antibodies used in this study were diluted in blocking buffer and applied for 60 minutes, followed by three washes with 1xPBS. DNA was counterstained with 1 µg/mL DAPI (Sigma) for 5 minutes. After a final washing step with dH_2O coverslips were mounted in Mowiol (Sigma) on microscopy slides. Images were acquired with a confocal laser scanning microscope (LSM-Meta 510, Zeiss) using a Plan-Apochromat 63x/1.4 Oil DIC objective. Pictures were further processed with Image J (National Institutes of Health).

Antibody	source	cat.#	host	dilution
LEM2	Human Protein Atlas	017340	rabbit	1:50
Emerin (8A1)	Glenn E. Morris	-	mouse	1:50
DDB1	GeneTex	GTX100232	rabbit	1:200
γH2AX	Upstate	05-636	mouse	1:300
V5	Invitrogen	R960-25	mouse	1:200
647-Streptavidin	Jackson ImmunoResearch	016-600-084	rabbit	1:100
53BP1	Novus Biologicals	NB100-305	rabbit	1:100

Table 1: Antibodies used for Immunofluorescence

The secondary antibodies conjugated to Alexa-488 were purchased from Molecular Probes and the secondary antibodies conjugated to Cy5 and TexasRed respectively were from Jackson Immuno Research.

2.3 Gel electrophoresis and western blotting

To obtain cell lysates, U2OS cells were scraped from petri dishes, pelleted, washed in 1xDPBS (Gibco) and lysed in high salt RIPA (25 mM Tris-HCl pH 7.4, 500 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS), supplemented with protease inhibitor cocktail mix (Roche). After sonication (Bandelin Sonopuls HD200; power MS 73/D, continuous pulse), the protein concentration of each sample was measured by Bradford assay and equal protein amounts were loaded on a SDS-PAGE (10µg total protein/lane/0,5mm 15 well comb).

Proteins were stained directly in the SDS-gel with GelCode Blue Safe protein stain (Thermo Scientific) or analyzed after transferring them to Nitrocellulose membrane (GE Healthcare). Western blot membranes were blocked with 5% low fat milk (Roth) in PBS containing 0,025% Tween20 (PBST). All primary antibodies (Table 2) were incubated for 1h with the membranes. Afterwards the membranes were washed 3 times with 1xPBST for 10 minutes and incubated with secondary horseradish peroxidase (HRP) coupled antibodies (Jackson ImmunoResearch) for 1 hour. After another three washes, the membranes were incubated with ECL prime (Amersham) or west pico chemiluminescent substrate (Pierce) according to manufactures instructions, to detect the bound secondary antibodies by chemiluminescent substrates. Signals were detected by exposure of light sensitive films (Thermo Scientific).

Antibody	source	cat.#	host	dilution
LEM2	Human Protein Atlas	017340	mouse	1:500
Emerin (8A1)	Glenn E. Morris	-	mouse	1:200
MAN1	GeneTex	GTX84220	mouse	1:500
γ-tubulin	Sigma-Aldrich (T6557)	MFCD00677366	mouse	1: 10000
DDB1	GeneTex	GTX100232	rabbit	1:1000
V5	Invitrogen	R960-25	mouse	1:40000
γH2AX	Upstate	05-636	mouse	1:200
Strep-HRP	Invitrogen	SN1004	-	1:10000

Table 2: Primary antibodies used for western blotting

2.4 Cloning strategy and plasmids

The pEMI-BirA*-gateway (GW) vector was generated using BirA* cDNA (Roux et al., 2012), which was amplified by PCR with primers containing a SfiI restriction site 5‘oligo and ligated in pJET1. The GW cassette was amplified using primers containing a SfiI restriction site within 3’ oligo from pDEST (Invitrogen) and ligated into pJET1-BirA*. The fusion product (BirA*-GW cassette) was cut from pJET-BirA-GW using the restriction enzyme SfiI and subsequently ligated into SfiI sites on the pEMI vector. All sequences were verified by sequencing prior to experimental use. In order to create pEMI-LEM2, pEMI-MAN1 and pEMI-Emerin, previously generated pEntry vectors containing the respective human cDNAs, were used for cloning pEMI-BirA*-LEM2, pEMI-BirA*-MAN1 and pEMI-BirA*-Emerin by GW cloning, according to the manufacturers instructions.

The pDEST51-hLEM2-mRFP vector was cloned by ligation of a PCR-generated mRFP ORF (open reading frame), cut with SpeI in frame into the previously made pDEST51-hLEM2 plasmid and digested with XbaI/PmeI.

2.5 BioID

Three V5 tagged U2OS cell lines, stably expressing doxycycline-inducible LEM proteins (LEM2, MAN1, Emerin) fused to BirA*, a promiscuous *Escherichia coli* biotin protein ligase, were incubated for 6 hours in complete media containing 1µg/ml doxycycline on 150 mm plates (~70% confluence). Cells were washed with 1xDPBS and incubated for additional 16 hours with 50µM biotin. After three 1xDPBS washes, the cells were scraped off the petri dish and lysed in high salt RIPA buffer on ice in the presence of protease inhibitor cocktail mix (Roche). The lysate was sonicated and mixed with 100µl Pierce streptavidin magnetic beads (Thermo Scientific) and incubated overnight at 4°C on an end-over-end mixer. The magnetic beads were collected and washed twice with high salt RIPA buffer, twice with wash buffer A (2% SDS in ddH2O), once with wash buffer B (0.1% deoxycholate, 1% Triton X-100, 500 mM NaCl, 1 mM EDTA, and 50 mM Hepes, pH 7.5), once with wash buffer C (250 mM LiCl, 0.5% NP-40, 0.5% deoxycholate, 1 mM EDTA, and 10 mM Tris, pH 8.1), and twice with wash buffer D (50 mM Tris, pH 7.4, and 50 mM NaCl) at 4°C. 10% of the beads were used for western blot analysis, the remaining 90% subjected to shotgun mass spectrometry (Figure 8).

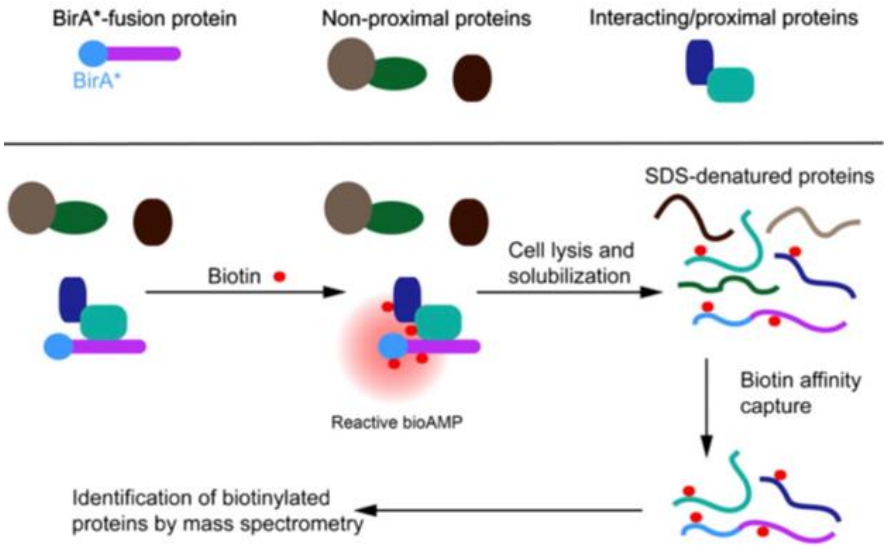


Figure 8: Schematic model for application of BioID
The principle of this technique is based on a genetically engineered E.coli biotin ligase, which biotinylates proximal Lysine residues without any target specificity. After the biotinylation process, the biotinylated proteins can be easily purified from whole cell lysates via streptavidin-binding, and subsequently identified by mass-spectrometry. Figure adapted from (Roux et al., 2012).

2.6 Shotgun Mass Spectrometry

The Mass Spectrometry Facility of the Max F. Perutz Laboratories carried out sample preparation and analysis. On-bead tryptic digests were measured using a Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific). Mass spectrometry data from the BioID experiment was evaluated using Scaffold Viewer (Proteome Software).

2.7 Gene knockdown by small interfering RNAs

U2OS cells were seeded in DMEM containing 10% FCS and 2 mM L-Glutamine 24 hours prior to transfection with 5µM siRNA pools (Dharmacon/GE) using DharmaFECT (Thermo Scientific) according to the manufacturers instructions. After additional 24 hours the cells were transfected for a second time using the same conditions as before and incubated for another 24 hours.

siRNA pools (mix of 4 different oligos)	**description**
LEM2	L-017941-02-0005, ON-TARGETplus Human LEMD2 (221496) siRNA - SMARTpool
MAN1	L-006306-01-0005, ON-TARGETplus Human LEMD3 (23592) siRNA - SMARTpool
Emerin	L-011025-00-0005, ON-TARGETplus Human EMD (2010) siRNA - SMARTpool
DDB1	L-012890-00-0005, ON-TARGETplus Human DDB1 (1642) siRNA – SMARTpool
Scrambled (Sc)	D-001810-10-20, ON-TARGET plus Non-targeting Pool

Table 3: siRNAs used for gene knockdown

2.8 Cell proliferation assay upon UV irradiation

For studying proliferation and determination of survival of U2OS cells after gene knockdown by small interfering RNAs combined with subsequent UV irradiation, gene knockdowns were performed as described in chapter 2.7. 48 hours following gene silencing, U2OS cells were harvested in complete medium. The total cell number was determined using a Casy Cell Counter (Schärfe Systems) and 50000 cells/well were seeded into a six well multi well dish. 24 hours after the seeding cells were washed once with 1xDPBS, covered with 500µl 1xDBPS and exposed to 5J/m^2 with a Stratalinker 2400 254 nm UV crosslinker (Stratagene). Afterwards the cells were cultured in complete medium for 96 hours and after 0, 24, 48, 72, 96 hours the cells were counted to determine their cell number at each time point.

2.9 Generation of stable transfected cell line

A stable U2OS cell line co-expressing LEM2-RFP and Fibrillarin-GFP was generated by transfection of the plasmids pEGFP-N1-Fibrillarin (from Dr. Pascal Roussel) and pDEST51-hLEM2-mRFP (AB455) into U2OS cells by Nanofectin (PAA) according to the manufacturers introductions. In order to obtain a homogenously expressing cell line a clonal isolation procedure was performed. After two weeks of selection to neomycin resistant cells by addition of G418 to the growth medium, cells were seeded at very low density (<100 cells/ml) on a 100mm petri dish allowing the selective isolation of single cell clones. The correct localization and expression of LEM2-RFP and Fibrillarin-GFP was examined using a Zeiss fluorescence microscope (Zeiss Axiovert 200M) prior to isolation of colonies using glass cloning cylinders (Sigma Aldrich). Clone isolation was repeated two times to obtain a monoclonal LEM2-RFP/Fibrillarin-GFP expressing cell line.

2.10 Live cell microscopy

The U2OS LEM2-RFP/Fibrillarin-GFP stable cell line was seeded in 35mm glass-bottom dishes (Ibidi) in Dulbecco's Modified Eagle Medium lacking phenol red. Live cell imaging was performed on a spinning disc microscope (Visitron Systems) using a Plan-Apochromat 63x/1,40 Oil DIC objective (Zeiss). The cells were kept at 37°C and 8,5% CO_2 (Pecon 37-2 Dual-channel Temperature controller, CO_2-controller and a temperature-controlled water bath) and imaged using a 63x objective heater against focal drift.

Images and sequences were analyzed with the VisiVIEW^{TM} imaging software (Visitron Systems) and further processed with Image J (National Institutes of Health).

2.11 Flow cytometric analysis of the cell cycle

Flow cytometric analysis of U2OS cells were performed by detaching and pelleting the cells by centrifugation at 1300 rpm for 5 minutes. Subsequently the pellet was washed and re-suspended in 1ml 1xDPBS. The cell suspension was fixed by drop wise pipetting into 4ml 85% EtOH, and were kept for at least 16 hours at -20°. The fixed cells were pelleted, washed with 1xDPBS and nucleic acids were stained by resuspending in 1ml 1xDPBS containing (40µg/ml) Propidium iodide, RNAs were removed by addition of RNaseA (40µg/ml) (Fermentas). After 30 minutes of incubation at 37°C, the cells were transferred through a mesh into a FACS tube and analyzed on a BD FACSCalibur (BD Bioscience). Flow cytometry data was evaluated using FlowJo (Tree Star Inc.).

3 Results

3.1 Identification of LEM protein binding partners using the BioID approach

3.1.1 The BioID approach

In order to investigate the interaction of inner nuclear membrane LEM domain-containing proteins, a proximity-dependent biotin labeling (BioID (see chapter 2.5)) technique was adapted for use in U2OS cells. The BioID approach offers a reliable technique to study low-affinity and transient interactions as well as protein complexes which are difficult to isolate, such as lamin-associated proteins and inner nuclear membrane proteins, under physiological lysis conditions. First, to generate U2OS cells, which express the LEM protein-BirA* fusion protein in a doxycycline inducible fashion, a Myc-BirA* module was subcloned into pEMI expression vector (Figure 9) which was made gateway (Life Technologies) cloning compatible before

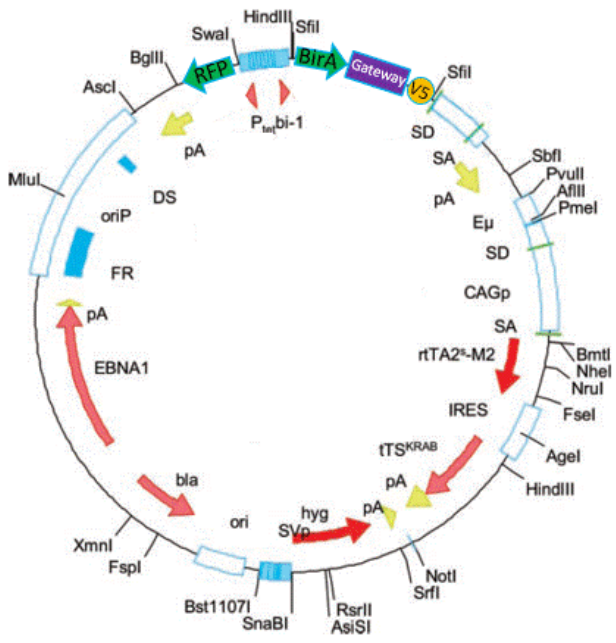


Figure 9: pEMI vector used for BioID approach.
After induction with doxycycline, a V5 tagged construct fused to the biotin protein ligase BirA* and a RFP co-reporter is expressed.

The resulting pEMI-BirA*-GW vector harbors a hygromycin B resistance gene and contains a bifunctional promoter allowing a doxycycline inducible expression of the V5-tagged BirA*-fusion proteins, and co-expression of a RFP reporter gene. The gateway cloning site allows the recombination-based transfer of gateway-compatible inserts encoding LEM2, MAN1 and Emerin, respectively into the respective vector. The expression of the RFP reporter gene enabled the easy, non-invasive identification of cells expressing the BirA*-fusion construct. The U2OS cells were transfected with the pEMI vectors and upon clonal selection, stable cell lines were generated for further investigations. The correct localization of either BirA*-LEM2, BirA*-MAN1 and BirA*-Emerin after doxycycline induction was checked by immunofluorescence analysis (Figure 10). In the presence of doxycycline and biotin, the distinct BirA*-fusion proteins biotinylated potential interactors in close proximity, which were detected with an Alexa-647 conjugated streptavidin (magenta). BirA*-LEM2, BirA*-MAN1 and BirA*-Emerin were correctly localized at the NE, as detected by anti-V5 staining (green). Furthermore, the RFP reporter enabled the identification of the BirA*-construct expressing cells. As expected, in the un-induced control cell line neither any RFP reporter nor V5 signal was detectable using immunofluorescence microscopy.

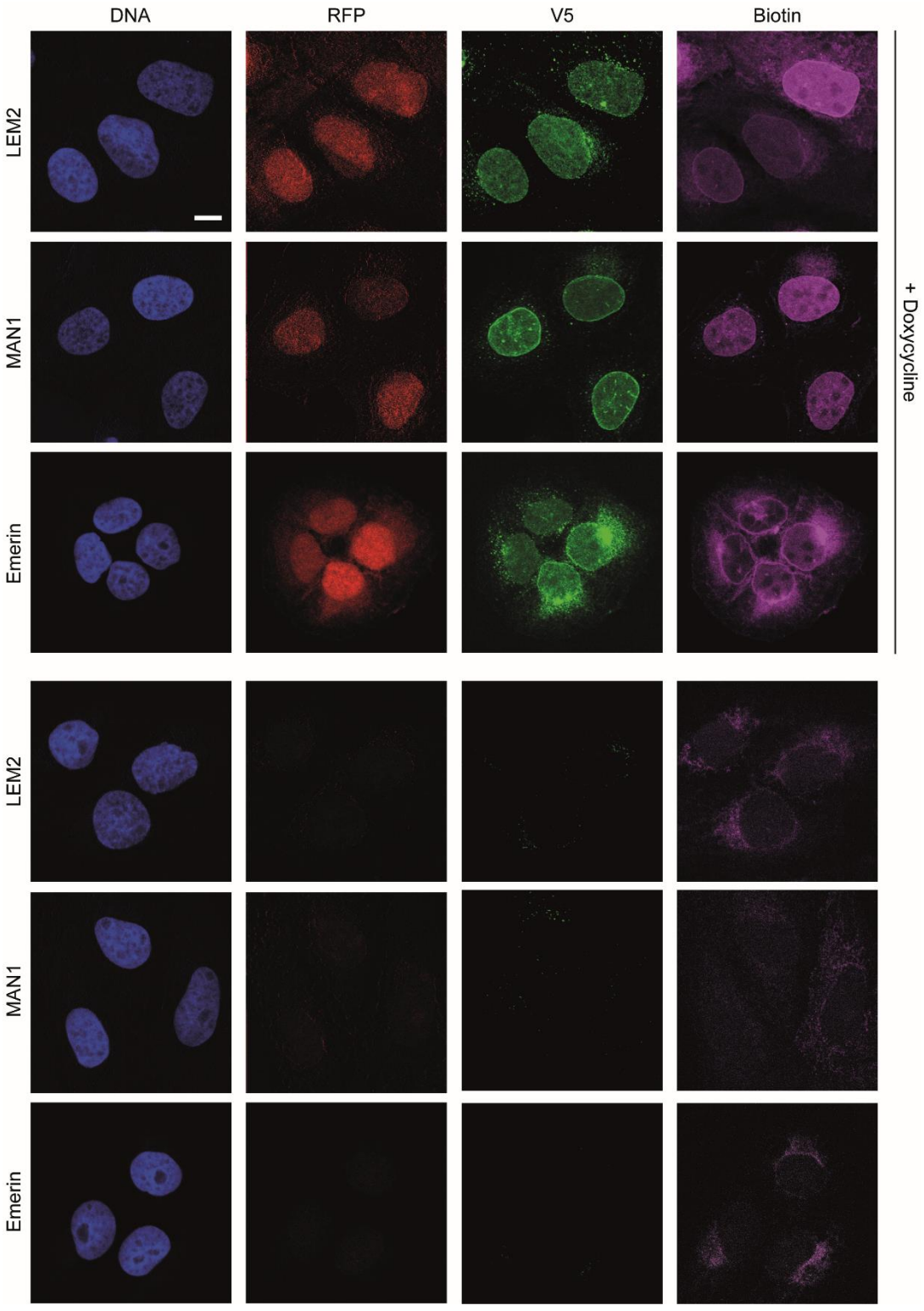


Figure 10 Expression of BirA* fusion proteins in U2OS cells
U2OS cell lines with stable integration of doxycycline-inducible BirA*-fusion constructs were established by standard procedures. Addition of doxycycline and biotin led to biotinylation of proteins nearby, which were detected with a 647-conjugated streptavidin (magenta). The BirA*-fusion constructs were detected by anti-V5 staining (green) and the RFP reporter (red) enabled the identification of the BirA*-construct expression. Bar, 10 µm.

Upon induction, the BirA*-constructs were easily detected by western blot analysis using the V5-tag at the expected molecular weights (LEM2: 91kDa; MAN1: 117kDa; Emerin: 69kDa) (Figure 11A). In addition, the enzymatic activities of BirA*-LEM2, BirA*-MAN1 and BirA*-Emerin were tested by identifying biotinylated proteins with streptavidin-HRP, including both endogenous and exogenous proteins, comparing cell lysates with and without doxycycline induction. The addition of doxycycline and biotin resulted in a strong activation of the BirA*- fusion proteins compared with uninduced cells, which show a weak signal of endogenously biotinylated proteins (Figure 11B). The results of immunofluorescence and western blot analysis showed that the biotin ligase BirA* was successfully fused to the three LEM proteins, which located correctly at the INM and the ligase biotinylated endogenous proteins nearby.

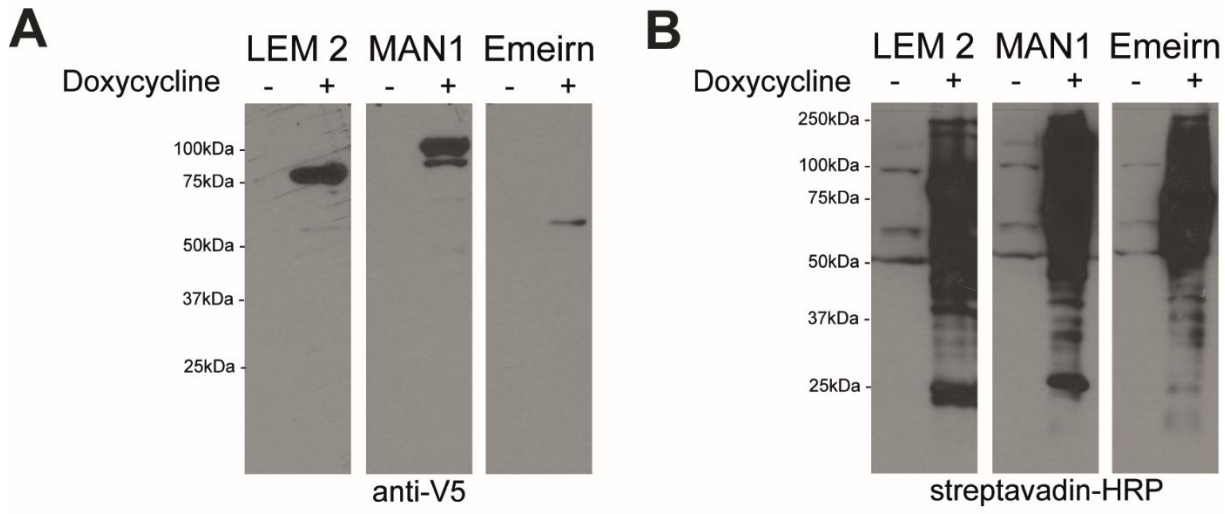


Figure 11: Confirmation of the inducible expression and the biotinylation of BirA*-constructs
Cell lines (lysates), each expressing different inducible constructs, were analyzed by immunoblotting after the addition of doxycycline and biotin. The three constructs were detected with an anti-V5 antibody (left) the biotinylation was detected by streptavidin-HRP (right). Streptavidin-HRP detected four known endogenously biotinylated proteins in the un-induced samples and exogenously biotinylated proteins in the induced samples. Expected molecular weights: LEM2 97 kDa, MAN1 117 kDa, Emerin 69 kDa.

3.1.2 Identifying the interactome of LEM2, MAN1 and Emerin: expected overlaps and surprising new specific interactions

In order to elucidate the interactome of LEM2, MAN1 and Emerin (Figure 12), four independent BioID experiments, for each LEM protein, were carried out.

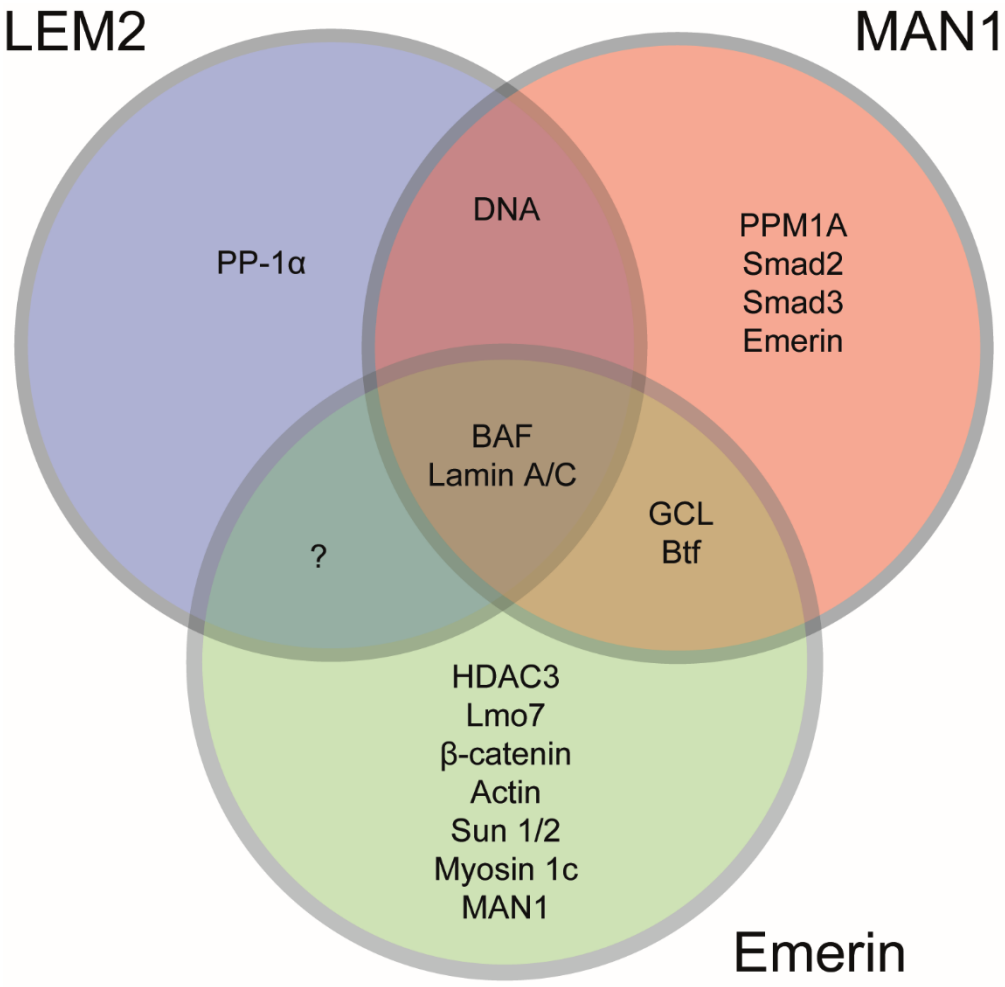


Figure 12: Interactome of LEM2-MAN1-Emerin
Venn diagram shows the network of the most important interaction partners of LEM2 (red), MAN1 (blue) and Emerin (yellow).
Previously reported interactions: LEM2 = PP-1α; MAN1 = PPM1A, Smad2, Smad3, Emerin; Emerin = HDAC3, Lmo7, β-catenin, Actin, Sun1/2, Myosin 1c, MAN1; LEM2-MAN1 = DNA; MAN1-Emerin = GCL, Btf; LEM2-MAN1-Emerin = BAF, Lamin A/C

The potential interactors of LEM proteins in the BioID experiments were captured on streptavidin-conjugated beads and directly trypsinized on the beads, to avoid ineffective elution and concomitant data-loss, and subsequently identified by mass spectrometry (MS).

In order to filter MS-hits with the highest likelihood and probability, the following restrictions were applied on the dataset, which consisted of four independent BioID experiments:

- Reproducibility: Proteins have to show up in at least two out of four runs;
- Significance: Proteins should have low-to-zero peptide-counts in the control sample;
- Probability: Proteins of proper cellular compartment and described molecular function.

Proteins unique to each BioID pulldown (BioID-LEM2, -MAN1 or -Emerin) and proteins which were identified in two or three pulldowns (LEM2-MAN1, LEM2-Emerin, MAN1-Emerin, or LEM2-MAN1-Emerin) respectively, were depicted in a Venn-diagram, which illustrates the overlap of potential binding partners between LEM2, MAN1 and Emerin (Figure 13A). Figure 13 B shows each subgroup (potential specific and overlapping binding partners) as a percentage of the total. The subgroup of potential common interaction partners is the largest group containing 91 (48% of the total) binding partners, followed by potential LEM2-specific binding partners (42) and Emerin-specific binding partners (20), respectively. Expectedly, LEM2 and MAN1 had a larger overlap in potential binding partners (21) than either LEM2 (3) or MAN1 (4) with Emerin, which is structurally unrelated.

Additionally, all filtered specific and common binding partners were further sub-categorized by their annotated molecular functions and their relative abundance presented as overview diagrams below (Figure 14, Figure 15 and Figure 16). To estimate if the identified proteins were localized to or function in specific cellular compartment, the cellular abundance of each protein was extracted from a quantitative proteome map of U2OS cells and grouped in four different abundance groups, according to the authors parameters (Beck et al., 2011):

- High- abundant proteins: >100000 copies per cell;
- Moderate-abundant proteins: 5000–100000 copies per cell;
- Low-abundant proteins: 500–5000 copies per cell;
- Very low-abundant proteins: <500 copies

Detailed lists of all specific and common binding partners are included in the appendix (chapter 6.1).

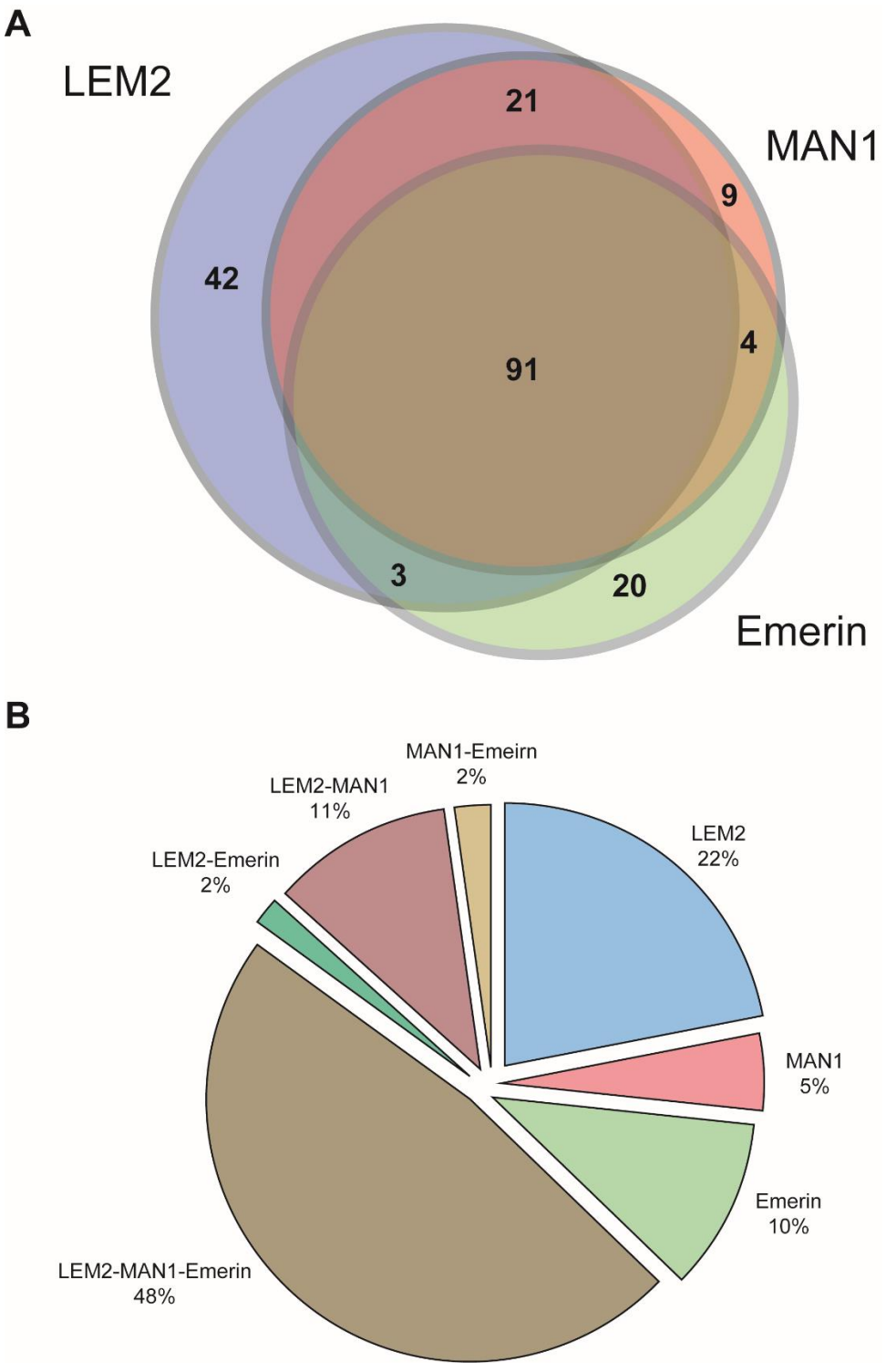


Figure 13: The distribution and overlaps of promising candidate proteins
(A) The Venn diagram shows number of shortlisted proteins, which were identified with BioID. The overlapping regions indicate the shortlisted common proteins for LEM2-MAN1, LEM2-Emerin, MAN1-Emerin, and LEM2-MAN1-Emerin.
(B) Chart shows the distribution of the shortlisted candidate proteins on a percentage basis.

3.1.3 Common interaction partners of LEM2, MAN1 and Emerin

The group of common interaction partners of LEM2, MAN1 and Emerin contained 91 proteins and represents the largest shortlisted group described here (Figure 14). Remarkably, it contains several already published and also expected binding partners like other LEM proteins (e.g. LAP2) or other expected binding partners of INM, such as Torsin-1A interacting protein 1 & 2, the lamin-B receptor (LBR), or several nuclear pore complex proteins (Nup160, Nup204, Nup85, Nup98-96, Nup188). Apart from the large group of functionally non-assignable proteins ("others") and proteins belonging to the NE (15%) the transcription-related proteins (14%) represented another large subgroup containing several different polymerases and transcription factors., followed by nucleolar proteins (10%) such as the rRNA processing nucleolar protein 6 or the Ribosome biogenesis protein BMS1 homolog. More comprehensively, proteins implicated in splicing and ubiquitination processes, respectively (9%) were significantly represented.

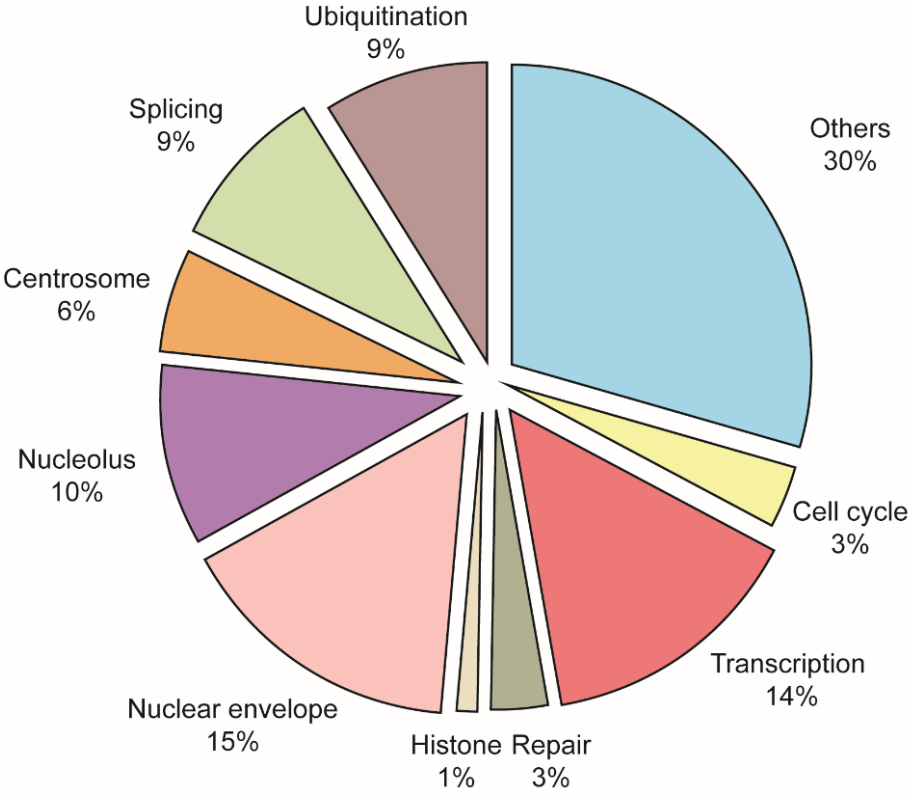


Figure 14: Classification of potential binding partners biotinylated by BirA*-LEM2, BirA*-MAN1 and BirA*-Emerin by their specific function and/or intracellular localization

Table 4 presents an extract of the shortlist, consisting of 10 top-scoring (in terms of peptide counts) hits of the potential common interaction partners. The top scoring protein, which was biotinylated by BirA*-LEM2, BirA*-MAN1 and BirA*-Emerin, was the E3 SUMO-protein ligase RanBP2, which is implicated in nuclear import and export pathways (Gareau et al., 2012). It has an abundance of about $1{,}74 \times 10^4$ copies per cell (moderate-abundant protein) (Beck et al., 2011), however its molecular weight is 358 kDa, which increases the statistical likelihood of becoming biotinylated. BioID identified several expected candidate interaction partners, for example the INM LEM protein LAP2β/γ and LAP2α (both moderate-abundant), which are implicated in maintaining the structural organization of the NE (Dechat et al., 2000), and the previously reported interaction partners lamin A/C (high-abundant protein) (Holaska et al., 2003). Table 4 also lists the cell cycle regulating protein G2/mitotic-specific cyclin-B1, the moderate-abundant core component of the condensing complex (Structural maintenance of chromosomes protein 4) and its regulatory subunit (Condensin complex subunit 1), the DNA-directed RNA polymerase II subunit RPB2 (moderate-abundant) or the high-abundant heterogeneous nuclear ribonucleoprotein H, which is implicated in mRNA processing events (Paul et al., 2006). The nuclear mitotic apparatus protein (NuMA) (moderate-abundant, 238 kDa), a protein which is implicated in maintaining the stability of spindle poles (Silk et al., 2009), was also identified as part of the BAF proteome (Montes de Oca et al., 2009). BAF was likely not detected because of 1) its low molecular weight (10 kDa), which reduces the likelihood to yield detectable peptides and 2) the N-terminal fusion of BirA* (next to the LEM domain) may have caused a steric hindrance for the LEM-BAF interaction. (Complete shortlist, see chapter 6.1.4)

Name	Function	Abundance	kDa
E3 SUMO-protein ligase RanBP2	Facilitates SUMO1 and SUMO2 conjugation	moderate	358
Lamina-associated polypeptide 2, isoforms beta/gamma	Involved in directing directing the assembly of the nuclear lamina	moderate	51
Lamina-associated polypeptide 2, isoform alpha	Involved in the structural organization of the nucleus	moderate	75
G2/mitotic-specific cyclin-B1	Essential for control of G_2/M transition.	moderate	48
DNA-directed RNA polymerase II subunit RPB2	DNA-dependent RNA polymerase	moderate	134
Heterogeneous nuclear ribonucleoprotein H	Component of the hnRNP complexes	high	14
Condensin complex subunit 1	Regulatory subunit of the condensing complex	moderate	157
Structural maintenance of chromosomes protein 4	Core component of the condensin complex	moderate	147
Nuclear mitotic apparatus protein 1	Required maintain and establish the mitotic spindle poles	moderate	238
Lamin A/C	Implicated in nuclear assembly, chromatin organization, gene expression	high	74

Table 4: 10 top-scoring potential common interaction partners

3.1.4 MAN1-specific candidates

The shortlist of potential specific binding partners of MAN1 contains 9 proteins (Figure 15) which are implicated, inter alia, in splicing regulation of pre-mRNAs (Splicing, 11%). Among these proteins the moderate-abundant WW domain-binding protein 11 or mitotic spindle checkpoint proteins like the MAD2B protein (cell cycle, 11%), which acts as an inhibitor of the anaphase-promoting complex (Chen and Fang, 2001) was identified. The subgroup of proteins, which are implicated in transcriptional regulation, is the biggest group.

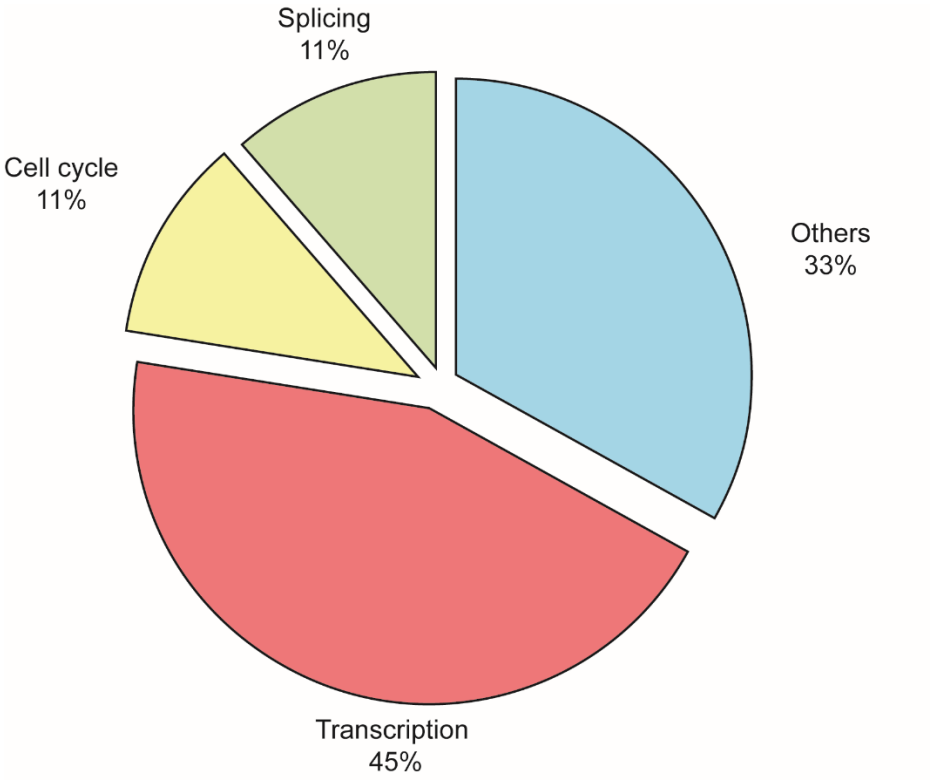


Figure 15: Classification of potential binding partners biotinylated by BirA*-MAN1 by their specific function and/or intracellular localization

Table 5 presents the shortlist of potential MAN1-specific binding partners. The potential interaction partners of MAN1, with Smad3 (low-abundant) leading the way, show again the powerfulness and the selectivity of the BioID approach. The signal transducer Smad3 (Mothers against decapentaplegic homolog 3), which mediates TGF-β signaling (Lin et al., 2005), is a previously reported binding partner of MAN1 and also represents one of the best hits in the MAN1 shortlist. The cell death suppressors as BCL2/adenovirus E1B 19 protein-interacting protein 2 also represents a top-scoring hit, which was biotinylated specifically by BirA*-MAN1. This low-abundant and small (36 kDa) protein is implicated in cell death suppression and interacts with Btf (Belcredito et al., 2001), a previously reported interactor of MAN1 and Emerin (Mansharamani and Wilson, 2005). The protein with the highest peptide-counts was the testis-specific Y-encoded-like protein 2 (very low abundant), which is able to inhibit cell proliferation by activating the antiproliferative autoantigen CDA1, through p53 pathways (Tu et al., 2007). Furthermore, listed in the list of MAN1-specific binding partners is the moderate-abundant WW domain-binding

protein 11 which is implicated in processing of pre-mRNAs (Ruegsegger et al., 1996). Other listed proteins are the transcription initiation factor IIE subunit beta (moderate-abundant), which recruits TFIIH to the preinitiation complex (Peterson et al., 1991), the non-catalytic exosome complex component RRP45 (moderate-abundant), the transcription factor GA-binding protein alpha chain and the poorly described nicotinate-nucleotide pyrophosphorylase, (low-abundant) which is implicated in quinolinic catabolism (Liu et al., 2007). (Complete shortlist, see chapter 0)

Name	Function	Abundance	kDa
Testis-specific Y-encoded-like protein 2	Part of the CASK/TBR1/TSPYL2 transcriptional complex	very-low	79
BCL2/adenovirus E1B 19 protein-interacting protein 2	Involved in the suppression of cell death	low	36
Mothers against decapentaplegic homolog 3 (Smad3)	Intracellular signal transducer and transcriptional modulator	low	48
Nicotinate-nucleotide pyrophosphorylase	Involved in the catabolism of quinolinic acid	low	31
Transcription initiation factor IIE subunit beta	Recruits TFIIH to the initiation complex	moderate	33
Exosome complex component RRP45	Non-catalytic component of the RNA exosome complex	moderate	49
GA-binding protein alpha chain	Transcription factor	moderate	51
WW domain-binding protein 11	Activates pre-mRNA splicing	moderate	70
Mitotic spindle assembly checkpoint protein MAD2B	Adapter protein which involved in different biological processes, e.g. regulating cellular response to DNA damage	low	24

Table 5: 9 potential MAN1-specific interactions partners

3.1.5 Emerin-specific candidates

The shortlist of potential Emerin-specific binding partners (extract see Table 6) consists of 20 proteins, and are grouped based on their biological and cellular functions (Figure 16).

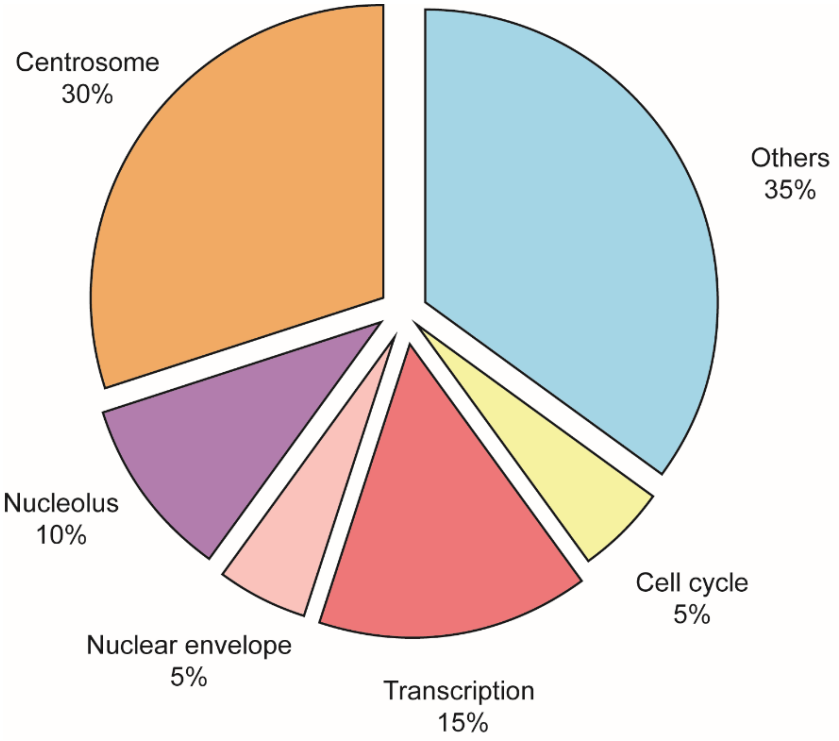


Figure 16: Classification of potential binding partners biotinylated by BirA*-Emerin by their specific function and/or intracellular localization

It is remarkable that several identified potential Emerin-specific binding partners are located at centrosomes, microtubules and microtubule organizing centres (MOC), like Kinectin, the centrosomal proteins 135 and 78 (both low-abundant) which are implicated in centriole biogenesis (Kim et al., 2008), the microtubular MAP7 domain-containing protein 3 (moderate-abundant) which localizes to the microtubules throughout mitosis (Sun et al., 2011) or the low-abundant proteins NEDD1 and Cytoskeleton-associated protein 2-like. This list of identified proteins fits and underlines previous observations that a small fraction of Emerin on the outer nuclear

membrane interacts directly with centrosomes and the microtubule network (Salpingidou et al., 2007). The group of transcription-related proteins (15%) consist of three transcription factors, such as the TATA-binding protein-associated factor 2N, which is implicated in transcription initiation step (Jobert et al., 2009). Furthermore, listed is the Transmembrane protein 43 (Nuclear envelope, 5%) which is implicated in maintaining the nuclear structure and retaining Emerin at the INM (Bengtsson and Otto, 2008). The best hit in all 4 runs was the 5-azacytidine-induced protein 2 (moderate-abundant) (Others, 35%) which is implicated in NF-kappa-B signaling (Sasai et al., 2005). Emerin has been discussed in connection with several diseases (Holaska, 2008). Indeed several proteins specifically biotinylated by BirA*-Emerin are linked to diseases caused by genetic disorders. Mutations of the chromatin associated proteins FAM111A (low-abundant) causes the rare dismorphologic Kenny-Caffey syndrome (Isojima et al., 2014) and mutations of FAM111B (low-abundant) cause hereditary fibrosing poikiloderma (Mercier et al., 2013). (Complete shortlist, see chapter 6.1.3)

Name	Function	Abundance	kDa
5-azacytidine-induced protein 2	Activates serine/threonine-protein kinase TBK1	moderate	122
Protein TANC2	Implicated in embryonic development	low	220
MAP7 domain-containing protein 3	assembly and stability of microtubules	low	98
TATA-binding protein-associated factor 2N	Transcription initiation	moderate	70
Centrosomal protein of 135	Involved in centriole biogenesis	low	133
Transmembrane protein 43	Essential for maintaining nuclear envelope structure	moderate	45
Nuclear fragile X mental retardation-interacting protein 2	Binds RNA	low	76
Protein PRRC2C	-	moderate	317
Protein FAM111A	Required for PCNA loading	low	70
Centrosomal protein of 78	G_2/M transition	low	78

Table 6: 10 top-scoring potential Emerin-specific interaction partners

3.1.6 LEM2-specific candidates

The identified shortlisted proteins of LEM2 cover a broad range of subgroups subdivided based on their biological and cellular functions (Figure 17). Beside a large number of undescribed and/or functionally non-classifiable proteins ("others"), the biggest groups consist of proteins which are implicated in transcription process (12%) such as the subunits of the DNA-directed RNA polymerase and nucleolar proteins (Nucleolus, 12%), respectively.

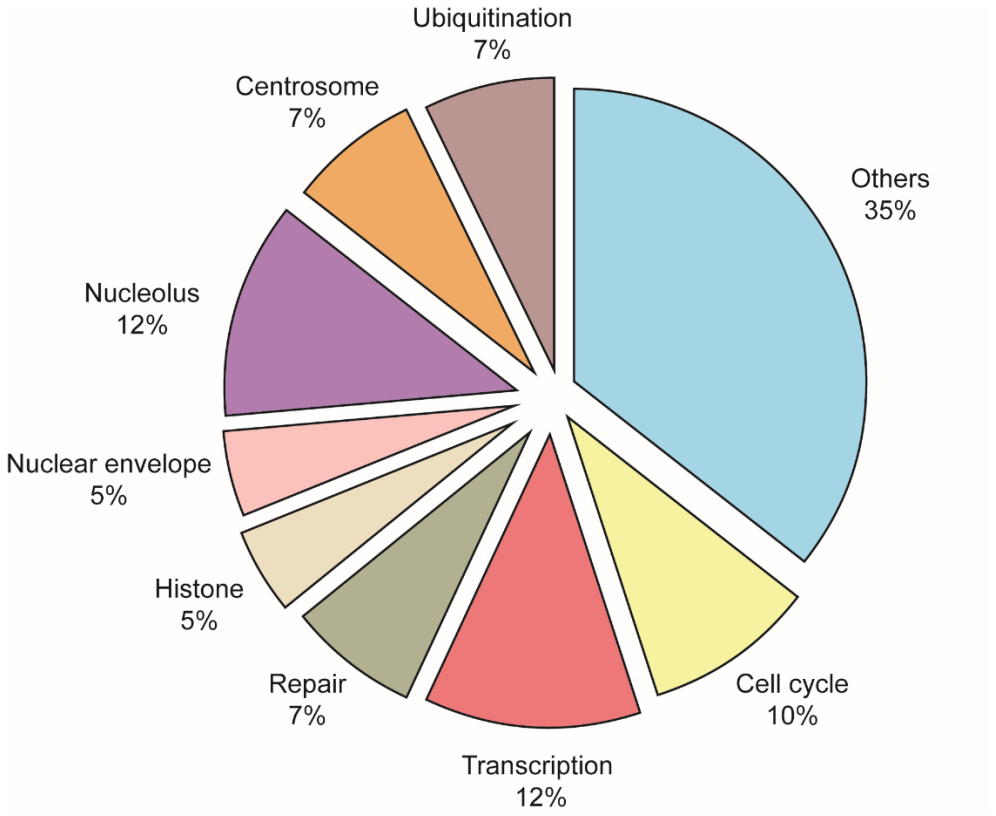


Figure 17: Classification of potential binding partners biotinylated by BirA*-LEM2 by their specific function and/or intracellular localization

Additionally the LEM2-specific binding partners include some proteins involved in histone modification (5%), such as the moderate-abundant N-alpha-acetyltransferase 40 which acetylates histones H4 and H2A (Hole et al., 2011) and centrosome binding (7%), such as the CLIP-associating protein 1 and cell cycle regulation (10%), such as the Cell division cycle protein 16 homolog which is a component of the anaphase promoting complex. In addition, proteins that are implicated in ubiquitination (7%) and nuclear envelope proteins (5%), such as the very-low

abundant protein Nesprin-1 showed up in the analysis. Nesprin-1 a protein of 1011 kDa, binds Emerin and lamin A *in vitro*, in this way stabilizing the INM (Mislow et al., 2002). However, despite its enormous molecular weight and its reported interaction with Emerin, it was biotinylated exclusively by BirA*-LEM2. PP-1α, the only previously reported interactor of LEM2, was biotinylated by all three BirA*-fusion proteins, therefore not shortlisted in the LEM2-specific hits. The transcriptional activator RNA-binding protein 4B (moderately-abundance), followed by Sestrin-2 (very low-abundant), which is implicated in reduction of peroxiredoxins (Budanov et al., 2004); the DNA damage-binding protein 1 (moderate-abundant) and the very low-abundant transcriptional regulator Zinc finger protein 10, represent the four best hits based on their peptide-counts. The moderate-abundant DNA damage binding protein 1 (Repair: 7%) is one of the best hits in all four BioID runs, which is one of the core proteins of the NER machinery. Additionally, two components of the COP9 signalosome complex (CSN) (Ubiquitination, 7%), were biotinylated by BirA*-LEM2, which negatively regulates the activity of DDB1 (Huang and D'Andrea, 2006). A list, containing 10 top-scoring hits of LEM2, (Table 7) exemplifies the described functional coverage of LEM2. (Complete shortlist, see chapter 6.1.1)

Name	Function	Abundance	kDa
RNA-binding protein 4B	Essential for activation of PER1 mRNA	moderate	40
Sestrin-2	Involved in peroxiredoxins reduction	very-low	54
DNA damage-binding protein 1	DNA damage repair (NER)	moderate	127
Zinc finger protein 10	Involved in transcriptional regulation.	very-low	66
Ribonucleoside-diphosphate reductase subunit M2 B	Repairs DNA in a in a p53/TP53-dependent manner	moderate	41
NEDD8	Important role in cell cycle, embryogenesis and NER	moderate	9
DNA-directed RNA polymerases I, II, and III subunit RPABC1	DNA-dependent RNA polymerase	moderate	25
Nesprin-1	Component of the LINC complexes	very-low	1011
COP9 signalosome complex subunit 8	Component of the COP9 signalosome complex	moderate	23
BTB/POZ domain-containing protein KCTD14	Protein homooligomerization	very-low	30

Table 7: 10 top-scoring potential LEM2-specific interactions partners

3.2 Impairment of DNA damage repair upon loss of LEM2

3.2.1 High representation of LEM2-“specific” binding partners, within components involved in nuclear excision repair

One of the top-scoring proteins of the identified possible LEM2-specific binding partners (see Table 7) was DDB1 which is well-known for its role in DNA damage repair (Marteijn et al., 2014). It forms a complex with DDB2 to recognize UV induced DNA damage and to recruit specific proteins of the nucleotide excision repair machinery to the DNA repair site (see chapter 1.4.1.1). Apart from DDB1, the BioID data contained a large number of proteins which play functional roles in different steps of the NER machinery (Figure 18 and Table 8). The BioID data showed that LEM2 had the highest scores for NER-associated proteins, as compared to MAN1 and Emerin. This interpretation is based on the number of peptides found in all 4 BioID runs and also concerning the number of runs, in which each of the proteins were identified.

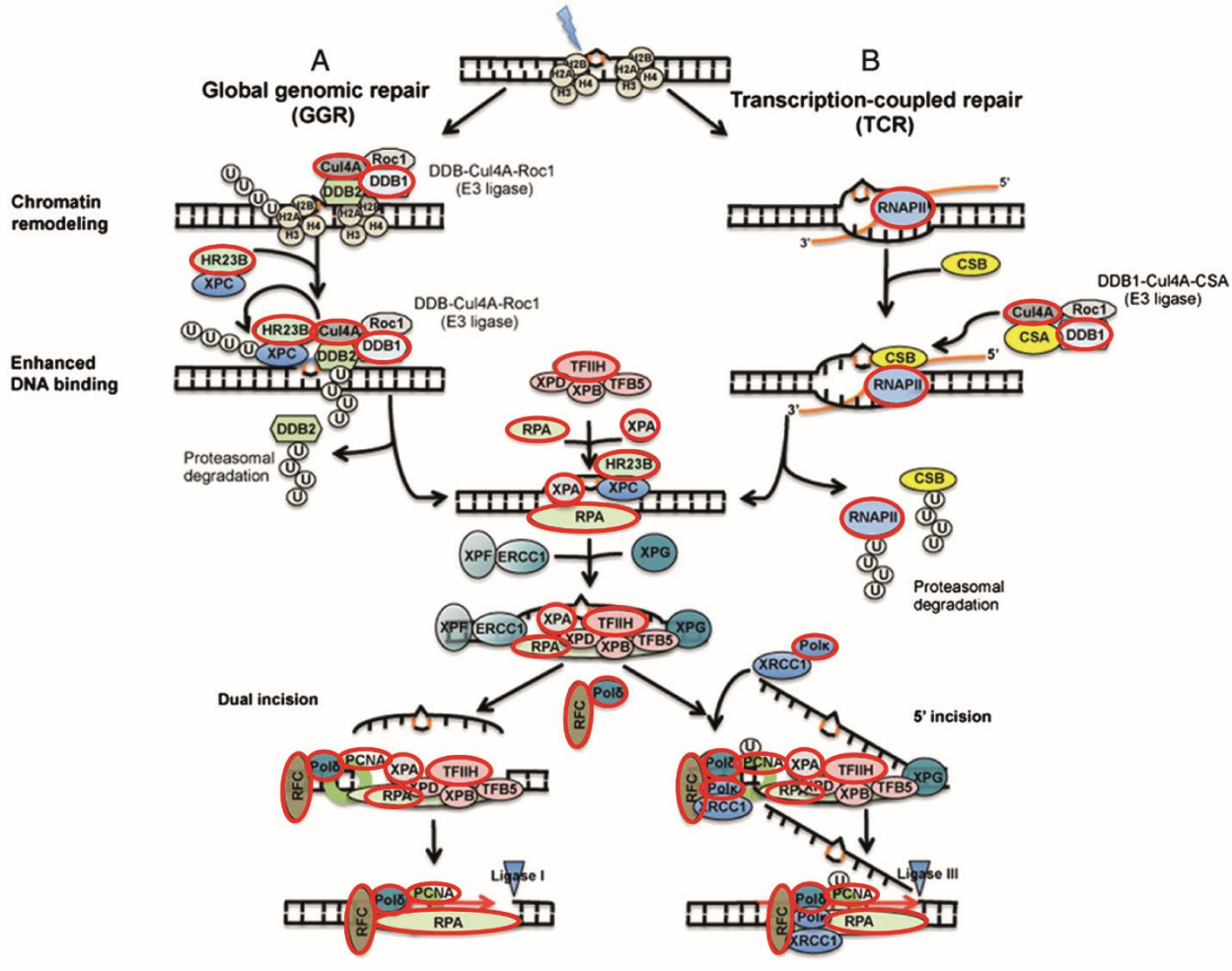


Figure 18: Nucleotide excision repair components identified BioID
Proteins identified by BioID, which are involved in the NER machinery. Biotinylated Proteins are indicated by red circles. Figure adapted from (Fei et al., 2011).

Name	LEM2			MAN1			Emerin		
	+	-	Runs	+	-	Runs	+	-	Runs
Cul4A	**6**	0	3	4	0	1	1	0	1
DDB1	**16**	0	4	0	0	0	0	0	0
DCAF13	**12**	1	4	2	2	2	6	1	2
DCAF7	**6**	0	4	4	4	2	0	0	0
COP9 (7SU)	**34**	3	4	8	4	1	3	0	1
H23B	1	0	1	1	0	1	0	0	0
TFIIH (3SU)	**16**	1	3	7	4	2	1	0	1
XPA 1&2	**22**	1	4	20	1	2	2	0	1
RPA	4	0	2	**6**	0	1	0	0	0
Polδ (4SU)	**57**	5	4	23	4	3	14	2	2
RFC (5SU)	**58**	6	4	26	6	3	26	8	3
PCNA	**16**	5	4	7	6	3	5	2	2
FLAP	**20**	1	4	7	3	3	8	4	3

Table 8: Shortlisted candidate proteins involved in the nucleotide excision repair machinery
The red color indicates the highest values of peptides, in all four BioID runs. Almost all values are higher for BioID-LEM2 than for BioID-MAN1 and BioID-Emerin
(+): induced cells; (-): un-induced cells; (Runs): indicates in how many runs the protein was identified

3.2.2 LEM2 KD cells show reduced viability after UV damage

The NER pathway is one of the main repair systems for removal of DNA lesions, induced by either UV irradiation, oxidative stress, or environmentally factors. In order to examine the relationship, the possible functional connection between proteins of the NER pathway and LEM2 proteins, U2OS cell viability was accessed following knockdown of LEM2, MAN1, Emerin and DDB1 by RNA-mediated interference (Figure 19). Western blot analysis (Figure 19B) was carried out to confirm the effectiveness of each knockdown. Immunofluorescence analysis (Figure 19A) of U2OS cells was carried out to further confirm the knockdown in cells and to observe potential changes of the NE morphology.

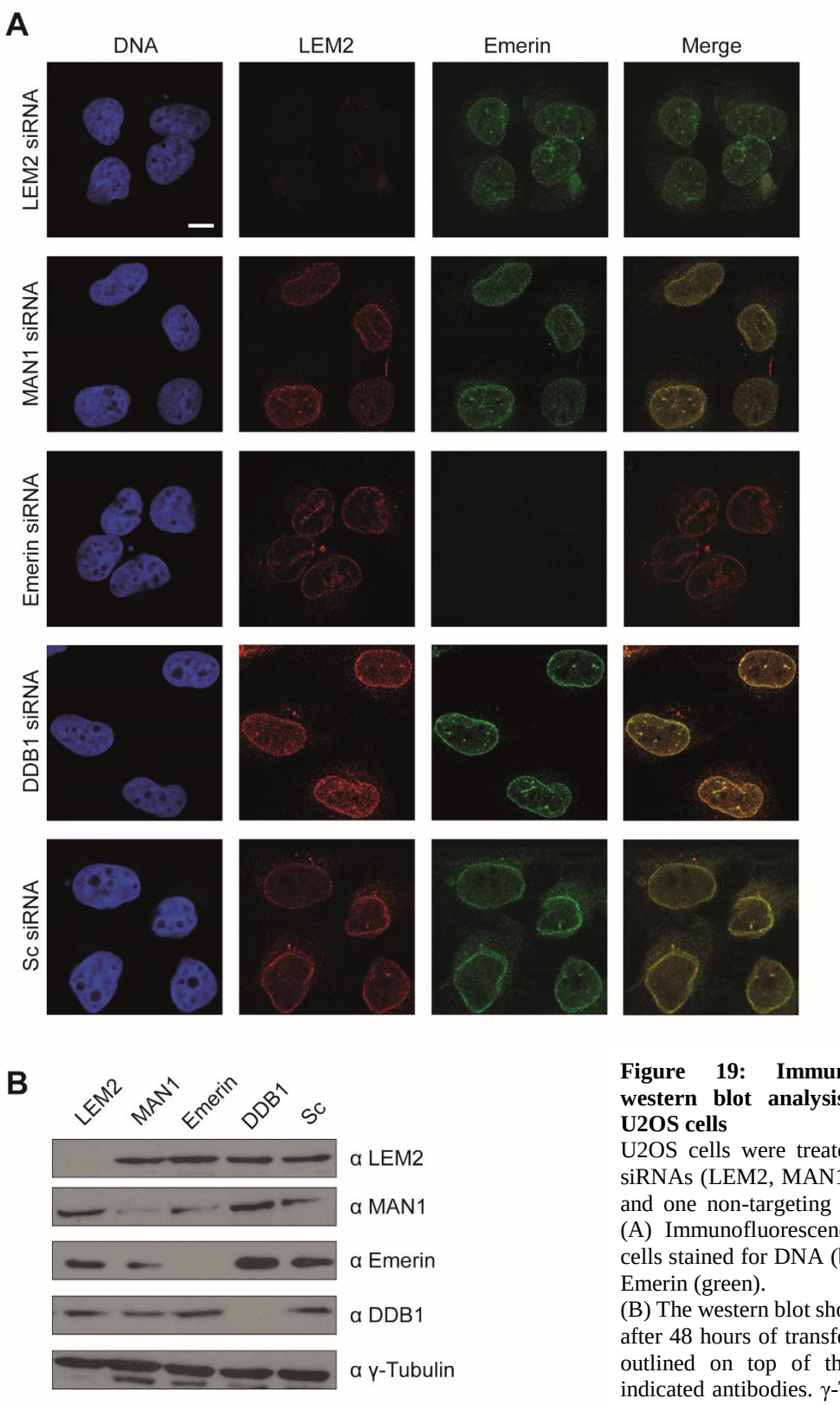


Figure 19: Immunofluorescence and western blot analysis of siRNA treated U2OS cells

U2OS cells were treated with four different siRNAs (LEM2, MAN1, Emerin, and DDB1) and one non-targeting negative control (Sc). (A) Immunofluorescence analysis of U2OS cells stained for DNA (blue), LEM2 (red) and Emerin (green).

(B) The western blot shows whole- cell lysates after 48 hours of transfection with siRNAs as outlined on top of the panel treated with indicated antibodies. γ-Tubulin was used as a loading control. Bar, 10µm.

48 hours after transfection, each protein was depleted to a nearly undetectable level, as monitored on western blots and observed by immunofluorescence microscopy. Unfortunately, due to the lack of appropriate antibodies, it was not possible to confirm the knockdown of MAN1 and DDB1 by immunofluorescence analysis. We revealed by immunofluorescence microscopy that the location of the other LEM proteins and NE structure were not affected by mentioned knockdown of the LEM protein individually. After 24 hours of siRNA treatment (1st time point: 0h), the cells were exposed to 5J/m² UV-C irradiation, which induced the formation of pyrimidine dimers, CPD and 6-4PPs in the DNA, followed by continued culturing for another 96 hours. Flow cytometric analysis of the cell cycle revealed a cell cycle profile, which showed that KD-cells arrested at G2/M, but continued to grow 72 hours after UV-treatment irrespective which siRNA (including the control) was used (Figure 20). This result is interpreted that the G_2/M arrest is only due to UV irradiation and not due to siRNA treatment.

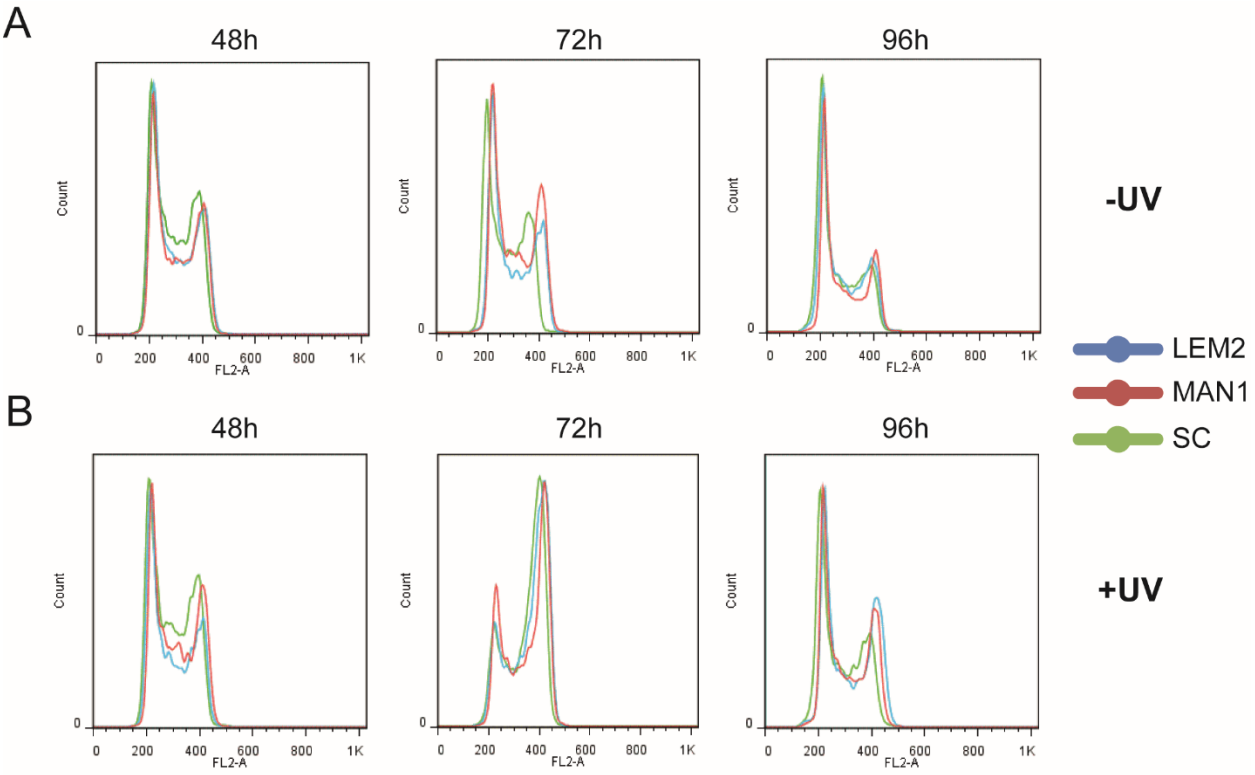


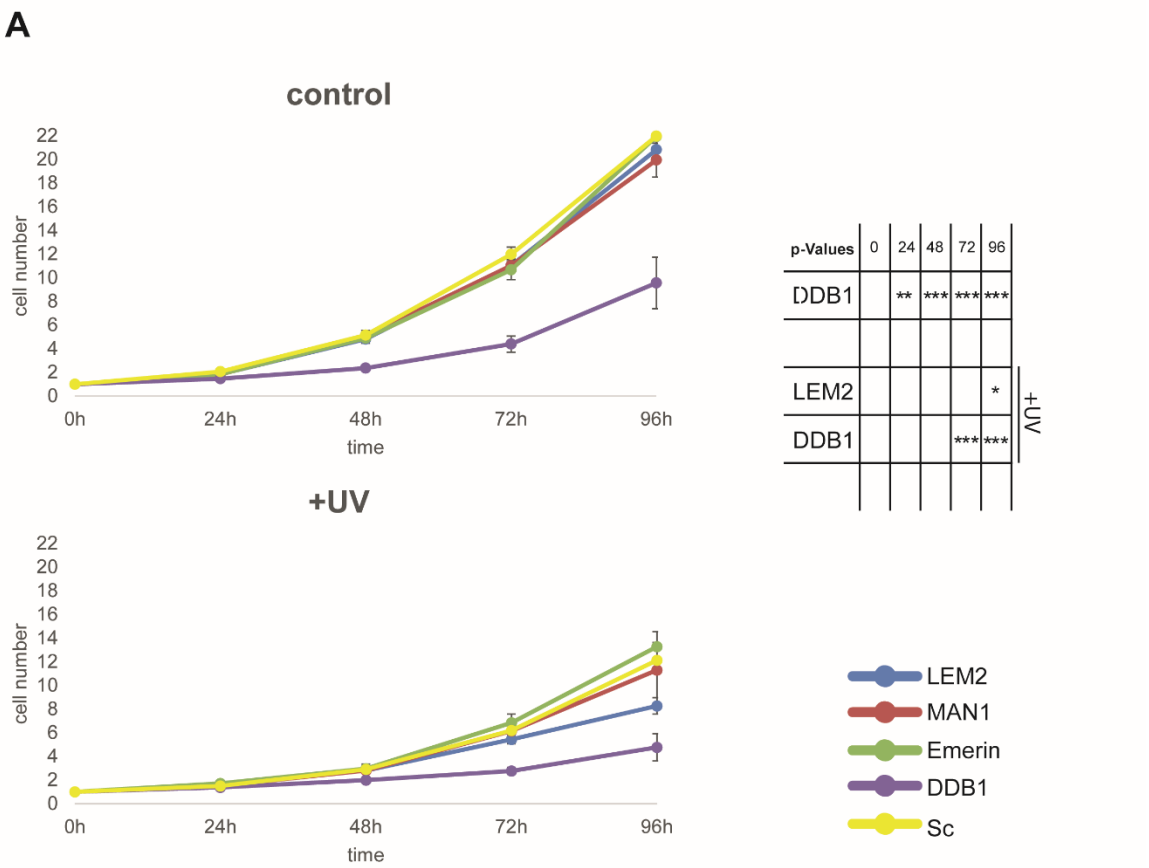
Figure 20: Flow cytometric analysis confirms G_2/M cell cycle arrest 72 hours after UV irradiation
Cell cycle profile of (A) untreated and (B) UV-treated U2OS cells after depletion of LEM2 (blue), MAN1 (red) and Sc control (green) respectively. KD-cells arrested at G_2/M but continue growing 72 hours after UV-treatment. Shown are timepoints 48h, 72h, and 96h +/- UV-treatment.

Every 24 hours the cells were counted and growth characteristics were analyzed by plotting the cell number over the cultivation time (Figure 21). In comparison to the UV-treated scrambled control (Sc), only LEM2 KD cells showed significantly (p=0,019) reduced viability after 96 hours. Knockdown of DDB1 showed significantly reduced viability compared to Sc-treated controls, in both cases, without and with UV treatment and can thus be used as an internal control.

In order to investigate if the reduced viability of LEM2 KD cells is specifically connected to the NER pathway, different treatments were done which induces different types of DNA damage, and cell viability was tested. Figure 21B shows growth curves of U2OS KD cells after bleomycin treatment, hydroxyurea (HU) and hydrogen peroxide (H_2O_2). Bleomycin treatment induces DNA single- and double-strand breaks, which are predominantly repaired by non-homologous end joining. Hydroxyurea interferes with DNA replication during S-phase, thereby causing stalled replication forks and consequently double strand breaks in G_2 which are repaired via HR repair. H_2O_2 has pleiotropic effects on cellular molecules, including single strand breaks and other kinds of DNA lesions which are repaired by NER, HR and by BER (Helleday et al., 2008). If KD of LEM2 would not specifically affect the NER pathway but has a more general effect in DNA repair, growth would be reduced significantly after each treatment.

Treatments of either HU or bleomycin revealed no difference in the proliferation pattern of the different KD cells, except for the DDB1 KD cells, which showed reduced viability also without any treatment. 96 hours after DNA damage induction, by hydroxyperoxide treatment, the number of LEM2 KD cells was reduced compared to those of MAN1- and Emerin-KD cells (cell number of Sc-control (96h) is probably reduced by technical problems).

Taken together, these results showed that knockdown of LEM2 in human cells reduced their viability siginificantly after UV irradiation, pointing to an impairment of NER upon loss of LEM2. Additionally, LEM2 KD cells showed slightly reduced viability after H_2O_2 treatment, which also induces DNA lesions partially repaired by NER. These results represent a strong evidence, that LEM2 is specifically involved in the repair of DNA damage by the efficacy of the NER pathway.



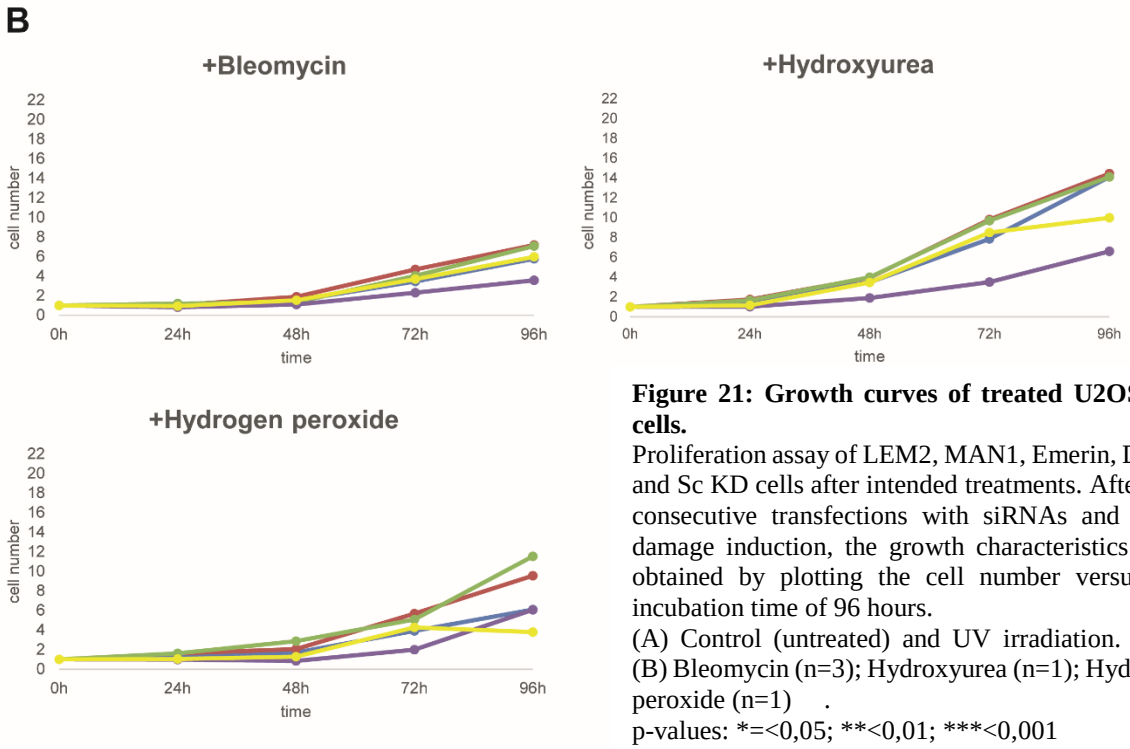


Figure 21: Growth curves of treated U2OS KD cells.

Proliferation assay of LEM2, MAN1, Emerin, DDB1 and Sc KD cells after intended treatments. After two consecutive transfections with siRNAs and DNA damage induction, the growth characteristics were obtained by plotting the cell number versus the incubation time of 96 hours.

(A) Control (untreated) and UV irradiation. (n=8)
(B) Bleomycin (n=3); Hydroxyurea (n=1); Hydrogen peroxide (n=1) .

p-values: *=<0,05; **<0,01; ***<0,001

3.2.3 Analysis DNA damage response of UV-treated KD-cells

Figure 21A shows that the growth curves of LEM2 and DDB1 KD cells start to drift apart from the growth curves of MAN1, Emerin KD cells, and Sc control cells, at the third time point (48h). In order to analyse DNA damage response of UV-treated KD cells biochemically, whole cell lysates of U2OS KD cells were analyzed by immunoblotting after 48 hours of UV treatment (Figure 22). The extent of DNA damage signaling was detected by γH2AX-antibody staining. Expectedly, UV-treatment caused a stronger γH2AX signal in DDB1- and LEM2- KD cells, compared to other samples (and control) supporting the hypothesis that LEM2 depleted cells have impaired NER and thus accumulate damage.

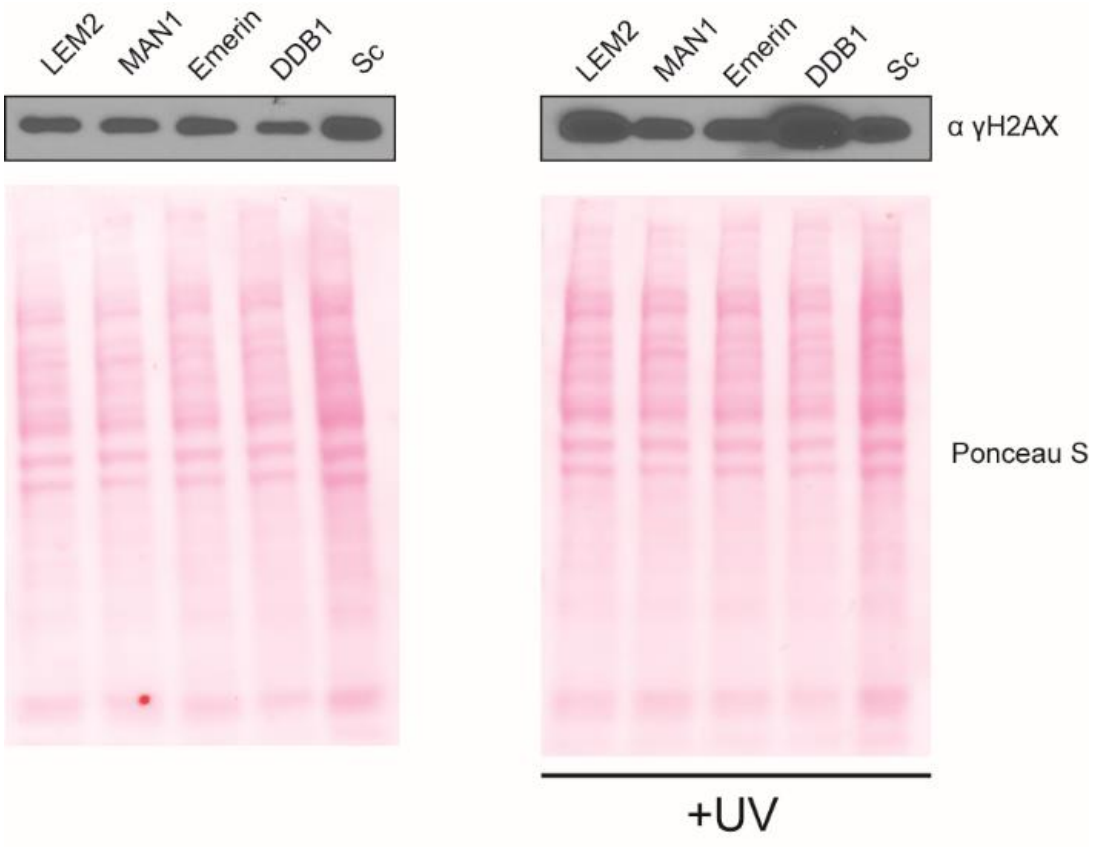


Figure 22: Western blot analysis of UV- treated U2OS KD cells.
Whole cell lysates were analyzed by western blot analysis, depicting the phosphorylation of histone H2AX (γH2AX) in response to 5J/m² after 48 hours. UV-treatment caused a stronger γH2AX signal in DDB1- and LEM2-KD cells, compared to other samples.

At the fourth time point (72h), when cell arrest in G_2/M following UV irradiation (Figure 20) immunofluorescence analysis was carried out to visualize UV treatment mediated DNA damage microscopically (Figure 23 (control) and Figure 24 (+UV)). KD cells were fixed and stained for γH2AX (green) and 53BP1 (red) to visualize DNA damage responses.

Except for DDB1 KD cells, LEM2, MAN1, Emerin KD cells and the scrambled-control, showed no or negligible differences in the number of DNA damage repair foci and the structure of the nuclear envelope in the absence of UV treatment (Figure 23). Expectedly DDB1 knockdown cells showed increased DNA damage signaling (γH2AX), compared to other cells and in addition, larger nucleoli, reflecting decreased growth characteristics as observed in viability assay experiments (Figure 21A).

In general UV-treated cells (Figure 24) showed, compared to untreated cells, increased γH2AX signaling and intensified 53PB1 staining, confirming the UV-effect. Expectedly, UV-irradiated DDB1 KD cells exhibited, besides slight structural NE deformations, several 53BP1 foci, and an intense and diffuse γH2AX signaling pattern, which reflects the accumulation of DNA lesions and an extensive impairment of DNA damage repair. Immunofluorescence of UV-treated LEM2 KD cells showed a slightly more intense γH2AX signaling pattern than that of the Sc control and that of MAN1 and Emerin KD cells, pointing to a stronger DNA damage response and an impairment of DNA damage repair. As expected, UV-treated DDB1 KD cells showed the highest number of DNA damage repair foci, reflecting the strongest DNA damage impairment, compared to other samples.

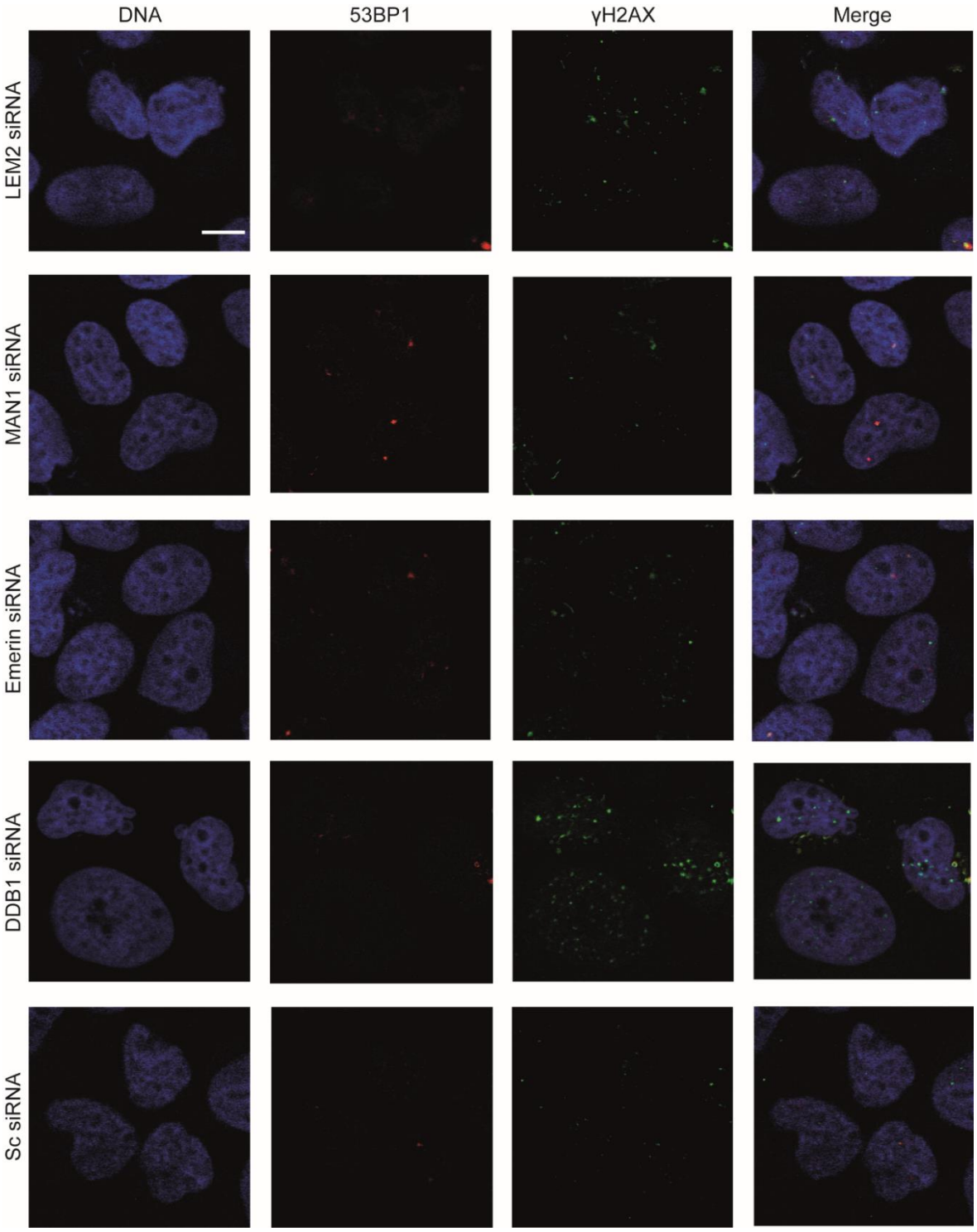


Figure 23: Immunofluorescence analysis of KD cells to visualize DNA damage response
Immunofluorescence analysis of U2OS KD cells, which were stained for DNA (blue), 53BP1 (red) and γH2AX (green). KD cells and scrambled-control, showed no or just slight differences in the number of DNA damage repair foci and the structure of the nuclear envelope in the absence of UV treatment. Bar, 10µm.

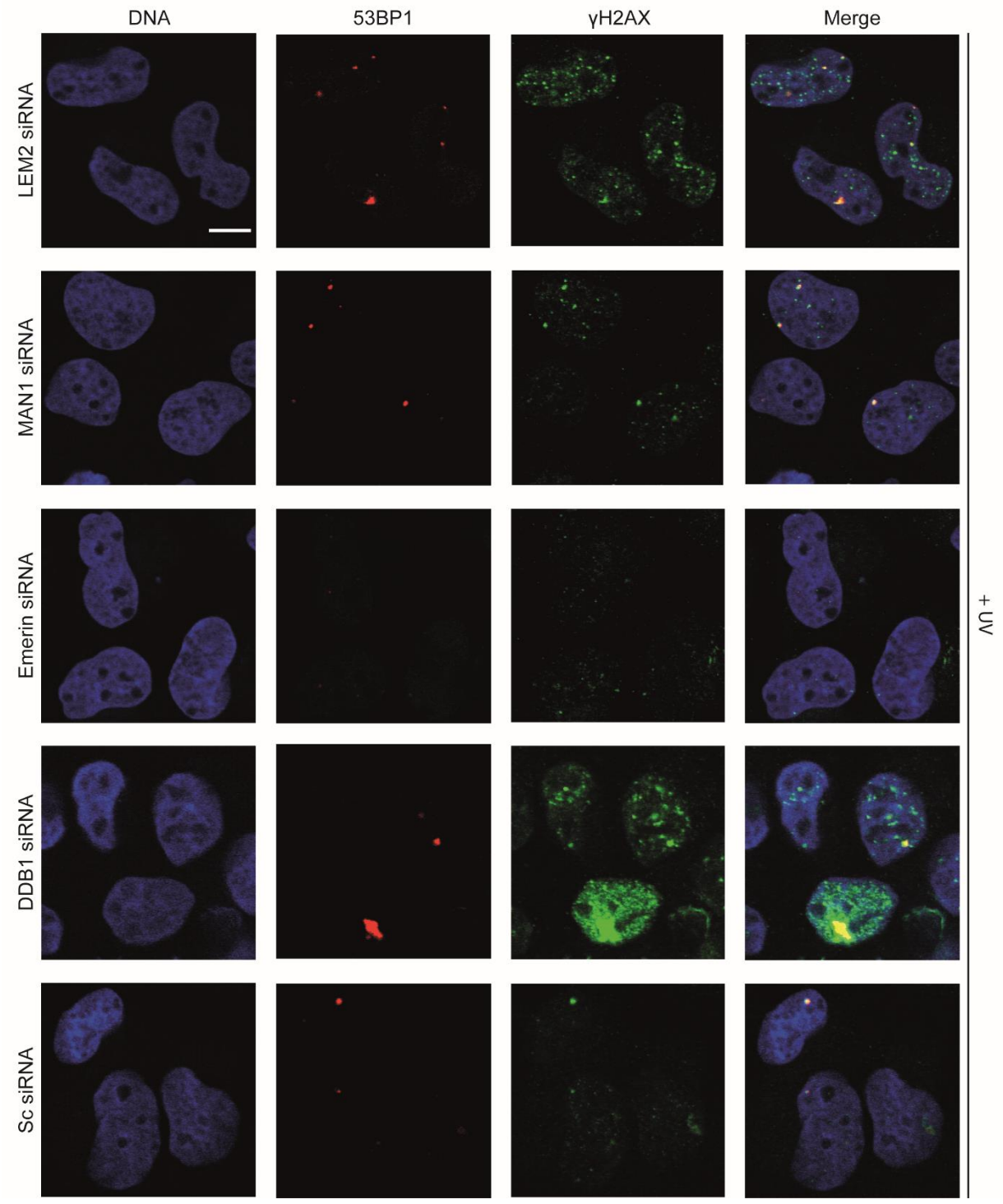


Figure 24: Immunofluorescence analysis of UV-treated KD cells to visualize DNA damage response
Immunofluorescence analysis of U2OS KD cells, which were stained for DNA (blue), 53BP1 (red) and γH2AX (green). UV-treated DDB1 KD cells showed the highest number of DNA damage repair foci and LEM2 KD cells showed a slightly more intense γH2AX signaling pattern than that of the Sc control and that of MAN1 and Emerin KD cells. Bar, 10µm.

3.3 Investigation of NE invaginations by live cell imaging

3.3.1 Nuclear envelope channels stay associated with nucleoli for several hours

The NE can form dynamic invaginations which can reach deep into the nucleoplasm. In mammalian cells, nucleoli are often directly associated with NE invaginations (Figure 1). There is increasing evidence that this unique structure may provide a platform with which the NE regulates specific molecular functions/processes in the nucleoplasm.

In order to obtain further insights into the dynamic and functional behavior of NE invaginations, U2OS cells stably expressing LEM2-RFP and Fibrillarin-GFP, respectively, were investigated using live cell imaging. Fibrillarin is a component of the nucleolar small nuclear ribonucleoprotein and therefore served to mark nucleoli. Live cell imaging microscopy allowed the identification of NE invaginations and their dynamics, excluding artefacts of cell fixation or the immunofluorescence staining.

U2OS cells expressing LEM2-RFP and Fibrillarin-GFP were seeded in a 35mm imaging dish with a glass bottom (Ibidi). The overexpression of the fluorophore-tagged proteins provided a strong signal, which enabled to work with lower and therefore, less harmful excitation light intensity. Due to this overexpression however, LEM2-RFP proteins also tend to diffuse and accumulate in the ER around the ONM of the NE.

During mitosis (Figure 25), the nuclear envelope breaks down and its proteins (including LEM proteins) get absorbed into the ER or diffuse into cytoplasm, (Hetzer, 2010) till the NE gets reassembled after sister chromatid separation at anaphase. In order to investigate how LEM2 localizes during the cell cycle, a live cell imaging experiment was carried out in which U2OS cells expressing LEM2-RFP and Fibrillarin-GFP were imaged. During early cell cycle stages, LEM2 localized mainly in the cytoplasm (Figure 25, time point 0m - 4m). Similar to other LEM domain containing proteins (Emerin and LAP2α) (Dechat et al., 2004), LEM2 re-localized to the NE at telophase, as soon as the NE started reassembling (time point, 9m).

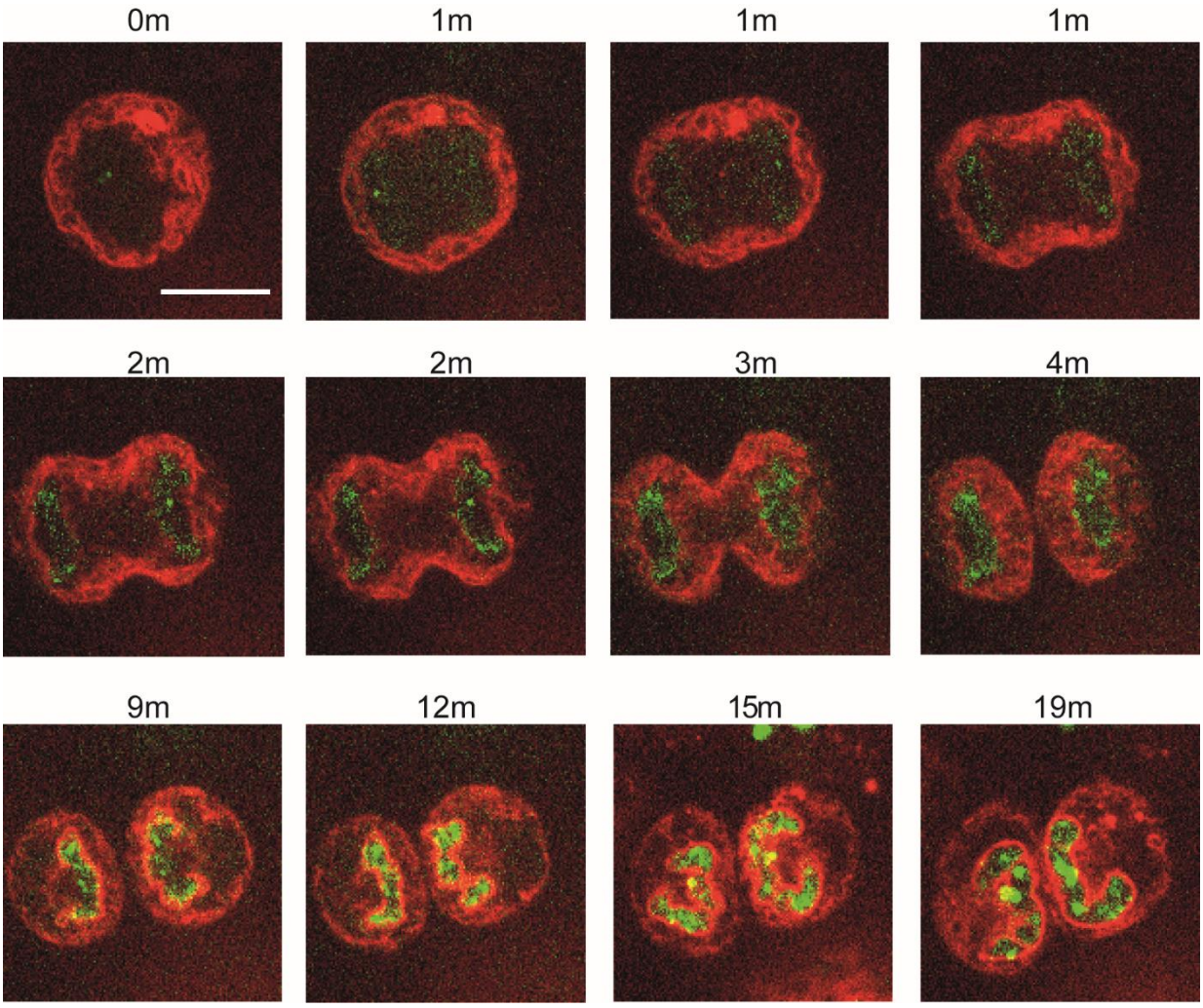


Figure 25: LEM2-dynamics during mitosis
representative images of different time points of a live cell imaging experiment. U2OS cell line expressing LEM2 (red) and Fibrillarin (green) was imaged to assay the dynamics of LEM2 during the cell cycle.
Time points: 0m= metaphase; 1m= anaphase; 2m-15m=telophase, 19m=G_1-phase. Bar, 10µm.

Figure 26 shows representative images at different time points of a live cell imaging experiment in which U2OS cells were imaged for more than 12 hours. The first time point (1h) shows three or two, several NE invaginations (arrows), which are associated to nucleoli. The whole cell, including the NE and nucleoli, changed orientation and localization over time. Despite positional movements, and repositioning of nucleoli and the NE, the NE invaginations remained associated with nucleoli for several hours. The last time point (12h) shows that the nucleolus at the left edge of the cell (asterisk at 12h time point) was assembled from three different nucleoli (asterisks at 1h time point).

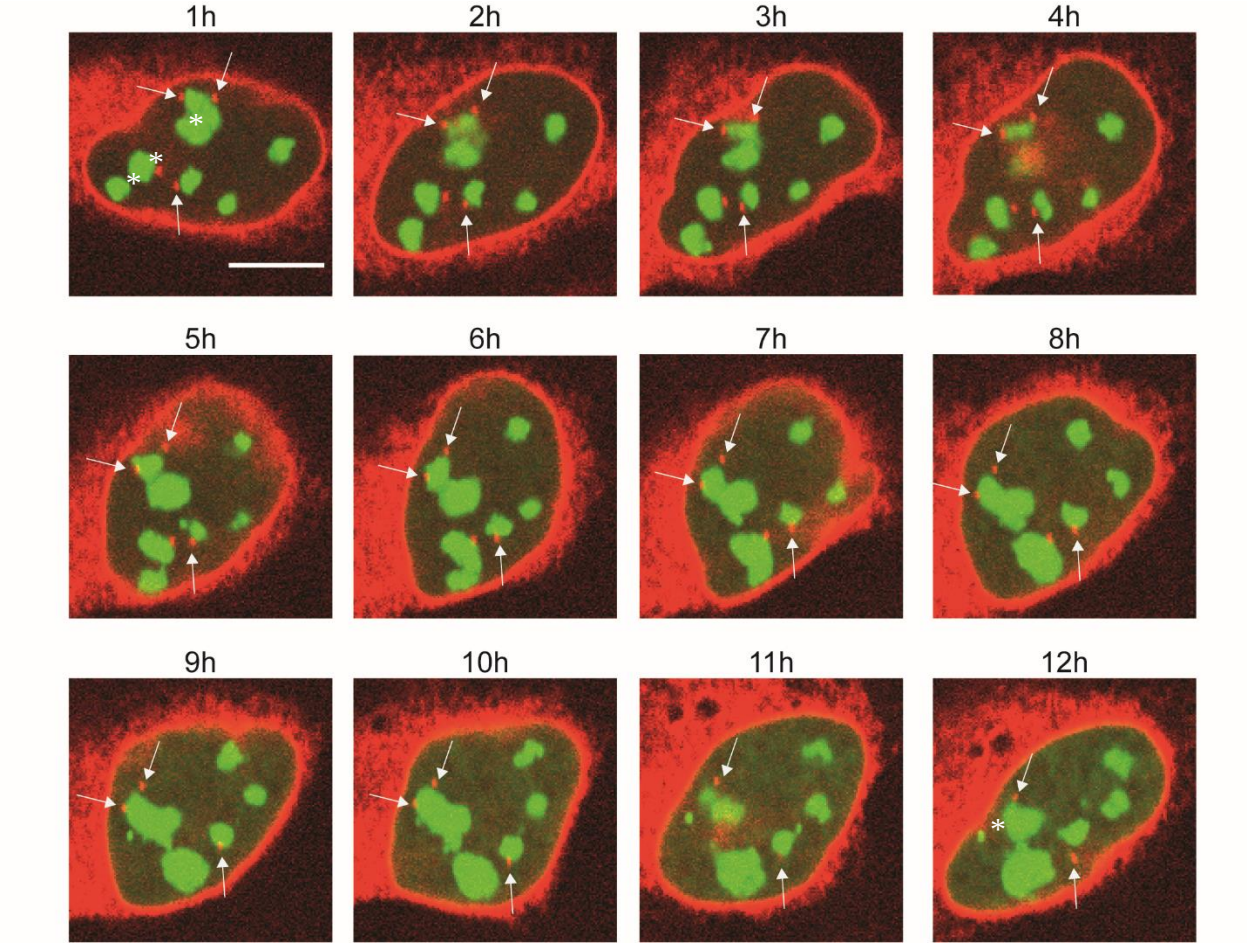


Figure 26: NE invaginations stay associated with nucleoli for several hours
Representative images of a live cell imaging experiment. U2OS cell line expressing LEM2 (red) and Fibrillarin (green) was imaged to assay the dynamics of NE invaginations. Arrows indicate NE invaginations, which stay associated with nucleoli for several hours. Asterisks indicate nucleolar reassembling. Bar, 10µm.

Figure 27 shows representative images of eight different time points of a live cell imaging experiment in which U2OS cells expressing LEM2-RFP and Fibrillarin-GFP were imaged for about two hours in mitosis and G_1-phase. The first two images show a mitotic U2OS cell at metaphase and anaphase. The third image (time point 0min), shows the same cell, which has already divided and reformed its NE.

Already 6 minutes after the NE is reformed, NE invaginations start to connect to nucleoli (arrows), which were completely lost during mitosis. This representative images clearly show, that despite enormous deformations of the NE, associations of nucleoli with NE invaginations were always detectable. The last time point (80min) shows nucleoli that assembled to large structures compared

to the 6min time point and most notably, all three NE invaginations (arrows) were still associated with these nucleoli.

Taken together, the results of both live cell imaging experiments (Figure 27 and Figure *28*) provide strong evidence for a possible role of NE invaginations in reassembling or re-localization of nucleoli. However, how this is achieved is a worthwhile focus for further research.

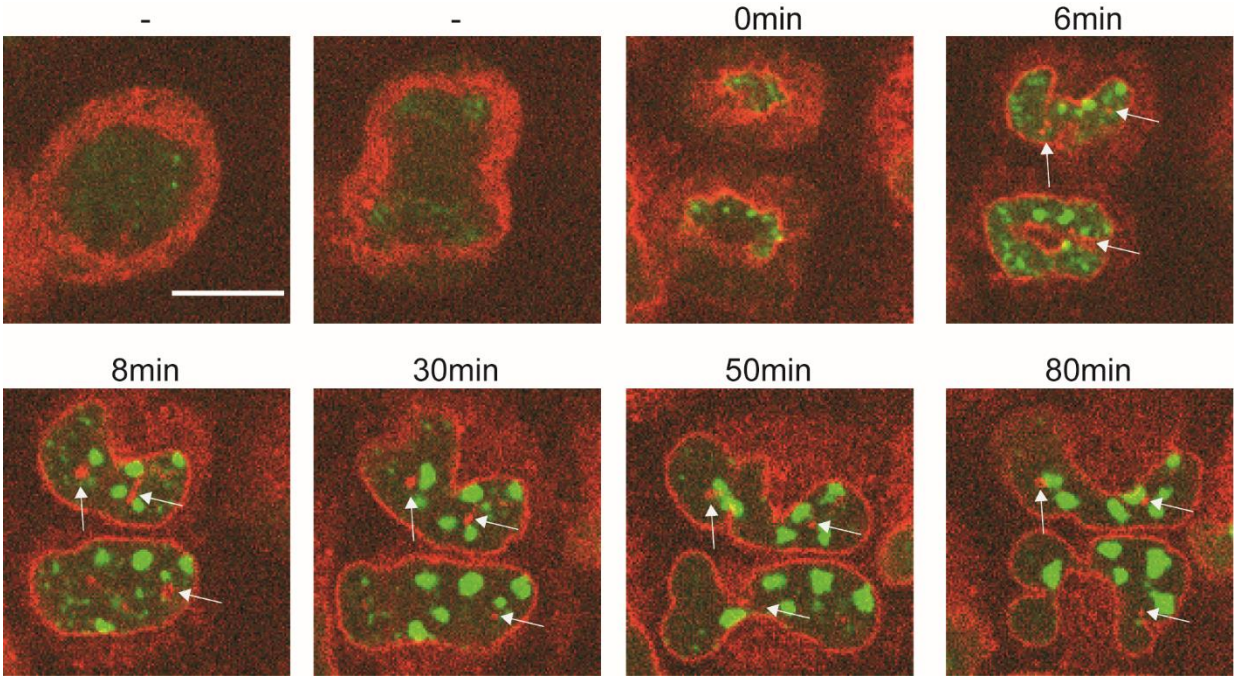


Figure 27: NE invaginations are associated with nucleoli shortly after the NE is reformed
Representative images of a live cell imaging experiment. U2OS cell line expressing LEM2 (red) and Fibrillarin (green) was imaged to investigate the dynamics of NE invaginations.
Arrows indicate NE invaginations, which are already connected to nucleoli shortly after mitosis is completed. Bar, 10µm.

4 Discussion

4.1 The BioID principle, a powerful approach to find potential interactors

The main part of this thesis centered on the identification of novel binding partners of LEM2, MAN1 and Emerin using the *in vivo* biotin-labelling method BioID. This method identified, besides several new candidate binding partners, also some expected and already reported interaction partners for each LEM protein. The BioID approach is a recently developed technique to screen for interactors in a proximity-dependent manner (Roux et al., 2012). A direct comparison of BioID with AP-MS (affinity purification coupled to mass spectrometry), showed that BioID identifies a larger interactome and more proteins of low abundance than AP-MS (Lambert et al., 2015). In principle, as with any biochemical or genetic method (e.g. AP-MS, yeast two-hybrid, co-immunoprecipitation), there are advantages but also general limitations which should be considered. In the classic approach, protein interactions are often identified in *in vitro* studies, or in a modified cellular environment that differs significantly from the normal cellular environment, e.g. lacking the post-translational modification machinery. Techniques, which are carried out under these non-physiological conditions, tend to generate biased and often incorrect results. Biochemical properties of the proteins, of course have a strong interference of the outcome of the approach. For example, proteins of interest can bind to their interactor with very low affinity or just transiently. These binding properties would not be sufficient to pull down candidate interaction partners for example in co-immunoprecipitation experiments. Another cause of candidate-loss, which is particularly relevant for low affinity interactions, is the insolubility of proteins, which makes it very difficult to map for interactors, because the solubilization of proteins requires such harsh conditions that would not preserve the interactions with partner proteins.

BioID is specifically suited to study low-affinity and transient interactions as well as protein complexes, which are difficult to isolate under physiological lysis conditions like in the case of inner nuclear membrane proteins or nuclear lamins. It detects potential interactors in their normal cellular environment, because the biotin ligase BirA* marks all proteins nearby, whether weak or transient interaction, in a promiscuous fashion before the proteins are solubilized. This

biotinylation event is no evidence for a direct physical interaction of proteins, because it marks all proteins nearby, in a labelling radius of ~ 20nm. However, it is in turn a suitable way to map, potential interaction partners, indirect interactors or bound protein complexes (Kim et al., 2014; Roux et al., 2012; Van Itallie et al., 2013).

One obvious limitation of BioID is that the BirA* fusion may affect localization and functional properties of the proteins to be studied. The genetically engineered *Escherichia coli* biotin ligase BirA* (~35kDa) is slightly larger than a GFP molecule (~27kDa). In this study, BirA* was fused to the N-terminus of the stably overexpressed proteins LEM2, MAN1 and Emerin. The BirA*-fusion proteins appeared nontoxic and normally localized in the inner nuclear membrane. In general, one can assume that, if a GFP tagged protein does not affect cellular localization of a protein it also should not be affected by tagging with BirA*. However, it cannot be ruled out that its fusion to the N-terminal region of the LEM proteins could affect their binding to potential interactors, by e.g. blocking/covering parts of the proteins binding region. In a future study, C-terminally fused BirA* constructs might be cloned and used to compare the interactome with currently available data set of N-terminal fusions. Additionally, the biotinylation of lysine residues, leads to a charge-loss and consequently could inhibit other secondary modifications and in turn alter the biochemical properties of the fusion protein and the potential binding partner. This could be one of the reasons why BAF, which is an expected candidate of LEM2, MAN1 and Emerin (Laguri et al., 2001), was not identified by BioID. Another reason for this may be attributed to the physical properties of BAF. Although BAF is an abundant protein with about $2x10^6$ copies per mammalian cell (Beck et al., 2011), its predicted protein size is just 10 kDa (Margalit et al., 2007). Apart from the fact that there is a bias towards large and highly abundant proteins, additional biochemical parameters are 1) how efficient a protein is cleaved by trypsin to yield detectable peptides and 2) how well those peptides are ionizable in mass spec. The complete BioID-dataset contains a huge amount of proteins highly unlikely to be directly linked to the LEM proteins, including e.g. extracellular proteins and cell membrane proteins, or unlikely binding partners like vesicular proteins or proteins of mitochondria or the endoplasmic reticulum. In this study, this could be explained by two reasons. First, to identify a multitude of essential candidate interaction partners, the biotin-labelling process was carried out for more than 22 hours without synchronizing the cells leading to an asynchronous cell population. During mitosis, the nuclear envelope breaks down and its proteins get absorbed into the ER or dispersed through the cytoplasm, leading to

displaced LEM proteins (Hetzer, 2010). Second, another major cause of contaminations is due to the overexpression of the LEM proteins leading to an accumulation of LEM proteins in vesicles, the cytoplasm or the endoplasmic reticulum (Volkova et al., 2012). Of course, it is possible that some e.g. cytoplasmic proteins could have access to LEM proteins, consequently being possible binding partners, however their concentration at the INM seems very unlikely.

4.2 Characterization of the identified interactome

Considering all advantages and limitations, the optimization of parameters, a strict selection criteria and the comparison of datasets allowed identifying common and specific candidates for each individual LEM protein. BioID revealed that three INM LEM proteins share a large number of potential interaction partners. Expectedly, LEM2 and MAN1 showed a larger overlap in binding partners than LEM2 or MAN1 with Emerin (Figure 13). The reason for this is that LEM2 forms a small LEM protein subgroup together with MAN1, due to their related structure properties. In comparison with the structurally unrelated LEM protein Emerin, which contains only one transmembrane domain and a LEM domain, LEM2 and MAN1 contain two transmembrane domains, one LEM domain and one C-terminal MSC motif (Brachner and Foisner, 2011). In species where LEM2 and MAN1 are expressed, they may share redundant functions, however also perform specific essential functions, which cannot be compensated by each other. For instance, lack of MAN1 causes embryonic lethality in mice by embryonic day 10.5 (E10.5) (Cohen et al., 2007) and lack of LEM2 caused embryonic lethality in mice by E11.5 (Tapia et al., 2015). Furthermore, it was reported that LEM2 siRNA treatment of U2OS cells lead to NE deformations and to a reduced cell survival, neither of which could be compensated by MAN1 (Ulbert et al., 2006). However, the results of the immunofluorescence, the viability assay experiments and the FACS analysis (Figure 19, Figure 20 Figure 21) of this study could not confirm this LEM2-specific phenotype. The shortlisted common proteins of LEM2-MAN1 contain some transcriptional regulators (RPB7, Transcription factor BTF3, Zinc finger protein 22), nuclear membrane proteins (SUN domain-containing protein 1 & 2, Nup37), some nucleolar proteins (eIF-3 p48, SPF27), to name a few. Sun1 & 2 are two unexpected potential binding partners of LEM2 and MAN1, because they are tow previously reported specific interaction partners of Emerin (Haque et al., 2010), which

stabilizes and promotes the nuclear actin cortical network by binding to the Sun1 & 2-containing LINC complex. The shortlisted common interactors of LEM2-MAN1 and MAN1-Emerin contain three or four, respectively potential binding partners of diverse function (see chapter 6.1.6 and 6.1.7). Candidate proteins of the common shortlist are discussed separately in chapter 4.2.4.

4.2.1 LEM2 and the nucleotide excision repair machinery

Based on the comparison of the BioID mass spectrometry results, common and specific candidates of LEM proteins were identified. The proteins identified by BioID-LEM2 can be divided in several sub-groups based on their molecular functions. Most notable is that this list contains several components of the NER machinery, with DDB1 the core-protein, belonging to the top-scoring candidates. Mammalian DDB1 is homologous to *Schizosaccharomyces pombe* Ddb1, which is functionally connected to the cell cycle checkpoint control (Bondar et al., 2003). Recent studies in *Schizosaccharomyces pombe* connect LEM2 and DDB1 identifying *lem2* as a novel S-phase checkpoint gene. In genome-wide screen to identify novel DNA checkpoint genes, deletion mutations of *lem2* and *ddb1* displayed a similar phenotype in the presence of HU, in which the cells failed to arrest in the cell cycle (Hayles et al., 2013).

Montes de Oca et al. revealed by co-immunoprecipitation in HeLa cells that DDB1, DDB2 and the ubiquitin ligase Cullin4A bind to BAF (Montes de Oca et al., 2009), which in turn binds to the LEM domain in LEM proteins (Laguri et al., 2001). Furthermore, they showed that Emerin binds to DDB2 and Cullin4A in a UV- and time-dependent manner and suggests that DNA damage responses might be coupled to changes of the nuclear periphery by BAF and Emerin (Montes de Oca et al., 2009). The BioID experiments of this study shows that the core protein DDB1 and the ubiquitin ligase Cullin4A were biotinylated by BirA*-LEM2 in three out of four or four out of four, independent BioID experiments respectively, whereas BirA*-MAN1 and BirA*-Emerin did not biotinylate DDB1, and Cullin4A only in one out of four runs. LEM2 samples showed the highest values of identified peptides of proteins involved in NER (Table 8). Furthermore, knockdown of LEM2 in human cells significantly reduced their viability after UV irradiation, and western blot analysis showed a stronger damage-signaling of LEM2-siRNA treated cells, whereas MAN1 and Emerin knockdown cells showed no or only little effect (Figure 21 and Figure 22).

Our model suggests many proteins of the NER pathway are recruited to the nuclear periphery but the physiological relevance remains unclear. One hypothesis could be that LEM2 regulates the activity of the NER pathway. A related function has been reported for the nuclear lamina or the nuclear periphery. The transcriptional activity of the genome can be regulated by lamina-associated domains (LADs), which are large developmentally regulated genome regions that are directed to the nuclear lamina by chromatin state and A-type lamins. In this way, the nuclear periphery is able to silence specific chromatin regions by peripheral contact or to activate transcriptional regions by dissociation from the periphery (Harr et al., 2015; Luperchio et al., 2014). Several studies propose a model in which the DNA double strand break repair is regulated by the spatial nuclear position of chromatin. In *Saccharomyces cerevisiae*, which lack "classical" lamins, the Nup84 complex organizes chromatin by anchoring telomere ends to the nuclear periphery. This tethering silences subtelomeric chromatin and enables efficient DNA double strand break repair through NHEJ. (Mekhail and Moazed, 2010; Therizols et al., 2006).

In mammals, it was proposed that lamin B1 modulates the transcription of specific genes encoding for proteins of the NER pathway. Lamin B1 silenced U2OS cells arrest in G_1 cell cycle phase, and exhibit a DNA damage repair delay upon UV irradiation (Butin-Israeli et al., 2013).

The results of this thesis shows that LEM2 loss causes an impairment of DNA damage repair, suggesting that LEM2 contributes to on effective response to UV-C induced DNA damage in wild type cells. The mass spectrometry results reveal that besides the core proteins DDB1 and Cullin4A, which recognizes DNA lesions, many other proteins of the NER pathway bind preferentially to LEM2 compared to MAN1 or Emerin. This would indicate that nearly the entire NER machinery is near BirA*-LEM2. Unfortunately, the BioID dataset and the results from the viability assay do not provide information if LEM2 recruits proteins of the NER pathway to the NE or if the LEM2 containing NE invaginations that reach deep into the nucleus associate to NER proteins. It also remains unclear if LEM2 binds directly or indirectly to the NER-machinery components. As mentioned before, BioID provides no evidence for direct interactions, thus it is possible that LEM2 binds indirectly (via BAF) to DNA and in this way could be in close proximity to the NER complex permanently, or as long, the DNA is repaired. Overall, the connection between LEM2 and NER proteins remains unsolved, however this study provides novel results which proposes a specific

functions for LEM2 and opens new ways in future research, in which the effect of LEM2 on NER proteins will be worth investigating.

4.2.2 Characterization of potential MAN1 binding partners

The shortlist of MAN1-specific interactors includes 9 proteins which are implicated mainly in transcriptional regulation. Included in the MAN1 specific shortlist is the previously reported MAN1 binding partner, Smad3. This interaction enables MAN1 to specifically repress activin, BMP or TGF-β signalling (Bourgeois et al., 2013; Pan et al., 2005). However, PPM1A or Smad2 or other components in this pathway were not biotinylated by BirA*-MAN1 (nor by BirA*-LEM2 or BirA*-Emerin). This could be due to the fact that MAN1 competes with the transcription factor FAST1 (forkhead activin signal transducer 1) for Smad2 binding and maybe because the binding affinity of MAN1 to PPM1A is weaker than that to R-Smads, leading to a displacement of PP1MA (Bourgeois et al., 2013). The death promoting transcriptional repressor Bcl-2-associated transcription factor, which binds to MAN1 and Emerin (Mansharamani and Wilson, 2005), was also identified by BioID However, it was not shortlisted because of the peptides found in all sample including control samples. However, BCL2/adenovirus E1B 19 kDa protein-interacting protein 2 (BNIP2), which suppresses apoptosis, binds to Bcl-2 and associated co-factors (Belcredito et al., 2001) was specifically biotinylated by BirA*-MAN1.

4.2.3 Emerin and its novel role in centrosome tethering

The shortlist of Emerin-specific binding partners, consists of many unreported but expected proteins. Salpingidou et al. (2007) found that a small fraction of Emerin proteins is located at the ONM and attaches centrosomes to the nuclear envelope by acting as a microtubule link. Emerin-null cells showed a mislocalization of the centrosomes, but not of the LINC complex, excluding the possibility that Emerin acts like the LINC complexes together with another protein across the NE. Microtubule co-sedimentation experiments and mass spectrometry confirmed that Emerin bind to microtubules, however, except from β-tubulin, no further interaction partners which may be part of the Emerin-centrosome-linkage were identified (Dabauvalle et al., 1999; Salpingidou et

al., 2007). Particularly noticeable in the shortlist of potential Emerin-specific binding partners is that a large percentage of proteins (30%) are centrosomal or microtubular proteins: The centrosomal protein of 78 kDa (Cep78) an uncharacterized protein and Cep135 which is implicated in centriole biogenesis and localization (Kim et al., 2008); the MAP7 domain-containing protein 3, which promotes the microtubular stability and their polymerization by binding to their C-terminal tail (Yadav et al., 2014); the gamma-tubulin ring complex member: NEDD1 (Neural precursor cell expressed developmentally down-regulated protein 1) which promotes microtubular nucleation from the spindle; and the Cytoskeleton-associated protein 2-like, which is required for correct mitotic spindle formation (Hussain et al., 2014). The top two candidates of the list, based on peptide-counts, are kinectin, which is the receptor of the motorprotein, kinesin (Abe et al., 2007), and the adapter protein 5-azacytidine-induced protein 2, which enhances the phosphorylation and consequently the activation of NF-kappa-B (Fujita et al., 2003). The transmembrane protein 43 (LUMA), which is required to retain Emerin at the INM and to maintain the NE shape (Bengtsson and Otto, 2008), was identified as an Emerin specific candidate, although it also binds to A- and B-type lamins. The BioID experiments of this study revealed for the first time several centrosomal candidates as potential binding partners of Emerin and offer a great opportunity and basis for further investigations.

4.2.4 Characterization of potential common interaction partners

Shortlisted candidate proteins (Figure 13) in the group of "common interactors" (binding to LEM2, MAN1 and Emerin) represent by far the biggest group. Many common interaction partners are probably near neighbours of the LEM proteins, hence nuclear membrane proteins. Alternatively, many indirect binding partners of the LEM proteins were identified which interact with lamins and/or BAF that in turn may bind LEM proteins. Common binding partners also confirmed the efficacy of the BioID approach, as it includes LEM and INM proteins, which were also detected by Roux et al. (2012): LAP2 α, β/γ, LBR, LAP1B (Torsin-1A-interacting protein 1) which is required for locating Torsin-1A at the nuclear membrane and the assembly of the nuclear lamina (Zhao et al., 2013) and Torsin-1A-interacting protein 2, which regulates the distribution of the Torsin-1A (Vander Heyden et al., 2009); and some nuclear pore complexes (Nup205, Nup160,

Nup188, Nup85, Nup98-Nup96) and nucleoporins (NDC1 and NUP188), just to name a few. One of the top-scoring proteins in this group was the nucleoporin E3 SUMO-protein ligase RanBP2, which binds the nuclear export factor, exportin-1, and is an essential part of the nuclear export pathway, (Gareau et al., 2012) and most likely involved in regulation of β-catenin by Emerin (Markiewicz et al., 2006). A small subgroup includes repair-proteins involved in DNA double strand break repair pathway NHEJ, e.g. the X-ray repair cross-complementing protein 5 (XRCC5) functions in V(D)J recombination and NHEJ by stabilizing the ends of DNA double strand breaks and increasing the affinity of the catalytic subunit of the DNA-dependent protein kinase to DNA (Ghosh et al., 2007; Li et al., 2011b; Zhou et al., 2012). The double-strand break repair protein MRE11A, is the core protein of the MRN-complex, whose recruitment leads to the processing of DNA ends (Giglia-Mari et al., 2011) and MRE11A provides the exonuclease activity (Paull and Gellert, 1998). Also included in the list of candidate proteins, are many proteins implicated in transcriptional regulation, signaling pathways, RNA and protein regulation or nucleolar proteins implicated in ribosome biosynthesis. The only previously reported potential specific binding partner of LEM2, PP-1α, which was co-immunoprecipitated with LEM2 but not with Emerin or ANKLE2 (Asencio et al., 2012), was biotinylated by all three BirA*-fusion proteins, and shortlisted in the common candidates list. It is possible that the BioID approach did not confirm the exclusive binding of PP-1α to LEM2, because the protein phosphatase complex (16 subunits) is implicated in dephosphorylating processes of hundreds of targets and binds to a variety of regulatory proteins (Ceulemans and Bollen, 2004; Cohen, 2002).

This substantial dataset of identified common interactors reflects the functional versatility of LEM proteins and furthermore, reinforces previously ascribed functions of LEM proteins. Nevertheless, this does not facilitate the identification of a common function of LEM2, MAN1 and Emerin without further systematical or hypothesis-driven investigations, however it may serve as a valuable starting point for further investigation in that direction.

4.3 NE invaginations – long lasting nucleoli associations

The NE is a double-membrane system, which separates the nucleoplasm from the cytoplasm. It contains invaginations, which can reach deep into the nucleus, however their function and its dynamic behavior is still poorly understood. This deep, often branching and "tube-like" invaginations are a common feature in some laminopathies, for example HGPS, where nuclei show an increased abundance of NE invaginations (Mounkes et al., 2003). Many vital processes such as gene expression, stress response regulation, genome organization and many signaling pathways are regulated by the NE. This fact makes it very interesting to further investigate NE invaginations at the molecular level. The dynamic study of living cells using live cell imaging provides an excellent opportunity to investigate the dynamics of NE invaginations. Previous immunofluorescence experiments confirmed the presence of lamin A/C and many other INM proteins and an enrichment of the LEM proteins LEM2 and Emerin in these nuclear envelope invaginations (data not shown). The results of this study provide valuable insight into the dynamics of nuclear envelope invaginations. Taken together, the live cell imaging experiments revealed that LEM2 is part of a complex connecting the nuclear envelope and nucleoli, which is built shortly after mitosis and remains over several hours. The movement of the cell does not influence the connection between the invaginations and the nucleoli. Furthermore, the change of the positon and the orientation of nucleoli do not affect this connection either.

What is the function of the connections between "tube-like" channels and nucleoli? It is known that NE invaginations are formed in many cell types and tissues (Fricker et al., 1997) and the association of NE invaginations with nucleoli is restricted to double-membraned (INM and ONM) type II invaginations which enclose a cytoplasmic core, containing ribosomes, microtubules and ER-markers (Malhas et al., 2011). Although more extensive investigations on NE invaginations are emerging, and many functions and regulatory mechanisms are reported to a certain degree, the mechanisms of nucleoli associations are still unknown. One consideration is that NE invaginations can function as transport channels.

There is increasing evidence that nuclear envelope channels are implicated in calcium signaling. This enables the cell to deliver calcium precisely to specific subcellular areas, resulting in controlled regulation of transcription and the cell cycle (Mauger, 2012). Additionally, it is known that many transcription factors are bound to the NE (Malhas and Vaux, 2009), which would

suggest an indirect transcriptional regulation by deep-reaching invaginations of the NE. The treatment of mouse embryonic fibroblasts with histone-deacetylase inhibitors (e.g. trichostatin A) increases number of transcriptional active genes and is correlated with increasing number of NE invaginations. Besides mRNA and rRNA export, nuclearporins bind to transcriptional active regions of the chromatin, or are able to organize the genome (Kalverda et al., 2010; Mekhail and Moazed, 2010), which could probably be potentially facilitated and specified by NE invaginations (Galiova et al., 2008). Quantitative proteomics and dynamic imaging of nucleoli has confirmed that nucleoli undergo reorganization in response to UV irradiation (Moore et al., 2011). Additionally, many nucleolar proteins and proteins which are implicated in NHEJ decrease transiently, which reflects its response to DNA damage.

Figure 28 shows immunofluorescence analysis of UV-C (15J/m^2) treated U2OS cells, which were stained for DNA (blue), LEM2 (red), fibrillarin (green) and γH2AX (magenta). Seven hours after UV irradiation, nucleoli (fibrillarin) reorganize and nucleoli disassemble, whereas LEM2 containing NE invaginations accumulate in the nucleoplasm (more intensely compared to untreated U2OS cells). After 24 hours, fibrillarin is dispersed and NE invaginations reformed to long thin invaginations. This experiment reflects that nucleoli (fibrillarin) and NE invaginations responded to UV irradiation.

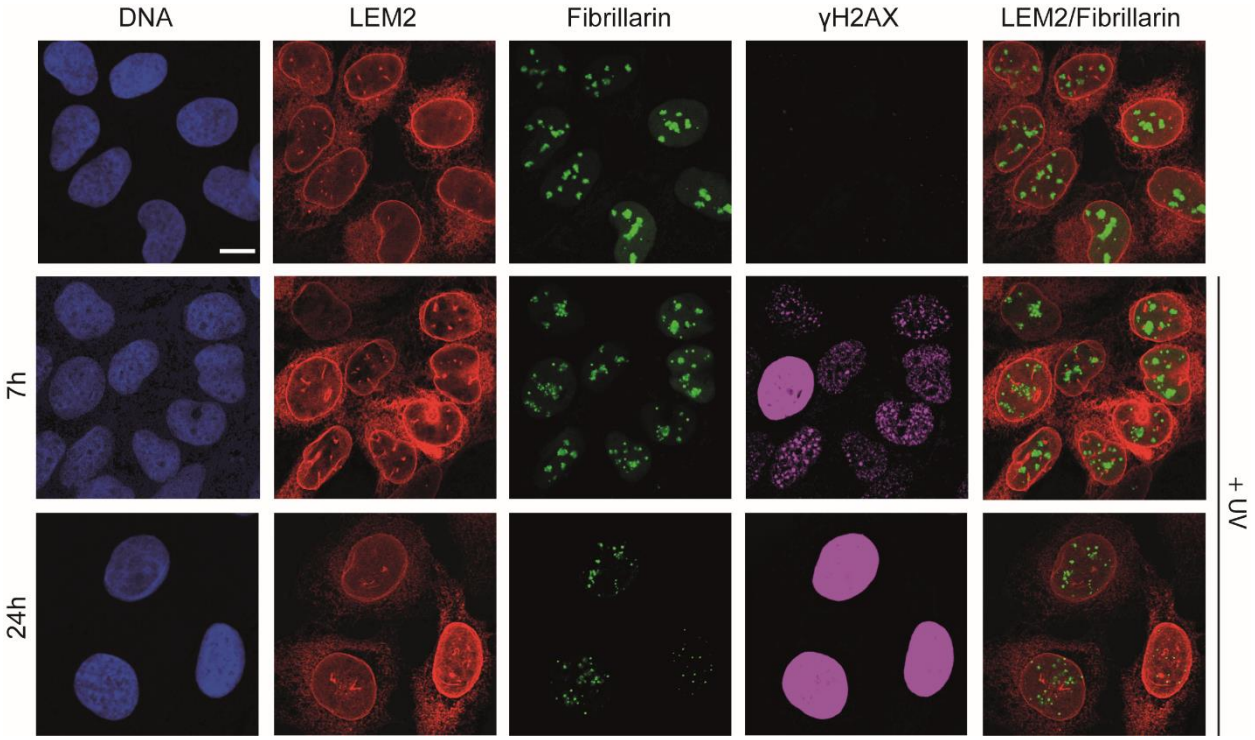


Figure 28: UV irradiation causes structural changes of nucleoli and more NE invaginations
Immunofluorescence analysis of UV-C (15J/m^2) treated U2OS cells. After UV irradiation nucleoli disassemble, fibrillarin translocates and LEM2 containing NE invaginations accumulate in the nucleoplasm. Cells were stained for DNA (blue), LEM2 (red), fibrillarin (green) and γH2AX (magenta). Bar, 10µm.

The protein LYRIC (shortlisted in common candidates), which is found in the ER membrane as well as in the NE and at nucleoli, represents a possible link between NE invaginations and nucleoli and consequently a conceivable link to ribosome export and biosynthesis (Britt et al., 2004). The serine/threonine-protein phosphatase PP1-alpha catalytic subunit (PP-1α), represents the only reported interaction partner of human LEM2 (Asencio et al., 2012), PP-1α, is primarily nuclear, but is targeted to nucleoli by different proteins like RRP1B, contributing to ribosome biogenesis (Chamousset et al., 2010), or NOM1 (Gunawardena et al., 2008). Furthermore, the disassembly and reassembly of nucleoli depend on phosphorylation and dephosphorylation of proteins of the transcription machinery and of the processing of ribonucleoproteins, which is controlled by CDK1-cyclin B kinases and PP1 phosphatases (Hernandez-Verdun, 2011). This could be another reason why NE invaginations associate to nucleoli. Another potential NE-nucleoli link reported before represents the MAN1 orthologue Heh1 of *Schizosaccharomyces pombe*, which is part of the CLIP (chromosome linkage INM protein) complex that physically associates the rRNA-bound cohibin

complex to the nuclear periphery. In this way Heh1 is implicated in stabilizing rRNA repeats by spreading and tethering rRNA repeats along the INM, consequently preventing their exit from nucleoli and limiting their contact to repair and recombination centers of the nucleoplasm (Mekhail and Moazed, 2010).

This study shows that LEM2 is part of persistent and stable NE invaginations, which associate to nucleoli before they are reassembled completely. LEM proteins have been previously linked to nucleoli reassembly. The fission yeast *Schizosaccharomyces japonicas* breaks up its nuclear envelope during mitosis, and requires MAN1 for its equal partitioning, the correct inheritance of nuclear pore complexes and the reformation of nucleoli (Yam et al., 2013). It may be possible that connections support nucleoli assembly, however some NE invaginations stay associated for more than 12 hours, which could be in the context of tethering of repetitive rRNA sequences. The existence of NE invaginations is undoubtedly an essential opportunity for the NE to carry out conventional functions deep inside the nucleoplasm. The extent to which LEM proteins or especially LEM2 contribute to this, remains an area of future research.

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6 Appendix

6.1 Potential interaction partners identified by BioID

6.1.1 Potential LEM2-specific binding partners

Description	AN	MW (kDa)	Copies/cell
Others			
Adrenocortical dysplasia protein homolog	gi|296439451	58	5,00E+02
BTB/POZ domain-containing protein KCTD14	gi|251757290	30	<5e2
COMM domain-containing protein 3	gi|51316114	22	2,46E+04
HEAT repeat-containing protein 6	gi|74724525	129	1,04E+04
Interferon-related developmental regulator 2	gi|327478577	55	3,46E+03
Leucine-rich repeat and calponin homology domain-containing protein 4	gi|49035987	73	6,05E+03
Mitogen-activated protein kinase 8	gi|2507195	48	6,84E+03
Nitrogen permease regulator 3-like protein	gi|18202492	64	6,97E+02
Prefoldin subunit 2	gi|12643887	17	5,75E+05
Protein phosphatase Slingshot homolog 3	gi|82582268	73	<5e2
Sentrin-specific protease 1	gi|215273882	73	5,20E+02
Sestrin-2	gi|13633882	54	<5e2
Tumor necrosis factor receptor type 1-associated DEATH domain protein	gi|6094511	34	2,46E+03
UPF0600 protein C5orf51	gi|190411549	34	7,11E+03
WD40 repeat-containing protein SMU1	gi|81911825	58	3,68E+04
Cell cycle			
Cell division cycle protein 16 homolog	gi|37537763	72	4,02E+03
Melanoma-associated antigen 11	gi|97536722	48	-
NEDD8	gi|2833270	9	1,65E+04
Ribonuclease H2 subunit A	gi|20981704	33	4,38E+04
Transcription			
DNA-directed RNA polymerase I subunit RPA2	gi|92090637	128	1,46E+04
DNA-directed RNA polymerases I, II, and III subunit RPABC1	gi|116242767	25	5,72E+04
Negative elongation factor C/D	gi|38372376	66	9,07E+03

Tribbles homolog 3	gi|28201830	40	
Zinc finger protein 10	gi|55977778	66	<5e2
Repair			
DNA damage-binding protein 1	gi|148529014	127	1,01E+04
Ribonucleoside-diphosphate reductase subunit M2 B	gi|74727333	41	1,01E+05
Tyrosyl-DNA phosphodiesterase 2	gi|67462008	41	
Histone			
Serine/threonine-protein kinase 19	gi|19860281	41	
N-alpha-acetyltransferase 40	gi|74727506	27	6,92E+03
Nuclear envelope			
1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-1	gi|12643814	139	-
Nesprin-1	gi|425906075	1011	<5e2
Nucleolus			
Pre-rRNA-processing protein TSR1 homolog	gi|121948971	92	1,01E+04
Probable ATP-dependent RNA helicase DDX31	gi|71153334	94	5,65E+02
Ribosomal RNA small subunit methyltransferase NEP1	gi|20532172	27	9,48E+04
RNA-binding protein 4B	gi|62511129	40	1,68E+04
Kallikrein-6	gi|3914480	27	-
Centrosome			
CLIP-associating protein 1	gi|74723323	169	1,43E+04
Dual specificity mitogen-activated protein kinase kinase 1	gi|400274	43	1,15E+05
UBX domain-containing protein 6	gi|30913412	50	8,56E+03
Ubiquitination			
Cell division cycle protein 23 homolog	gi|254763423	69	1,17E+04
COP9 signalosome complex subunit 4	gi|55976582	46	7,82E+04
COP9 signalosome complex subunit 8	gi|55976572	23	9,33E+04

6.1.2 Potential MAN1-specific binding partners

Description	AN	MW (kDa)	Copies/cell
Others			
BCL2/adenovirus E1B 19 protein-interacting protein 2	gi|6093506	36	9,80E+02
Exosome complex component RRP45	gi|147744559	49	1,67E+04
Nicotinate-nucleotide pyrophosphorylase [carboxylating]	gi|296439291	31	3,04E+03
Transcription			
GA-binding protein alpha chain	gi|729553	51	6,07E+03
Mothers against decapentaplegic homolog 3	gi|51338669	48	2,18E+03
Testis-specific Y-encoded-like protein 2	gi|74752604	79	5,00E+02
Transcription initiation factor IIE subunit beta	gi|135232	33	1,11E+04
Cell cycle			
Mitotic spindle assembly checkpoint protein MAD2B	gi|12643889	24	5,23E+02
Splicing			
WW domain-binding protein 11	gi|74735242	70	5,16E+04

6.1.3 Potential Emerin-specific binding partners

Description	AN	MW (kDa)	Copies/cell
Others			
5-azacytidine-induced protein 2	gi|74718550	122	6,41E+03
Casein kinase I isoform epsilon	gi|1346369	47	2,30E+03
Protein FAM111A	gi|125991848	70	5,66E+02
Protein FAM111B	gi|74758524	85	2,17E+03
Protein PRRC2B	gi|308153415	243	-
Protein PRRC2C	gi|341942262	317	9,34E+03
Protein TANC2	gi|189029946	220	5,00E+02
Cell cycle			
Nuclear fragile X mental retardation-interacting protein 2	gi|74713454	76	3,63E+03
Transcription			
Protein MCM10 homolog	gi|126215746	98	-
TATA-binding protein-associated factor 2N	gi|8928305	62	2,27E+04

Tudor domain-containing protein 3	gi|29337133	73	5,00E+02
Nuclear envelope			
Transmembrane protein 43	gi|74733151	45	3,34E+04
Nucleolus			
Protein MAK16 homolog	gi|71152029	35	9,47E+03
Protein SDA1 homolog	gi|296452964	80	7,45E+03
Centrosome			
Centrosomal protein of 135	gi|296434460	133	6,38E+02
Centrosomal protein of 78	gi|74742229	76	1,35E+03
Cytoskeleton-associated protein 2-like	gi|311033474	84	500
Kinectin	gi|34098465	156	8,93E+04
MAP7 domain-containing protein 3	gi|158705880	98	2,72E+03
Protein NEDD1	gi|74762597	72	5,42E+02

6.1.4 Potential LEM2-MAN1-Emerin binding partners

Description	AN	MW (kDa)	Copies/cell
Others			
Aladin	gi|20137527	60 kDa	1,83E+04
ATP-binding cassette sub-family F member 3	gi|114149223	80 kDa	1,71E+04
Bifunctional purine biosynthesis protein PURH	gi|23831360	65 kDa	3,48E+05
Condensin complex subunit 1	gi|259016362	157 kDa	5,31E+04
Calponin-2	gi|6226844	34 kDa	4,03E+05
Calponin-3	gi|6225157	36 kDa	1,44E+06
Coilin	gi|585632	63 kDa	1,02E+04
Hematological and neurological expressed 1-like protein	gi|71152128	20 kDa	1,98E+05
Lamina-associated polypeptide 2, isoform alpha	gi|1174689	75 kDa	3,14E+04
Leucine zipper protein 1	gi|97072093	120 kDa	3,22E+04
Melanoma-associated antigen 10	gi|317373386	41 kDa	-
Melanoma-associated antigen 11	gi|97536722	48 kDa	-
Melanoma-associated antigen 12	gi|14286145	35 kDa	-
Methyltransferase-like protein 2B	gi|317373413	43 kDa	4,23E+03
mRNA cap guanine-N7 methyltransferase	gi|74735378	55 kDa	4,49E+04
PCI domain-containing protein 2	gi|85681034	46 kDa	3,56E+04
PDZ and LIM domain protein 4	gi|20141642	35 kDa	2,85E+05
Proteasome subunit beta type-1	gi|130853	26 kDa	3,69E+05
Protein CMSS1	gi|215274008	32 kDa	1,17E+04
Protein MB21D2	gi|118572625	56 kDa	6,70E+03
Serine/threonine-protein phosphatase PP1-alpha catalytic subunit	gi|49065778	38 kDa	1,51E+05
Structural maintenance of chromosomes protein 4	gi|30173386	147 kDa	4,97E+04
SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A5	gi|57014128	122 kDa	5,76E+04
Thioredoxin-like protein 1	gi|18202039	32 kDa	1,92E+05
Transitional endoplasmic reticulum ATPase	gi|6094447	89 kDa	1,38E+06
UPF0428 protein CXorf56	gi|74733589	26 kDa	1,92E+03
Zinc finger protein 638	gi|71153483	221 kDa	2,93E+03
Cell cycle			
G2/mitotic-specific cyclin-B1	gi|116176	48	5,30E+04
KH domain-containing, RNA-binding, signal transduction-associated protein 1	gi|62511098	48 kDa	1,18E+05
PEST proteolytic signal-containing nuclear protein	gi|67460963	19 kDa	2,56E+05
Transcription			
Catenin delta-1	gi|14916543	108 kDa	6,51E+04
CCAAT/enhancer-binding protein zeta	gi|308153621	121 kDa	1,43E+04
DNA polymerase delta catalytic subunit	gi|50403732	124 kDa	1,96E+04

DNA-directed RNA polymerase II subunit RPB2	gi|401012	134 kDa	3,82E+04
DNA-directed RNA polymerases I and III subunit RPAC140 kDa polypeptide	gi|3914807	39 kDa	1,39E+04
Melanoma-associated antigen 1	gi|1170855	34 kDa	
Metastasis-associated protein MTA2	gi|29840793	75 kDa	5,08E+04
Prolactin regulatory element-binding protein	gi|55977881	45 kDa	3,18E+04
RelA-associated inhibitor	gi|92090607	89 kDa	2,46E+04
Scaffold attachment factor B1	gi|116242782	103 kDa	2,00E+04
SNW domain-containing protein 1	gi|2500813	61 kDa	1,51E+04
Transcription factor p65	gi|62906901	60 kDa	2,68E+04
Transcription termination factor 2	gi|73920148	130 kDa	4,34E+03
Repair			
DnaJ homolog subfamily B member 1	gi|1706473	38 kDa	9,62E+04
Double-strand break repair protein MRE11A	gi|17380137	81 kDa	7,34E+03
X-ray repair cross-complementing protein 5	gi|125731	83 kDa	8,26E+05
Histone			
Small nuclear ribonucleoprotein Sm D3	gi|51338667	14 kDa	6,53E+06
Nuclear envelope			
E3 SUMO-protein ligase RanBP2	gi|83305554	358 kDa	1,74E+04
Lamina-associated polypeptide 2, isoforms beta/gamma	gi|1174690	51 kDa	2,61E+04
Lamin-B receptor	gi|20141468	71 kDa	1,13E+04
Lamin-A/C	gi|125962	74 kDa	1,51E+06
Nuclear pore complex protein Nup160	gi|238054372	162 kDa	2,56E+04
Nuclear pore complex protein Nup205	gi|296439283	228 kDa	2,65E+04
Nuclear pore complex protein Nup85	gi|74733394	75 kDa	3,45E+04
Nuclear pore complex protein Nup98-Nup96	gi|308153660	198 kDa	1,68E+04
Nucleoporin NDC1	gi|97180263	76 kDa	1,36E+03
Nucleoporin NUP188 homolog	gi|74743623	196 kDa	7,63E+03
Ran GTPase-activating protein 1	gi|1172922	64 kDa	3,16E+05
Testis-expressed sequence 10 protein	gi|71153593	106 kDa	9,61E+03
Torsin-1A-interacting protein 1	gi|90101788	66 kDa	1,50E+04
Torsin-1A-interacting protein 2	gi|74751288	51 kDa	1,55E+04
Nucleolus			
Nucleolar protein 6	gi|62900709	128 kDa	5,82E+03
Transducin beta-like protein 3	gi|223634675	89 kDa	7,55E+04
HEAT repeat-containing protein 1	gi|71153494	242 kDa	1,98E+04
ATP-dependent RNA helicase DDX18	gi|20532388	75 kDa	1,42E+05
DNA-directed RNA polymerases I, II, and III subunit RPABC3	gi|20178325	17 kDa	1,84E+04
Probable dimethyladenosine transferase	gi|27151492	35 kDa	1,31E+04

Ribosome biogenesis protein BMS1 homolog	gi|27151474	146 kDa	1,83E+04
RNA 3'-terminal phosphate cyclase-like protein	gi|90103518	41 kDa	1,27E+04
SPATS2-like protein	gi|160185484	62 kDa	1,05E+05
Centrosome			
Cytoskeleton-associated protein 5	gi|212276513	226 kDa	4,97E+04
Guanine nucleotide-binding protein G(k) subunit alpha	gi|120996	41 kDa	-
Nuclear mitotic apparatus protein 1	gi|145559510	238 kDa	6,71E+04
Targeting protein for Xklp2	gi|13124096	86 kDa	2,88E+04
Transforming acidic coiled-coil-containing protein 1	gi|59800391	88 kDa	1,39E+04
Splicing			
DBIRD complex subunit ZNF326	gi|73622105	66 kDa	9,89E+04
Gem-associated protein 4	gi|322510030	120 kDa	5,74E+03
Poly(U)-binding-splicing factor PUF60	gi|74761960	60 kDa	2,59E+05
Polypyrimidine tract-binding protein 1	gi|131528	57 kDa	1,74E+06
Pre-mRNA-processing factor 6	gi|24212088	107 kDa	2,48E+04
Probable ATP-dependent RNA helicase DDX20	gi|12643886	92 kDa	1,41E+04
Serine-threonine kinase receptor-associated protein	gi|12643951	38 kDa	4,45E+05
Zinc finger CCCH-type antiviral protein 1	gi|223634727	101 kDa	3,97E+04
Ubiquitination			
26S protease regulatory subunit 6B	gi|20532409	47 kDa	3,53E+05
Melanoma-associated antigen B2	gi|317373424	35 kDa	5,42E+04
Melanoma-associated antigen D4	gi|41017391	81 kDa	5,00E+02
Probable E3 ubiquitin-protein ligase TRIML2	gi|74714998	44 kDa	6,41E+02
Ubiquitin fusion degradation protein 1 homolog	gi|160332310	35 kDa	7,40E+04
Ubiquitin-associated protein 2	gi|74745207	117 kDa	5,39E+03
Ubiquitin-associated protein 2-like	gi|109940042	115 kDa	7,76E+04
Ubiquitin-like modifier-activating enzyme 1	gi|24418865	118 kDa	7,75E+05

6.1.5 Potential LEM2-MAN1 binding partners

Description	AN	MW (kDa)	Copies/cell
Others			
Charged multivesicular body protein 7	gi|73917782	51 kDa	1,87E+04
Condensin-2 complex subunit H2	gi|74709496	68 kDa	2,49E+03
Glycogen phosphorylase, liver form	gi|6648082	97 kDa	3,26E+04
mRNA export factor	gi|3122666	41 kDa	8,94E+04
Nucleoplasmin-3	gi|6647592	19 kDa	7,35E+04
Proteasome subunit beta type-4	gi|116242733	29 kDa	1,34E+05
Protein LTV1 homolog	gi|74751878	55 kDa	3,91E+03
Sorting nexin-9	gi|12643956	67 kDa	3,60E+03
Transportin-3	gi|166215035	104 kDa	1,08E+04
WD repeat-containing protein 54	gi|74761551	36 kDa	2,20E+03
Transcription			
40S ribosomal protein S29	gi|50403626	7 kDa	1,87E+05
DNA-directed RNA polymerase II subunit RPB7	gi|50403597	19 kDa	7,48E+04
Protein SGT1	gi|17368102	73 kDa	3,92E+03
Transcription factor BTF3	gi|115143	22 kDa	1,45E+06
Zinc finger protein 22	gi|20455530	26 kDa	
Nuclear envelope			
Nucleoporin Nup37	gi|27923820	37 kDa	1,57E+04
SUN domain-containing protein 1	gi|32700069	90 kDa	1,41E+03
SUN domain-containing protein 2	gi|29337242	80 kDa	5,11E+03
Nucleolus			
Eukaryotic translation initiation factor 6	gi|3122258	27 kDa	2,81E+05
Splicing			
Pre-mRNA-splicing factor SPF27	gi|62899888	26 kDa	7,31E+04
U2 small nuclear ribonucleoprotein A'	gi|31077165	28 kDa	1,25E+05

6.1.6 Potential LEM2-Emerin binding partners

Description	AN	MW (kDa)	Copies/cell
Others			
Activity-regulated cytoskeleton-associated protein	gi|74738627	45 kDa	<5e2
Nucleolus			
Ribosome maturation protein SBDS	gi|28380824	29 kDa	9,37E+04
Cell cycle			
Aurora kinase A	gi|27923855	46 kDa	3,63E+04

6.1.7 Potential MAN1-Emerin binding partners

Description	AN	MW (kDa)	Copies/cell
Other			
Eukaryotic peptide chain release factor GTP-binding subunit ERF3A	gi|121688	56 kDa	5,29E+04
Repair			
Activating signal cointegrator 1 complex subunit 3	gi|158518649	251 kDa	5,31E+03
Transcription			
Activating signal cointegrator 1	gi|116242828	66 kDa	1,15E+03
Zinc finger protein 512	gi|229462786	65 kDa	<5e2

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7.1 Abbreviations

(6–4)PP	6–4 photoproduct
aa	amino acid
AP1	activator protein 1
BAF	barrier-to-autointegration factor
BER	base excision repair
BMP	bone morphogenetic protein
Btf	bcl-2-associated transcription factor
Cep	centrosomal protein
CLIP	chromosome linkage INM protein
CPD	cyclobutane pyrimidine dimer
DDB1	DNA damage-binding protein 1
DNA	deoxyribonucleic acid
DNA-PK	DNA-activated protein kinase
E10.5	embryonic day 10.5
eIF-3	eukaryotic translation initiation factor 3
ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
GCL	germ cell less
GFP	green fluorescent protein
H_2O_2	hydrogen peroxide
Heh1	helix-extension-helix motif 1
HR	homologous recombination
HRP	horseradish peroxidase
HU	hydroxyurea
INM	inner nuclear membrane
KD	knockdown
LAP	lamin associated polypeptide
LBR	lamin-B receptor
LEM	LAP2-Emerin-MAN1
LINC	linker of nucleoskeleton and cytoskeleton
Lmo7	LIM domain only 7

MS	mass spectrometry
MRE11	meiotic recombination 11
MRN	MRE11-RAD50-NBS1
mRNA	messenger RNA
MSC	MAN1-SRC1p-C-terminal
NE	nuclear envelope
NER	nucleotide excision repair
Nesprins	nuclear envelope spectrin repeat proteins
NHEJ	non-homologous end joining
NPC	nuclear pore complex
ONM	outer nuclear membrane
PCNA	proliferating cell nuclear antigen
PI3	phosphoinositide 3-kinase
PP-1α	protein phosphatase PP1-alpha catalytic subunit
PPM1A	protein phosphatase 1A
pRB	retinoblastoma protein
RFP	red fluorescent protein
RNA	ribonucleic acid
ROS	reactive oxygen species
RPA	replication protein A
RRM	RNA recognition motif
r-Smads	receptor-regulated
Sc	scrambled
siRNA	small interfering RNA
Smad	mothers against decapentaplegic
TGF-β	transforming growth factor
V(D)J	variable (diversity) joining
XPA/B/D/G	xeroderma pigmentosum A/B/D/G
XRCC	X-ray repair cross-complementing protein

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7.3 Curriculum vitae

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