Journal of Cellular Biochemistry 96:1185–1192 (2005)

Inner Nuclear Membrane and Signal Transduction

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Abstract Recent research has shown that the inner nuclear membrane is a site for regulation of signal transduction from the plasma membrane to the nucleus. This has coincided with discoveries showing that mutations in extrinsic and intrinsic inner nuclear membrane proteins cause a variety of inherited diseases. In most instances, the mechanisms by which mutations in inner nuclear membrane proteins cause disease are not understood. In at least one case, however, an alteration in signal transduction appears to underlie disease pathogenesis. J. Cell. Biochem. 96: 1185–1192, 2005.

Key words: nuclear envelope; nuclear lamina; lamin; MAN1; transforming growth factor-beta; bone morphogenic protein

THE INNER NUCLEAR MEMBRANE

The nuclear envelope is composed of the nuclear membranes, nuclear lamina, and nuclear pore complexes (Fig. 1). The nuclear membranes are divided into three connected but morphologically distinct domains called outer, pore, and inner. The outer nuclear membrane is generally similar in composition to the rough endoplasmic reticulum, with which it is directly continuous. The pore membranes connect the inner and outer nuclear membranes at numerous points and are associated with nuclear pore complexes. The nuclear lamina is composed of intermediate filament proteins called lamins, which polymerize to form the filamentous lamina primarily localized at the inner surface of the inner nuclear membrane [Gerace et al., 1978; Aebi et al., 1986; Fisher et al., 1986; Goldman et al., 1986; McKeon et al., 1986]. While the lamins and lamina likely have several functions, one generally agreed upon is mechanical support for the nuclear envelope.

Integral proteins of the inner nuclear membrane are synthesized on the rough endoplasmic reticulum and reach the inner nuclear membrane by lateral diffusion in the interconnected endoplasmic reticulum, outer, pore, and inner nuclear membranes [Soullam and Worman, 1993, 1995; Ellenberg et al., 1997; Östlund et al., 1999]. Binding to fixed nuclear structures is responsible for the retention of proteins in the inner nuclear membrane [Soullam and Worman, 1993, 1995; Ellenberg et al., 1997; Östlund et al., 1999; Ohba et al., 2004]. Most of the characterized integral proteins of the inner nuclear membrane bind to nuclear lamins and/or chromatin components, example being lamin B receptor [Worman et al., 1988; Ye and Worman, 1994, 1996; Ye et al., 1997] and isoforms of lamina-associated polypeptide 2 [Foisner and Gerace, 1993]. Data obtained from a proteomics analysis of purified nuclear envelopes suggest that approximately 80 transmembrane proteins reside in the inner nuclear membrane of interphase cells [Schirmer et al., 2003]. Most of these proteins remain to be characterized in detail.

Grant sponsor: National Institutes of Health; Grant numbers: AR048997, DK062351; Grant sponsor: Muscular Dystrophy Association; Grant sponsor: American Diabetes Association; Grant sponsor: Human Frontiers Science Program.

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Received 19 August 2005; Accepted 22 August 2005

DOI 10.1002/jcb.20650

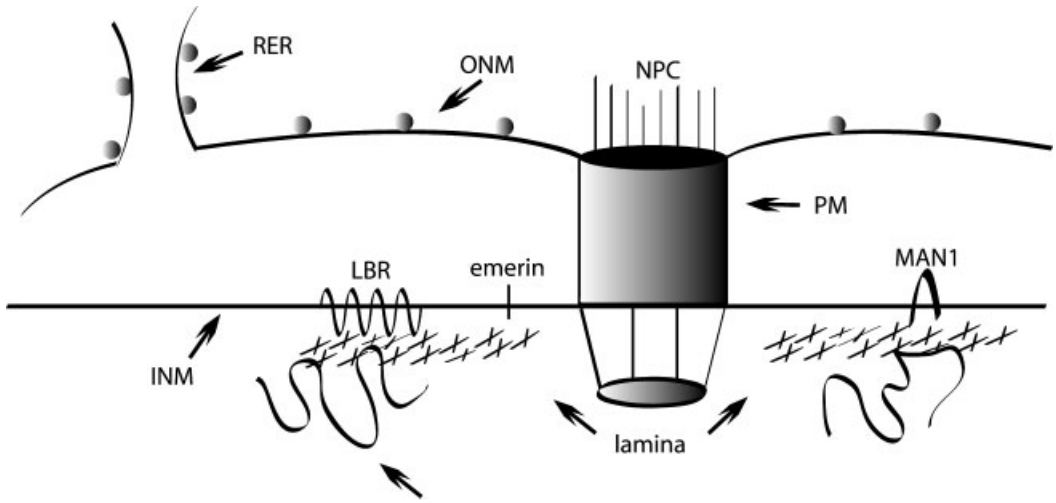

Fig. 1. Schematic diagram of a section of the nuclear envelope showing the inner nuclear membrane (INM), pore membrane (PM), and outer nuclear membrane (ONM), which is continuous with the rough endoplasmic reticulum (RER). Representative integral proteins of the inner nuclear membrane shown are lamin B receptor (LBR), emerin and MAN1. A single nuclear pore complex (NPC) is also shown.

INHERITED DISEASES CAUSED BY MUTATIONS IN INNER NUCLEAR MEMBRANE PROTEINS

Over the past decade, geneticists have shown that mutations in nuclear lamins and integral proteins of the inner nuclear membrane cause a wide variety of diseases, some of which are fairly tissue-specific (Table I). Several of these diseases result from mutations in A-type lamins. The major somatic cell A-type lamins are lamins A and C, which arise by alternative splicing or RNA encoded by the *LMNA* gene [Fisher et al., 1986; McKeon et al., 1986; Lin and Worman, 1993]. Lamins A and C are expressed in most terminally differentiated cells, making the fact that mutations in these proteins cause tissue-specific disorders somewhat surprising.

Mutations in three integral proteins of the inner nuclear membrane have also been shown to cause inherited human diseases. Mutations in emerin cause X-linked Emery–Dreifuss muscular dystrophy [Bione et al., 1994]. Mutations in lamins A and C cause a phenotypically identical but autosomal dominantly inherited form of Emery–Dreifuss muscular dystrophy [Bonne et al., 1999]. Interestingly, lamins A and C and emerin have been shown to interact in vitro and in vivo [Fairley et al., 1999; Sullivan et al., 1999; Clements et al., 2000; Sakaki et al., 2001]. Heterozygous mutations in lamin B receptor cause Pelger–Huët anomaly, a benign alteration of neutrophil nuclear morphology [Hoffmann et al., 2002]. However, homozygous mutations cause neonatal lethal HEM/Greenberg skeletal dysplasia, which is associated with a loss of 3 beta-hydroxysterol delta 14-reductase sterol reductase activity [Waterham et al., 2003]. This finding is consistent with a portion of lamin B receptor being similar in sequence to

TABLE I. Chronology of Major Discoveries Showing That Mutations in Inner Nuclear Membrane Proteins Cause Inherited Diseases

- Bione et al. [1994] show that emerin mutations cause X-linked Emery–Dreifuss muscular dystrophy
- Bonne et al. [1999] show that lamin A/C mutations cause autosomal dominant Emery–Dreifuss muscular dystrophy, Fatkin et al. [1999] and Muchir et al. [2000] show mutations in related skeletal and cardiac muscle disorders
- Cao and Hegele [2000], Shackleton et al. [2000], and Speckman et al. [2000] show lamin A/C mutations cause Dunnigan-type partial lipodystrophy
- De Sandre-Giovannoli et al. [2002] show a lamin A/C missense mutation cause recessive Charcot-Marie-Tooth disorder type 2
- Novelli et al. [2002] show that a lamin A/C homozygous missense mutation causes mandibuloacral dysplasia
- Hoffmann et al. [2002] show that mutations in lamin B recptor cause Pelger–Huët anomaly
- Waterham et al. [2003] show that mutations in lamin B receptor cause autosomal recessive HEM/Greenberg skeletal dysplasia
- De Sandre-Giovannoli et al. [2003] and Eriksson et al. [2003] show prelamin A splicing mutations in Hutchinson–Gilford progeria syndrome
- Hellemans et al. [2004] show that mutations in MAN1 (LEMD3) cause osteopoikilosis, Buschke–Ollendorff syndrome, and inherited melorheostosis

known human, animal, plant, and yeast sterol reductases [Schuler et al., 1994; Holmer et al., 1998] and the demonstration that it has delta 14-sterol reductase activity when expressed in budding yeast [Silve et al., 1998]. Heterozygous mutations in MAN1 cause osteopoikilosis, Buschke–Ollendorff syndrome and inherited melorheostosis [Hellemans et al., 2004]. This seminal discovery helped establish that the inner nuclear membrane is a subcellular site for regulating signal transduction from the plasma membrane to the nucleus.

MAN1: AN INTERGRAL PROTEIN OF THE INNER NUCLEAR MEMBRANE

The "MAN antigens" were originally identified using serum ("MAN antiserum") from a subject with a collagen vascular disease that labeled the nuclear envelope when used in indirect immunofluorescence microscopy [Paulin-Levasseur et al., 1996]. The antibodies in this serum recognized three predominant antigens that co-isolated with nuclear lamins in cell fractionation experiments. The MAN1 antigen with an approximate molecular mass of 60 kDa was subsequently identified as lamina-associated polypeptide 2-beta [Lang et al., 1999]. Using "MAN antiserum" to screen expression libraries, a partial cDNA for the largest recognized antigen was obtained and isolation of overlapping cDNAs led to the characterization of MAN1 [Lin et al., 2000].

MAN1 has an approximate molecular mass of 97 kDa and two predicted transmembrane segments that presumably span the inner nuclear membrane (Fig. 2). The amino-terminal domain preceding the first transmembrane segment contains approximately 450 amino acids and faces the nucleoplasm. The first approximately 50 amino acids of MAN1 is a LEM domain, a helix-loop-helix motif found in lamina-associated polypeptide 2, emerin, MAN1 and several other nuclear proteins [Lin et al., 2000; Cai et al., 2001; Laguri et al., 2001]. The LEM domain has been shown to bind to barrier-to-autointegration factor [Cai et al., 2001]. The carboxyl-terminal domain following the second transmembrane segment of MAN1 contains approximately 230 amino acids and faces the nucleoplasm. The human gene encoding MAN1 was localized to chromosome 12q14 [Lin et al., 2000].

The amino-terminal nucleoplasmic domain of MAN1 is necessary for its retention in the inner nuclear membrane [Wu et al., 2002]. This domain interacts with lamin A, lamin B1 and emerin [Mansharamani and Wilson, 2005]. MAN1 can diffuse relatively freely in the endoplasmic reticulum membrane but its lateral diffusion is decreased in the inner nuclear membrane [Wu et al., 2002]. Hence, MAN1 is likely retained in the inner nuclear membrane of interphase cells as a consequence of its amino-terminal domain binding the nuclear lamina and possibly other protiens.

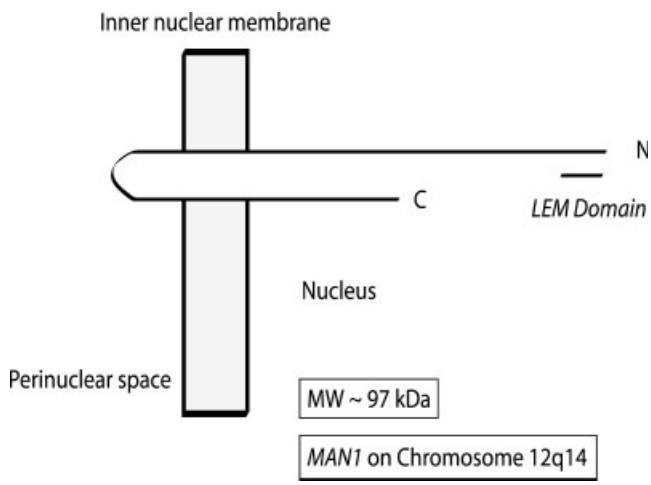

Fig. 2. Diagram showing topology of MAN1 in the inner nuclear membrane. The protein has two transmembrane segments and an amino-terminus (N) and a carboxyl-terminus (C) that face the nucleoplasm. A LEM domain is located at the amino-terminus. The approximate molecular mass of MAN1 is 97 kDa and the *MAN1* gene is on human chromosome 12q14.

MAN1 REGULATES SMAD SIGNALING

Smads are intracellular mediators of signaling by the transforming growth factor-beta (TGF-beta) family of cytokines [Shi and Massagué, 2003]. Two major subfamilies are the TGF-beta and bone morphogenic protein (BMP) subfamilies. Binding of BMP or other members of its subfamily to their plasma membrane receptors leads to phosphorylation and nuclear translocation of Smad1. Binding of TGF-beta or other members of its subfamily to their plasma membrane receptors leads to phosphorylation and nuclear translocation of Smad2 and Smad3. In the nucleus, Smad1, Smad2, and Smad3 interact with Smad4 and other cofactors to form complexes that regulate transcription from numerous target genes.

There are several positive and negative regulators of Smads. Examples of well-studied negative regulators are Sno and Ski [Luo, 2004]. Convergent studies from independent laboratories using different approaches have now shown that MAN1 is a negative regulator of signal transduction mediated by Smad1, Smad2, and Smad3. This work established that the inner nuclear membrane is a novel site of signal transduction regulation.

Evidence showing that MAN1 is involved in regulating Smad-mediated signal transduction came from a search for interacting proteins. Lin et al. [2005] carried out yeast two-hybrid screens with the nucleoplasmic carboxyl-terminal and amino-terminal domains of MAN1. While screens with the amino-terminal domain did not identify specific interactors, screens with the carboxyl-terminal domain yielded cDNA clones that encoded Smad2. In vitro binding and direct yeast two-hybrid interaction assays showed that the carboxyl-terminal domain of MAN1 bound to the MH2 domain of Smad2 and Smad3. "MAN antiserum" also co-immunoprecipitated Smad2 from cellular protein extracts, demonstrating an in vivo interaction. Overexpression of MAN1 in cultured cells inhibited activation of TGF-beta responsive reporter genes and antagonized TGF-beta induced proliferation arrest of mink lung epithelial cells. These results implicated MAN1 as a negative regulator of Smad2 and Smad3.

Searches for new proteins that interacted with Smads similarly provided evidence that MAN1 is an inhibitor of their activities. Raju et al. [2003] performed a yeast two-hybrid screen using *Xenopus* Smad1 as bait and identified the frog MAN1 orthologue, which they called SANE for Smad1 antagonistic effector. The carboxyl-terminal domain of this *Xenopus* protein bound to the MH2 domain of Smad1. They further demonstrated that the protein was expressed in early embryos in a pattern that overlapped with Smad1, that its overexpression blocked BMP-induced Smad1 phosphorylation and that it antagonized BMP/Smad1 signaling in *Xenopus* embryos and a mammalian model of bone formation. Pan et al. [2005] identified MAN1 using an affinity-purification binding assay to identify proteins that bound to mammalian Smad3. They showed that the carboxyl-terminal domain of MAN1 associates with the MH2 domains of Smad1, Smad2, and Smad3 but not Smad4. In their assays, overexpression of MAN1 inhibited phosphorylation of Smad1, Smad2, and Smad3, their heterodimerization with Smad4 and their nuclear translocation. They also demonstrated that MAN1 repressed transcriptional activation of the TGF-beta, BMP, and activin-responsive reporter genes.

MAN1 was also identified as a Smad1 binding protein that antagonizes BMP signaling in a functional screen for cDNAs that neutralized ectoderm formation in *Xenopus* development [Osada et al., 2003]. Osada et al. [2003] further demonstrated that the *Xenopus* MAN1 orthologue antagonized BMP signaling downstream of its receptor in animal cap development and BMP reporter gene activation assays. They also showed that the ectoderm neutralizing and BMP-antagonizing activities of MAN1 resided in the carboxyl-terminal region and that this region bound to Smad1, Smad5, and Smad8. Interference of endogenous *Xenopus* MAN1 with antisense morpholino oligonucleotides stimulated a BMP-mediated pathway.

Additional evidence that MAN1 is a negative regulator of TGF-beta and BMP signaling came from a positional cloning study to identify the gene responsible for a the rare human allelic variant disorders osteopoikilosis, Buschke–Ollendorff syndrome and inherited, non-sporadic melorheostosis [Hellemans et al., 2004]. A genome-wide linkage analysis in three affected families followed by the identification of a microdeletion in an unrelated individual with these diseases allowed for mapping of the responsible gene to chromosome 12p14, which contained the *MAN1* gene. All affected

individuals investigated were heterozygous with respect to truncating mutations in MAN1 (which these authors referred to as LEMD3). Hellemans et al. [2004] also showed that MAN1 interacted with BMP and TGF-beta receptor-activated Smads and antagonized both signaling pathways in human cells. Cells from human subjects with the MAN1 mutations had enhanced expression of genes activated by BMP and TGF-beta. Individuals with osteopoikilosis, Buschke–Ollendorff syndrome and inherited melorheostosis have sclerosing bone lesions and sometimes skin and connective tissue abnormalities. These pathological abnormalities are consistent with enhanced BMP and TGF-beta signaling.

Antagonism of TGF-beta and BMP signaling by MAN1 may simply result from sequestration of Smad1, Smad2, and Smad3 at the inner nuclear membrane by binding to MAN1 (Fig. 3). This would compete with their binding to gene regulatory regions. However, some results suggest that MAN1 may play a more complex role, blocking Smad phosphorylation or inducing its dephosphorylation, in turn affecting nuclear translocation. While future studies may reveal additional data as to how MAN1 antagonizes Smad-mediated signaling, the results so far clearly demonstrate that the inner nuclear membrane is a subcellular location where signal transduction is regulated.

CONCLUDING SPECULATION

Several different experimental approaches have revealed that inner nuclear membrane protein MAN1 is a negative regulator of Smad-mediated TGF-beta and BMP signal transduction. They have also provided a plausible pathogenic mechanism as to how mutations in an inner nuclear membrane protein cause human disease. Could mutations in other inner nuclear membrane proteins similarly cause diseases by affecting signal transduction pathways?

Lammerding et al. [2004] have shown that fibroblasts from lamin A/C null mice have nuclear structural defects and attenuated NF-kappaB-regulated transcription in response to mechanical or cytokine stimulation. Lamin A/C null mice develop cardiomyopathy and skeletal muscle abnormalities similar to human Emery–Dreifuss muscular dystrophy [Sullivan et al., 1999; Nikolova et al., 2004]. Hence, abnormal signal transduction may play a role the development of striated muscle disease with loss of A-type lamins. Emerin, an inner nuclear membrane protein that is mutated in X-linked Emery–Dreifuss muscular dystrophy, appears to have overlapping functions with MAN1 in *Caenorhabditis elegans* [Liu et al., 2003]. While emerin itself may not bind to Smads, data suggest that MAN1, emerin and lamins interact at the inner nuclear membrane [Mansharamani

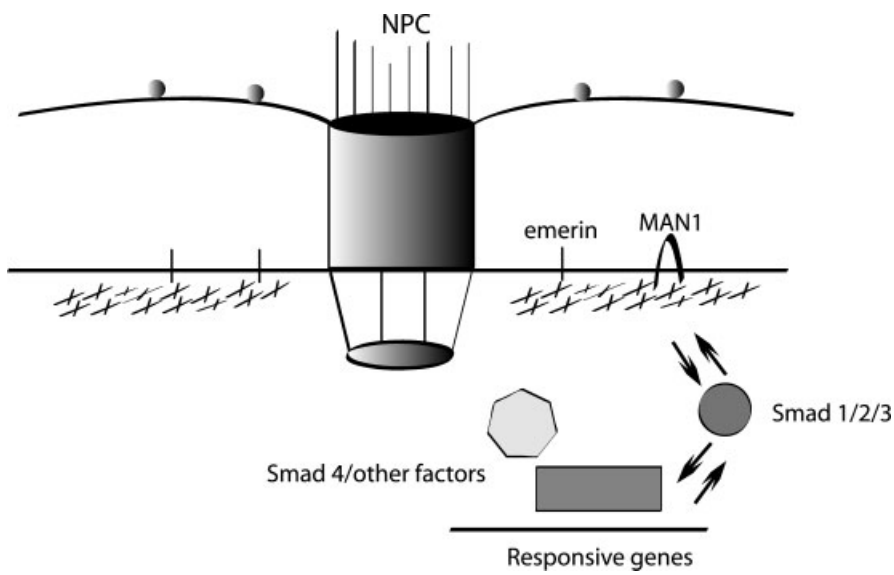

Fig. 3. Diagram showing simple mode for how MAN1 may regulate the effects of Smad1, Smad2, and Smad3 on responsive genes. Nuclear Smad1, Smad2, and Smad3 can interact with Smad4 and factors to activate or repress responsive genes. MAN1 in the inner nuclear membrane can competitively interact with these Smads, antagonizing their effects on gene expression. MAN1 interacts with lamins and emerin and emerin also may interact directly with MAN1, providing a possibly link as to how mutations in A-type lamins and emerin may also affect Smad-mediated signal transduction.

and Wilson, 2005]. As a result of these interactions, mutations in emerin and lamins could therefore alter the localization of function of MAN1, consequently affecting regulation of Smads. Smad 2 and Smad 3 also mediate signal transduction by myostatin, a negative regulator of muscle growth [Grobet et al., 1997; McPherron et al., 1997]. Hence, defective antagonism of Smad activity at the inner nuclear membrane could potentially lead to striated muscle abnormalities, such as those caused by mutations in emerin and lamins A and C. This hypothesis remains to be tested. As research on the inner nuclear membrane and inherited diseases caused by mutations in its proteins continues, it is likely that additional regulatory steps in signal transduction will be identified at this subcellular location.

ACKNOWLEDGMENTS

The author appreciates the assistance of Ms. Priscilla Vazquez with the artwork.

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