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Integral Nuclear Membrane Proteins in Vertebrate Development

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ABSTRACT

The nuclear envelope is composed of the outer and inner nuclear membranes, nuclear lamina, and nuclear pore complexes. Recent studies show that the nuclear envelope is more than a capsule for protecting genetic information or a gatekeeper for molecular traffic between the cytoplasm and nucleus. The outer and inner nuclear membranes contain unique integral transmembrane proteins. The number of such transmembrane proteins is estimated as many as 80. However, a dozen of them have been characterized in detail. Nesprins, the first outer nuclear membrane proteins identified, are involved in nuclear positioning by connecting the nuclear envelope to the cytoskeletal system. A growing number of integral inner nuclear membrane proteins have been implicated in diverse cellular functions, such as gene regulation, chromatin organization, and signal transduction through interactions with their binding partners. In addition, mutations in the nuclear envelope proteins cause a wide variety of human diseases. Analysis of experimentally altered animal models of the integral nuclear membrane proteins is essential to understanding their functional roles at the whole-organism level and pathogenesis of the diseases caused by mutations in these proteins. However, only several such animal models are available in vertebrates. This review highlights recent progress in the field of integral nuclear membrane proteins from the view of vertebrate development.

Keywords: development, gene regulation, LEM domain, muscular dystrophy, nuclear envelope, nuclear membrane, signal transduction
Abbreviations: **BAF**, barrier to autointegration factor; **CNS**, central nervous system; **ER**, endoplasmic reticulum; **EDMD**, Emery-Dreifuss muscular dystrophy; **INM**, inner nuclear membrane; **MEF**, mouse embryonic fibroblast; **NLS**, nuclear localization signal; **NMJ**, neuromuscular junction; **NPC**, nuclear pore complex; **ONM**, outer nuclear membrane

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INTRODUCTION

Eukaryotic cells are characterized by the presence of a nuclear envelope that separates the cytoplasm from the nucleus. The nuclear envelope is composed of three major components: the nuclear membranes, nuclear lamina, and nuclear pore complexes (NPCs; **Fig. 1**). Two concentric nuclear membranes, the outer nuclear membrane (ONM) and inner

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Invited Review

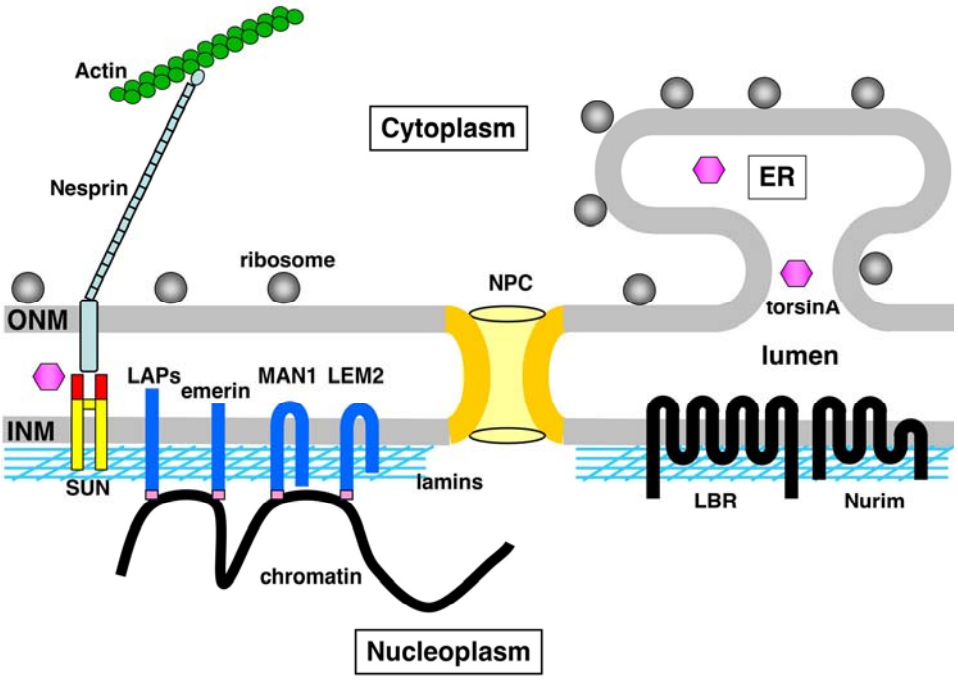

Fig. 1 Schematic diagram of the nuclear envelope with integral nuclear membrane proteins. Three major components of the nuclear envelope: the nuclear membranes, nuclear lamina, and a nuclear pore complex (NPC) are depicted. The LEM domain proteins (LAPs, emerin, MAN1, and LEM2), LBR, nurim, and a SUN protein are shown as representatives for inner nuclear membrane (INM) proteins. Nesprin resides in the outer nuclear membrane (ONM) and is composed of three domains; an N-terminal calponin homology domain (circle), a long stretch of spectrin repeats, and a C-terminal KASH domain (longitudinal box). A luminal domain (SUN domain, red boxes) of SUN interacts with the KASH domain of nesprin. Because the number of transmembrane segments of the SUN protein varies depending on reports, a single transmembrane segment is depicted for convenience. Note that SUN acts as a dimer. Pink boxes, LEM domains.

nuclear membrane (INM), are connected at the nuclear pore membrane, which is associated with NPCs. NPCs are large proteinaceous assemblies that serve as the channels for directional transport of soluble macromolecules across the nuclear envelope. In vertebrates, the NPC comprises multiple copies of approximately 30 distinct proteins (nucleoporins), including two integral proteins, gp120 and POM121, which are thought to tether the NPCs to the pore membrane. The nuclear lamina is a meshwork mainly composed of type V intermediate filaments formed by A-type and B-type lamins.

The ONM is continuous with the membrane of the endoplasmic reticulum (ER), and, like the rough ER, is covered with ribosomes engaged in protein synthesis. Although it has long been assumed that the protein composition of the ONM is identical to that of the ER, the recently identified ANC-1/MSP-300/nesprin (nuclear envelope spectrin repeats) family of proteins includes several that localize to the ONM. The nesprin family of proteins regulates nuclear positioning by establishing a unique linkage between the ONM and the cytoskeletal systems in *Caenorhabditis elegans*, *Drosophila*, and vertebrates (Starr and Fischer 2005; Tzur *et al*. 2006b).

The INM also contains at least 50 different integral membrane proteins (Gruenbaum *et al*. 2005). However, a dozen of them, including lamin B receptor (LBR), lamina-associated polypeptides (LAPs), emerin, MAN1, LEM2, and SUN domain proteins, have been characterized in detail (**Table 1**). INM proteins generally have one or more nucleoplasmic domains, which interact with nuclear lamins and/or chromatin, and a single or multiple transmembrane segments. Several INM proteins share a conserved structural motif of approximately 40 amino acid residues, termed the LEM domain, which was first identified in LAP2, emerin, and MAN1 (Lin *et al*. 2000). The LEM domain binds to barrier-to-autointegration factor (BAF), a small diffusible molecule that mediates various functions by interacting with chromatin and transcription factors (Segura-Totten and Wilson 2004).

How do INM proteins localize to the INM? After being synthesized on the rough ER, INM proteins are thought to localize to the INM by two major mechanisms: 1) lateral

Table 1 Characterized vertebrate integral nuclear membrane proteins.

Protein	Other names/ Orthologs	TM[1]	Topology	Domains
dullard	NET56	2	NE[2]	Phosphatase
emerin		1	INM2	LEM, APC
LAP1s		1	INM	
LAP2s	Thymopoietin	1	INM[4]	LEM
LBR		8	INM	Sterol reductase
LEM2	NET25	2	INM	LEM
LEMD1	LEM5	1	NE[2]	LEM
LUMA		3-4	NE[2]	
MAN1	XMAN1, SANE	2	INM	LEM, Winged-helix, UHM
Nesprin-1	Syne-1, Myne-1, enaptin/ANC-1, MSP-300	1	ONM	CH, spectrin repeats, KASH
Nesprin-2	Syne-2, Myne-2, NUANCE	1	ONM	CH, spectrin repeats, KASH
Nesprin-3	C14orf49	1	ONM	Spectrin repeats, KASH
nurim		6	INM	
RFBP		9	INM	ATPase
SUN1	Matefin	1-3[3]	INM	SUN
SUN2		1-3[3]	INM	SUN
UNCL		1	NE[2]	LEM-like, RRM

[1] TM, number of (putative) transmembrane segments.
[2] These proteins are localized to the nuclear envelope, but their decisive localization to the INM or ONM is unknown.
[3] The number of transmembrane segments of the SUN proteins varies depending on reports.
[4] LAP2α and LAP2ζ, isoforms lacking a transmembrane segment, are present in the nucleoplasm.
Abbreviations: APC, adenomatous polyposis coli; CH, calponin homology; INM, inner nuclear membrane; KASH, Klarsicht-Anc1-Syne-1 homology; NE, nuclear envelope; ONM, outer nuclear membrane; RRM, RNA recognition motif; UHM, U2AF homology motif.

diffusion and retention, and 2) energy-dependent active transport. In the first mechanism, newly synthesized INM proteins diffuse freely in the ER membrane and pass along the pore membrane through the lateral channels of NPCs to reach the INM (Ellenberg *et al*. 1997; Ostlund *et al*. 1999). Binding to nuclear structures such as lamins and chromatin retains the incoming proteins in the INM. Because of steric constraints of the lateral channels, only globular proteins with a mass less than about 60 kDa are allowed to diffuse along the pore membrane domain through the channels (Soullam and Worman 1995).

The idea of the second mechanism comes from a recent study demonstrating that transport of integral proteins to the INM requires energy-dependent active restructuring of the NPC (Ohba *et al*. 2004). Most mammalian INM proteins such as LBR, LAP1, LAP2β, emerin, MAN1, and LEM2 have nuclear localization signal (NLS)-like sequences. NLSs in Heh1 and Heh2, yeast orthologs of MAN1 and LEM2, respectively, mediate Ran GTPase cycle-dependent and karyopherin/importin-dependent targeting of these proteins to the INM (King *et al*. 2006).

In this review, I give an overview of recent advances in unraveling the roles of integral nuclear membrane proteins from the view of development. For most proteins discussed, the interaction with nuclear lamins is essential for their localization to the nuclear membrane and for their functions. Because there are several excellent reviews on lamins (Burke and Stewart 2002; Goldman *et al*. 2002; Hutchison 2002; Gruenbaum *et al*. 2005; Worman and Courvalin 2005), I do not discuss them in detail here.

OUTER NUCLEAR MEMBRANE PROTEINS

The KASH domain protein family

The nucleus and other organelles occupy specific locales within a cell. How is the position of these structures determined? Exploring an answer to this question has identified KASH (Klarsicht, ANC-1, and Syne homology) domain proteins that localize to the ONM. Eukaryotic cells have three cytoskeletal filament systems: actin filaments, intermediate filaments, and microtubules. Recent studies have revealed that the KASH domain proteins are central components of complexes that interconnect the ONM to the cytoskeletal filament systems and the INM to the intranuclear filament systems (Starr and Fischer 2005; Tzur *et al*. 2006b).

Nesprin-related proteins regulate nuclear positioning

Analysis of *anc-1*, a *C. elegans* mutant with mispositioned nuclei, has identified the prototype for the nesprinprotein, ANC-1 (Starr and Han 2002). The body of an adult worm is covered by large syncytial hypodermal cells with more than 100 evenly spaced nuclei. In the *anc-1* mutant, the nuclei float freely within the cytoplasm because of defects in nuclear anchorage. Four nesprin genes (nesprins 1-4) have been identified in mammals so far. Nesprin-1 is also known as Syne-1 (synaptic nuclear envelope-1; Apel *et al*. 2000), Myne-1 (myocyte nuclear envelope-1; Mislow *et al*. 2002b) or enaptin (for "to attach" in Greek; Padmakumar *et al*. 2004), and nesprin-2 is also called Syne-2, Myne-2, or NUANCE (NUcleus and ActiN Connecting Element; Zhen *et al*. 2002). The nesprin-1 and nesprin-2 genes each encode a large isoform ("giant") plus many shorter isoforms, some of which localize to the INM and interact with A-type lamins and emerin (Mislow *et al*. 2002a; Zhang *et al*. 2005).

A comparison of ANC-1 and its related proteins in *Drosophila* MSP-300 and mammalian Syne-1 has identified three characteristic domains that engage in nuclear anchorage (Apel *et al*. 2000; Starr and Han 2002; Zhang *et al*. 2002): 1) a paired N-terminal calponin homology (CH) domain, 2) a large central region consisting of repetitive sequences, and 3) a C-terminal conserved KASH domain with a single putative transmembrane segment. The CH domain is implicated in actin binding, and consistent with this, ANC-1, MSP-300, and nesprin giant isoforms bind actin filaments *in vitro* (Volk 1992; Starr and Han 2002; Zhen *et al*. 2002; Padmakumar *et al*. 2004). The central region is composed of mainly spectrin repeats in MSP-300 and nesprins, whereas in *C. elegans*, it comprises unique repetitive sequences. The KASH domain is sufficient for nuclear envelope localization and directly interacts with the luminal domain of SUN proteins, a family of INM proteins (see below). In *C. elegans*, ANC-1 fails to localize to the nuclear envelope in *unc-84* mutant backgrounds in which a SUN domain protein, UNC-84, is mutated (Starr and Han 2002). In mammals, nesprins directly interact with SUN domain proteins (Padmakumar *et al*. 2005; Crisp *et al*. 2006). These results suggest that the ANC-1/MSP-300/nesprin proteins are a central component of the complex that connects actin filaments at their N termini and the INM at their C termini (Starr and Fischer 2005; **Fig. 2A**).

Over half of Emery-Dreifuss muscular dystrophy

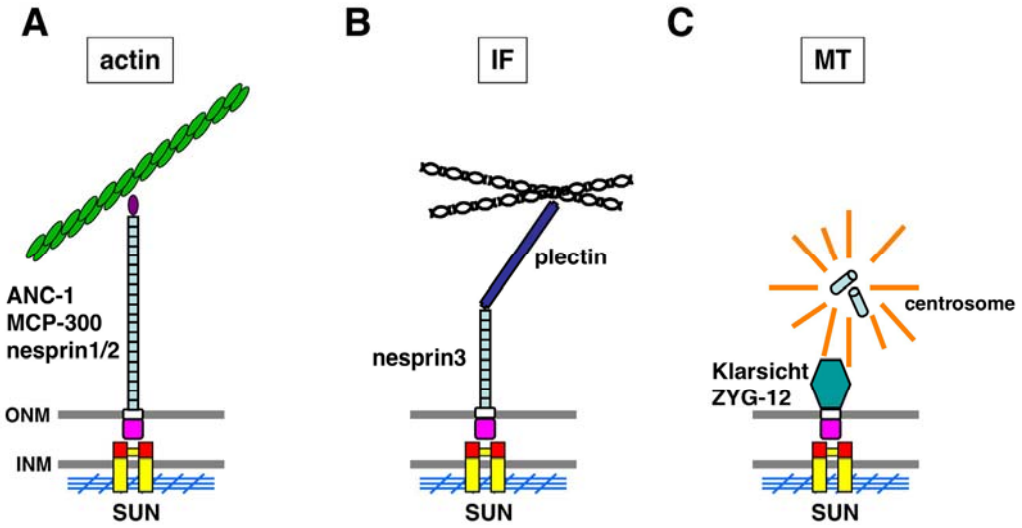

Fig. 2 Complexes connecting the nuclear envelope and the cytoskeletal filament systems. Eukaryotic cells have three cytoskeletal filament systems; actin filaments (green), intermediate filaments (IFs, black), and microtubules (MTs, orange). KASH domain proteins (ANC-1, MSP-300, nesprins, Klarsicht, and ZYG-12) and SUN domain proteins form molecular bridges between the cytoskeleton and nuclear components. (**A**) Nesprin-1 and nesprin-2 tether nuclei to actin filaments through the calponin homology domain (purple). (**B**) nesprin-3 connects IFs and the nuclear envelope via plectin. (**C**) Klarsicht and ZYG-12 link the nucleus to centrosomes via interaction with MTs. Note that in vertebrates, a protein corresponding to Klarsicht or ZYG-12 has not yet been identified.

(EDMD) patients have no mutations in emerin or A-type lamins, suggesting that other proteins are involved in the pathogenesis of this complex disease. Because nesprin-1 and nesprin-2 are both highly expressed in skeletal muscle and bind to A-type lamins and emerin, nesprins are thought to be a promising candidate mutated protein in EDMD patients with unknown etiology. As predicted, a small number of unique sequence variants in the nesprin-1 and nesprin -2 genes have recently been identified in some EDMD patients (pers. comm., Dr. CM Shanahan, University of Cambridge).

The third member of nesprin, nesprin-3, is involved in connecting the nucleus to intermediate filaments (**Fig. 2B**). Nesprin-3 has been identified as a binding protein for plectin, a member of the plakin family of cytoskeletal cross-linkers (Wilhelmsen *et al*. 2005). Nesprin-3 has a stretch of spectrin repeats and a C-terminal KASH domain, but unlike nesprin-1 and nesprin-2, nesprin-3 lacks the N-terminal CH domain for actin binding. Two nesprin-3 variants, nesprin-3α and nesprin-3β, are both ubiquitously expressed and their green fluorescent protein (GFP)-tagged forms localize to the ONM (Wilhelmsen *et al*. 2005). Nesprin-3 overexpression in keratinocytes results in a strong recruitment of plectin to the ONM, where keratin-6 and keratin-4 intermediate filaments also colocalize. Plectin associates with integrin α6β4 at hemidesmosomes at the plasma membrane. These results suggest the presence of a complex comprising nesprin-3, plectin, intermediate filaments, and integrins that connects the ONM to the plasma membrane (Wilhelmsen *et al*. 2005).

In mouse, nesprin-4 has been identified (pers. comm., Dr. B. Burke, University of Florida). Interestingly, nesprin-4 is particularly expressed in secretory epithelial cells. By analogy with nesprins 1-3, nesprin-4 may interact with cytoskeletal components.

The KASH domain family proteins are also involved in forming a complex that connects the ONM and microtubules (**Fig. 2C**). *Drosophila* Klarsicht and *C. elegans* ZYG-12 were the first KASH domain proteins discovered that link the nucleus to centrosomes (Malone *et al*. 2003; Patterson *et al*. 2004), probably through their interaction with microtubules. In *Drosophila*, wild-type photoreceptor (R cells) nuclei in compound eyes are apical and the centrosome is located just above them. In *klarsicht* mutants, most of the R cell nuclei lose their connection to the centrosome and are located basally (Mosley-Bishop *et al*. 1999). *C. elegans* centrosomes are closely associated with the nuclear envelope. In *zyg-12* mutants, the attachment of the centrosome to the nucleus is severely perturbed, resulting in early embryonic death because of DNA segregation defects (Malone *et al*. 2003). Both Klarsicht and ZYG-12 attach to microtubules through dynein (Malone *et al*. 2003; Fischer *et al*. 2004). In mammals, KASH domain proteins involved in the link between the nuclear envelope and microtubules have not been identified.

UNC-83 is a *C. elegans* KASH domain protein involved in nuclear migration (Starr *et al*. 2001). The switch between nuclear anchorage and nuclear migration must be tightly regulated. Like ANC-1, UNC-83 also requires interaction with UNC-84 to localize to the ONM (McGee *et al*. 2006), suggesting the location where ANC-1 and UNC-83 compete for binding to UNC-84 (Starr and Han 2003): the association between ANC-1 and UNC-84 anchors the nucleus in a certain position, whereas the association between UNC-83 and UNC-84 induces nuclear migration.

Syne-1 regulates nuclear positioning at the neuromuscular junction (NMJ)

Because most studies of the role of nesprins at the synapse and the NMJ were performed under the name of Syne, I use the term Syne in this and the following sections. Syne-1 is involved in nuclear anchorage at the NMJ. Mammalian myoblasts fuse to form syncytial muscle fibers with hundreds of myonuclei located at the periphery of individual muscle fibers. When innervated, the NMJ, a highly organized synapse for chemical neurotransmission, is formed. The NMJ is composed of the presynaptic membrane of the motor nerve terminal and the postsynaptic membrane of the muscle fiber. Transcriptionally specialized myonuclei expressing genes for synaptic proteins such as the acetylcholine receptors aggregate beneath the postsynaptic membrane (i.e. synaptic nuclei).

Table 2 Human diseases caused by mutations in integral nuclear membrane proteins.

Protein	Disease	Form of inheritance	OMIM[1]
emerin	Emery-Dreifuss muscular dystrophy	X-linked	310300
LAP2α	Familial dilated cardiomyopahty	autosomal dominant	188380
LBR	Pelger-Huët anomaly	autosomal dominant	169400
	Greenberg skeletal dysplasia	autosomal recessive	215140
MAN1	Osteopoikilosis	autosomal dominant	166700
	Buschke-Ollendorff syndrome	autosomal dominant	166700
	Melorheostosis	autosomal dominant	155950
Nesprin-1	Cerebellar ataxia	autosomal recessive	610743

[1] Online Mendelian Inheritance in Man.

Overexpression of the KASH domain acts dominant-negatively to cause mislocalized nuclei in *C. elegans* (Starr and Han 2002). Using a similar dominant-negative approach, transgenic mice expressing the KASH domain of Syne-1 in skeletal muscles were generated (Grady *et al*. 2005). Fewer myonuclei accumulate beneath the postsynaptic membrane in the transgenic mice than in controls, and in the transgenic mice, some NMJs have no synaptic nuclei but an increased number of nuclei at the periphery of synapses (i.e. perisynaptic nuclei; Grady *et al*. 2005). Syne-2 KASH-domain transgenic mice display essentially a similar dominant-negative phenotype to that of Syne-1 transgenic mice (Zhang *et al*. 2007). Interestingly, no functional defects occur in the NMJs of the transgenic mice, despite a substantial reduction in the number of synaptic nuclei.

Syne-1 and *Syne-2* KASH-domain knockout mice have been recently generated (Zhang *et al*. 2007). In *Syne-1*$^{-/-}$ mice, non-synaptic nuclei in syncytial skeletal muscle cells disorganize to form clusters, and the number of synaptic nuclei was dramatically reduced due to defects in nuclear anchorage. In contrast, synaptic and non-synaptic nuclei were properly positioned in *Syne-2*$^{-/-}$ mice.

Although neither Syne-1 nor Syne-2 single deficiency affects viability and fertility, *Syne-1* and *Syne-2* double knockout mice (*Syne* DKO) die of respiratory failure shortly after birth (Zhang *et al*. 2007). Phrenic nerves governing the diaphragm have longer branches in *Syne* DKO mice. However, because the muscle morphology and NMJ structure are surprisingly normal in *Syne* DKO, the cause of the respiratory failure and lethality remains obscure (Zhang *et al*. 2007).

In humans, mutations leading to premature termination of SYNE-1 cause a newly discovered form of autosomal recessive cerebellar ataxia (Gros-Luis *et al*. 2007; **Table 2**). Consistent with this, mouse *Syne-1* is expressed in Purkinje cells. Although abnormal positioning of myonuclei at the NMJ occurs in affected individuals, muscle phenotypes are not observed clinically or electrophysiologically (Gros-Luis *et al*. 2007).

A recent systemic cancer genome study with stringent criteria has clarified that the *SYNE1* and *SYNE2* genes are mutated at significant frequency in colorectal and breast cancers, respectively (Sjoblom *et al*. 2006). The role of mutated SYNEs in tumorigenesis would be one of the most intriguing questions to be investigated.

Brain-specific Syne-1 isoforms regulate postsynaptic receptor endocytosis

CPG2 and CPG2b (Candidate Plasticity Gene 2), brain-specific shorter isoforms of Syne-1 lacking both the CH and KASH domains, regulate the internalization of glutamate receptors at synapses (Cottrell *et al*. 2004). Glutamate receptors mediate excitatory synaptic transmission. Modification of the number of surface glutamate receptors is critical for synaptic plasticity and is regulated by clathrin-mediated endocytosis. In cultured glutamatergic neurons, CPG2 localizes specifically to the postsynaptic side of excitatory synapses and is present in the vicinity of clathrin-coated vesicles. CPG2 knockdown by RNA interference (RNAi) disrupts glutamate receptor internalization, resulting in an increase in the number of surface receptors. These results suggest that CPG2 is a crucial component of the postsynaptic endocytic process needed for proper synaptic transmission.

INNER NUCLEAR MEMBRANE PROTEINS

SUN domain proteins

How are large nesprin isoforms targeted to the ONM? Another *C. elegans* mutant with the Anc phenotype, *unc-84*, has provided a clue to answer this question. UNC-84 is characterized as a nuclear envelope protein required for nuclear migration and anchorage during *C. elegans* development (Malone *et al*. 1999; Lee *et al*. 2002). UNC-84 has a single transmembrane domain and a C-terminal SUN domain, which was first identified in the *Schizosaccharomyces pombe* spindle pole body protein Sad1p and UNC-84 (Malone *et al*. 1999).

ANC-1 and UNC-83 fail to localize to the nuclear envelope in the null *unc-84* allele and in alleles with mutations in the SUN domain of UNC-84 (Starr *et al*. 2001; Starr and Han 2002). In addition, UNC-83 physically interacts with the SUN domain of UNC-84 *in vitro* (Starr *et al*. 2001; McGee *et al*. 2006). These results indicate that the KASH domain proteins (ANC-1 and UNC-83) interact with the SUN domain protein (UNC-84) and provide an experimental basis for a "bridging" model in which UNC-84 interacts with the luminal domain of an ONM protein to form a structural "bridge" through the nuclear envelope (Lee *et al*. 2002).

A direct physical interaction between the KASH domain and the SUN domain has been also proved in mammals. There are four SUN domain proteins in mammals, but SUN1 and SUN2 have been studied in more detail. SUN1 and SUN2 are INM proteins with their SUN domains located in the perinuclear space (Hodzic *et al*. 2004; Padmakumar *et al*. 2005). The KASH domains of nesprin-1 and nesprin-2 directly associate with the SUN domain of SUN1, and SUN1 and SUN2 are required for the nuclear envelope localization of nesprin-2 (Padmakumar *et al*. 2005; Crisp *et al*. 2006). Taken together, KASH/SUN domain pairs act as a connecting link between the nucleus and cytoplasm.

In *C. elegans*, the SUN-domain protein Matefin/SUN has a novel function: its expression is restricted to germ cells from mid-embryogenesis to adulthood and is essential for germ cell maturation and survival (Fridkin *et al*. 2004). Matefin/SUN also acts as a nuclear envelope receptor for the proapoptotic protein CED-4, the ortholog of mammalian Apaf-1 (Tzur *et al*. 2006a). Translocation of CED-4 from the mitochondria to the nuclear envelope is a crucial for apoptosis in *C. elegans*. Moreover, Matefin/SUN is required for centrosome attachment to the nuclear envelope through its interaction with ZYG-12 (Malone *et al*. 2003). The role of mammalian SUNs in germ cell development and apoptosis is unknown. Human disorders caused by mutations in SUNs or their genetically altered mice have not been reported.

Lamin B receptor (LBR)

As its name implies, LBR was originally identified in avian erythrocyte nuclear membranes as a protein specifically bound to B-type lamins (Worman *et al*. 1988). LBR is an INM protein with an N-terminal nucleoplasmic domain followed by a hydrophobic domain with eight putative transmembrane segments (Worman *et al*. 1990; Ye and Worman 1994; **Fig. 1**), which have sterol-reductase activity (Silve *et al*. 1998). The nucleoplasmic domain of LBR interacts with an ortholog of *Drosophila* heterochromatin protein 1 (Ye and Worman 1996; Ye *et al*. 1997), DNA (Ye and Worman 1994), histones H3/H4 modified by methylation and acetylation to various degrees (Makatsori *et al*. 2004), and chromatin-associated protein HA95 *in vitro* as well as B-type lamins, suggesting that LBR is involved in chromatin organization.

Heterozygous mutations in the gene encoding LBR cause Pelger-Huët anomaly (PHA) in granulocytes (Hoffmann *et al*. 2002; **Table 2**). PHA nuclei are hypolobulated and contain coarser and denser heterochromatin. Homozygous mutations in the LBR gene cause an autosomal recessive fetal chondrodystrophy called hydrops-ectopic calcification-'moth-eaten' (HEM) or Greenberg skeletal dysplasia (Waterham *et al*. 2003; **Table 2**). In cultured skin fibroblasts of an affected fetus, the concentration of an intermediate of sterol biosynthesis is elevated (Waterham *et al*. 2003), which is consistent with a lack of sterol reductase activity. However, it is not clear whether the fetal lethality is attributable to alterations in chromatin organization or to deficiency in the sterol reductase activity caused by LBR mutations.

Mice with the ichthyosis (*ic*) phenotype have homozygous mutations in *Lbr* (Shultz *et al*. 2003). These mice show a blood phenotype similar to PHA along with alopecia, syndactyly, and hydrocephalus.

B-type lamins, "ligands" for LBR, are also implicated in human disorders. Adult-onset autosomal dominant leukodystrophy, a slowly progressive, fatal neurological disorder characterized by symmetrical myelin loss of the central nervous system (CNS), is caused by a tandem genomic duplication of the gene encoding lamin B1 (Padiath *et al*. 2006). Mutations in lamin B2 are associated with acquired partial lipodystrophy (Hegele *et al*. 2006). The role of the interactions between LBR and B-type lamins in myelination and adipocyte differentiation is unknown.

Lamina-associated polypeptides (LAP)

LAP1 and LAP2 were originally identified using monoclonal antibodies against isolated nuclear envelopes (Senior and Gerace 1988; Foisner and Gerace 1993). LAP2 was also discovered in thymocytes and named 'thymopoietin' (Harris *et al*. 1995). Subsequent characterization of their cDNAs clarified that the human LAP1 and LAP2 genes give rise to at least three and six isoforms, respectively, by alternative splicing: LAP1A-C for LAP1 and LAP2α-ζ for LAP2 (Furukawa *et al*. 1995; Berger *et al*. 1996; Holmer and Worman 2001). LAP2s show no sequence similarity to LAP1s, and only LAP2s have the LEM domain. Except for LAP2α and LAP2ζ, all other LAPs have a single transmembrane segment and localize to the INM. LAP2α and LAP2ζ lack a transmembrane segment and are present diffusely within the nucleus (**Fig. 1**). As their names imply, LAP1s and LAP2s are associated with the nuclear lamina by directly binding to lamins through their nucleoplasmic domains. A single mutation in the C-terminal lamin-binding domain of LAP2α is associated with dilated cardiomyopathy (Taylor *et al*. 2005; **Table 2**)

Although the functional role of LAP1 is poorly understood, LAP1 has been rediscovered as an interacting protein for the AAA+ (ATPases associated with various cellular activities) protein torsinA (Goodchild and Dauer 2005). TorsinA is an ER luminal protein (**Fig. 1**), and a glutamic acid deletion of torsinA (ΔE-torsinA) causes early-onset

torsion dystonia (or DYT1 dystonia), an autosomal dominant trait characterized by sustained involuntary twisting movements in the legs and/or arms. The disease-causing ΔE-torsinA and a mutant with defects in ATP hydrolysis (EQ-torsinA) relocate from the ER to the perinuclear space near the INM (Gonzalez-Alegre and Paulson 2004; Goodchild and Dauer 2004; Naismith *et al*. 2004), where they accumulate because of aberrantly strong interactions with LAP1 (Goodchild and Dauer 2005). The AAA+ proteins act as molecular chaperones that alter the conformation of substrates (Vale 2000). TorsinA is proposed to regulate the luminal interactions between INM and ONM proteins (Gerace 2004), which may include nesprin-SUN complexes (**Fig. 1** and see above), and this general chaperone function may be disrupted by disease-causing torsinA mutations. It is still unknown whether the luminal domain of LAP1 interacts with ONM proteins, such as nesprins.

TorsinA null and homozygous disease-causing torsinA "knock-in" mice die soon after birth with no obvious developmental abnormalities (Goodchild *et al*. 2005). However, neurons from these mutant mice show severe nuclear membrane abnormalities, whereas the nuclear membranes of non-neuronal cells appear normal (Goodchild *et al*. 2005). Similarly disturbed nuclear membranes are observed in EQ-torsinA-overexpressed cultured cells (Naismith *et al*. 2004). The relationship between the neuronal-selective nuclear membrane abnormalities and lethality has not been clarified.

TorsinA also binds to a newly identified ER resident protein, LULL1 (luminal domain like LAP1). LULL1 is structurally similar to LAP1 and is presumed to have arisen from *LAP1* gene duplication (Goodchild and Dauer 2005). LULL1 was independently identified as NET9 (Chen *et al*. 2006b), which is enriched in the nuclear envelope. LULL1/NET9 is upregulated during C2C12 myoblast differentiation (Chen *et al*. 2006b). The role of LULL1/NET9 in myogenesis and the pathogenesis of DYT1 dystonia remains to be elucidated.

Emerin

Emerin was identified as a protein encoded by the gene mutated in X-linked EDMD (X-EDMD; Bione *et al*. 1994; **Table 2**). X-EDMD is characterized by contractures of the elbow, neck, and Achilles tendons, slowly progressive muscle wasting, and defects in the cardiac conduction system, which frequently cause fatal cardiac attacks. Emerin is an INM protein (Manilal *et al*. 1996; Nagano *et al*. 1996) with an N-terminal LEM domain (Lin *et al*. 2000) and a C-terminal single transmembrane segment (**Fig. 1**). Because of its involvement in X-EDMD and the diversity of its binding partners (**Table 3**), emerin is one of the most intensively studied INM proteins. Although the molecular mechanism underlying X-EDMD pathophysiology is still unknown, recent studies suggest roles for emerin in myogenesis, nuclear architecture, and signal transduction.

Table 3 Emerin binding proteins.

Proteins	Possible functions	References
actin	Enhancement of polymerization	Holaska *et al.* 2004
A-type lamins	Nuclear integrity; Gene regulation	Clements *et al.* 2000; Lee *et al.* 2001; Sakaki *et al.* 2001
BAF	Chromatin binding, HIV infection	Segura-Totten and Wilson 2004 ; Jacque *et al.* 2006
β-catenin	Regulation of Wnt signaling	Markiewicz *et al.* 2006
Btf	Apoptosis	Haraguchi *et al.* 2004
GCL	Transcription repression	Holaska *et al.* 2003
MAN1	Regulation of Smad signaling	Mansharamani *et al.* 2005
Lmo7	Gene regulation	Holaska *et al.* 2006
nesprins	Nuclear envelope anchorage	Mislow *et al.* 2002a; Zhang *et al.* 2005
YT521-B	mRNA splicing	Wilkinson *et al.* 2003

Emerin in myogenesis and muscle regeneration

It is rational to assume that emerin is implicated in muscle development. Emerin and its major structural partners, A-type lamins, are not required to form muscle during development because *emerin*-null people have muscles and *LMNA*-null mice are born normal. However, these proteins are important later for the regeneration (Bakay *et al*. 2006; Melcon *et al*. 2006) or function of skeletal muscle precursor cells, or satellite cells (Frock *et al*. 2006).

Myoblasts with reduced expression of emerin by RNAi display impaired differentiation kinetics and compromised differentiation potential with suppressed expression levels of MyoD and desmins (muscle-specific intermediate filaments). Readdition of MyoD in the emerin-reduced myoblasts restores their differentiation potential (Frock *et al*. 2006). Similar results are also observed in myoblasts lacking A-type lamins (Frock *et al*. 2006). These results suggest that emerin and A-type lamins are involved in myogenesis by regulating the expression of a subset of myogenic factors.

In contrast to humans with emerin mutations, *emerin*-null mice show no anomalies in their skeletal or cardiac muscles (Melcon *et al*. 2006). However, loss of emerin compromises the muscle regeneration program (Bakay *et al*. 2006; Melcon *et al*. 2006). The differentiation of skeletal myoblasts largely depends on the phosphorylation status of the retinoblastoma protein (Rb) and acetylation status of MyoD (Puri *et al*. 2001). In normal undifferentiated myoblasts, Rb is hyperphosphorylated to ensure the E2F-dependent proliferation program, whereas MyoD, which forms a heterodimer with E proteins to bind an E-box sequence present in the regulator regions of its downstream target genes, forms a complex with class I histone deacetylases (HDACs) to repress the differentiation program. Upon differentiation, Rb is dephosphorylated and displaces HDAC1 from MyoD; the resulting Rb-HDAC1 complex then represses the E2F-dependent proliferation program. LAP2α and LAP2β, which can recruit HDACs and Rb to the nuclear lamina (Markiewicz *et al*. 2005; Somech *et al*. 2005), are thought to help establish repressive complexes. Conversely, to activate the differentiation program, MyoD must be acetylated by histone acetyltranferases.

Emerin deficiency leads to inappropriate timing of both Rb phosphorylation and myoD acetylation during muscle regeneration (Bakay *et al*. 2006; Melcon *et al*. 2006). In wild-type regenerating myoblasts, dephosphorylation of Rb is finished by day 4 after degeneration. In contrast, Rb remains in a higher phosphorylation state at day 4 in *emerin*-deficient conditions (Melcon *et al*. 2006), leading to a failure of downstream activation of MyoD targets at the appropriate time. It is unknown why emerin deficiency leads to the prolongation of hyperphosphorylated Rb levels.

Recently, a regulator of the expression of emerin and EDMD-relevant factors has been reported. Lim domain only 7 (Lmo7) was identified as an emerin-binding protein (Holaska *et al*. 2006). Endogenous Lmo7 is diffusely present in the nucleus and cytoplasm, being enriched at the cell surface and nuclear envelope, and requires emerin for its nuclear localization. Lmo7 may act as a shuttling protein between the cytoplasm and nucleus in a Crm/exportin-dependent manner. Interestingly, Lmo7 is required for emerin gene transcription. Consistent with this, RNAi-mediated downregulation of Lmo7 leads to misregulation of the genes known to be compromised in X-EDMD patients and *emerin*-null mice. Because mice harboring a genomic deletion including the *Lmo7* gene show a dystrophic phenotype, Lmo7 might be directly relevant to the pathogenesis of EDMD.

Emerin regulates nuclear morphology and signal transduction

Two mutually compatible hypotheses to explain the mechanism underlying X-EDMD have been proposed. The structural hypothesis proposes that emerin deficiency leads to increased nuclear fragility to render the nuclear envelope vulnerable to mechanical stress. The gene-regulation hypothesis proposes that emerin deficiency disrupts the interactions between emerin and a variety of transcription factors on the nuclear envelope to compromise gene regulation. Although *emerin*-null mice phenotypically do not serve as a good model for human X-EDMD, they have provided a clue to understand the molecular mechanism underlying the pathogenesis of X-EDMD, as discussed above. In human cells, *emerin*-deficient fibroblasts have an increased prevalence of irregularly shaped nuclei. However, the nuclear morphological abnormalities do not affect the nuclear mechanics or induce nuclear fragility; instead, *emerin*-null nuclei show an attenuated mechanotransduction response (Lammerding *et al*. 2005). In *emerin*-null cells, the expression of *egr*-1 (the mechanosensitive gene) and *iex-1* (the anti-apoptotic gene) decreases in response to mechanostress, and prolonged mechanical stimulation increases apoptosis in *emerin*-deficient cells. These results suggest that X-EDMD is not caused by increasing nuclear fragility, but by altered transcriptional regulation (Lammerding *et al*. 2005).

Emerin regulates β-catenin signaling by restricting its accumulation in the nucleus (Markiewicz *et al*. 2006). Emerin directly interacts with β-catenin through an adenomatous polyposis coli (APC)-like domain located in the nucleoplasmic domain of emerin. When emerin was expressed in cultured cells, nuclear β-catenin accumulation and β-catenin-dependent reporter transactivation was inhibited, whereas an emerin mutant lacking the APC-like domain increased nuclear accumulation and stimulated β-catenin activity. Treatment with leptomycin B, an inhibitor of Crm-1-dependent nuclear export, increases the amount of nuclear β-catenin in emerin-transfected cells, suggesting that emerin influences the nuclear export of β-catenin. Human fibroblasts from X-EDMD patients display an autostimulatory growth phenotype for a limited time in culture. This unusual growth phenotype correlates with enhanced nuclear accumulation and activity of β-catenin. Because Wnt signaling is involved in a wide variety of developmental processes (Clevers 2006), it will be intriguing to see whether emerin is involved in these processes.

MAN1

MAN1 was identified as one of three antigens recognized by autoantibodies from a patient with a collagen vascular disease (Paulin-Levasseur *et al*. 1996; Lin *et al*. 2000). A protein sequence comparison of MAN1 with two other INM proteins, LAP2β and emerin, led to the discovery of the LEM domain (described above). MAN1 also has two transmembrane domains followed by an evolutionally conserved C-terminal domain composed of two distinct segments: a winged helix-like globular domain, which directly binds to DNA *in vitro* (Caputo *et al*. 2006), and a protein-interaction domain termed, the U2AF homology motif (also known as the RNA recognition motif; Kielkopf *et al*. 2004).

MAN1 regulates SMAD signaling

SMAD proteins are central intracellular mediators of signaling for the transforming growth factor (TGF)-β superfamily of cytokines (Massague *et al*. 2005), which include two major types of signaling molecules: TGF-β/activin/nodal and bone morphogenetic proteins (BMPs). Upon ligand binding, the receptor-associated SMADs (R-SMADs) are phosphorylated by the active type I receptor serine/threonine kinases. Different utilization of R-SMADs determines the signaling specificity: TGF-β/activin/nodal signaling is mediated by SMAD2 and SMAD3, whereas BMP signaling is mediated by SMAD1, SMAD5, and SMAD8. The phosphorylated R-SMADs then form a heteromeric complex with the co-SMAD, SMAD4, and this complex then translocates into the nucleus where, along with other cofactors, it regulates the expression of numerous downstream target genes.

Xenopus laevis has played an important role in identifying a novel function of MAN1 in SMAD signaling. *Xenopus* orthologs of MAN1 was identified as a neuralizing factor (XMAN1; Osada *et al*. 2003) and as a SMAD1-interacting protein (SANE for SMAD1 antagonistic effecter; Raju *et al*. 2003). They induce neural tissue with anterior characterisrics in ectodermal explants without inducing mesoderm (i.e., neuralization), and a secondary axis containing somites in the ventral mesoderm (i.e., dorsalization). Both neuralization and dorsalization are good indicators of BMP antagonism during *Xenopus* neural development. Accordingly, XMAN1 inhibits BMP signaling and suppresses the expression of downstream targets of BMP signaling and BMP-dependent reporter activation downstream of the type I BMP receptor. The inhibitory activity of XMAN1 is mediated by direct interaction between its C-terminal region containing the U2AF homology motif and the MH2 (mad homology 2) domain of SMAD1 (Osada *et al*. 2003; Raju *et al*. 2003).

Human MAN1 also inhibits TGFβ/activin/nodal signaling by directly interacting with SMAD2 and SMAD3 through the C-terminal domain (Hellemans *et al*. 2004; Lin *et al*. 2005; Pan *et al*. 2005). In cultured cells, overexpression of MAN1 inhibits TGFβ-responsive reporter activation and antagonizes the arrest of TGFβ-induced cell proliferation. In the *Xenopus* embryo, XMAN1 inhibits mesoderm induction by TGFβ/activin/nodal signaling (Ishimura *et al*. 2006). The involvement of MAN1 in the regulation of TGFβ superfamily signaling has helped to identify the pathogenesis of human disorders caused by heterozygous loss-of-function mutations in MAN1, such as osteopoikilosis, Buschke-Ollendorff syndrome, and melorheostosis (Hellemans *et al*. 2004; **Table 2**). These diseases are characterized by hyperostotic bones and skin abnormalities such as disseminated connective tissue nevi. Notably, the associated mutations involve the deletion of the C-terminal SMAD-interacting domain. Fibroblasts from affected individuals, cultured cells with reduced level of MAN1 by RNAi, and mouse embryonic fibroblasts (MEFs) from *Man1*-deficent embryos show increased responsiveness to TGFβ1 and BMP (Hellemans *et al*. 2004; Pan *et al*. 2005; Cohen *et al*. 2007). The simple interpretation is that the bone and skin phenotypes observed in patients with MAN1 mutations may reflect enhanced BMP and TGFβ signaling.

MAN1 regulates vascular development

Two research groups independently generated *Man1*-deficient mice lacking the SMAD-interacting domain using an embryonic stem cell line containing a gene-trap insertion into the *Man1* locus (Ishimura *et al*. 2006; Cohen *et al*. 2007). Homozygous *Man1*-mutants die during early embryogenesis because of defects in vascular development. Although endothelial cells normally differentiate to build a primary capillary plexus in the mutants, subsequent vascular remodeling to form a well-branched mature capillary network does not occur in the yolk sac and in the embryo proper (**Fig. 3**). In addition, the recruitment of vascular smooth muscle cells to the vascular wall is severely disturbed in the mutants.

In *Man1*-deficient embryos, the regulation of SMAD signaling is severely perturbed (Ishimura *et al*. 2006; Cohen *et al*. 2007), probably because of a lack of the SMAD-interacting domain. Nuclear phosphoSMAD2 levels increased in mutant embryos and MEFs. In contrast, phosphoSMAD1 signals are similar to those in wild-type embryos, suggesting that SMAD2/3 signaling is preferentially enhanced in

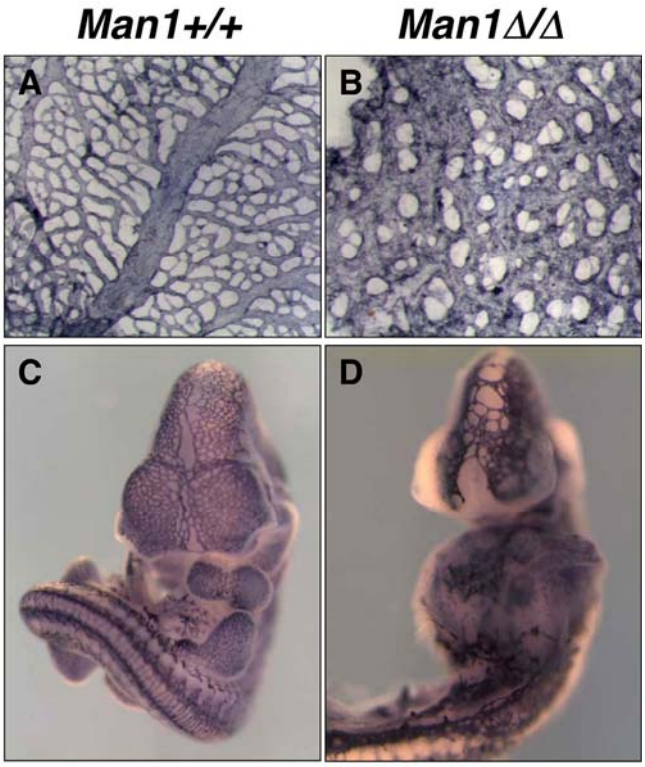

Fig. 3 Defective vascular remodeling in *Man1*-deficient embryo. Whole-mount immunostaining of the yolk sac (A, B) and embryo proper (C, D) at E10.0. with an antibody to platelet endothelial cell adhesion molecule 1. *Man1Δ/Δ* indicates homozygous *Man1*-deficient.

Man1-deficient embryos (Ishimura *et al*. 2006; Cohen *et al*. 2007). Accordingly, the expression of the downstream targets for SMAD2/3 signaling is up-regulated in the mutant embryos and MEFs, whereas SMAD1 targets are unchanged. Abnormally augmented SMAD2/3 signaling results in ectopic deposition of fibronectin, a major downstream target of this signaling, which may lead to the decreased migration of endothelial and vascular smooth muscle cells.

The mechanism by which SMAD2/3 signaling is preferentially augmented in *Man1*-deficient embryos is currently known. The expression of *TGFβ1* is also upregulated in the mutants (Ishimura *et al*. 2006), suggesting that SMAD2/3 signaling is autoactivated. MAN1 may regulate the entry or exit of R-SMADs by modulating their phosphorylation status on the INM. It will be important to examine whether MAN1 cooperates with recently identified nuclear SMAD phosphatases (Chen *et al*. 2006a; Knockaert *et al*. 2006; Lin *et al*. 2006).

MAN1 in other tissues

Heterozygous *Man1* mutant mice are viable and fertile, and do not show overt bone or skin abnormalities (Ishimura *et al*. 2006; Cohen *et al*. 2007), in contrast to human patients with heterozygous MAN1 mutations. Early embryonic death of *Man1*-deficient embryos because of the vascular defects hampers the examination of the role of MAN1 in these tissues. SANE inhibits BMP-mediated osteoblast differentiation *in vitro* (Raju *et al*. 2003).

MAN1 is expressed in the skeletal muscles and heart (Lin *et al*. 2000), both of which are primarily affected in EDMD. Whether MAN1 plays a role in myogenesis is unknown, but recent data that MAN1 directly interacts with emerin raise the possibility that emerin cooperates with MAN1 by positively or negatively regulating MAN1 binding to R-SMADs (Mansharamani and Wilson 2005). Myostatin, a member of the TGF-β superfamily, negatively regulates muscle growth by inhibiting myoblast proliferation and differentiation through SMAD3 (Langley *et al*. 2002). The role of MAN in myostatin signaling is unknown.

MAN1 transcripts are enriched in the CNS in *Xenopus* and mice (Osada *et al*. 2003; Ishimura *et al*. 2006; Cohen *et al*. 2007). In *Xenopus*, knockdown of XMAN1 by antisense morpholino oligos leads to anterior truncations and the suppression of anterior neural markers (Osada *et al*. 2003). Although initiation of neural induction and early anterior-posterior patterning of the CNS seems to be normal in *Man1*-deficient embryos (Ishimura *et al*. 2006), MAN1 may play an undefined role in later neural development.

LEM2

LEM2 was isolated *in silico* as a novel member of the LEM domain protein subfamily (Brachner *et al*. 2005) and was found to be identical to NET25, independently identified by a proteomics approach (Chen *et al*. 2006b). LEM2 is structurally related to MAN1 in that it contains containing an N-terminal LEM domain, two putative transmembrane domains, and a so-called MAN1-Src1p-C-terminal (MSC) domain (equivalent to the winged-helix-like domain described above; **Fig. 1**), although LEM2 lacks the SMAD-interacting domain present in MAN1. Consistent with this, LEM2 does not inhibit BMP signaling in *Xenopus* ectodermal explants (S. Osada, unpublished data). Targeting of LEM2 to the nuclear envelope requires interaction with A-type lamins, and NLSs on LEM2 may be involved in its energy-dependent localization to the INM (King *et al*. 2006). LEM2 overexpression induces patched accumulation of LEM2 on the nuclear envelope and tubular structures that interconnect nuclei of adjacent cells with unknown function (Brachner *et al*. 2005). In contrast, LEM2 knock-down by RNAi leads to abnormally shaped nuclei and reduced cell survival (Ulbert *et al*. 2006), suggesting that a proper level of LEM2 is essential for normal morphology of the nuclear envelope.

Although *LEM2* is expressed in various mammalian tissues (Brachner *et al*. 2005), a recent study shows that *LEM2* is expressed with strong preference in skeletal muscle and that its expression is significantly up-regulated during C2C12 myoblast differentiation (Chen *et al*. 2006b). In *C. elegans*, Ce-emerin and Ce-LEM2 (originally termed Ce-MAN1) are cooperatively involved in postmitotic nuclear assembly and cell survival (Liu *et al*. 2003). These results suggest that LEM2 plays an important role in myogenesis by itself or in collaboration with emerin, and that loss of LEM2 might be involved in pathogenesis of a human laminopathy-like disease.

Other LEM Proteins

Three additional LEM domain proteins, LEM3, LEM4, and LEM5, were isolated *in silico* in the human genome (Lee *et al*. 2004). LEM3 is an ortholog of *C. elegans* Lem3 with unknown function (Lee *et al*. 2000). Because LEM3 has no transmembrane domain, it is predicted to be soluble. LEM3 has three ankyrin repeats near its N terminus and a LEM domain in the middle of the protein. LEM4 has an N-terminal transmembrane domain followed by a LEM domain and two ankyrin repeats. The presence of ankyrin repeats, which mediate protein-protein interactions, in both LEM3 and LEM4, suggests that identification of their binding partners would help elucidate their functions.

LEM5 was independently found as LEM domain-containing 1 (LEMD1), which was isolated as one of the genes frequently up-regulated in colorectal carcinomas (CRCs) by a genome-wide cDNA microarray analysis (Yuki *et al*. 2004). The *LEMD1* gene gives rise to six alternatively spliced transcripts (A-F). The longest transcript A encodes a protein with an N-terminal LEM domain and a C-terminal putative transmembrane domain. *LEMD1* transcripts are enriched in testes, but only transcript F is expressed in CRCs. When LEMD1 and BAF were transiently co-expressed in cultured cells, they are co-localized at the nuclear envelope, suggesting that, like other LEM domain proteins, LEMD1 interacts with BAF through its LEM domain. Exclusive ex-

pression of *LEMD1* in CRCs and testes raises the possibility that LEMD1 is a novel member of testis-cancer antigens (Zendman *et al*. 2003), which are considered promising targets for tumor immunotherapy. The role of LMED1 in tumorigenesis remains to be elucidated.

Nurim

Nurim (nuclear rim) was identified as a novel nuclear membrane protein from a visual screen of a GFP-fusion library for identifying proteins localized to specific subcellular compartments (Rolls *et al*. 1999). Nurim is a six transmembrane-spanning protein (Hofemeister and O'Hare 2005). The most C-terminal transmembrane domain is likely to be bipartite and forms a hairpin turn, making the N and C termini reside on the same side of the membrane (**Fig. 1**). Because it remains associated with the nuclear membrane even after nuclear lamins are completely extracted, unlike other INM proteins, nurim does not seem to require interaction with lamins for its nuclear envelope localization. Although its role is still unknown, nurim shows significant similarity to the enzyme family of isoprenylcysteine carboxymethyltransferases, which are involved in the processing of proteins containing a C-terminal CAAX motif such as Ras oncoprotein and nuclear lamins (Hofemeister and O'Hare 2005). It would be interesting to see whether nurim is involved in the processing of lamins or the etiology of laminopathies.

Dullard

Dullard was first identified as a novel gene expressed in neural tissues by a whole mount *in situ* hybridization screening in *Xenopus* (Satow *et al*. 2002). *Dullard* mRNA is maternally present and is expressed exclusively in the animal hemisphere at blastula stages. It becomes restricted to neural tissues, branchial arches, and pronephros at neurula stages. Morpholino-mediated depletion of dullard causes abnormal neural development with defective neural tube closure and reduced head development. Consistent with this, the expression of early and late neural markers is suppressed severely in morpholino-injected embryos, suggesting that dullard is required for neural development in *Xenopus* (Satow *et al*. 2002; 2006).

Dullard is a C-terminal domain serine/threonine phosphatase (Satow *et al*. 2006; Kim *et al*. 2007) and dephosphorylates the mammalian phosphatidic acid phosphatase, lipin (Satow *et al*. 2006; Kim *et al*. 2007). Lipin produces diacylglycerol, a major second messenger in signal transduction, in mammalian cells (Carman and Han 2006).

Because inhibition of BMP signaling is essential for neural induction in *Xenopus*, BMP inhibitory activity of dullard was examined in *Xenopus* ectoderm and mammalian cultured cells to find that dullard inhibits BMP signaling by two different mechanisms (Satow *et al*. 2006). Dullard preferentially interacts with the BMP type II receptor (BMPRII) to recruit it to the caveolin-dependent endocytic pathway and promotes ubiquitin-mediated degradation of the BMPRII. Although the phosphatase activity of dullard seems to be required for the degradation process, the mechanism by which dullard facilitates ubiquitination of the BMPRII is unknown. Dullard also directly binds to the BMP type I receptor (BMPRI) and dephosphorylates it, although dullard shows higher binding affinity to BMPRII than to BMPRI. Dullard also promotes dephosphorylation of BMP-responsive R-SMADs. Because it is not present in the nucleus, dullard unlikely acts as a nuclear R-SMAD phosphatase (Satow *et al*. 2006).

Endogenous dullard is localized to the nuclear envelope (Kim *et al*. 2007), consistent the observation with that dullard is identical to NET56, which was identified as a putative nuclear envelope protein by a proteomics approach (Schirmer *et al*. 2003). However, dullard seems to function as a BMP inhibitor exclusively in the cytoplasm (Satow *et al*. 2006). Additional work is needed to clarify the relationship between the subcellular localization of dullard and its phosphatase activity in BMP signaling and phosphatidate signaling. The role of dullard in mammalian development has not been reported.

Nemp1

Nemp1 (nuclear envelope integral membrane protein 1) was isolated from a systemic screening for genes involved in *Xenopus* neural development (pers. comm., Dr. M. Taira, University of Tokyo). Its name is derived from the fact that Nemp1 localizes exclusively to the nuclear rim, when transiently expressed in COS cells, although its precise localization in the nuclear envelope has not been determined.

Nemp1 shows a unique expression pattern during *Xenopus* development: its transcript is enriched in the eye anlagen during neurulation and becomes restricted to the eyes at the tailbud stages. Nemp1 orthologs have been identified throughout the animal kingdom, suggesting that the function of Nemp1 is evolutionally conserved. The role of Nemp1 in eye development is under investigation.

UNCL

A genetic screen for *C. elegans* mutants resistant to the acetylcholinesterase inhibitor, levamisole, identified *unc-50*, in which muscle nicotinic acetylcholine receptors (nAchRs) fail to assemble, resulting in an "uncoordinated (*unc*)" phenotype. UNCL (UNC+L, Roman numeral for 50) was identified as a mammalian ortholog of UNC-50 (Fitzgerald *et al*. 2000). UNCL has a LEM-like domain and an RNA recognition motif (RRM) near its N terminus followed by five putative transmembrane domains. When transiently expressed in cultured cells, UNCL shows a nuclear rim-staining pattern that is resistant to 1% Triton X-100 extraction, suggesting that UNCL is an INM protein. Consistent with the presence of an RRM, UNCL binds RNA homopolymers *in vitro*. Coexpression of UNCL with neuronal nAchRs in *Xenopus* oocytes and cultured cells increases the expression of functional cell surface receptors, implying that UNCL is required for nAchR assembly. Because UNCL is expressed in tissues devoid of nAchR, UNCL may have a broader role. Human disorders caused by mutations in UNCL or UNCL-deficient mice have not yet been reported.

LUMA

LUMA was identified as a putative integral membrane protein with a mass of 45 kDa from the Triton X-100-resistant nuclear envelope fraction using a subcellular proteomics approach (Dreger *et al*. 2001). LUMA is predicted to have three or four transmembrane segments, and, when transiently expressed, is localized to the nuclear rim. Its precise topology within the nuclear envelope, its expression profiles during development, and its function have not yet been reported.

RING finger binding protein

RING finger binding protein (RFBP) was identified as a binding partner for the RING motif of an SWI/SNF-related transcription factors (Mansharamani *et al*. 2001). RFBP is an atypical type IV P-type ATPase, which may act as a phospholipids pump. RFBP seems to be integrated into the INM through nine putative transmembrane segments. *RFBP* mRNA is expressed in various adult mouse tissues and its expression is hormonally regulated. The function of RFBP is unknown.

NETs

Nuclear envelope transmembrane proteins (NETs) were identified from liver by a subtractive proteomics approach (Schirmer *et al*. 2003). The screen identified 67 novel putative NETs, although the decisive localization in the nuc-

lear envelope has not been clarified for many of them.

Expression of six NETs (NETs 9, 14a, 25, 32, 37, and 39) increase significantly in mouse C2C12 cells during differentiation from myoblasts to myotubes (Chen *et al*. 2006b). All six NETs are highly expressed in adult mouse skeletal muscle. NET9 and NET25 are identical to LULL1 and LEM2, respectively. NET32 has an SPFH consensus sequence and may be implicated in the formation of lipid rafts, membrane macrodomains that mediate signal events. NET37 and NET39 are strongly induced during C2C12 differentiation and show homology to glycosidase and phosphatidic acid phosphatase type 2, respectively. NETs exclusively enriched in liver, adipocytes, or blood cells were also isolated using tissue-specific proteomics (Schirmer and Gerace 2005).

CONCLUDING REMARKS

As described here, there has been great progress in clarifying the functional roles of integral nuclear membrane proteins in nuclear structure, nuclear positioning, gene transcription, and signal transduction, and in understanding their roles in development of the skeletal muscle, the vascular system, the neuromuscular junctions, and the CNS. However, most of these proteins are also present in other tissues where they may have similar or novel functions. To discover their functions and clarify their roles in other tissues will require identification of their binding partners, particularly the partners whose expression is tissue-specific.

Generation of animal models deficient in or expressing mutant proteins using homologous recombination or a transgenic approach will be useful in analyzing the functional roles of integral nuclear membrane proteins at the whole-organism level and in understanding the pathogenesis of human diseases caused by mutations in these proteins. However, as exemplified by nesprins, genes encoding some integral nuclear membrane proteins express a variety of splicing variants. In addition, genetically altered mice do not always recapitulate the features of human disease.

More work is needed to understand how nuclear envelope proteins influence transcriptional repression and activation at the whole-genome level. In *Drosophila*, approximately 500 genes that depend on B-type lamins for their correct expression have been identified by a genome wide approach (Pickersgill *et al*. 2006). These genes are transcriptionally silent and late replicating, and they form clusters in the genome, which, interestingly, are expressed coordinately during development.

The delay in activation of the muscle regeneration program in *emerin*-null mice and sustained enhancement of SMAD2/3 signaling in *Man1*-deficient mouse embryos tell us the importance of "timing" during development. The regulation of timing relates closely to the determination of "competence", the ability to respond to stimuli at the right time and right place during development. In *Xenopus*, the responsiveness of the ectoderm to activin is strictly restricted (Grimm and Gurdon 2002) and inappropriate stimulation by activin fails to induce mesodermal tissues.

Future studies in the field of integral nuclear membrane proteins will undoubtedly expand our knowledge about how intracellular structures (nuclear membranes, cytoskeletons, mitochondria, and Golgi apparatus) communicate with each other to execute physiological functions, and how disruption of this communication causes a variety of diseases.

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