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The nuclear envelope LEM-domain protein emerin

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Emerin, a conserved LEM-domain protein, is among the few nuclear membrane proteins for which extensive basic knowledge—biochemistry, partners, functions, localizations, posttranslational regulation, roles in development and links to human disease—is available. This review summarizes emerin and its emerging roles in nuclear "lamina" structure, chromatin tethering, gene regulation, mitosis, nuclear assembly, development, signaling and mechano-transduction. We also highlight many open questions, exploration of which will be critical to understand how this intriguing nuclear membrane protein and its "family" influence the genome.

Emerin: One Charted "Island" in the Unexplored Archipelago of Nuclear Membrane Proteins

The nuclear envelope (NE) comprises two membranes (inner and outer) with embedded nuclear pore complexes (NPCs) and underlying networks of nuclear intermediate filaments formed by A-type and B-type lamins.[1,2] The NE membrane proteome is large and diverse, and includes both ubiquitous and potentially cell type-specific ("unique") proteins. Nearly 200 unique NE transmembrane ("NET") proteins were identified in proteomic studies of three cell types: rat liver (67 proteins),[3] rat skeletal muscle (29 proteins)[4] and human leukocytes (87 proteins).[5] Among leukocyte NETs, 27% were unique to either resting or activated leukocytes.[5] Different subsets of NETs contribute to spatial control of the genome,[6] cell cycle regulation,[7] or cytoskeletal organization,[4] through unknown mechanisms. The functions of most NE membrane proteins are unexplored. The exceptions include NE membrane proteins that possess either a KASH-domain or SUN-domain (which form LINC [links the nucleoskeleton and cytoskeleton] complexes),[2] and the LEM-domain family of proteins, named for founding members Lap2, emerin and Man1.[8,9] This review will focus on emerin, an extensively studied LEM-domain protein.

Emerin is a Conserved LEM-Domain Protein

The LEM-domain is a ~40-residue helix-loop-helix fold conserved both in eukaryotes and in prokaryotic DNA/RNA-binding proteins.[10] With one exception (Lap2 proteins have a second LEM-domain that binds DNA),[10] eukaryotic LEM-domains have one known function: they directly bind a conserved chromatin protein named barrier-to-autointegration factor (BAF).[11-14] Atomic structures have been solved for the emerin LEM-domain (residues 1–47) either alone,[15,16] or in complex with BAF.[15]

Human LEM-domain proteins are encoded by seven genes.[9,17] These proteins and genes have acquired many names; for clarity this review will employ the "generally used" protein names indicated in **Table 1**. LEM-domain proteins are conserved in both multicellular and single-celled members of the *Opisthokont* lineage of eukaryotes, which includes fungi and multicellular animals ("metazoans").[18] For example the nematode worm *C. elegans* genome encodes three proteins orthologous to human emerin, Lem2 and Ankle1.[19] The evolution of the LEM-domain has been thoughtfully discussed.[20,21] In metazoans, at least, the LEM-domain appears to have a fundamental role in tethering chromatin to the NE. The fission yeast *S. pombe*, which lacks lamins and BAF, encodes two LEM-domain proteins orthologous to Lem2 and Man1.[21,22] Man1 enriches with Swi6 (orthologous to human heterochromatin protein 1 [Hp1]) near telomeres.[23] Overexpression of the LEM- ("Heh"-) domain of either Man1 or Lem2 causes chromatin to compact near the spindle pole body,[22] consistent with the competitive release of telomeres (not centromeres) from sites of attachment at the NE.[23] The conservation of LEM-domain proteins in yeast, apparently independently of both BAF and lamins, suggests LEM-domain proteins are intrinsically important for genome organization and nuclear structure.

Emerin Contributes to Nuclear "Lamina" Structure and Function in Multicellular Animals

Emerin and several other LEM-domain proteins (e.g., Lap2β, Lem2, Man1) are integral membrane proteins that localize predominantly at the NE inner membrane. These LEM-domain proteins bind directly to lamins (nuclear intermediate filament proteins) and BAF,[24] together forming a major component of NE-associated nucleoskeletal structure known as the nuclear "lamina"[2,25] (**Fig. 1**). The structural inter-dependence of this "trio" of components was revealed by downregulating either lamin or BAF-1, or both Emr-1 and Lem-2, in *C. elegans* embryos— if any one component was missing, the other two failed to co-assemble, with severe consequences for mitotic chromosome segregation and postmitotic

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Table 1. Human LEM-domain gene and protein nomenclature

NCBI Gene Symbol	HGNC Gene Symbol (name)	Gene AKA	Gene Aliases	Generally used protein name(s)
LEMD1	LEMD1 (LEM domain containing 1)	LEM1	LEMP-1, CT-50	Lem1, Lem5
LEMD2	LEMD2 (LEM domain containing 2)	LEM2	NET25, dJ482C21.1	Lem2
LEMD3	LEMD3 (LEM domain containing 3)	MAN1	MAN1	Man1
LEMD4	TMPO (Thymopoietin)	LAP2	TP, LAP2, CMD1T, LEMD4, PRO0868, MGC61508	Lamina associated polypeptide 2 (Lap2α, β, γ, δ, ε or ζ)
LEMD5	EMD (Emerin)		LEMD5, STA, EDMD	Emerin
LEMD6	ANKLE1 (Ankyrin repeat and LEM domain containing 1)	LEM3	LEM3, LEMD6, ANKRD41, FLJ39369	Ankle1 or Lem3
LEMD7	ANKLE2 (Ankyrin repeat and LEM domain containing 2)	LEM4	LEMD7, FLJ22280, FLJ36132, KIAA0692	Ankle2

NCBI, National Center for Biotechnology Information; HGNC, Human Gene Nomenclature Database.

nuclear assembly.[26-28] In mammalian cells certain LEM-domain proteins localize intriguingly, and dynamically, during mitosis. Lap2α (which is soluble, not membrane anchored) and BAF co-localize on telomeres during anaphase, and during telophase form "core" structures on chromatin at specific regions of nuclear envelope assembly near the spindle pole.[29] These "core" structures transiently recruit and concentrate BAF, emerin and A-type lamin(s), and are distinct from neighboring "non-core" regions enriched in Lap2β, LBR and B-type lamins.[29-31] "Core" regions are NPC-free, whereas "non-core" regions are NPC-rich.[32] The mitotic roles of emerin and other LEM-domain proteins in mammals are major open questions. Further exploration is needed both to define these mitotic roles, which may be shared by multiple LEM-domain proteins (**Table 1**), and to determine their impact on genome activity, since mitosis appears to be crucial for LEM-domain proteins to establish functional (repressive) contact with silent chromatin.[33]

Emerin and Other LEM-domain Proteins Organize and Tether Chromatin at the NE

Functional overlap is a major theme for LEM-domain proteins. In *C. elegans* the two NE-localized LEM-domain proteins, Emr-1 and Lem-2, have overlapping roles in nuclear structure, mitosis and development,[34] and are co-essential for viability.[26] The two fission yeast proteins, Lem2 and Man1, localize at the NE inner membrane and are co-essential for nuclear structure.[22] In mice, emerin and Lem2 have overlapping roles, along with Man1, in the regulation of MAP kinase signaling during myoblast differentiation.[35] Emerin can also bind the N-terminal domain of Man1 directly,[14] but whether or how this affects their functions is unknown. Among 16 proteins that bind emerin directly (discussed extensively below) are three "shared" partners (in addition to lamins and BAF) that also bind at least one other LEM-domain protein. These shared partners are Btf (BCL-associated transcription factor 1 [BCLAF1]),[36] germ cell-less (GCL)[37] and the chromatin-silencing enzyme HDAC3 (histone deacetylase 3). HDAC3 directly binds emerin[38] and Lap2β.[39] HDAC3 and the transcription factor cKrox co-mediate silent chromatin tethering to Lap2β at the NE; as noted above, these tethering complexes are established during mitosis,[33] when the nucleoskeleton undergoes complex and dynamic reorganization and then reassembles nuclear structure.[2] In all, four LEM-domain proteins—emerin,[40] Lap2β,[33] Otefin (in *Drosophila*)[41] and Lem-2 (in *C. elegans*)[42]—along with A- and B-type lamin filaments[40,43] mediate chromatin organization and tethering at the NE (**Fig. 2**). These discoveries are providing unique and unexpected insight into genome biology.[44]

Discovery of Emerin: Loss of Function Causes Emery-Dreifuss Muscular Dystrophy

The emerin gene (originally *STA*; renamed *EMD*) was identified in 1994 by genetic mapping[45] of X-linked recessive Emery-Dreifuss muscular dystrophy (X-EDMD; Emery and Dreifuss, 1966).[46] EDMD is characterized by contractures of major tendons, slowly progressive skeletal muscle wasting and weakness, and dilated cardiomyopathy with potentially lethal ventricular conduction system defects that can cause sudden cardiac arrest.[47] In rare cases, *EMD* mutations cause limb-girdle muscular dystrophy or severe cardiac conduction defects.[48,49] Two years after X-EDMD was genetically mapped came a surprise: emerin was revealed as a NE membrane protein.[50,51] Emerin was the harbinger of a new category of human disease ("laminopathies") caused by mutations in lamins or lamin-binding proteins.[52,53] Indeed the emerin and lamin "proteomes" have become a rich source of candidate disease genes. For example EDMD is also caused by mutations in at least five other genes: *LMNA* (A-type lamins; numerous mutations reported),[54] *SYNE-1* (nesprin-1; three reported mutations),[55] *SYNE-2* (nesprin-2; one reported mutation),[55] *TMEM43* (LUMA; two reported mutations)[56] or *FHL1* (four-and-a-half LIM domains 1; seven reported mutations).[57] Four of these proteins (FHL-1 is untested) interact

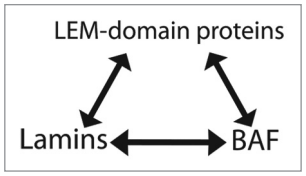


Figure 1. Nuclear "lamina" structure has three fundamental components: lamins, LEM-domain proteins and BAF (barrier-to-autointegration factor). These components bind each other with nanomolar affinity in vitro (see text). In *C. elegans*, loss of any one component (lamin or BAF, or two LEM-domain proteins [Emr-1 and Lem-2]) disrupts co-assembly of the other two and hence blocks nuclear reassembly after mitosis.

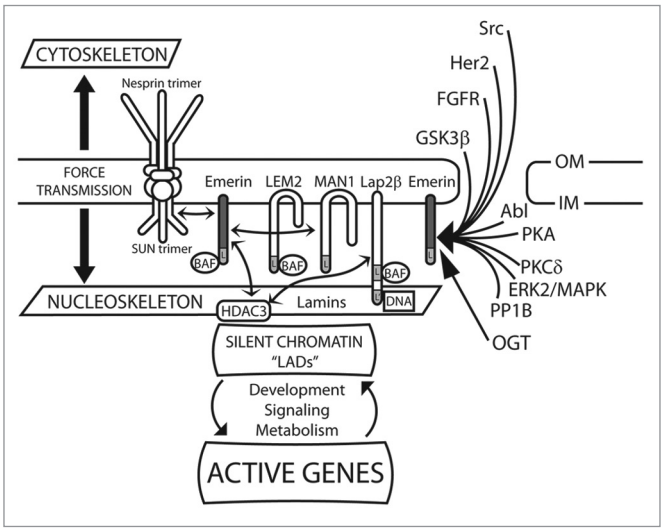


Figure 2. Schematic depiction of the regulation, partners and selected functions of emerin at the nuclear envelope. Depiction of emerin and other LEM-domain proteins (Lem2, Man1 and Lap2β) at the inner membrane (IM) of the nuclear envelope. Double-headed arrows connect direct binding partners, including emerin (dark gray), SUN-domain proteins, BAF, HDAC3 and Man1. Direct binding to lamins is not indicated. Emerin has roles in signaling, mechano-transduction, nuclear architecture, chromatin tethering and gene regulation. Also depicted are enzymes and pathways that directly target or regulate emerin. "L" indicates the LEM-domain. "L-prime" [L'] in Lap2β indicates the DNA-binding "LEM-like" domain. OGT, *O*-GlcNAc transferase. OM, outer membrane.

with each other, and with emerin, suggesting EDMD disease is caused by the disruption of NE-anchored "links the nucleoskeleton and cytoskeleton" (LINC) complexes that include these proteins[2,55,58] (**Fig. 2**). In contrast to nesprins, SUN-proteins and lamin A, all of which directly transmit mechanical force,[59,60] emerin is required to sense force and activate the downstream mechano-sensitive genes *IEX-1* and *EGR-1*.[61] The mechanisms by which emerin senses and signals mechanical force are unknown.

Emerin is expressed in essentially all tissues.[62] This suggests the relative mildness of EDMD disease, which mainly affects striated muscle, may be due to the presence of another LEM-domain protein(s) that "backs up" or compensates for emerin loss, as seen for emerin and Lem2 during mouse myoblast differentiation.[35] "Backup" function might also be provided by unidentified isoform(s) of Lap2, since the Lap2 gene (*TMPO*; **Table 1**) is upregulated in EDMD patient muscle,[63] and a mutation in LAP2α is genetically linked to cardiomyopathy.[64]

Emerin Biogenesis and Nuclear Envelope Localization

Emerin is a 254-residue type-II integral membrane protein with a proposed 23-residue hydrophobic (transmembrane) domain near the C-terminus, and a tiny (11-residue) lumenal domain. Consistent with this domain organization, newly synthesized (presumably soluble) emerin polypeptides in the cytoplasm are inserted into the endoplasmic reticulum (ER) membrane post-translationally,[65,66] possibly mediated by ATP-dependent TRC40/Asna-1 complexes that mediate the "guided entry of tail-anchored" (GET) pathway.[67,68] Once inserted, emerin diffuses throughout the contiguous membranes of the ER/NE, including the "pore membrane" surrounding each NPC, where the outer and inner NE membranes are connected. Extensive FLIP and FRAP studies showed membrane-anchored emerin easily "slides past" the NPC because its cytoplasmically-exposed domain is small (~25 kD).[66,69] Proteins with larger exposed domains (> 60 kD) fail to accumulate at the inner membrane.[70] Alternative mechanisms to reach the inner membrane[71,72] may not apply to emerin; emerin lacks "FG-repeats" and its predicted nuclear localization signal (residues 35–47)[73,74] is not required for nuclear import,[75] as discussed below (**Fig. 3**). Having reached the inner membrane, evidence suggests emerin is retained and accumulated by binding A-type lamins,[76] for which human emerin has high (40 nM) affinity in vitro (**Fig. 4**).[77] Note that emerin retention might alternatively or additionally require another partner disrupted by loss of A-type lamins. By FRAP analysis the diffusion constant of GFP-emerin at the NE is three times slower than the ER (0.10 ± 0.01 vs. 0.32 ± 0.01 μm^2/second respectively).[66] In cells that lack A-type lamins, emerin is more mobile and distributes equally throughout the NE and ER,[69,76] supporting diffusion–retention models for emerin localization at the NE inner membrane.[66,69]

On the other hand, subpopulation(s) of emerin appear to localize elsewhere, including the NE outer membrane and the plasma membrane (see below). Outer membrane localization might be achieved by binding to high-affinity partners (e.g., nesprin-1 isoforms; **Fig. 4B**) at the NE outer membrane. Unconventional destinations (e.g., plasma membrane) might be achieved, we speculate, either by (1) direct posttranslational insertion of nascent emerin into alternative membrane(s), (2) diffusion onto ER vesicle membranes and trafficking to the plasma membrane, or (3) a hypothetical mechanism that "hides" the hydrophobic domain and thereby allows nascent emerin to associate as a soluble protein with partners outside the nucleus.

Unconventional Locations for Emerin Include the Intercalated Discs (ICDs) of Cardiomyocytes

Emerin localizes predominantly at the NE in skeletal and cardiac muscle.[50,51] Similarly, immuno-gold EM labeling and digitonin

studies showed emerin is abundant at the NE inner membrane in HeLa cells (e.g., ref. 78) and COS7 cells (e.g., refs 66 and 75). Emerin has also been detected in the cytoplasm (presumably ER) of various tissues and cell types,[51,74,79,80] the NE outer membrane[81] and ER (consistent with its known biogenesis) and—most unexpectedly—the plasma membrane. The main caveat, in each case, is the specificity of emerin detection, since antibodies might recognize similar or identical epitopes on other proteins including other LEM-domain proteins, some of which are located in the cytoplasm (e.g., Lap2α and Lap2ζ).[9]

Emerin was detected at the NE outer membrane and on ER-Golgi intermediate compartment ("ERGIC") vesicles in human dermal fibroblasts, and can also bind β-tubulin directly in vitro.[81] The microtubule organizing center ("centrosome"), normally located ~1.5 μm from the NE, was more distant in emerin-null X-EDMD patient fibroblasts and in emerin-downregulated human dermal fibroblasts (average distance > 3.0 μm).[81] Whether emerin influences centrosome positioning via LINC complexes, emerin-dependent gene misregulation or other mechanisms, and its potential implications for mitosis are open questions.

Endogenous emerin was detected at the plasma membrane in rat cardiomyocytes and in heart tissue from human, rat and mouse.[82-84] One affinity-purified polyclonal antibody detected emerin at adhesive junctions of ICDs in human heart cryosections, both by indirect immunofluorescence and immunogold EM.[82] Another study screened 15 monoclonal emerin antibodies (mAbs) by indirect immunofluorescence staining of ICDs: two were clearly positive, and five were faintly positive;[83] note that lack of staining is inconclusive since specific epitopes might be masked by ICD-specific partners or posttranslational modifications. Further evidence was obtained using an affinity-purified antibody (APS20) raised in sheep against rat emerin residues 114–183, which detected the adherens junctions of rat heart tissue by immuno-gold EM labeling and indirect immunofluorescence staining.[84] These findings suggest at least two locations for emerin in cardiomyocytes: the NE inner membrane and the adherens junctions of ICDs. How emerin localizes or functions at adherens junctions, which mechanically interconnect neighboring cardiomyocytes,[85] and whether loss of this function contributes to EDMD heart pathology, are critical open questions. Of note, two direct partners of emerin, namely β-catenin[84] and Lmo7,[86] also localize at cardiac adherens junctions.

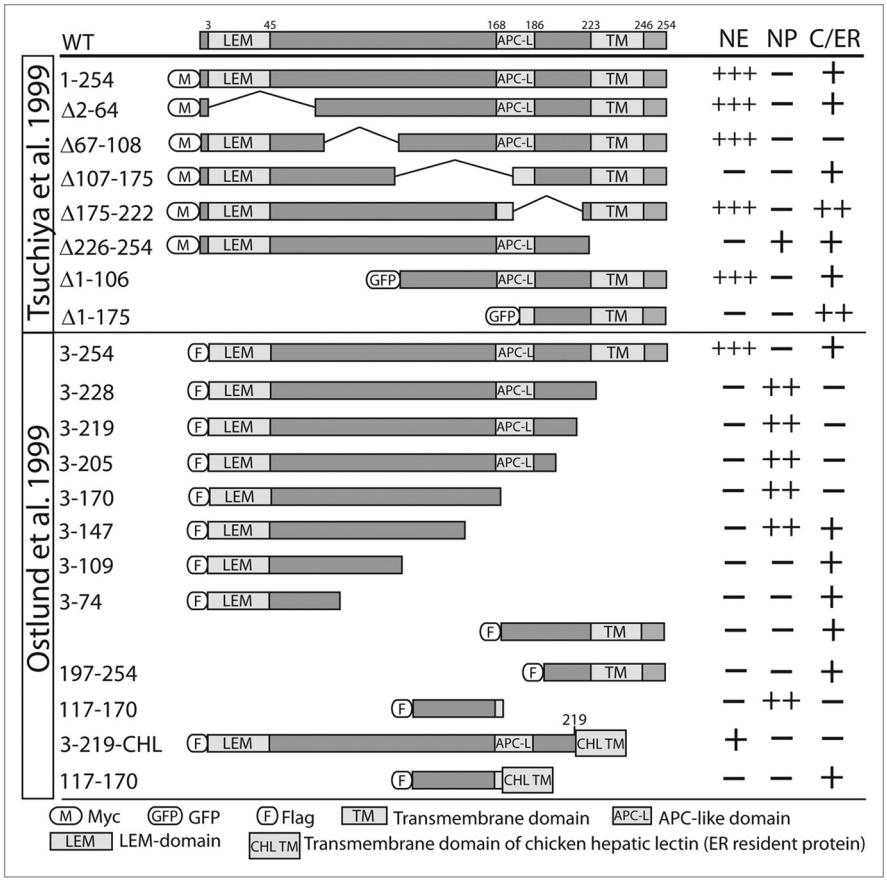
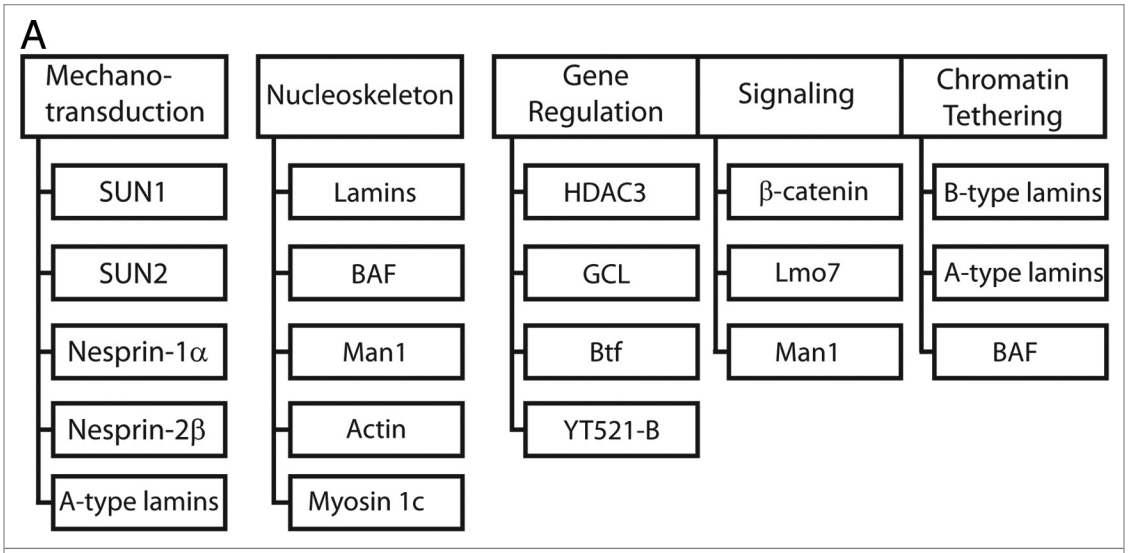


Figure 3. Localizations of epitope-tagged emerin polypeptides in cells. Summary of the localization of epitope-tagged emerin polypeptides (truncations, internal deletions or chimeras) expressed transiently in mammalian cells.[66,75] *WT*, wildtype emerin; *M*, myc-tag; *GFP*, green fluorescent protein; *F*, flag tag. Plus or minus indicate polypeptide localization predominately at the nuclear envelope (NE), nucleoplasm (NP) or cytoplasm/endoplasmic reticulum (C/ER), compared with full-length emerin (residues 1–254 or 3–254).

NE-Targeting Regions Identified by Expressing Emerin Polypeptides in Cells

In two studies, cells that expressed truncated or internally-deleted emerin polypeptides were stained by indirect immunofluorescence to assess potential localization in the cytoplasm/ER ("C/ER") or nucleoplasm ("NP"), or enrichment at the NE,[66,75] as summarized in **Figure 3**. Emerin residues 3–219 (comprising almost the entire nucleoplasmic domain) concentrate at the NE inner membrane when fused to the transmembrane and lumenal domains of chicken hepatic lectin (CHL), a type II integral membrane protein normally found in the ER, endosomes and plasma membrane (**Fig. 3**, Emerin-CHL).[66] Emerin residues 107–175 were required to block emerin aggregation in the cytosol (**Fig. 3**, Δ107–175).[75] NE enrichment was reportedly unaffected by loss of residues 2–64, residues 1–106 or residues 175–222, and was slightly improved by deleting residues 67–108 (**Fig. 3**).[75] This study also showed the full C-terminal half of emerin (residues 107–254) was sufficient to enrich at the NE (**Fig. 3**, GFP-Δ1–106).[75]



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	Partner	Affinity	Reference
High Affinity	Nesprin-1α	4 nM	Mislow et al., 2002
	GCL	30 nM	Holaska et al., 2002
	Lamin A	40 nM	Holaska et al., 2002
	Btf	100 nM	Haraguchi et al., 2004
	Lmo7	125 nM	Holaska et al., 2006
	BAF	200 nM	Holaska et al., 2002
	F-actin	300-500 nM	Holaska et al., 2004
Low Aff.	HDAC3	7.3 uM	Demmerle et al., 2012
Not Meas.	YT521b	?	Wilkinson et al., 2003
	MAN1	?	Mansharamani & Wilson, 2005
	Nesprin-2	?	Zhang et al., 2005
	Nuclear myosin IC	?	Holaska & Wilson, 2005
	β-catenin	?	Markiewicz et al., 2006
	β-tubulin	?	Salpingidou et al., 2007
	SUN1	?	Haque et al., 2009
	SUN2	?	Haque et al., 2009

Figure 4. Direct binding partners of emerin. (**A**) Proteins that bind emerin directly in vitro, grouped based on their known or proposed functions in mechanotransduction, nucleoskeleton, gene regulation, signaling or chromatin tethering. (**B**) Direct partners and equilibrium binding affinity for human emerin in vitro, if known.

Emerin residues 3–205 and 3–147 accumulated in the nucleoplasm, whereas residues 3–109 remained in the cytoplasm; this suggested residues 110–147 either possess a non-canonical NLS, or associate with an NLS-bearing partner ("piggyback" import; **Fig. 3**).[66] Residues 117–170 are indeed sufficient for import (**Fig. 3**),[66] but the mechanism and relevance to membrane-anchored emerin are unknown. Piggyback mechanisms are possible, since mutations in this region (residues 117–170) disrupt binding to HDAC3, actin and lamin A (see below), all of which are imported into the nucleus as soluble proteins. In summary, the mechanisms of emerin enrichment at the NE are not yet understood, and are likely to involve a partner(s) other than lamins (e.g., nuclear protein 4.1R, discussed later).

Direct Partners and a Mutagenesis-Based Map of Functional Regions in Emerin

In addition to binding lamin A and BAF (nuclear "lamina" components), human emerin directly binds at least 14 other proteins (**Fig. 4**). Emerin has no known secondary structure other than its LEM-domain (residues ~4–44)[16] and transmembrane domain (residues 223–246). To identify functional regions, two sets of clustered Ala-substitution mutations were generated in recombinant emerin ("m-series" mutations): one set targeted residues homologous or identical between emerin and LAP2β, postulated to mediate conserved or shared functions (**Table 2**).[13] The second set of mutations targeted residues that differ between emerin and LAP2β; these were predicted to disrupt emerin-specific functions (**Table 3**).[37] Also tested were four human mutations (S54F, Q133H, P183H, deletion of residues 95–99 [Δ95–99]) that were unusual: each is sufficient to cause emerin-null EDMD disease, even though the mutant protein localizes normally and is expressed at normal or near-normal (~60%) levels.[87,88] Emerin polypeptides bearing these various mutations have been tested in vitro for binding to as many as eight different partners: BAF, lamin A, GCL, Btf, YT521-b, Lmo7, HDAC3, F-actin and Man1 (**Fig. 5A**). This research yielded a functional map based on the locations of mutations that disrupt binding to each partner (**Fig. 5B**).

Emerin Biochemistry and the Functional Implications of Known Partners

Few NE membrane proteins other than emerin have been studied at the biochemical level. The equilibrium binding affinity of emerin has been measured for eight partners in vitro (**Fig. 4B**). Human emerin binds with relatively high (4–500 nM) affinity to each of seven partners (nesprin-1α, GCL, lamin A, Btf, Lmo7, BAF and F-actin) and with lower affinity to HDAC3 (7.3 μM; **Fig. 4B**). Competition studies showed BAF and GCL compete with each other for binding to emerin, whereas GCL and lamin A can co-bind emerin.[37] Six distinct emerin-containing multiprotein complexes were purified from HeLa cell nuclei, suggesting emerin might scaffold a variety of multi-protein complexes at the NE.[89] We are still far from understanding these complexes or their functions. However studies of proteins that bind emerin, particularly lamin A, BAF, HDAC3 and GCL, discussed below, are beginning to illuminate daily "life" (protein-protein interactions) at the nuclear envelope.

Emerin binds structural components of both the NE (e.g., SUN1, SUN2, nesprins)[90,91] and the nucleoskeleton (lamins, actin)[77,92] (**Fig. 4**). Emerin also directly binds signaling transcription factors including β-catenin and Lmo7 (**Fig. 4**).[93,94] These various partners suggest emerin might integrate a variety of mechanical and signaling "inputs," and by unknown mechanisms convert these inputs into situation- or tissue-appropriate changes in gene activity. Indeed genes that are normally activated in response to mechanical force, fail to activate in emerin-deficient cells.[61] Selected partners and their functional implications for emerin and "life at the NE" are summarized below.

Table 2. Summary of mutations in "conserved" emerin residues identical or homologus in Lap2β[13]

Name	Wildtype	Mutated
M11	11EL	11AA
M24	24GPVV	24AAA
M30	30TR	30AA
M34	34YEKK	34AAA
S54F*	54S	54F
m70	70DADMY	70AAMA
m76	76LPKKEDAL	76PAKADAA
m112	112GPSRAVRQSVT	112AASRAVAAAVA
m141	114SSSEEECKDR	141AASAEECKAA
m164	164ITHYRPV	164AAHARPA
m179	179LS	179AA
m196	196SS	196AA
m207	207RP	207AA
m214	214GAGL	214AAGA

*Sufficient to cause Emery-Dreifuss muscular dystrophy.

Table 3. Summary of mutations in emerin-specific residues not conserved in Lap2β[37]

Name	Wildtype	Mutated
45A	45RRR	45AAA
45E	45RRR	45EEE
Δ95–99*	95YEESY	Deleted
Q133H*	133Q	133H
m122	122TS	122AA
m145	145EE	145AA
m151	151ER	151AA
m161	161YQS	161AAA
m175	175SSL	175AAA
P183H*	183P	183H or 183T
m192	192SSSSS	192ASAAA
m198	198SSWLTR	198AAAAA
m206	206IRPE	206AAPA

*Sufficient to cause Emery-Dreifuss muscular dystrophy.

Lamin A

Lamins A and C (lamins A/C) are major alternatively-spliced products of the *LMNA* gene that polymerize to form A-type nuclear intermediate filaments that concentrate near the inner nuclear membrane and are a major component of the nucleoskeleton.[2] A-type lamins have roles in chromatin tethering, epigenetic regulation, DNA damage repair, mechanotransduction, replication and development.[1,95] Lamin A has 28 reported direct partners besides emerin including Lem2,[96] Man1[14] and BAF.[37,97] A-type lamins are regulated by phosphorylation, SUMOylation, *O*-GlcNAcylation and other modifications (see ref. 97). Over 350 missense mutations in the *LMNA* gene have been linked to over

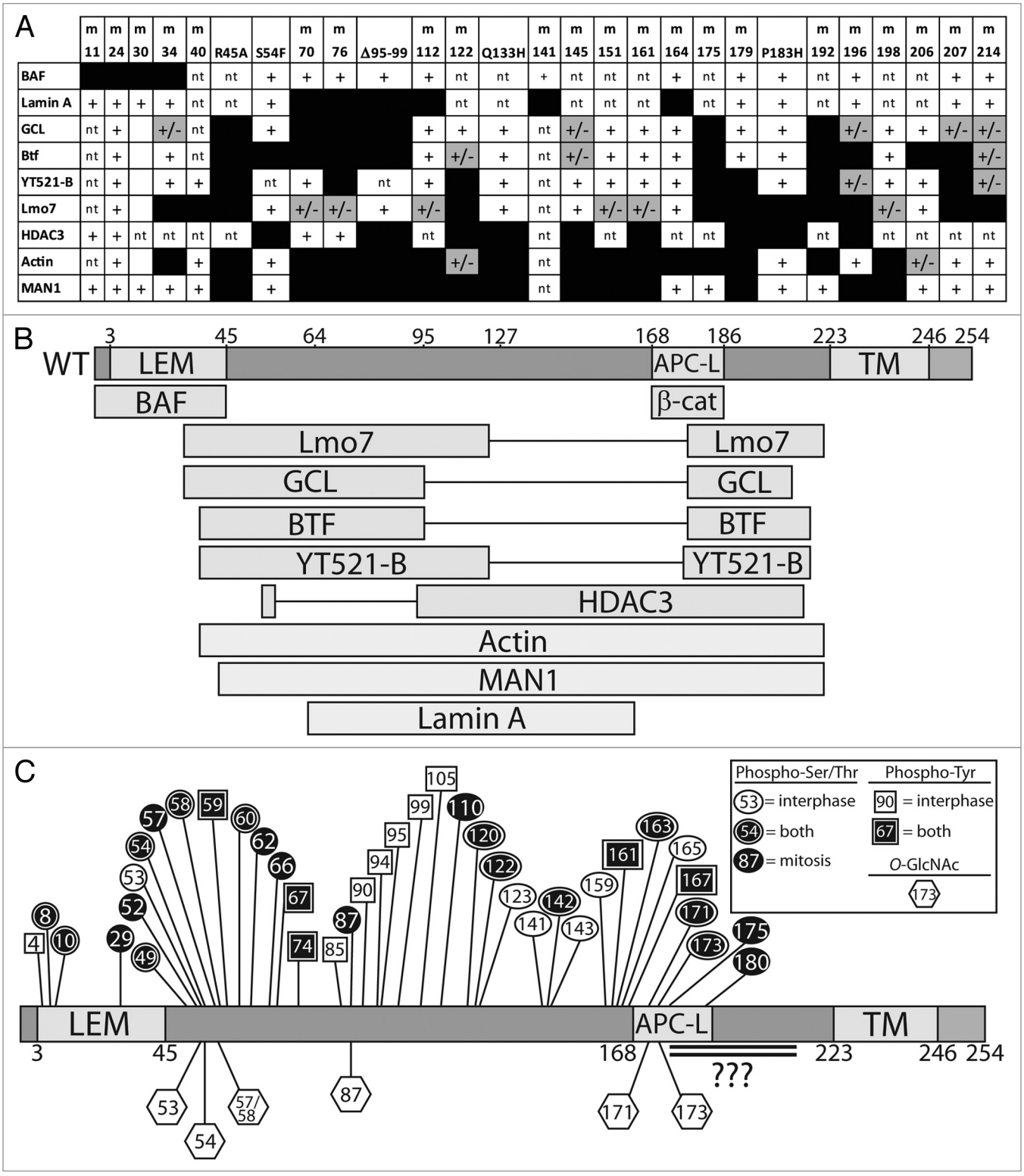

Figure 5. Functional map based on emerin missense mutations that disrupt binding to specific partners. (**A**) Summary of binding results for each named partner, tested for binding to each "m-series" (clustered Ala substitutions) or EDMD-causing mutations in emerin (mutations specified in **Tables 2 and 3**). Scoring: normal binding (+), weakened binding (± and gray), and undetectable binding (black box). nt, not tested. (**B**) Results from (A) mapped schematically to the emerin polypeptide. APC-L, APC-like domain. TM, transmembrane domain. (**C**) Schematic diagram of known phosphorylation sites in emerin (see **Fig. 6**). Hexagons, *O*-GlcNAc sites; circles, phospho-Ser/Thr; squares, phospho-Tyr; white, asynchronous cultures; black, mitotic cultures and conditions. Black with outline, sites identified in both asynchronous and mitotic cells. Double-underlined region has at least two *O*-GlcNAc sites and potentially other modifications that are uncharacterized due to the large size of the corresponding trypic peptide and poor recovery by mass spectrometry.

13 human diseases including EDMD, lipodystrophy, atypical Werner syndrome and Hutchinson-Gilford progeria syndrome (HGPS).[98] Emerin missense mutations that disrupt binding to lamin A (**Fig. 5A and B**) are located centrally in emerin (residues 70–164), and affect residues that are identical or conserved in Lap2β (**Table 2**).[13]

Barrier-to-Autointegration Factor

The conserved LEM-domain of emerin (and other LEM-domain proteins) confers direct binding to an essential chromatin protein named Barrier-to-Autointegration Factor (BAF).[24,99] BAF is an 89-residue (10 kD) protein that is highly conserved in multicellular eukaryotes.[99] A homozygous missense mutation (A12T) in human BAF, which appears to destabilize the BAF protein (> 90% reduced protein level), causes Nestor-Guillermo progeria syndrome (NGPS).[100,101] This syndrome is proposed to arise from reduced BAF protein. Emerin does not localize efficiently at the NE in NGPS cells,[100] suggesting the mislocalization of emerin (and perhaps other LEM-domain proteins) also contributes to this syndrome. NGPS patients and HGPS patients share some clinical features including accelerated skin aging, lipoatrophy, osteoporosis and osteolysis.[100] By contrast NGPS patients have no apparent cardiovascular defects, diabetes, or elevated blood triglycerides, which might explain why they are living longer (the two published patients were 24 and 32 y); HGPS patients die in their early teens from stroke or heart failure.[102] Further study of NGPS may provide much-needed insight into BAF, a core component of nuclear lamina networks that "bridges" DNA, directly binds LEM-domain proteins, histones H3, H4 and selected linker histones[103,104] and influences histone posttranslational modifications.[105] BAF can also form higher-order complexes with DNA and Lap2β in vitro,[106] and was detected in two distinct emerin-containing complexes isolated from HeLa cells.[89] In *C. elegans*, BAF has a fundamental role in attaching chromatin to the NE inner membrane via LEM-domain proteins; this role is untested in mammalian cells.

BAF is essential for chromosome segregation, cell cycle progression and post-mitotic nuclear assembly.[27,107,108] As noted above, BAF localizes to the so-called "core" regions of anaphase chromosomes at the earliest stages of nuclear assembly and is essential to recruit lamin A and emerin to this region of the reforming nuclear envelope.[30,31] Genetic analysis of *baf-1* null *C. elegans* showed BAF also has tissue-specific functions and is required for the maturation and survival of the germline, cell migration, vulva formation and muscle maintenance.[109]

BAF is required for the nuclease activity of a newly characterized human LEM-protein, Ankle1, expressed at highest levels in hematopoietic tissue (**Table 1**).[11] BAF can bind chromatin and the LEM-domain of Ankle1 simultaneously.[11] A loss of function mutation of the *C. elegans* ortholog, Lem3, which (like Ankle1) is a nuclease, causes phenotypes after irradiation that are similar to BAF-null worms,[27] including defects in chromosome segregation and anaphase bridge progression.[110] Interestingly *C. elegans* that lacked either emerin (*emr-1* null) or Lem2 (*lem-2* null) were also hypersensitive to DNA damage.[110] Together these studies show BAF and LEM-domain proteins (including *C. elegans* emerin) have critical role(s) in the DNA damage response and genome integrity. There are hints that human emerin might also influence the DNA damage response, since emerin and BAF co-associate with the DNA repair proteins CUL4A and DDB2 within minutes after cell exposure to UV (UV) light.[111] HeLa cells that overexpress laminopathy-causing mutations in GFP-lamin A,[112] and HeLa cells downregulated for emerin, both show reduced phosphorylation of H2AX ("γ-H2AX" response) after treatment with inter-strand DNA crosslinking agents such as camptothecin (Berk and Wilson, unpublished results).

HDAC3

Emerin associates with all core components of the nuclear co-repressor (NCoR) complex,[89] which represses genes by stably binding chromatin. One NCoR component is HDAC3, which deacetylates specific Lys residues in the histone H4 tail to promote NCoR interaction with chromatin. Emerin also binds HDAC3 directly.[38] In the mutation-based functional map of emerin, HDAC3 is sensitive to diverse mutations (**Fig. 5A and B**), and is the only known partner that is disrupted by all four "special" EDMD-causing mutations.[38] Furthermore emerin association increases the enzyme activity (V_{max}) of HDAC3 by 2.5-fold in vitro, suggesting emerin enhances HDAC3-dependent gene silencing.[38] This finding is consistent with an epigenetic phenotype (globally increased H4K5 acetylation) seen in emerin-downregulated cells and emerin-null mouse fibroblasts.[38] Thus HDAC3-emerin association may be fundamentally important for tissue-specific gene repression. The LEM-domain protein Lap2β also binds HDAC3 directly,[39] and influences the levels of histone H4 acetylation.[113] Notably Lap2β interaction with HDAC3 is required for the NE tethering of Lamina Associated Domains (LADs) of DNA, specifically LADs enriched in cKrox binding sites (GAGA sequence).[33] These findings support overlapping roles for emerin and Lap2β in tissue-specific gene silencing and tethering at the NE.

Btf and GCL

Less is known about two other "shared" partners, Btf and GCL. Btf (*BCLAF1*), which binds emerin[36] and Man1,[9,14] is a poorly understood, multifunctional protein with roles in DNA damage response,[114,115] apoptosis,[36,116,117] transcriptional regulation,[118] and development.[119] In response to DNA damage, Btf localizes to sites of damage[114] and can interact with protein kinase C δ to form a complex that activates the p53 promoter.[115] Most Btf is sequestered in the cytoplasm by anti-apoptotic proteins Bcl-2 and Bcl-xL, but then accumulates at the NE after apoptosis is induced.[36,116] Other work suggests Btf is an mRNA splicing factor[36,120,121] that associates with ribonucleoprotein complexes.[118,120] The phenotypes of Btf-null mice include polydactyly, deficient ex vivo T cell activation, and postnatal death due to improper lung development.[119] Emerin also has poorly understood roles in regulating mRNA splice site selection by another partner, named YT521B.[122] Why Btf associates with emerin is unknown, but one

can speculate that mRNA splicing or possibly apoptosis might be misregulated in emerin-deficient muscle.

GCL, which binds Lap2β, emerin and Man1,[14,37,39] is a conserved protein that directly binds the DP subunit of E2F and DP heterodimers, which activate genes required for entry into S-phase. Emerin or Lap2β, in conjunction with GCL, effectively co-repress E2F and DP-dependent promoters in vivo.[37,39,123] These same genes are well known targets of repression by Rb, which directly binds the E2F subunit. This co-repression of E2F-DP dependent promoters by emerin and GCL implicates emerin (and other LEM-domain proteins) in proliferation control. One study reported increased proliferation in emerin-null human fibroblasts.[93] Interestingly GCL also associates with, and can recruit to the NE, a family of primate-specific "cancer-testis antigen" proteins named GAGE,[124] which are normally expressed only in male germ cells, but are highly upregulated in many cancers.[125] Whether GAGE influences the functions of GCL or LEM-domain proteins during cancer are open questions. Whether emerin and its LEM-domain brethren share other partners is unknown, due to gaps in knowledge about most other LEM-domain proteins.

SUN-Domain Proteins and Nesprins

Emerin binds directly to SUN-domain proteins and nesprins,[90,91] the core integral membrane components of LINC complexes.[126] LINC complexes transmit mechanical force across the NE to the nuclear lamina nucleoskeleton,[127] and help maintain a uniform distance between the inner and outer membranes of the NE (for a review, see ref. 2). Emerin binding to nesprins was studied using relatively short isoforms, nesprin-1α and nesprin-2β, which are partly or fully included within most related isoforms.[90,128] However it is uncertain which if any nesprin isoforms localize at the NE inner membrane.[129] By contrast SUN-domain proteins localize almost exclusively at the NE inner membrane. Emerin binds the nucleoplasmic domains of SUN1 (SUN1 residues 223–302) and SUN2 (specific residues unmapped).[91] Much more work is needed to understand how LINC complexes contact lamins and emerin.

F-actin and Nuclear Myosin 1c

Emerin directly binds (and caps) the pointed end of actin filaments, stabilizing F-actin in vitro.[92] Emerin also binds the molecular motor, nuclear myosin Ic, directly in vitro even when myosin is "burning" ATP.[89] These results suggest emerin might anchor "cortical" actin-myosin networks near the NE. Emerin also associates (at least indirectly; direct binding not tested) with the multifunctional structural protein 4.1R in vivo, which directly binds actin and spectrin to form a ternary complex,[130] and is required for mitotic spindle function and nuclear assembly.[131,132] The nuclear localizations of emerin and 4.1R are mutually dependent: loss of 4.1R decreases emerin retention at the INM and vice-versa, and loss of either protein increases accumulation of β-catenin in the nucleus.[93,133] The nucleoskeletal roles of actin, myosin, spectrin and 4.1 are major understudied areas of cell biology.[2]

Emerin is required for the nuclear accumulation and activity of the mechanosensitive transcription factor, megakeryoblastic leukemia 1 (MKL1).[134] MKL1 localizes predominantly in the cytoplasm, but moves into the nucleus in response to mechanically-induced increases in actin polymerization.[135,136] In the nucleus, MKL1 and serum response factor (SRF) co-activate genes encoding cytoskeletal proteins.[137] *Lmna*$^{-/-}$ and *Emd*$^{-/y}$ mouse embryonic fibroblasts (MEFs) have reduced MKL1 nuclear localization after mechanical stimulation. Ectopic overexpression of GFP-emerin in *Lmna*$^{-/-}$ or *Emd*$^{-/y}$ MEFs rescues MKL1 nuclear localization. Three emerin mutants incapable of binding actin (clustered Ala-substitutions m151 or m164, and "special" EDMD-causing mutation Q133H) failed to rescue MKL1 nuclear accumulation,[134] suggesting emerin association with actin is required. However other functions of emerin may also contribute to the nuclear accumulation of MKL1: the emerin m151 and Q133H mutations also disrupt binding to the LEM-domain protein Man1[14] (see **Table 3; Fig. 5A**) and have not yet been tested for binding to lamin A, and the m164 mutation also disrupts binding to lamin A[13] (**Table 2; Fig. 5A**). Whether MKL1 binds emerin directly is unknown.

Emerin Binds β-catenin and Lmo7 and Regulates Signaling from the Cell Surface

Emerin directly binds two signaling transcription factors that shuttle between the cell surface and nucleus: one is the well-known protein β-catenin,[93] which mediates Wnt signaling; the other is Lim-domain-only 7 (Lmo7).[94] Emerin binds β-catenin directly, and emerin-null fibroblasts accumulate high levels of β-catenin in the nucleus, grow rapidly, and improperly continue proliferating in low serum.[93] This suggests emerin normally attenuates Wnt signaling. Similarly, Lmo7 is widely distributed in the cytoplasm and cell surface (adherens junctions) and also shuttles into the nucleus where its accumulation appears to be inhibited by emerin.[86,94] Lmo7 also co-localizes with p130Cas at focal adhesions, and is inhibited by p130Cas (Wozniak et al., forthcoming). Lmo7 is a transcription factor that activates many genes including the emerin gene; evidence suggests Lmo7 binding to emerin protein feedback-regulates emerin gene expression.[94] Lmo7 is expressed at elevated levels in heart and muscle, and Lmo7-null mice have dystrophic muscle (JM Holaska, personal communication), suggesting Lmo7 association with emerin is highly relevant to EDMD disease.[138,139] Lmo7 activates the promoters of myogenic genes (encoding MyoD, Myf5, Pax3) whose expression is critical for early myogenic differentiation. These genes are turned off after myotubes form, concomitant with increased emerin expression; emerin is proposed to both recruit Lmo7 away from these promoters, and to drive Lmo7 exit from the nucleus.[140]

Studies of Emerin–Null EDMD Patient Cells and Mouse Models

Emerin-null mice display subtle defects in motor coordination and muscle regeneration and mild atrioventricular conduction

defects with age.[141,142] This unfortunately has limited their use as an EDMD model.

In EDMD patient muscle and emerin-null mice, loss of emerin misregulates certain muscle-specific and heart-relevant genes regulated by Rb and MyoD.[63,142,143] MyoD-dependent genes are crucial for muscle development and repair. Some genes misregulated in EDMD (including *CREBBP*, *NAP1L1* and *RBL2*), and the emerin gene itself, depend for their transcriptional activation on Lmo7.[94] In regenerating emerin-null mouse muscle, Rb remains inappropriately hyper-phosphorylated, and cells that should arrest during differentiation instead continue proliferating.[142] The mechanisms by which Rb–MyoD-regulated pathways depend on emerin are unknown, but might involve loss of emerin-dependent gene tethering at the NE. Rb/MyoD-regulated pathways are also required in muscle stem ("satellite") cells, which express emerin and have long-term roles in muscle homeostasis, repair and regeneration.[144] Loss of emerin in satellite cells is proposed to reduce their capacity to repair and regenerate muscle tissue,[145] due in part to increased nuclear fragility[54] and reduced mechano-transduction.[61]

Loss of emerin also affects genes regulated by the JNK, MAPK, NF-κB, integrin, Wnt and TGFβ signaling pathways.[54] The nuclear localization and activity of ERK1/2 (a MAPK) increases in emerin-null mouse hearts.[143] Thus emerin is proposed to block or attenuate the nuclear accumulation of at least three signaling proteins: ERK1/2, Lmo7 and β-catenin. The mechanism(s) by which emerin inhibits nuclear accumulation of these key regulators are important open questions. A different LEM-domain protein, Man1, inhibits TGFβ/BMP signaling during vertebrate development by directly binding and inhibiting R-Smads.[146-149] Man1 is proposed to inhibit TGFβ/BMP signaling by stimulating dephosphorylation of Smads and hence favoring nuclear export.[150] Interestingly β-catenin export is mediated by two proteins: 14-3-3 (ε and other isoforms) and the Wnt pathway inhibitor Chibby.[151,152] We speculate emerin might promote β-catenin export by "scaffolding" its co-association with Chibby and 14-3-3, since 14-3-3 isoforms β, ε and θ were recovered as potential components of two emerin-containing complexes.[89]

Mechanical Properties of Emerin-Deficient Nuclei and Selective "Pruning" of "Bad" Nuclear Structures by Autophagy

Muscle sections from seven X-linked EDMD patients revealed severe nuclear shape disruptions in a subset of nuclei in fibroblasts, smooth muscle and skeletal muscle. The frequency of abnormal nuclei in muscle tissue increased with patient age, and on average 20–25% of nuclei were misshapen in a manner consistent with apoptosis.[153,154] Remarkably, prior to apoptosis, muscle cells first deploy a strategy in which the structurally abnormal (e.g., blebbed or herniated) regions of emerin-null nuclei, including chromatin, are specifically recognized and selectively destroyed by autophagy.[141,155] When autophagy was experimentally blocked, the entire nucleus underwent apoptosis.[141,155] The pathway(s) that identify structurally defective regions of the NE and trigger "surgical" removal via autophagy are unknown.

In cultured MEFs, the emerin-null condition increases the percentage of cells with nuclear morphology defects, but not as much as the *lmna*-null condition.[61] Emerin-null MEFs also have higher rates of apoptosis after continuous mechanical strain, possibly because they fail to activate the mechanosensitive gene *IEX-1*, which protects against apoptosis.[61] Reduced NE elasticity in emerin-null MEFs may also contribute to nuclear fragility in EDMD patients.[156] Collectively these findings suggest emerin contributes to nuclear architecture and is important to maintain the structural integrity and function of the NE.

Extensive Emerin Phosphorylation during Mitosis and Interphase

Human emerin has at least 39 published sites of phosphorylation in vivo (25 Ser, 4 Thr, 11 Tyr) as summarized in **Figures 5C and 6**.[157-176] Although the responsible kinases/pathways and functional consequences of emerin phosphorylation remain poorly understood, one broad theme is emerging: emerin appears to be a major target of phosphorylation not only during mitosis but also during interphase.

Emerin, lamins and other key NE proteins are hyperphosphorylated during mitotic prophase to trigger and regulate their mutual detachment, nucleoskeletal reorganization and NE disassembly.[65,173,177,178] Emerin is phosphorylated at 26 sites (including tyrosines) during mitosis,[162,174-176] with up to 83–94% stoichiometry.[162,175]

Emerin is also phosphorylated during interphase at 30 reported sites (**Figs. 5C and 6**). Only a few kinases, and one phosphatase, that target emerin have been identified,[84,163,167,179-181] as summarized in **Table 4**. Emerin is phosphorylated on Ser49 and at least one other residue by PKA.[163] Emerin is also targeted by the metabolically important kinase GSK3β at unknown site(s) in vitro.[84]

A new pathway was reported in postsynaptic neurons, wherein nascent RNPs exit the nucleus directly through the NE; this pathway involves PKC-dependent hyperphosphorylation of lamin A.[182] This pathway is proposed to be exploited in cells infected with Herpes simplex virus type 1, where emerin is targeted by the virus-encoded kinase US3 and cellular PKCδ as a mechanism for the virus to directly bud and exit at the NE.[179,183] Emerin is also hyperphosphorylated by the Kaposi sarcoma associated herpesvirus.[184] The MAPK pathway kinase ERK2 phosphorylates emerin directly both in vitro, and during nuclear egress of vesicular stomatitis virus G-protein-pseudotyped human and feline immunodeficiency viruses.[185] This virus-induced hyper-phosphorylation causes emerin to mislocalize and might represent a viral strategy to hijack the host RNP-egress pathway.[179,182,183]

Emerin is nearly 10-fold Tyr hyper-phosphorylated in NIH3T3 cells that overexpress Her2.[167,181] At least two nonreceptor Tyr kinases target emerin directly: Src specifically phosphorylates at least three residues (Y59, Y74, Y95) and Abl targets at least one (Y167).[167] The Src-regulated sites are critical for BAF binding, since the triple Y to F substitution (at Y59, Y74 and Y95) decreases BAF binding by 70% in vivo.[167] All three sites are phosphorylated during interphase (**Fig. 6**).[158,166] Emerin is also

Table 4. Enzymes that target emerin

			Identified sites	Assay
Ser/Thr kinases	**PKA**	Roberts et al., 2006	S49	in vitro, MS
	PKCδ	Leach et al., 2007	(nd)	in vivo, inhibitors
	ERK2/MAPK	Bukong et al., 2010	(nd)	in vivo, inhibitors, in vitro
	GSK3β	Wheeler et al., 2010	(nd)	in vitro
Tyrosine kinases	**Src**	Tifft et al., 2009	Y59, Y74, Y95	in vitro, MS/in vivo
	Abl	Tifft et al., 2009	Y167	in vitro, MS
	Her2	Tifft et al., 2009, Bose et al. 2006	(nd)	in vivo
Phosphatases	**PTP$_1$B**	Yip et al., 2012	(nd)	in vivo
Glyco-transferases	**OGT**	Berk et al., submitted	S53, S54, S57/S58, S87, S171, S173	in vitro, MS
	OGT	Berk et al., submitted	S53, S54, S173	in vivo

*MS, mass spectrometry. nd, not determined.

ubiquitinylated at K88,[186,187] but the specific context and consequences of this modification, like phosphorylation, are unknown. These diverse modifications, especially Tyr-phosphorylation and *O*-GlcNAcylation (discussed below), suggest emerin is regulated by tissue-specific signaling, potentially as a mechanism for "crosstalk" regulation of gene expression at the NE.

One potential therapy for EDMD, being developed by Worman and colleagues, is based on their discovery that MAP kinase signaling is overactive in *lmna*- or emerin-deficient mouse hearts; presymptomatic treatment with an ERK kinase inhibitor prevented dilated cardiomyopathy in an autosomal-dominant *Lmna* EDMD mouse model.[188] Additional pharmacological strategies to treat EDMD may emerge from a better understanding of the kinases and other enzymes that regulate emerin itself or other (potentially "compensating") LEM-domain proteins.

Emerin is Sweet: Regulation by *O*-linked β-*N*-acetylglucosamine Transferase

Emerin is directly regulated by *O*-GlcNAc transferase ("OGT"; UDP-*N*-acetylglucosamine-peptide β-*N*-acetylglucosaminyltransferase), an essential enzyme that attaches a single β-*N*-acetylglucosamine sugar to Ser/Thr residues of target proteins.[189] The OGT-null condition in mice is lethal at embryonic stage E4.5, and in embryonic stem cells.[189,190] Similarly, mice null for OGA (β-*N*-acetylglucosaminidase), the enzyme that removes *O*-GlcNAc, have delayed development and die at birth, with severe defects in mitosis.[191] OGT is a pleiotropic enzyme with critical roles in the cellular stress response,[192-194] mitosis,[195] epigenetic regulation[196] and transcriptional regulation.[197] *O*-GlcNAcylation is highly dynamic, influences target proteins at many different levels and can compete or cooperate with phosphorylation to regulate specific sites.[189] Emerin is highly *O*-GlcNAcylated in mammalian cells; in vitro studies identified five sites (Ser53, Ser54, Ser87, Ser171 and Ser173) and revealed at least three additional sites.[198] Two sites (Ser53 and Ser54) are each individually critical for overall emerin *O*-GlcNAcylation not only in cells but also in vitro, suggesting potential control of emerin conformation. A third site (Ser173) is proposed to function as a molecular "switch": *O*-GlcNAcylation at Ser173 promotes emerin binding to BAF, whereas Ser173 phosphorylation promotes emerin hyperphosphorylation and reduces BAF association in cells.[198] All five identified *O*-GlcNAc sites in emerin are phosphorylated during mitosis (**Fig. 5C**), suggesting OGT and mitotic kinases might compete for control of emerin.

Concluding Remarks

Basic biochemical, cellular and genetic studies of emerin have yielded unprecedented insight into the structure and function of the nuclear envelope and nuclear lamina networks, and the fundamental roles of the conserved LEM-domain protein family. Perhaps most surprising, and frustrating in terms of EDMD disease therapy, is the sheer variety of functions to which emerin contributes—from mitosis and chromosome segregation, to silent chromatin tethering, mechano-transduction and signaling. However emerin's roles in signaling have encouragingly suggested the first potential pharmacological treatment for EDMD. Further biochemical studies and human gene mapping studies will continue to complement each other: proteins that bind emerin represent candidate EDMD genes, and each mapped EDMD gene is a vital clue to understanding both human laminopathy disease and NE-dependent mechanisms of signaling and genome control. Given the many open questions in this young field, we wish to emphasize the dual importance of continuing both directly EDMD-relevant, and basic curiosity-driven, research. Both strategies are crucial to understand emerin and its fellow LEM-domain proteins, and uncover their overlapping vs. unique roles in human physiology that might lead to new therapies.

Figure 6 (See opposite page). Published human emerin phosphorylation sites. X indicates emerin phosphorylation sites identified in asynchronous or mitotic cells. Grey columns indicate emerin-specific studies; other columns are high-throughput studies. (S), Ser. (T), Thr. (Y), Tyr. These results are compiled from references 157–176.

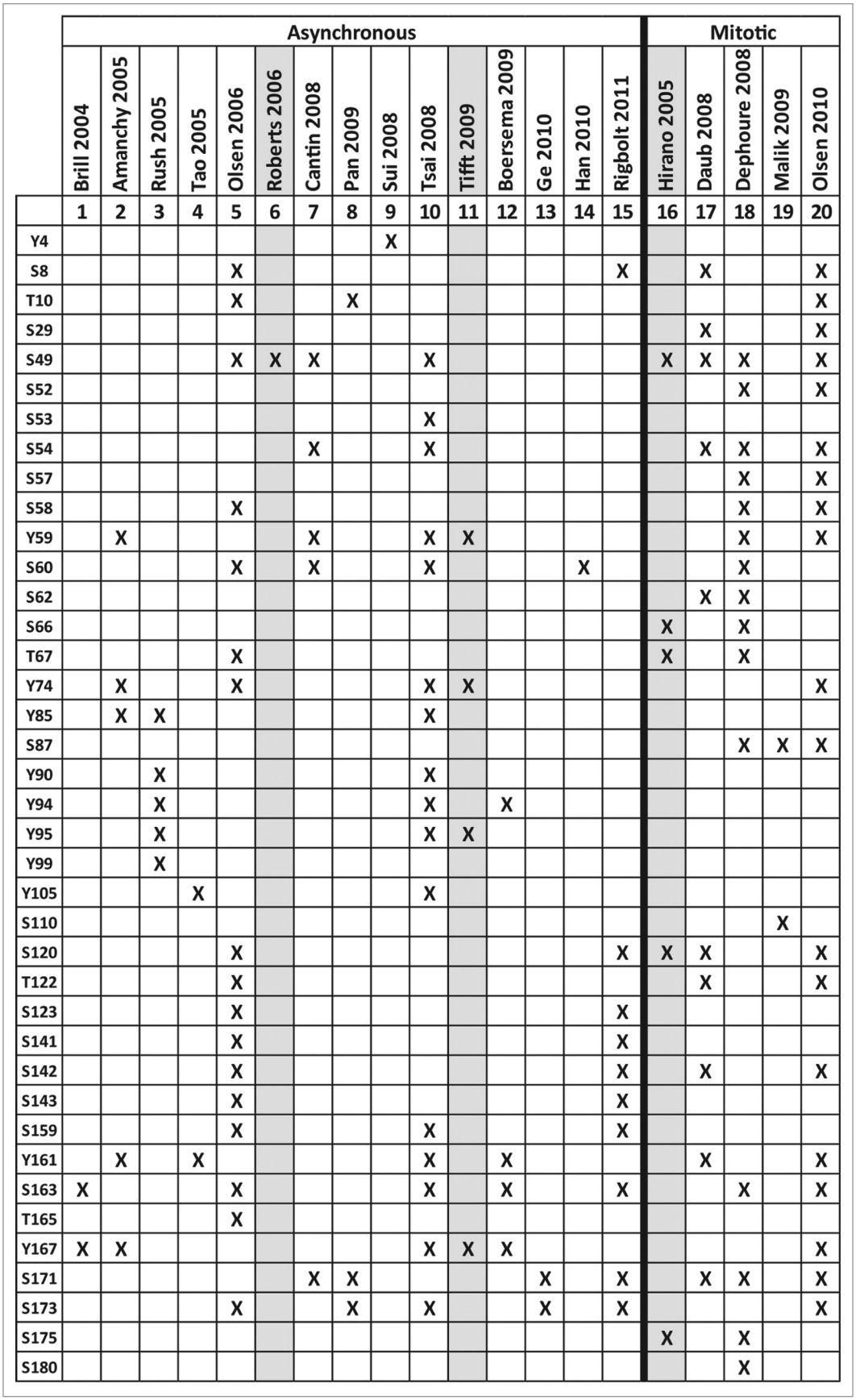

Figure 6. For figure legend, see page 308.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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