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Networking in the nucleus: A spotlight on LEM-domain proteins

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Abstract

Proteins resident in the inner nuclear membrane and underlying nuclear lamina form a network that regulates nuclear functions. This review highlights a prominent family of nuclear lamina proteins that carries the LAP2-emerin-MAN1-domain (LEM-D). LEM-D proteins share an ability to bind lamins and tether repressive chromatin at the nuclear periphery. The importance of this family is underscored by findings that loss of individual LEM-D proteins causes progressive, tissue-restricted diseases, known as laminopathies. Diverse functions of LEM-D proteins are linked to interactions with unique and overlapping partners including signal transduction effectors, transcription factors and architectural proteins. Recent investigations suggest that LEM-D proteins form hubs within the nuclear lamina that integrate external signals important for tissue homeostasis and maintenance of progenitor cell populations.

Introduction

The nuclear lamina is an extensive protein network that lies underneath the inner membrane of the nuclear envelope. This network establishes mechanical support for the nucleus and provides a platform for protein interactions that contribute to gene regulation, DNA replication and genome stability [1–3]. The major constituents of the nuclear lamina are the A- and B-type lamins, which scaffold potentially hundreds of inner nuclear membrane proteins [4, 5] [Schirmer and Worman, this issue] including membrane components of the nuclear lamina. Multiple human diseases are caused by loss of individual nuclear lamina proteins, highlighting the importance of this network.

The LAP2-emerin-MAN1-domain (LEM-D) protein family has prominent roles within the nuclear lamina. The defining feature of this family is the ~40 amino acid domain that binds Barrier-to-Autointegration Factor (BAF or BANF1), a metazoan histone-binding and sequence-independent DNA-binding protein [6, 7]. The LEM-D shares sequence similarity with domains that bind DNA including the SAP [SAF/Acinus/PIAS] domain and HeH [helix-extension-helix] domain (reviewed [8, 9]). Indeed, LAP2 carries both a LEM-D and a

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LEM-like domain that is structurally LEM-related but directly binds DNA [7]. Interestingly, inner nuclear membrane proteins with LEM-related domains are found in unicellular organisms, such as yeast, that lack BAF and lamins. These observations suggest that the LEM-D evolved from an ancestral DNA binding protein involved in bridging chromatin to the nuclear periphery [10].

Complexity of the LEM-D protein family

LEM-D proteins fall into three groups based on membrane topology and other features (Table 1, Figure 1, [10][11, 12]). Group I LEM-D proteins have an amino-terminal LEM-D and large nucleoplasmic domain; some are not membrane proteins but most have a single transmembrane domain at their carboxyl-terminus. Representatives of this class include LAP2 and emerin. Group II LEM-D proteins carry amino-terminal LEM-Ds, two internal transmembrane domains, and carboxyl-terminal winged-helix 'MSC' (MAN1/Src1p/C-terminal motif) domains that directly bind DNA [13]. Representatives of this group include LEM2 and MAN1. In addition, MAN1 carries an RRM-like protein interaction domain named UHM (U2AF homology motif; Figure 1, [13, 14]). Group III LEM-D proteins carry internal LEM-Ds and multiple ankyrin repeats, although the relative positioning of these domains differs (Figure 1). Representatives of this group include LEM3/Ankle1 and LEM4/Ankle2. LEM3/Ankle1 has no transmembrane domain but carries an active carboxyl-terminal endonuclease domain [15, 16]. The subcellular localization of Group III LEM-D proteins contrasts with other LEM-D proteins. LEM3/Ankle1 shuttles between the cytoplasm and nucleoplasm [15], whereas LEM4/Ankle2 has a transmembrane domain and localizes throughout the endoplasmic reticulum in human cells and at the nuclear envelope in worms [17]. The structural complexity and distinct subcellular localizations of LEM-D proteins underlie their functional diversity.

The composition of the LEM-D protein family differs between organisms. Yeast have a minimal LEM-like family comprised solely of Group II proteins, while metazoans have an expanded family that includes at least one member from each group (Table 1). Further, single LEM-D genes can generate multiple isoforms. For example, *LAP2* (also known as *TMPO*) encodes isoforms that share the amino terminal LEM-D and LEM-like domains, but differ in their carboxyl termini. Most LAP2 isoforms carry a carboxyl-terminal transmembrane domain and localize to the inner nuclear membrane, such as LAP2 β , whereas others such as LAP2 α lack the transmembrane domain, localize to the nucleoplasm and associate with distinct partners [18]. Thus evolutionary expansion of the LEM-D family was accompanied by functional diversification.

Analysis of emerin reveals a dynamic LEM-D protein network at the nuclear lamina

LEM-D proteins interact with proteins of diverse functions. This is best illustrated by studies of emerin [19], a LEM-D protein that interacts with proteins involved in mechanotransduction, cellular architecture, transcriptional regulation, and chromatin tethering [20]. Emerin also displays self-associations, involving interactions between the amino terminal LEM-D and internal residues that are predicted to form an emerin-emerin

network within the nuclear lamina ([21]; Figure 2). The considerable emerlin interactome requires conformational plasticity, which may be imparted by large regions of predicted intrinsic disorder within emerlin [21].

Post-translational modifications (PTMs) influence protein interactions of LEM-D proteins (Figure 2A). Emerlin is extensively modified by phosphorylation and *O*-linked glycosylation (*O*-GlcNAc) [20]. Competing PTMs of internal residues impact BAF binding to emerlin, with *O*-GlcNAc modification increasing BAF association [22]. As BAF has the capacity to increase nuclear lamina stability and alter histone modifications [23], changes in LEM-D association with BAF likely lead to global changes in gene expression. Interestingly, BAF localization and mobility are regulated by environmental stress, which was linked to the strength of the emerlin-BAF interaction [24]. Taken together, these findings suggest external signals are transmitted through PTMs of emerlin, leading to changes in the emerlin interactome and hence nuclear lamina function. Interactions with partners are also controlled by internal signaling; for example cell cycle-dependent modifications of MAN1 disrupt association with BAF [25]. These findings emphasize that the nuclear lamina includes a dynamic LEM-D protein network that responds to cell signaling.

LEM-D proteins bridge chromatin at the periphery

LEM-D proteins contribute to the tethering of genomic regions to the nuclear periphery. In yeast, LEM-related proteins recruit repressive subtelomeric domains and ribosomal DNA repeats to the nuclear periphery [26–28]. In metazoans, LEM-D proteins associate with regions of high repeat density and low gene number that are enriched in repressive epigenetic marks [29–31], all features matching those defined for lamin associated domains (LADs) of chromatin [31]. Strikingly, direct comparisons of LEM-D-associated regions and LADs revealed a 90% overlap [29–31], suggesting that LEM-D proteins may contribute to LAD establishment or maintenance. Shared interactions with BAF provide one mechanism by which LEM-D proteins might tether LADs to the periphery (Figure 2A). Although the mechanisms of BAF function remain unclear, new studies underscore its relevance to chromatin and laminopathy [Jamin and Wiebe, this issue]. BAF-independent mechanisms may also exist, highlighted by the discovery of specialized DNA sequences known as lamina-associating sequences (LASs) that target LADs to the nuclear periphery [32]. LASs are enriched for the GAGA DNA motif, which is bound by transcription repression complexes that contain cKrox, HDAC3 and the LEM-D protein LAP2 β (Figure 2B). Reduced levels of cKrox or HDAC3 disrupted the peripheral association of LASs, whereas reducing LAP2 β had a more modest effect, likely due to redundancy with other nuclear lamina proteins. Interestingly, reduced levels of BAF had no detectable effect on the localization of studied LASs [32]. These results suggest LEM-D proteins have the capacity to tether specific regions of the genome to the nuclear lamina and may use multiple mechanisms.

Chromatin condensation plays a critical role in gene positioning at the nuclear periphery [33]. As such, proteins that promote the formation of repressed chromatin may contribute to LAD maintenance. Several findings support such a role for LEM-D proteins. Emerlin and LAP2 β each directly bind to the histone deacetylase HDAC3 [19, 34], and emerlin

association increases HDAC3 deacetylase activity [35]. Further, emerin-dependent targeting of a reporter gene to the nuclear periphery causes transcriptional silencing and histone deacetylation [36]. Endogenous genes behave similarly: emerin and HDAC3 are required for the nuclear lamina localization and proper temporal expression of genes required for muscle differentiation [37], and emerin-deficient fibroblasts have reduced levels of peripheral heterochromatin [38]. The extensive co-association of LEM-D proteins and LADs suggests that LEM-D proteins maintain LADs, at least in part, by recruiting partners that contribute to repressed chromatin states.

Loss of LEM-D proteins causes tissue-restricted phenotypes

The first LEM-D gene linked to human disease was *emerin* (*STA* renamed *EMD*), the gene responsible for X-linked recessive Emery-Dreifuss muscular dystrophy (EDMD) [39]. Hallmark features of EDMD begin in early childhood with joint contractures and slowly progressive weakness of humeroperoneal muscles, followed by cardiac conductance defects and dilated cardiomyopathy in adulthood. These characteristics suggest emerin is dispensable for muscle development, but needed for muscle maintenance. EDMD is also caused by mutations in genes that encode emerin-binding proteins such as A-type lamins and two LINC (links the nucleoskeleton and cytoskeleton) complex components named nesprin-1 and nesprin-2 [40–42] [Razafsky and Hodzic, this issue]. Mutations in other widely expressed *LEM-D* genes also cause tissue-restricted human diseases. For example mutations in *LAP2* cause dilated cardiomyopathy, and *MAN1* mutations cause skin and bone density disorders [2, 9]. The overlapping disease phenotypes caused by mutations in *LMNA* and LEM-D genes places LEM-D diseases within the wider classification of laminopathies.

Studies in model organisms have uncovered multiple functions for LEM-D proteins. Mutations in genes encoding emerin homologues have been generated in worms, flies, and mice [43–47]. Studies of these mutants uncovered general roles in tissue homeostasis. For example, *emerin*-null mice display mild defects in motor coordination, delayed muscle regeneration and altered cardiac conductance [46, 47]. These defects are associated with compromised muscle satellite stem cells and blocked differentiation of *emerin*-null myoblasts [48]. Similarly, loss of the fly emerin homologue Otefin blocks differentiation of the adult germline stem cell daughters, leading to stem cell death [43]. In fact, growing evidence suggests that LEM-D proteins are broadly required for stem cell function and cell differentiation. In mice, *MAN1* loss results in early embryonic lethality [49, 50]. These mutants showed defective vascular development and abnormal heart morphogenesis [49, 50] [51], consistent with evidence from cultured cells that *MAN1* and *Lem2* are required for myogenic differentiation [52]. Finally, mice lacking the nucleoplasmic *LAP2α* isoform are viable and morphologically normal, but specific progenitor cell populations in regenerative tissues were seen to overproliferate [53], a phenotype also observed in cultured myoblasts [54]. Together, genetic analyses of *LEM-D* mutants provide evidence for roles in tissue homeostasis and maintenance of stem cell populations, consistent with the age-dependent progression of the corresponding human diseases.

LEM-D proteins regulate transcription factor function

Altered tissue homeostasis in *LEM-D* mutants is associated with mis-regulation of signaling pathways (Figure 3). Multiple LEM-D proteins directly interact with signaling effectors [9, 55, 56]. For example, emerin directly binds and regulates the flux of β -catenin into the nucleus (Figure 3A, [57]). In the absence of emerin, levels of nuclear β -catenin increase, resulting in up-regulation of target genes [55, 57]. Similarly, emerin regulates nuclear envelope localization of other transcription factors, including LIM Domain Only 7 (Lmo7), a transcriptional activator involved in myogenic differentiation [58]. The role of emerin in signal transduction is not unique. MAN1 and its homologues directly interact with receptor associated Smads (rSmads) [11, 59–62], causing down-regulation of TGF- β signaling (Figure 3A). The MAN1-rSmad interaction is linked to rSmad dephosphorylation that may be promoted by MAN1 interaction with a phosphatase [56]. Finally, the nucleoplasmic LAP2 α preferentially binds and anchors the hypophosphorylated retinoblastoma protein (pRb) in the nucleus, conferring efficient pRb repressor activity [9]. These findings reinforce proposals that LEM-D-associated human diseases arise due to the misregulation of signaling pathways important for stem cell renewal and differentiation [9, 40, 63].

The loss of LEM-D proteins may alter mechanosensory signal transduction. Nuclear deformities are common upon LEM-D protein loss, especially in tissues undergoing mechanical strain [64]. Observations that emerin binds and regulates actin and myosin 1c, and co-immunoprecipitates with myosin IIB, suggest that nuclear and cytoskeletal changes are coupled [19, 65]. Mechano-signal transduction is altered in *emerin*-deficient cells [2, 66–68], linked to changes in nuclear localization of the mechano-sensitive transcription factor, megakaryoblastic leukemia 1 (MKL1, Figure 3B) [67]. Interestingly, emerin undergoes tyrosine phosphorylation in nuclei exposed to force [66], suggesting that changes in mechano-responsive gene expression may involve remodeling of the extensive emerin interaction network in the nuclear lamina (Figure 3B). Connections between mechano-signal transduction and emerin help explain why tissues undergoing repeated mechanical strain are among those affected by LEM-D protein loss.

Functional redundancy restricts the impact of individual LEM-D protein loss

LEM-D proteins have overlapping functions. This conclusion is supported by two lines of evidence. First, LEM-D mutants share tissue-specific defects. For example, LAP2 α -deficient and emerin-deficient muscles both have higher numbers of satellite stem cells and altered myoblast differentiation associated with mis-regulation of the Rb1/E2F pathway [47, 54]. Second, loss of two LEM-D proteins causes phenotypes significantly more severe than loss of single LEM-D proteins [44, 45, 52, 60, 69]. Functional redundancy is perhaps expected between LEM-D proteins in the same group, but not between LEM-D proteins in different groups due to their limited homology outside of the LEM-D. However functional overlap between group I and group II LEM-D proteins was first described in worms [44, 45] and later found in other organisms [43, 52, 60, 69]. A reasonable explanation for this overlap is the additive loss of the common interaction with BAF. Yet, mounting evidence suggests that redundancy extends beyond BAF association. Genetic studies in flies showed that loss

of any two of the three nuclear lamina LEM-D proteins is lethal; the timing of death varies depends upon which specific LEM-D proteins were lost [69]. Furthermore, the mutant phenotypes of *lem-d* double mutants differed from those of *baf* mutants, emphasizing that shared functions of the fly LEM-D proteins are not limited to BAF recruitment.

Functional redundancy among LEM-D proteins may reflect their shared protein interactomes. For example, emerin and MAN1 each interact with the death promoting transcriptional repressor Bcl-2-associated transcription factor; emerin and LAP2 each bind the histone deacetylase HDAC3, and all three proteins interact with the transcriptional regulator germ cell-less [20, 35, 70]. On one hand, these findings suggest that loss-of-function phenotypes associated with defects in a single LEM-D protein may be tissue-restricted because other members of the LEM-D family are present at sufficient levels in other tissues to maintain critical functions of the nuclear lamina. Nevertheless, defects caused by loss of individual LEM-D proteins also suggest certain functions are unique and cannot be compensated by other family members [43, 52, 60, 69]. Thus, only some LEM-D protein functions are shared with other family members.

Non-LEM-D proteins may also compensate for loss of a single LEM-D protein. This feature is illustrated by recent studies of Lamina-associated polypeptide 1 (LAP1, [71]). Conditional deletion of mouse *LAP1* causes muscular dystrophy and early lethality, pathologies that worsen in the absence of emerin. Analysis of emerin and LAP1 levels in mouse and human skeletal muscle revealed that mouse muscle has significantly higher levels of LAP1 than human muscle, suggesting that the subtle muscle phenotype of *emerin*-null mice may result from compensation by high levels of LAP1. Interestingly, the nuclear envelope proteome differs greatly between tissues [5, 72]. Thus tissue-specific defects caused by loss of single LEM-D proteins may involve a constellation of nuclear membrane proteins and their capacity for functional compensation.

Concluding remarks

Genetic, cellular and biochemical results from multiple organisms have significantly advanced our understanding of the complex LEM-D protein family. These studies reveal that LEM-D proteins have both shared and unique functions in tissue homeostasis and the maintenance of progenitor cell populations. Interactions with chromatin and signaling effectors predict that LEM-D proteins function as hubs that integrate external signals, which ultimately contribute to the regulation of gene expression. Studies in model organisms in particular are expanding our knowledge of the LEM-D protein networks in biological contexts directly relevant to human LEM-D-associated diseases, a promising advance towards therapeutics.

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Figure 1. The human LEM-D protein family

Shown are the features and subcellular localization of human LEM-D proteins representing each structural group (I, II, III). Most human LEM-D proteins are anchored in the inner nuclear membrane (INM) of the nuclear envelope (NE). Exceptions include LAP2 α in the nucleoplasm, the nucleo-cytoplasmic shuttling protein Ankle1 (LEM3), and Ankle2 (LEM4), which localizes at the endoplasmic reticulum in human cells or the NE in *C. elegans*. LEM-D proteins interact with lamins, forming the nuclear lamina (NL) network. In addition to their LEM domain (LEM-D), members of this family are characterized by the presence or absence of other features including a transmembrane (TM) domain(s), LEM-like domain (LEM-like), MSC (MAN1-Src1-p C-terminal) domain, UHM (U2AF homology motif) domain, GIY-YIG endonuclease domain or ankyrin (ANK) repeats. The arrow indicates nucleocytoplasmic shuttling of Ankle1 (LEM3). NPC, nuclear pore complex. ONM, outer nuclear membrane.

Figure 2. LEM-D proteins dynamically influence chromatin attachment to the nuclear envelope (A). Emerin-lamin and emerlin-emerlin interactions are regulated by post-translational modifications (PTMs). Multiple PTM sites have been mapped throughout emerlin, including serine, threonine and tyrosine (phosphorylation, P in yellow circles) and *O*-linked β -*N*-acetylglucosamine modification (*O*-GlcNAcylation; G in blue hexagons). PTMs are predicted to influence the emerlin interactome, with implications for recruitment of BAF and organization of lamina-associated domains (LADs). (B). LEM-D proteins use multiple mechanisms to organize chromatin at the nuclear periphery. LEM-D proteins bind BAF (left). LEM-D proteins associate with HDAC3 to maintain repressive chromatin (middle). The LEM-D protein LAP2 β is part of a transcriptional repressor complex that includes HDAC3 and cKrox. cKrox binds GAGA-like DNA elements within Lamina-Associated Sequences (LASs) of LADs, thereby targeting LADs to the nuclear periphery (right). The properties of LEM-D proteins suggest this family contributes to LAD establishment and maintenance.

Figure 3. LEM-D proteins regulate signal transduction

(A). MAN1 directly interacts with Smads causing down-regulation of TGF- β signaling, and emerlin directly interacts with β -catenin causing down-regulation of Wnt signaling. Down-regulation of target genes may be due to sequestration of signaling effectors at the nuclear lamina, or increased nuclear export of the effectors. Dashed arrows denote regulation of signaling effector access to their target genes. (B) Emerin has roles in mechanosensory signal transduction. Emerin binding to actin is proposed to influence the nuclear accumulation of MKL1 (left). Mechanical force exerted on LINC complexes leads to increased phosphorylation of tyrosine residues in emerlin, which may remodel the emerlin-interaction network (right).

Table 1

LEM domain proteins from yeast to humans

Organism	Group	Proteins	Tissues Affected	Processes Impacted	Ref
<i>S. pombe</i>	II	Lem2	n/a	Telomere localization	[26]
		Man1	n/a	Telomere localization	[26]
<i>S. cerevisiae</i>	II	Heh1 (Src1)	n/a	Telomere localization and repression, rDNA stability	[28]
		Heh2	n/a	Telomere localization and repression, rDNA stability	[27, 28]
<i>C. elegans</i>	I	Ce-emerin	None	Chromatin anchoring, gene repression	[29, 45, 73]
	II	MAN1	Global (semi-lethal)	Mitosis	[45]
	III	LEM3	Global	DNA damage response	[16]
		LEM-4L	Global	Nuclear envelope reformation	[17]
<i>D. melanogaster</i>	I	Otefin	Female germline stem cells	Stem cell homeostasis, Germline differentiation, Signal transduction	[43]
		Bocksbeutel [α , β]	Salivary glands	Structural integrity of the nucleus	[69]
	II	dMAN1	Wings, testes, ovaries, muscle	Signal transduction	[11, 59]
	III	dLEM3 (CG8679)	Unknown	Unknown	
<i>H. sapiens</i>	I	Emerin	Heart, striated limb muscles	Stem cell homeostasis, muscle differentiation, Transcriptional regulation, Signal transduction, Structural integrity of the nucleus, Actin dynamics, Mechano-sensitive response, Centrosome positioning	[20]
		LAP2 ($\alpha, \beta, \gamma, \delta, \epsilon, \delta^*$)	Heart	Stem cell homeostasis, Muscle differentiation, Transcriptional regulation, DNA replication, Nuclear envelope reassembly	[9]
		LEM1 (CT50, LEMP-1) (1,2,3,4)*	Unknown	Unknown	[20]
	II	MAN1 (LEM3) (1,2)	Bone and skin	Signal transduction, Transcriptional regulation	[74]
		LEM2 (NET25) (1,2)*	Muscle cells	Signal transduction	[52]
III	Ankle1 (LEM3, ANKRD41) (1,2,3,4)*	unknown	DNA damage response	[15]	
	Ankle2 (LEM4)	unknown	Nuclear envelope reformation	[17]	

* Predicted isoforms