Distinct functional domains in emerin bind lamin A and DNA-bridging protein BAF

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SUMMARY

Loss of emerin, a lamin-binding nuclear membrane protein, causes Emery-Dreifuss muscular dystrophy. We analyzed 13 site-directed mutations, and four disease-causing mutations that do not disrupt emerin stability or localization. We show that emerin binds directly to barrier-to-autointegration factor (BAF), a DNA-bridging protein, and that this binding to BAF requires conserved residues in the LEM-motif of emerin. Emerin has two distinct functional domains: the LEM-domain at the N-terminus, which mediates binding to BAF, and a second functional domain in the central region, which mediates binding to lamin A. Disease mutation Δ95-99 mapped to the lamin-binding domain and disrupted lamin A binding in vitro. Two other disease-linked residues, Ser54 and Pro183, mapped outside the BAF and lamin-binding domains, suggesting that emerin may have additional functional domains relevant to disease. The disease-linked emerin proteins all remained active for binding to BAF, both in vitro and in vivo, suggesting that disease can result from the loss of specific molecular interactions between emerin and either lamin A or putative novel partner(s). The demonstration that emerin binds directly to BAF, coupled to similar results for LAP2, provides proof in principle that all LEM-domain nuclear proteins can interact with BAF, with interesting implications for chromatin attachment to the nuclear envelope.

Key words: Barrier to autointegration factor, Emery-Dreifuss muscular dystrophy, lamin A, lamin-associated polypeptide 2, LEM-domain, nuclear envelope, nuclear lamina, MAN1.

INTRODUCTION

Emery-Dreifuss muscular dystrophy (EDMD) is characterized by a triad of symptoms: progressive muscle weakening, contractures of the Achilles and other tendons, and potentially life-threatening cardiac conduction defects (Emery, 1989). EDMD is inherited through mutations in either of two genes, *STA* (Bione et al., 1994) or *LMNA* (Bonne et al., 1999), which encode nuclear lamina proteins named emerin and A-type lamins, respectively (Cohen et al., 2001). Mutations in *LMNA* can also give rise to other diseases (Bonne et al., 2000), including dilated cardiomyopathy and lipodystrophy. The mechanisms of these diseases, collectively termed laminopathies, are not understood (Wilson et al., 2001; Morris, 2001).

Human emerin is a 254-residue integral protein of the nuclear inner membrane (Manilal et al., 1996; Nagano et al., 1996; Yorifuji et al., 1997). Emerin belongs to a family of nuclear proteins defined by a ~40-residue motif termed the LEM-domain (Lin et al., 2000). The LEM-domain family is growing and includes MAN1 (Lin et al., 2000), lamina associated polypeptide-2 (LAP2) (Foisner and Gerace, 1993), otefin (Goldberg et al., 1998; Wolff et al., 2001) and Lem-3 (Lin et al., 2000; Lee et al., 2000). Emerin and the β-isoform of LAP2 have a second region of high homology at their transmembrane domains, and are similar throughout their lengths. Both emerin and LAP2β interact with lamins. LAP2β interacts specifically with lamin B1 (Foisner and Gerace, 1993), whereas emerin interacts with both A- and B-type lamins (Fairley et al., 1999; Clements et al., 2000). In *LMNA*-knockout mice, emerin becomes localized to both the nuclear envelope and ER, suggesting that A-type lamins contribute to (but are not essential for) the nuclear localization of emerin (Sullivan et al., 1999). Localization at the inner nuclear membrane appears to be important for emerin's function, since a mutation that prevents emerin from reaching the inner membrane causes disease (Fairley et al., 1999).

The homology between LAP2β and emerin suggested to us that these proteins might have related functions. In addition to binding lamin B, LAP2β also interacts with chromatin in vitro (Foisner and Gerace, 1993). A novel binding partner for LAP2β on chromatin was identified in a yeast two-hybrid screen (Furukawa, 1999); this partner, barrier-to-autointegration factor (BAF), is an essential, highly conserved DNA-bridging protein of unknown function (Lee and Craigie, 1998; Chen and Engelman, 1998; Zheng et al., 2000). The LEM-domain is essential for LAP2β to bind BAF (Furukawa, 1999; Shumaker et al., 2001) and BAF-DNA complexes (Shumaker et al., 2001). Because emerin has a LEM domain, we tested the hypothesis that emerin binds BAF. Our results for wildtype emerin and a collection of site-directed emerin mutants strongly support this model, and define at least two proposed functional domains within emerin.

MATERIALS AND METHODS

Antiserum production and immunoblots

Polyclonal antibodies against recombinant human emerin were raised in rabbit serum 2999, using untagged wildtype emerin residues 1-222 as antigen. Immunizations and serum production were done by Covance Research Products (Denver PA). For immunoblots, recombinant emerin proteins in bacterial lysates were resolved by electrophoresis in 10% SDS-PAGE gels, transferred to nitrocellulose (Schleicher and Schuell), blocked in PBS/0.1% Tween-20 (PBST) containing 5% nonfat dry milk, and probed with serum 2999 (1:1000 dilution). Bound antibodies were detected using HRP-conjugated goat anti-rabbit antibodies (1:50,000 dilution; Pierce) and enhanced chemiluminescence (Amersham/Pharmacia Biotech).

Site-directed mutagenesis

An emerin cDNA was generated by PCR by E. Abrams and J. Beneken from a human heart cDNA library obtained from R. Reed (Johns Hopkins School of Medicine). The starting point for site-directed mutagenesis was a cDNA encoding wildtype human emerin residues 1-222, subcloned into the pET11c vector (Novagen). All mutations were made using the QuickChange site-directed mutagenesis kit (Stratagene), following the manufacturer's instructions, and verified by full-length double-stranded DNA sequence analysis (data not shown). GFP-emerin constructs were made as described (Haraguchi et al., 2001).

Emerin expression and blot overlay assays

Each emerin construct was transformed into *E. coli* strain BL21 (DE3). Transformed cells containing each plasmid were grown to an OD_{600} of 0.6, and emerin expression was induced by 0.4 mM IPTG for four hours. Cells were pelleted for 5 minutes at 14,000 ***g***, and resuspended in 2× SDS sample buffer. Proteins from unfractionated bacterial lysates were separated on 10% SDS-PAGE gels, transferred to nitrocellulose membranes (Schleicher and Schuell), and blocked for 1 hour in PBST containing 5% nonfat dry milk. Blots were then washed twice in BRB (Blot Rinse Buffer; 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.1% Tween-20) for 5 minutes at 22-24°C, and incubated with 20 μCi of ^{35}S-cysteine/methionine labeled probe protein (either BAF or lamin A; see below) diluted 1:200 into BRB containing 0.1% fetal calf serum (final volume, 10 ml). The lamin A construct in vector pET7a was a kind gift from Robert Moir and Robert Goldman (Northwestern University, Chicago). Blots were incubated overnight with ^{35}S-labeled in vitro-transcribed/translated probe protein at 4°C, washed twice in BRB, dried and exposed to Hyperfilm MP (Amersham/Pharmacia Biotech). Emerin mutant proteins m76 and m141 consistently migrated more slowly than other recombinant emerins on SDS-PAGE.

Synthesis of ^{35}S-Cys/Met labeled proteins and immunoprecipitation

We used the T7 promotors on expression vectors pET11c (for emerin and emerin mutants), pET7a (for lamin A) and pET15b (for BAF) to drive the expression of ^{35}S-cysteine/methionine-labeled emerin, lamin A and BAF proteins using the T_NT Quick Coupled Transcription/Translation System (Promega Corp., Madison WI), according to the manufacturer's protocol. Proteins were transcribed/translated individually for 90 minutes at 30°C. For use as probes in blot overlay experiments, each protein was diluted 1:200 into BRB/0.1% FCS and used as described above. For immunoprecipitation experiments, labeled proteins (10 μl each from a 50 μl T_NT reaction) were incubated (individually, or mixed as indicated) for 30 minutes at 22-25°C to allow binding. We then added 300 μl of immunoprecipitation (IP) buffer (20 mM Hepes pH 7.9, 150 mM NaCl, 10 mM EDTA, 2 mM EGTA, 0.1% NP-40, 10% glycerol, 1 mM DTT, 1 mM PMSF and 20 ug/ml leupeptin) to each sample. To immunoprecipitate ^{35}S-labeled emerin, 4 μl of serum 2999 (immune or pre-immune) was added to each reaction and incubated one hour on ice. BAF was immunoprecipitated using rabbit serum 3000. We then added 50 μl of washed protein A Sepharose beads (Amersham/Pharmacia Biotech), incubated overnight at 4°C, centrifuged at 5000 ***g*** for 5 minutes to pellet the beads, and washed the pellets five times with ice-cold IP buffer. Bound proteins were removed from beads by boiling in 40 μl 2× SDS sample buffer, subjected to 17% SDS-PAGE, dried and exposed to Hyperfilm (Amersham/Pharmacia Biotech).

GFP-emerin plasmid construction

GFP-emerin was a gift of Yuichi Tsuchiya and Kiichi Arahata. To make a GFP fusion to emerin-m24, emerin-S54F and emerin-Δ95-99 that included the transmembrane domain, the coding region of pET11c-emerin-m24, pET11c-emerin-S54F and pET11c-emerin-Δ95-99 was first PCR-amplified using primers 5′-CGTCC-GGACTCAGATCCATGGACAACTAC-3′ and 5′-GCGGATCCC-TGGCGATCCTGGCCCAG-3′. Secondly, the PCR product was digested with *Bsp*EI and *Bam*HI, and inserted in the pEGFP-C1 vector at the *Bsp*EI and *Bam*HI sites. Finally, this construct was digested with *Sac*I and *Bam*HI, and ligated with the *Sal*I/*Bam*HI fragment from full-length GFP-emerin plasmids that include the transmembrane domain. To make a GFP-fusion to emerin-P183T and emerin-P183H that included the transmembrane domain, the coding region of GFP-emerin was PCR-amplified using the following primers; 5′-CGGAGCTCCCTGGACCTGTCCTATTATACTACTTCCTCCTC-3′ and 5′-GGATCCGGTGGATCCCGGGCCCGCGGTACCGTAGAC-3′ for emerin-P183T, and 5′-CGGAGCTCCCTGGACCTGTCC-TATTATCATACTTCCTCCTC-3′ and 5′-GGATCCGGTGGATCCC-GGGCCCGCGGTACCGTAGAC-3′ for emerin-P183H. The PCR product was digested with *Sac*I and *Bam*HI, and ligated with the *Sac*I/*Bam*HI fragment from full-length GFP-emerin plasmids. The DNA sequence of all fusion plasmids were confirmed using an ABI377 DNA sequencer (Applied Biosystems, Norwalk, CT).

GFP-emerin expression and indirect immunofluorescence staining in HeLa cells

HeLa cells were cultured in a 35 mm glass-bottom culture dish as described previously (Haraguchi et al., 1997). Transfection of the plasmid DNA encoding the wildtype and various mutations of GFP-emerin was performed with LipofectaminePlus (Gibco BRL, Rockville, MD) according to the manufacturer's protocol except that the incubation time of the cells with the reagent complexes was reduced to 1.5 hours. Cells were cultured for 2 days under regular culture conditions before being subjected to live microscopic observation, as described previously (Haraguchi et al., 2000).

RESULTS

We hypothesized that residues conserved between emerin and LAP2β might be important for emerin function, and therefore targeted many of these conserved residues for mutation (Fig. 1). We first generated a nearly full-length recombinant human emerin protein consisting of residues 1-222, ending just before the transmembrane domain. We then used site-directed mutagenesis to construct 13 mutant emerin proteins, each carrying a cluster of alanine substitutions in residues that are identical between human emerin and human LAP2β. Mutant clusters were numbered according to their most N-terminal altered residue (Fig. 1).

Emerin binds directly to BAF in vitro

To test the hypothesis that emerin binds BAF, we first used in vitro-transcribed/translated wildtype human BAF to probe blots of immobilized emerin ('blot overlay' experiments).

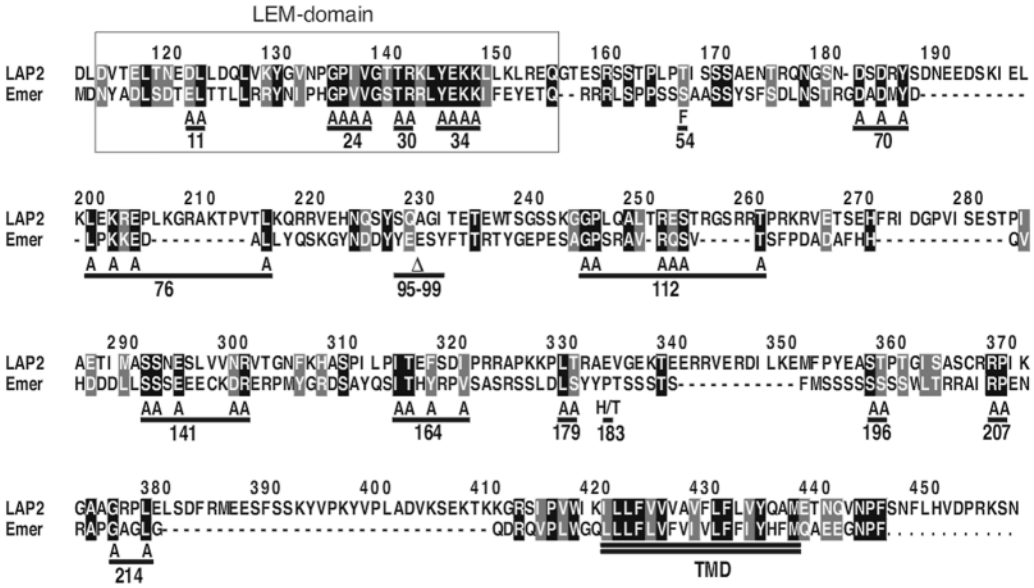

Fig. 1. Mutagenesis of human emerin, targeting residues conserved with human LAP2β. Shown are the aligned amino acid sequences of human emerin and human LAP2β, starting with the LEM-domain of each protein (residue 1 of emerin; residue 110 of LAP2β). Numbers on the top line refer to the LAP2β sequence. The regions mutated in this study are indicated by lines; residues changed to alanine are indicated by A. The number below each line refers to the amino acid sequence of emerin, and names each cluster of mutations according to its most N-terminal altered residue. TMD, transmembrane domain.

Lysates from bacteria that expressed recombinant emerin proteins (wildtype or mutant residues 1-222) were resolved by SDS-PAGE, transferred to membranes and first probed with antibodies against emerin (Fig. 2A), to demonstrate the presence of each recombinant protein. Recombinant wildtype emerin (residues 1-222) migrated with an apparent mass of 27 kDa in SDS-PAGE. Parallel blots were probed with ^{35}S-labeled human BAF, synthesized in coupled transcription/translation reticulocyte lysates. Supporting our hypothesis, wildtype BAF bound to wildtype recombinant emerin on the blots (Fig. 2B, W). This interaction was specific since BAF did not bind to several mutant emerins. All four LEM-domain mutations (m11, m24, m30, m34) significantly reduced emerin binding to BAF (Fig. 2B). Mutations in the central and C-terminal regions of emerin did not disrupt the emerin-BAF interaction (Fig. 2B; m70 to m214). These results suggested that emerin interacts directly with BAF, and that residues within the LEM-domain of emerin are required to bind BAF.

We used co-immunoprecipitation experiments to independently confirm the emerin-BAF interaction. ^{35}S-labeled wildtype and mutant emerin proteins were incubated with ^{35}S-labeled human BAF, and precipitated using polyclonal antibodies against human emerin (Fig. 3). Supporting the blot overlay results, wildtype emerin (residues 1-222) co-immunoprecipitated with wildtype BAF (Fig. 3, WT). Co-immunoprecipitation results confirmed that LEM-domain residues were essential for emerin and BAF to interact in solution, since LEM-domain mutants m11 to m34 all failed to co-immunoprecipitate with BAF (Fig. 3). The other emerin mutants all co-immunoprecipitated with BAF from solution (Fig. 3). Based on these two independent lines of evidence we concluded that emerin binds directly to BAF in vitro, and that this binding is mediated by residues in the LEM-domain.

Central region of emerin binds lamin A

Emerin interacts with A-type lamins in vitro (Fairley et al., 1999; Clements et al., 2000). To map the binding region for lamin A, we tested our emerin mutants for binding to lamin A in blot overlay experiments. Five mutations (m70, m76, m112, m141 and m164) reduced emerin binding to lamin A; all five mapped outside the LEM-domain, in the central region of emerin (Fig. 2C). All other mutant emerins bound to lamin A at least as well as wildtype emerin. Several emerin mutants, notably m24 in the LEM-domain, reproducibly bound to lamin A better than wildtype emerin (Fig. 2C), as estimated by densitometry analysis (data not shown; see Discussion). When

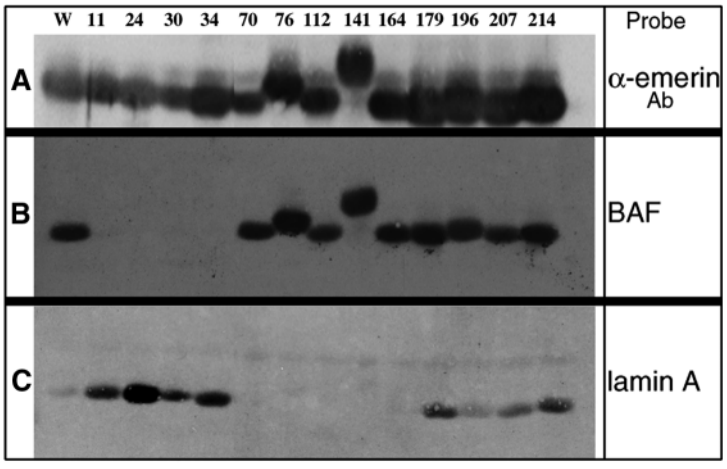

Fig. 2. Blot overlay assays for emerin binding to BAF or lamin A. Bacterial lysates containing wildtype (W) or mutant emerin residues 1-222 were separated on gels, blotted and probed with: (A) anti-emerin antibodies, (B) ^{35}S-labeled BAF, or (C) ^{35}S-labeled lamin A. Mutants are numbered according to Fig. 1.

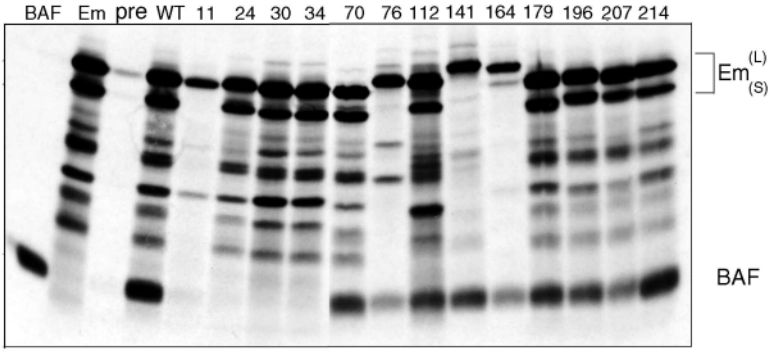

Fig. 3. Solution binding as assayed by co-immunoprecipitation. Wildtype BAF, wildtype emerin (WT, residues 1-222) and mutant emerin proteins (numbered as in Fig. 1), were synthesized and ^{35}S-labeled in vitro using coupled transcription/translation reactions, and then immunoprecipitated using immune (lanes Em, WT, 11-214) or preimmune (pre) antiserum against emerin, or anti-BAF antisera (BAF). In vitro translation of emerin yielded a 27 kDa long form (L), and often also yielded a prominent 23 kDa short form (S) (Östlund et al., 1999), assumed to arise by translation initiation at an internal site, as well as several smaller bands.

blots were probed with ^{35}S-labeled lamin C, similar results were seen but the signals were significantly weaker than for lamin A (data not shown). Note that in competitive co-immunoprecipitation assays, emerin prefers lamin C (Vaughan et al., 2001). The first 566 residues of lamins A and C are identical, but their C-termini differ (Lin and Worman, 1993).

Disease-associated emerin mutations

Most human emerin mutations yield cells that are null for emerin protein. However, in four cases, comprising point mutations S54F, P183H and P183T, and a small deletion (ΔYEESY; www.path.cam.ac.uk/emd/mutation.html), the mutant protein is stable and localized at the nuclear envelope, rather than being degraded like most other mutant emerins (Fairley et al., 1999; Ellis et al., 1999; Haraguchi et al., 2001). To determine if disease-causing mutations disrupted emerin binding to BAF or lamin A, three of these 'stable' mutations were introduced into recombinant emerin (residues 1-222). We changed serine 54 to phenylalanine (S54F; referred to as 'S54P' in Fairley et al.) (Fairley et al., 1999), proline 183 to histidine (P183H) (Ellis et al., 1999), and deleted five residues to create the ΔYEESY mutation (referred to here as Δ95-99) (Fairley et al., 1999). All three mutant proteins were tested for direct binding to BAF and lamin A. Our controls were wildtype emerin, mutant m24 (defective in binding BAF) (Fig. 2; Fig. 3) and mutant m141 (defective in binding lamin A; Fig. 2). Mutants S54F and P183H both interacted with BAF in blot overlay (Fig. 4A) and co-immunoprecipitation assays (Fig. 4B), and also interacted with lamin A in blot overlay assays (Fig. 4A). Thus, these mutations did not disrupt binding to either BAF or lamin A in vitro, consistent with their positions within the proposed functional map of emerin (Fig. 5). By contrast, mutation Δ95-99 had no effect on emerin binding to BAF, but significantly reduced its binding to lamin A (Fig. 4A, lam A). This result strongly supported the proposed lamin-binding domain of emerin, where residues 95-99 map (Fig. 5). These findings suggested that mutation Δ95-99 might cause disease by specifically disrupting emerin attachment to lamins.

The above results showed that disease-causing emerin mutants are active for binding to BAF in biochemical assays. To independently confirm these results, we tested the disease-linked emerin mutants for binding to BAF in living cells. Each disease mutation, plus the alternative P183T allele (Ellis et al., 1999), was incorporated into full-length emerin with Green Fluorescent Protein attached to the N-terminus of emerin (GFP-emerin; see Materials and Methods). Each mutant protein was transiently expressed and localized in living HeLa cells. All four mutants localized predominantly to the nuclear envelope during interphase, with weak ER staining, and were indistinguishable from wildtype emerin-GFP (Fig. 6A), as expected (Fairley et al., 1999). These interphase results showed that our fusions to GFP did not disrupt localization. We then followed the HeLa cells as they progressed through mitosis, to determine if the mutant emerins were able to interact with BAF in living cells, based on a novel in vivo assay (Haraguchi et al., 2001). BAF recruits emerin to co-localize at the 'core' region of telophase chromosomes for about two minutes near the end of mitosis; this 'core' localization appears to be critical for the assembly of both emerin and A-type lamins (but not B-type lamins) into re-forming nuclear envelopes (Haraguchi et al., 2001). 'Core' localization was present for wildtype emerin-GFP (Fig. 6B), and absent in the negative control (Fig. 6B, mutant m24), as expected. Notably, all four disease-linked mutations were recruited to the 'core' region (Fig. 6B), demonstrating their ability to bind BAF in vivo. These proteins subsequently redistributed uniformly over the nuclear envelope, like wildtype emerin, and continued to localize normally after exiting mitosis (data not shown). The apparently normal

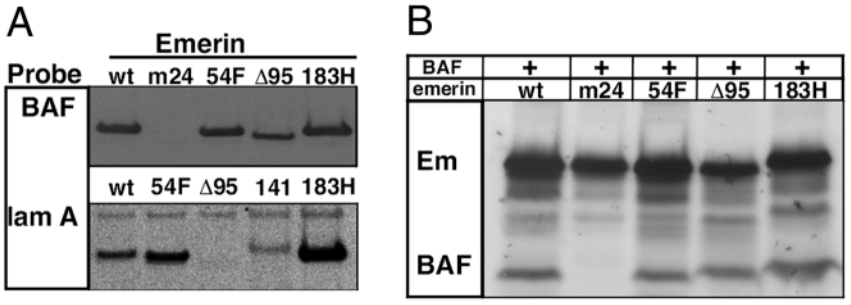

Fig. 4. Effects of disease-associated mutations S54F, Δ95-99 and P183H on emerin binding to BAF and lamin A. (A) Bacterial lysates containing wildtype (wt) emerin protein, disease-linked emerins (S54F, Δ95-99, P183H) or alanine-substitution mutants (m24 and m141) were separated on gels, blotted and probed with ^{35}S-labeled BAF or ^{35}S-labeled lamin A. (B) Wildtype and mutant emerin proteins were synthesized as ^{35}S-labeled proteins in vitro, mixed with ^{35}S-labeled BAF, and immunoprecipitated with immune (shown) or preimmune (not shown) antibodies against emerin (see Materials and Methods).

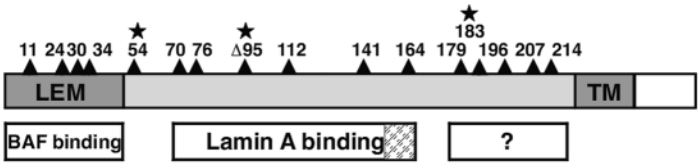

Fig. 5. Functional domains of emerin defined in this study. Emerin is depicted schematically, showing the LEM-domain (LEM), transmembrane domain (TM), and position of each cluster of mutations (inverted triangles; numbered as in Fig. 1). Mutations are positioned to scale along the polypeptide sequence. Domains defined in this study are the BAF binding domain (residues 1-50, which include the LEM-domain), the lamin-binding domain (residues 70-178) and a proposed third domain of unknown function (residues 179-222). Stars indicate the positions of human mutations that cause Emery-Dreifuss muscular dystrophy. Shading at the right end of the proposed lamin-binding domain indicates less severely reduced binding of lamin A to mutant m164.

recruitment of disease-causing emerin proteins to the 'core' region of assembling nuclear envelopes strongly supported our in vitro findings that these mutant proteins are active for binding to BAF. We propose that these mutations cause disease at the molecular level, by specifically disrupting emerin interactions with partners other than BAF during interphase.

DISCUSSION

Our discovery that emerin interacts with BAF in vitro brings a potentially important new player into the picture for Emery-Dreifuss muscular dystrophy. BAF is essential for life in *C. elegans* (Zheng et al., 2000), where it is expressed in every cell (M. Segura and K.L.W., unpublished). BAF is proposed to be a DNA-bridging protein, based on the unique ability of BAF dimers to assemble into discrete nucleoprotein complexes consisting of six BAF dimers plus multiple dsDNAs (Zheng et al., 2000). Cells that lack emerin also lack emerin-BAF interactions, which might contribute to the molecular mechanism of disease. In cells, emerin and BAF are strikingly colocalized for about two minutes during telophase, at the 'core' region of telophase chromosomes (Haraguchi et al., 2001). In cells that transiently express an exogenous mutant BAF, emerin fails to localize at the core and is absent from the subsequent assembled nuclei, suggesting a role for BAF in recruiting and stabilizing emerin during nuclear assembly (Haraguchi et al., 2001).

Proposed functional domains of emerin

Our strategy of mutagenizing small clusters of conserved residues was highly effective. Every cluster of mutations from residues 11 to 179 disrupted binding to either BAF or lamin A, but not both, demonstrating that residues conserved between emerin and LAP2β are indeed critical for emerin function. We propose that the exposed (nucleoplasmic) region of emerin has at least two independent domains, comprising an N-terminal BAF-binding domain and a central lamin-binding domain, and might also have additional domains relevant to disease (Fig. 5). These domains are each discussed below.

The most N-terminal domain of emerin is the LEM motif (residues 1-43), which is here demonstrated to bind BAF. Consistent with this model, residues 1-65 (but not residues 1-37) of emerin are sufficient to localize emerin to the 'core' region of telophase chromosomes in vivo (Haraguchi et al., 2001). Our discovery that emerin binds BAF is also strongly supported by the recently solved solution structure of the constant region of LAP2 (Cai et al., 2001); this work showed that the LEM-domain folds independently into a conserved backbone structure (Cai et al., 2001; Laguri et al., 2001) with surface features that complement a hydrophobic binding pocket on the BAF dimer interface (Cai et al., 2001). The ability of wildtype emerin and four disease-linked emerin proteins to bind BAF, both in vitro and in living cells, strongly suggests that (a) BAF interactions are central to emerin function, and (b) for these particular mutant alleles, disease may arise from disrupted binding to a partner other than BAF, such as lamin A or a hypothetical novel partner.

Residues 70-178 comprise the proposed lamin A-binding domain. This domain includes residues 117-170, which function as a nuclear membrane retention signal for emerin (Östlund et al., 1999), supporting our proposal that this region interacts directly with lamins. Furthermore, EDMD-associated mutation Δ95-99, which failed to bind lamin A in vitro, is more susceptible to biochemical extraction from nuclei, consistent with weakened binding to lamins (Ellis et al., 1998). Emerin mutant Δ95-99 is localized at the nuclear envelope in EDMD patients (Fairley et al., 1999) and when expressed in HeLa cells (our results). This proper localization could be explained at least two ways: this mutant might somehow remain competent to bind lamin A in vivo, even though it fails to bind lamin A in vitro. Alternatively, other partners (e.g. B-type lamins, BAF or novel partners) might contribute to its localization in vivo. Two findings support the idea that emerin localization in humans depends on a partner other than lamin A, or multiple partners. First, emerin localization at the nuclear envelope is completely lost in *C. elegans* embryos that are depleted of their only lamin (B-type; Gruenbaum et al., unpublished), suggesting that lamins per se are essential for emerin localization. Second, emerin and lamin A both fail to associate with assembling nuclear envelopes in cells that express a dominant mutant BAF (Haraguchi et al., 2001), implying that BAF is key to localizing both emerin and lamin A. Together, these findings indicate that emerin recruitment and retention at the nuclear envelope is complicated, involving distinct sequential interactions with BAF, A-type lamins and B-type lamins. We suggest that emerin mutant Δ95-99 is recruited appropriately by BAF, but its function is then compromised by defective binding to lamin A. Thus in patients who express emerin Δ95-99, emerin interactions with A-type lamins may be abnormal.

Residues 179-222 define a potential third domain, which was not required to bind either BAF or lamin A. Based on the effectiveness of our mutagenesis strategy, and the fact that mutations P183H and P183T cause disease, we propose that this third region has a novel function. Interestingly, residues 176-222 are sufficient to localize the transmembrane domain of emerin at the nuclear envelope (Haraguchi et al., 2001), implying that the predicted 'third' domain of emerin might interact with a partner found at or near the inner nuclear

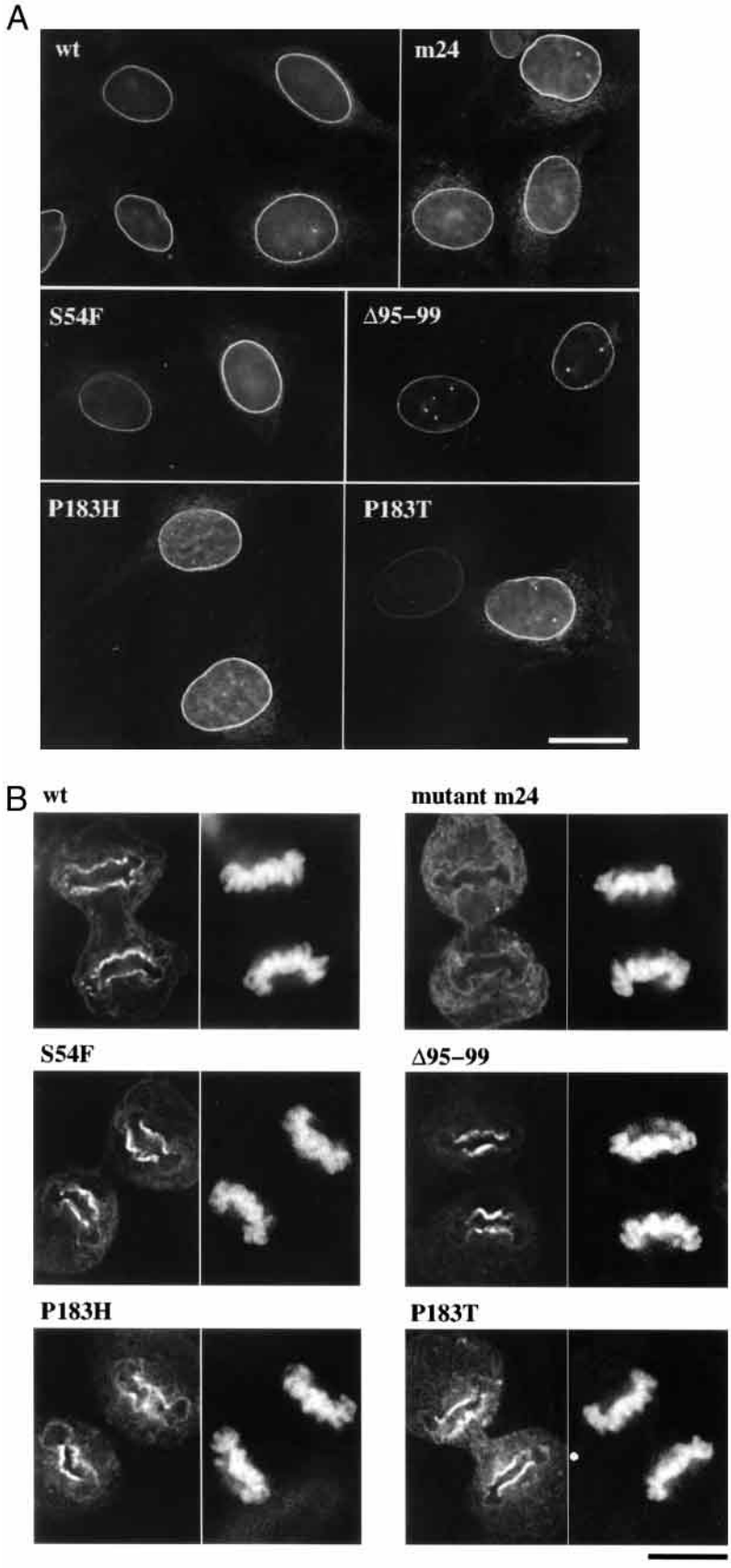

Fig. 6. Localization of GFP-fused emerin mutants S54F, Δ95-99, P183H and P183T in living HeLa cells. HeLa cells were transiently transfected to express the indicated emerin mutant as a GFP-fusion protein. (A) GFP fluorescence during interphase. (B) GFP fluorescence in living cells 5-7 minutes after the metaphase-anaphase transition, when wildtype emerin localizes to the 'core' region of telophase chromosomes. Bars, 10 μm.

membrane. Mutations at disease-linked residue P183 had no affect on emerin binding to BAF or lamin A, either in vitro or in living HeLa cells. We therefore propose that mutations at P183 (located within the putative third domain) cause disease by disrupting emerin binding to an unidentified new partner.

Our findings show both in vitro and in vivo that the nucleoplasmic region of emerin has at least two modular structural domains, which mediate its binding to BAF and lamin A. Because two disease-associated residues (S54 and P183) both lie outside the BAF-binding and lamin-binding domains, we speculate that these mutations might disrupt emerin regulation, or define additional functional domains. An important future question will be to determine whether emerin interacts with its partners simultaneously, or if binding to one partner can displace or enhance binding to another partner. Based on the enhanced lamin-binding activity of some LEM-domain mutants, particularly m24, we speculate that these domains might influence each other intramolecularly. As precedent for intramolecular regulation of LEM proteins, we note that the binding affinity of LAP2 for BAF is reduced three- to ninefold when the BAF-binding constant region of LAP2 is linked to the 'variable' regions of different LAP2 isoforms (Shumaker et al., 2001).

Implications for nuclear infrastructure

Our discovery that emerin binds BAF in a LEM-domain-dependent manner, coupled to parallel results for LAP2 (Furukawa, 1999; Shumaker et al., 2001), strongly suggest that all LEM proteins can bind BAF. Since BAF binds nonspecifically to double-stranded DNA (Zheng et al., 2000), our findings have important implications for chromatin organization in the nucleus. LEM proteins, as a family, are collectively positioned to play major roles in chromatin attachment to the nuclear inner membrane and lamin filaments. Emerin and other LEM proteins are expressed in nearly all cells (Lin et al., 2000), and some are abundant: the molar ratio of LAP2 to lamin B in rat liver nuclei has been estimated at 2-5%, enough to position one LAP2β molecule every 25-50 nm along lamin filaments (Foisner and Gerace, 1993). Furthermore, the abundant α isoform of LAP2 co-localizes with lamin A throughout the nuclear interior (Dechat et al., 2000; Moir et al., 2000), meaning that the proposed attachments between LEM proteins and BAF are not restricted to the nuclear periphery, but could also extend throughout the nuclear interior. Further study

of emerin-BAF interactions will be critical for understanding chromatin organization in the nucleus, and the disease mechanism of EDMD.

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