Transcriptional Repressor Germ Cell-less (GCL) and Barrier to Autointegration Factor (BAF) Compete for Binding to Emerin in Vitro*

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Emerin belongs to the “LEM domain” family of nuclear proteins, which contain a characteristic ~40-residue LEM motif. The LEM domain mediates direct binding to barrier to autointegration factor (BAF), a conserved 10-kDa chromatin protein essential for embryogenesis in Caenorhabditis elegans. In mammalian cells, BAF recruits emerin to chromatin during nuclear assembly. BAF also mediates chromatin decondensation during nuclear assembly. The LEM domain and central region of emerin are essential for binding to BAF and lamin A, respectively. However, two other conserved regions of emerin lacked ascribed functions, suggesting that emerin could have additional partners. We discovered that these “unassigned” domains of emerin mediate direct binding to a transcriptional repressor, germ cell-less (GCL). GCL co-immunoprecipitates with emerin from HepG2 cells. We determined the binding affinities of emerin for GCL, BAF, and lamin A and analyzed their oligomeric interactions. We showed that emerin forms stable complexes with either lamin A plus GCL or lamin A plus BAF. Importantly, BAF competed with GCL for binding to emerin in vitro, predicting that emerin can form at least two distinct types of complexes in vivo. Loss of emerin causes Emery-Dreifuss muscular dystrophy, a tissue-specific inherited disease that affects skeletal muscles, major tendons, and the cardiac conduction system. Although GCL alone cannot explain the disease mechanism, our results strongly support gene expression models for Emery-Dreifuss muscular dystrophy by showing that emerin binds directly to a transcriptional repressor, GCL, and by suggesting that emerin-repressor complexes might be regulated by BAF. Biochemical roles for emerin in gene expression are discussed.

Emery-Dreifuss muscular dystrophy (EDMD) is characterized by progressive muscle weakening, contractures of major tendons, and defects in cardiac conduction that can be life-threatening (1). EDMD is inherited through mutations in two different genes: LMNA and STA. LMNA encodes A-type lamins, which are developmentally regulated nuclear filament proteins, and STA encodes an integral nuclear membrane protein named emerin (2, 3). Emerin mutations cause the X-linked recessive form of EDMD (4, 5). Emerin is expressed in most cell types examined thus far, except non-myocytes of the heart (6). Because emerin is expressed in most cells, but its loss affects only a few specific tissues, emerin was proposed to have roles in tissue-specific gene expression (7).

Emerin belongs to the LEM domain family of nuclear proteins, which contain a characteristic ~40-residue LEM motif (8, 9). Other family members include LAP2, MAN1, otefin, LEM-3, and SANE (5, 10–12). Most LEM proteins are localized to the nuclear inner membrane. The LEM domains of LAP2β and emerin mediate their direct binding to barrier to autointegration factor (BAF) (13–15). BAF is a 10-kDa, highly conserved chromatin protein essential for the viability of dividing cells (16). In mammalian cells, BAF recruits emerin to chromatin during nuclear assembly (17), and this recruitment is somehow essential for localizing emerin at the reforming nuclear envelope. BAF also has important roles in higher-order chromatin structure during nuclear assembly (18). The LEM domain of emerin is essential for binding to BAF, and the central region of emerin is essential to bind lamin A (15). These results, coupled with distinct binding regions for BAF and lamins on LAP2β (14, 19), suggest that BAF links chromatin directly to membrane-anchored LEM proteins and indirectly to lamins. However, two conserved regions of emerin lacked any ascribed function, suggesting that emerin has additional unknown partners (15).

We now report that these “unassigned” domains of emerin mediate direct binding to germ cell-less (GCL) a transcriptional repressor that is conserved from Caenorhabditis elegans to humans. Nili et al. (20) showed previously that GCL binds LAP2β. GCL also binds directly to the DP3 subunit of E2F-DP heterodimers and thereby represses E2F-DP-dependent gene transcription (20, 21). Independently, E2F-DP-dependent genes are repressed by retinoblastoma protein, which binds to E2F and recruits histone-modifying complexes (22). Interestingly, LAP2β can inhibit a reporter gene regulated by E2F-DP, suggesting that LAP2β itself is a repressor; furthermore, co-expression of both LAP2β and GCL repressed transcription as effectively as retinoblastoma protein (20). Because GCL binds to a region in LAP2β that is conserved with emerin, we proposed and tested the hypothesis that GCL also binds emerin. We determined the binding affinities of emerin for GCL, BAF, and lamin A and studied their oligomeric interactions biochemically. Our findings support molecular models in which emerin and its partners form at least two types of complexes in vitro. Our results suggest that emerin has distinct roles in vivo, depending on whether it is complexed with BAF or a transcription factor.

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The abbreviations used are: EDMD, Emery-Dreifuss muscular dystrophy; BAF, barrier to autointegration factor; GCL, germ cell-less; Iz, interaction zone; BSA, bovine serum albumin.
**EXPERIMENTAL PROCEDURES**

**Protein Purification—**Human emerin (residues 1–222) was cloned into pET15 plasmid (Novagen, Inc., Madison, WI), and alanine substitution mutagenesis was performed as described previously (15). Wild-type emerin and all mutant proteins were expressed in Escherichia coli strain BL21(DE3)-plysS as described previously (15). Induced bacteria were collected by centrifugation, resuspended in PBS, and sonicated (30 s, five times each). The resulting homogenate was centrifuged (20 min, 40,000 g, 4 °C). The pellet was washed twice with PBS; resuspended in 8 M urea, 500 mM NaCl, and 20 mM HEPES (pH 7.4); and recenter- fuged for 20 min at 40,000 g. The supernatant was recovered, treated with 10 volumes of 500 mM NaCl, 20 mM HEPES (pH 7.4), and recenter- fuged for 20 min at 40,000 g. This supernatant was loaded on a Superdex 75 preparative gel filtration column (Amersham Biosciences), and 1.5-ml fractions were collected. Enermin elutes at a position consistent with monomers (data not shown). Fractions containing emerin protein were snap-frozen in liquid nitrogen. 35S-labeled proteins were synthesized *in vitro* using the TnT® Quick Coupled Transcription/Translation System according to the manufacturer’s instructions (Promega, Madison, WI). The cDNA encoding human GCL was a kind gift from Aom Sirinukulyanun (20).

**Binding Assays—**Purified recombinant human emerin (residues 1–222) was covalently attached to Affi-Gel-15 beads (Bio-Rad Laboratories, Inc., Hercules, CA) according to the manufacturer’s instructions. Beads were washed three times in binding buffer (20 mM HEPES, pH 7.4, 110 mM potassium phosphate, 2 mM magnesium acetate, and 0.5 mM EGTA) and incubated with either [35S]GCL or [35S]BAF for 2 h at 22–24 °C. Beads were washed five times with binding buffer, eluted with SDS sample buffer, and subjected to SDS-PAGE, and bound proteins were detected by autoradiography.

Co-immunoprecipitations were performed as described previously (15) using 3.5 mg nuclear extract/experiment. HeLa cell nuclear extracts were generated by standard cell fractionation techniques, as described previously (23). Antibodies against human GCL were produced in rabbits (44438) immunized against a GCL peptide corresponding to residues 408–420, linked to keyhole limpet hemocyanin. Micro- titer well binding assays were done essentially as described previously (24), with the following changes. To measure affinities accurately, the amount of emerin per microtiter well was titrated 10–20-fold: we added lamin A, GCL, or BAF at several concentrations ranging from 0.5 nM to 10 μM to microtiter wells containing either 1, 5, 10, 20, or 50 pmol of emerin. These affinity curves were confirmed by computer modeling (data not shown). When testing for GCL binding to our collection of emerin mutants, subsaturating amounts of [35S]-GCL (50 μM) were used, with 15–25 pmol of emerin typically present per well. Wells were not allowed to dry at any time during these assays. In all cases, bound [35S]-labeled proteins were extracted with 5% SDS and counted in a scintillation counter.

To detect three-way complexes, 150 nM lamin A plus 250 nM BAF, 160 nM lamin A plus 54 nM GCL, or 5 μM BAF plus 60 nM GCL was mixed and immediately added to 10 pmol of wild type emerin. BAF inhibition of GCL binding to emerin was quantitated by first mixing 60 nM [35S]-GCL with increasing concentrations of purified recombinant BAF (50 nM to 10 μM), prepared as described previously (18), and immediately adding the mix to 1.5 pmol of emerin immobilized on microtiter wells. Wells were washed five times with binding buffer, eluted with SDS, and separated on SDS-PAGE, and proteins were detected by autoradiography.

**Reverse Transcription-PCR—**Multitissue panels I and II (Clontech) were used as templates to probe with oligonucleotide primers specific for a LAP2β-specific exon or GCL, according to the manufacturer’s instructions. The 5′ LAP2β primer was 5′-CCTACAGCTGTTAAT-ACTGAGACTGAATG-3′, and the 3′ primer was 5′-GGTCAACATGAA-TGGAATATTATTGAACTGGAGATTCC-3′. The 5′ GCL primer was 5′-CCGGGATC- CGATGAAATTATTTGAGACTGCTGGATTC-3′, and the 3′ primer was 5′-GGTGCTGAGGTTPCCCGATCTGCTGGTGGGC-3′. Control prim- ers for glyceraldehyde-3-phosphate dehydrogenase were provided by the manufacturer.

**Quantitation of BAF—**HeLa cells (105) were resuspended in sample buffer, and 10-cell equivalents were subjected to SDS-PAGE along with 1, 2.5, 5, and 10 ng of recombinant purified human BAF. After transfer to nitrocellulose, blots were probed with an antibody against human BAF diluted 1:1000 (18). We calculated the concentration of BAF near the inner nuclear membrane by modeling the nucleus as a sphere 5 μm in diameter and defining the interaction zone (IZ) to extend 200 nm from the inner membrane. We calculated the volume of the IZ by subtracting the volumes of two spheres, where R1 is the radius to the inner membrane (2.5 μm), and R2 (2.3 μm) equals R1 minus the interaction distance (200 nm). The volume of the IZ of the nuclear envelope was V2 = V1 – V2 = 14.1 fl (10–15 liters), where V1 = 4/3πR12 and V2 = 4/3πR22.

**RESULTS**

Because the GCL-binding region in LAP2β was conserved in emerin, we tested the hypothesis that GCL can also bind emerin. We synthesized full-length [35S]-labeled human GCL and BAF and incubated each protein separately with recombinant human emerin conjugated to Affi-Gel beads. Beads conjugated with BSA served as negative controls. The emerin beads specifically bound BAF and also bound efficiently to GCL (Fig. 1A). The equilibrium affinity of the emerin-GCL interac- tion was measured using a microtiter well binding assay. Recombinant soluble purified emerin (residues 1–222) was immo- bilized in microtiter wells, and different concentrations of soluble [35S]-GCL were added to each well. In this assay, the affinity of GCL for emerin was 30 nM (range, 20–60 nM; n = 30; Fig. 1B), and the stoichiometry of interaction was ~0.8–1 mol GCL/mol emerin (data not shown). These results demonstrated that emerin can bind a transcriptional repressor, GCL, with high affinity *in vitro.* GCL was previously shown to be enriched at the nuclear envelope in *Drosophila* and mammalian cells (20, 21, 25). To determine whether GCL and emerin interacted *in vivo,* we did co-immunoprecipitation experiments. HeLa nu- clear extract was incubated with either protein A beads alone or protein A beads plus anti-emerin antibody. These beads were then washed and resolved by SDS-PAGE, transferred to nitrocellulose, and probed with antibodies against GCL. GCL co-immunoprecipitated with emerin; comparatively little GCL was pulled down by the protein A beads alone (Fig. 1C). Coimmunoprecipitation of GCL with emerin was evidently effi- cient because GCL was present at almost undetectable levels in the starting lysate (Fig. 1C, load). Thus, emerin and GCL appear to interact both *in vitro* and *in vivo.*

We then mapped the GCL-binding region in emerin. The microtiter well binding assay was used to test [35S]-GCL binding to a collection of emerin mutants (15), each containing a cluster of alanine substitutions in residues highly conserved between human LAP2β and emerin (Fig. 1D, mutations are noted in black). We immobilized either BSA, recombinant wild type emerin (residues 1–222), or emerin mutant proteins in micro- titer wells and added [35S]-GCL to the solution in these wells. After washing, the bound [35S]-GCL was removed using 5% SDS and counted (Fig. 1E, graph). To control for the amount of emerin present in each well, immobilized emerin proteins were eluted from parallel wells using 10% SDS, separated by SDS-PAGE, transferred to nitrocellulose, and probed with an anti- emerin antibody (Fig. 1D, top). At the concentration of [35S]-GCL used for these studies (50 nM), emerin was in 3–5-fold excess. Mutants 24, 112, 164, and 179 bound 68% of wild type emerin (Fig. 1E). Mutants 34, 196, 207, and 214 had weak binding (35–45% of wild type). The weakest binding among “conserved mutations” was seen for mutants 70 and 76 (Fig. 1E). The background binding of [35S]-GCL to BSA ranged from 16–23% of the wild type emerin signal, suggesting that mu- tants 34–76 and 196–214 were severely reduced in binding to GCL. To refine the putative GCL-binding domain of emerin, we generated 11 new emerin mutants by alanine substitutions, specifically targeting clusters of residues that are dissimilar between LAP2β and emerin (Table I; Fig. 1D, mutations are shown in gray). We reasoned that emerin-specific residues might contribute uniquely to its affinity for GCL and that these new mutations might better define the GCL-binding domain. Indeed, several emerin-specific mutations disrupted binding quite effectively. When tested in the well binding assay, mu-
FIG. 1. **Emerin Binds GCL**

**A**, affinity binding assays. Affi-Gel beads coupled to recombinant human emerin (residues 1–222) or BSA were incubated with either 35S-GCL or 35S-BAF (see "Experimental Procedures"). **B**, affinity of GCL for emerin, determined by adding increasing concentrations of GCL to constant amounts of emerin immobilized in microtiter wells (see "Experimental Procedures"). Double reciprocal plots and computer modeling were used to accurately determine the affinity constant. **C**, GCL co-immunoprecipitates with emerin from HeLa nuclear extract. Extracts were incubated with either protein A beads alone or protein A beads plus antibodies against human emerin. Pelleted beads were washed, extracted, resolved by SDS-PAGE, and immunoblotted using antibodies against human GCL (top panel) or emerin (bottom panel). **D**, aligned amino acid sequences of human LAP2β (top row, starting at residue 112) and full-length emerin (bottom row), showing mutations used to map the putative GCL-binding domain in emerin. In most cases, underlined residues were replaced by alanine (A) as indicated, in clusters. Mutations in residues conserved between emerin and LAP2β are indicated in black (15). New mutations in emerin-specific residues are shown in gray. Mutant clusters are numbered according to their most N-terminal altered residue, as detailed in Table I. Arrow indicates the last residue (222) of recombinant emerin protein. **E** and **F**, quantitative microtiter well binding assays using immobilized wild type (WT) emerin (residues 1–222) and emerin mutants (numbered as described in D). Emerin proteins immobilized in microtiter wells were incubated with 35S-GCL, washed, and counted. Top panels show emerin proteins extracted from parallel wells and immunoblotted to verify similar amounts of emerin/microtiter well.

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tants 122, 133, 151, 161, 198, and 206 each bound $^{35}$S-GCL to at least 65% wild type levels (Fig. 1, graph). Control blots verified that similar amounts of mutant emerin protein were immobilized in each well (Fig. 1, top). Mutant 145 was compromised (~50% wild type activity) but still bound detectably to $^{35}$S-GCL. Importantly, mutants 45A, 45E, 175, and 192 had very low or background levels of binding to $^{35}$S-GCL (Fig. 1F).

| Name of mutation | Wild type residues | Mutant     |
|------------------|--------------------|------------|
| 45A              | 45RRR              | 45AAA      |
| 45E              | 45RRR              | 45EEE      |
| 175              | 175SWSLTR          | 175AAAAA   |
| 206              | 206IRPE            | 206AAPA    |

Collectively, these results identified two regions in emerin essential for binding to GCL: (a) residues 34–83, which overlap the LEM domain, and (b) residues 175–196 and 207–217 near the transmembrane domain of emerin. This results also implicated serines at positions 175–176 and 192–196 of human emerin as potentially critical for binding GCL.

Most disease-causing mutations in the emerin gene cause cells to be null for emerin protein. However, there are special points in emerin (point mutants S54F, Q133H, P183H, P183T, and deletion Δ95–99) that cause EDMD, wherein the mutant proteins are stable and properly localized at the nuclear envelope (15). The Δ95–99 mutation disrupts emerin binding to lamin A in vitro but has no effect on BAF binding. Mutants S54F and P183H bind normally to both lamin A and BAF in vitro (15). To determine whether any disease-causing mutations disrupted emerin binding to GCL, we used $^{35}$S-GCL to probe mutants S54F, Δ95–99, and P183H immobilized on microtiter wells. Similar amounts of emerin protein were present in each well, as determined by Western blotting of parallel microtiter wells (Fig. 2A, a-emer). BAF bound to emerin mutants S54F and P183H at levels similar to wild type (75–85% of wild type). In contrast, GCL binding to Δ95–99 was reduced to levels barely above the BSA negative control. The Δ95–99 disease mutation therefore disrupts emerin binding to two partners: (a) transcriptional regulator GCL, and (b) lamin A (15).

We considered the possibility that the Δ95–99 mutation causes emerin to misfold, nonspecifically disrupting its binding to both partners. However, any such misfolding must be spatially limited because the Δ95–99 mutant is soluble and can still bind BAF (15) and therefore has a properly folded LEM domain. Alternatively, GCL and lamin A might both require this region of emerin and might theoretically compete for binding to emerin. Our GCL binding results for all emerin mutants are mapped schematically in Fig. 2B, relative to previous results for BAF and lamin A (15). Residues required to bind GCL mapped primarily to the two “unascertained” regions of emerin that flank the lamin-binding region, with some overlap of both the BAF- and lamin A-binding domains. Thus, this transcription factor represents an important new type of binding partner for emerin.

The identification of a transcription factor as a binding partner for emerin raised important questions about the relationship between GCL and emerin’s other partners. To test the hypothesis that GCL, BAF, or lamin A might compete with each other for binding to emerin, we first needed to determine the equilibrium binding affinities of BAF and lamin A for emerin. BAF dimers bind emerin with a $K_d$ of 200 nM (range, 100–550 nM; n = 23; Fig. 2C) and stoichiometry of ~0.75–1 mol BAF dimer/mol emerin in microtiter well binding assays (Fig. 2C; data not shown). Lamin A bound emerin with higher affinity (40 nM; range, 30–80 nM; n = 19; Fig. 2D) and a stoichiometry of ~0.85–1.1 mol lamin A dimer/mol emerin (Fig. 2D; data not shown). Thus, all three partners had reasonable affinities for emerin ($K_d$ of 200, 40, and 30 nM for BAF, lamin A, and GCL, respectively).

We then did competition assays to determine whether BAF competed with GCL for binding to emerin. Microtiter wells with a constant amount of immobilized emerin were incubated with buffer containing a constant amount of $^{35}$S-GCL plus increasing concentrations of BAF (Fig. 3A). Under these conditions, BAF blocked the binding of GCL to emerin, with 50% inhibition of binding at 4.1 μM BAF ($K_d$, Fig. 3A). This competition depended specifically on the BAF-emerin interaction because BAF failed to compete for GCL binding to emerin LEM domain mutant m24 (Figs. 1D and 3B) (15). We concluded that BAF binding to the LEM domain of emerin displaces GCL by reducing its affinity because GCL also depends on residues at the C terminus of the LEM domain. Because the affinity of BAF for emerin (200 nM) is about 7-fold weaker than that of GCL (30 nM), it was reasonable that higher levels of BAF (4.1 μM) were required to inhibit GCL binding. To determine whether these concentrations were physiologically relevant, we did Western blots comparing HeLa cell lysates against known amounts of purified recombinant BAF and determined that HeLa cells contain ~7 nM endogenous BAF (Fig. 3C). However, based on the relative fluorescence intensities for BAF in cultured Xenopus cells, determined by indirect immunofluorescence (18), we estimate that ~30% of cellular BAF is concentrated near the nuclear inner membrane. We therefore modeled the nucleus as a sphere (diameter, 5 μm) and defined the interaction zone as extending 200 nm from the inner membrane (Fig. 3C, I2).

Based on these estimates, the concentration of BAF dimers near the nuclear envelope is ~9 μM (see “Experimental Procedures”). However, local (molecular scale) concentrations of BAF could be even higher because BAF dimers oligomerize in the presence of DNA in vitro (16). In contrast, immunoblotting results for GCL suggested that the concentration of GCL near the nuclear envelope is lower, ~1 μM (data not shown). Thus, the above-determined affinities of emerin for BAF and GCL are likely to be physiologically relevant and collectively predict that BAF has the potential to inhibit GCL binding to emerin at the nuclear envelope in cells.

Does lamin A influence GCL binding to emerin? To answer this question, we did binding competition assays in microtiter wells, using appropriate concentrations of each protein.Emerin was immobilized in wells and incubated with buffer containing premixed 54 nM $^{35}$S-GCL plus 160 nM $^{35}$S-lamin A (GCL does not bind detectably to lamin A; data not shown). This concentration of lamin A ensured that over 90% of emerin molecules would be occupied by lamin A, and thus any GCL signal could be validly attributed to the formation of three-way complexes. Interestingly, lamin A and GCL did not compete for binding to emerin but instead bound efficiently and simultaneously to emerin in vitro (Fig. 3D). We concluded that emerin is capable of forming stable complexes with lamin A and GCL at the nuclear envelope, if BAF is absent or negatively regulated. This finding supported “scaffolding” models in which lamin A anchors emerin at the inner nuclear membrane yet does not interfere with emerin binding to the transcriptional repressor.

Can BAF itself form stable complexes with emerin and lamin A? To answer this question, we used a relatively low concen-
tration of lamin A (150 nM dimers) because lamin A binds weakly but directly to BAF with a $K_{d}$ of $1 \mu$M (Fig. 3E). In the presence of 150 nM lamin A, only one in five BAF molecules would be prebound to lamin A (see Fig. 3E). Under these conditions, we found that BAF (at 250 nM) and lamin A (at 150 nM) bound simultaneously and quantitatively to emerin (Fig. 3F). This work strongly supported our previous model that emerin can bind independently, through distinct domains, to both BAF and lamin A (15).

Because GCL can interact with two different LEM proteins (emerin and LAP2β), the tissues susceptible to EDM disease may be determined, at least in part, by the natural presence or absence of LEM domain proteins, such as LAP2β, whose functions overlap with emerin. To determine whether LAP2β or GCL was present in tissues affected by EDM, we performed quantitative reverse transcription-PCR analysis of mRNA from 16 human tissues using LAP2β- or GCL-specific primers. LAP2β expression is relatively low but detectable in heart and skeletal muscle, which are strongly affected by the loss of emerin (Fig. 3G). The low expression of LAP2β mRNA in these tissues was consistent with previously determined low levels of LAP2β protein (26). Human GCL mRNA was abundant in only one tissue examined (testis). GCL was undetectable in eight tissues (liver, spleen, thymus, prostate, ovary, small intestine, colon, and peripheral blood leukocytes), and low expression of GCL was detected in heart, brain, placenta, lung, skeletal muscle, kidney, and pancreas, consistent with expression patterns seen in adult mice (27). Thus, GCL is expressed in a tissue-specific manner and is present (albeit at low levels) in two tissues (heart and skeletal muscle) affected in EDMD patients. Obviously, the expression pattern for GCL does not explain the tissue specificity of EDM disease. However, as discussed below, GCL is the first of many transcription factors that may bind emerin (see “Discussion”). Thus, models based on GCL-emerin interactions may be generally applicable to the disease mechanism.

**DISCUSSION**

Our biochemical results suggest that emerin and lamin A are stable partners at the nuclear envelope and can form stable oligomeric complexes with either BAF or GCL, but not both. Interestingly, the stability of BAF-emerin-lamin A complexes might be increased by the low but direct affinity of BAF for lamin A. Based on our estimated physiological concentrations of BAF and GCL, we propose that BAF-mediated chromatin attachment to emerin will dominate and exclude GCL in the absence of modifications or regulators that change their measured affinities. Because GCL localizes to the nuclear envelope (20, 21), we propose that its binding is regulated by posttranslational modifications of BAF, GCL, emerin, or all three, as discussed below. Our findings have important implications for emerin function and for the disease mechanism of Emery-Dreifuss muscular dystrophy.

The structures of GCL and emerin (outside the LEM domain) are not yet known. However, based on our analysis of 24 mutations distributed throughout the nucleoplasmic domain of
Fig. 3. Binding competition studies. A, concentration of BAF dimers that reduced GCL binding to emerin by 50% (K_i), determined by incubating immobilized emerin with 60 nM ^35S-GCL plus increasing concentrations of BAF. B, wild type emerin or mutant m24, which cannot bind BAF (15), was immobilized in microtiter wells and incubated with 60 nM ^35S-GCL plus or minus 5 μM BAF. C, quantitation of BAF in HeLa cells. 10^6 HeLa cell equivalents and increasing amounts of recombinant human BAF protein were run on SDS-PAGE, transferred to nitrocellulose, and probed with antibodies against BAF (top panel). The amount of BAF in 10^6 HeLa cells is ~8 ng. Diagram (bottom) shows how we calculated the volume of a 200-nm IZ near the inner nuclear membrane (INM) for a 5-μm-diameter nucleus (see “Experimental Procedures”); the concentration of BAF at the nuclear envelope was estimated at ~9 μM. D, lamin A and GCL can simultaneously bind to emerin. 160 nM ^35S-lamin A (LmnA), 54 nM ^35S-GCL (GCL), or both (LmnA + GCL) were incubated with immobilized emerin, washed, eluted, run on SDS-PAGE, and autoradiographed. Top and bottom arrows indicate lamin A and GCL, respectively. E, affinity of lamin A for BAF, determined by adding increasing ^35S-lamin A to constant amounts of BAF immobilized in wells (see “Experimental Procedures”). Double reciprocal plots were used to accurately determine the affinity constant. F, lamin A and BAF form stable complexes with emerin. 150 nM ^35S-lamin A (LmnA), 250 nM ^35S-BAF (BAF), or both (LmnA + BAF) were incubated with immobilized emerin, washed, eluted, and subjected to SDS-PAGE and autoradiography. Top and bottom arrows indicate ^35S-labeled lamin A and BAF, respectively. G, tissue-specific expression of GCL and LAP2β. Reverse transcription-PCR was performed on cDNA from the indicated tissues using primers specific for LAP2β, GCL, or the positive control glyceraldehyde-3-phosphate dehydrogenase (G3PDH). H, models for docking of BAF-DNA complexes (III) or transcription repressor GCL (active, I; inactive, II) to emerin-lamin complexes at the inner nuclear membrane (INM). ONM, outer nuclear membrane.
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Emerin, we suggest that GCL might contact two surfaces on emerin, which we broadly designate repressor binding domain (RBD)-1 (residues 94–83) and RBD-2 (residues 175–217; Fig. 3H). Alternatively, emerin might fold to form a single repressor binding domain surface (not depicted). As modeled in Fig. 3, the binding of GCL to emerin may recruit E2F-DP heterodimer-chromatin complexes to the nuclear envelope and thereby repress transcription (Fig. 3H, model I). Alternatively, the re-recruitment of GCL to emerin may cause GCL-E2F-DP3 complexes to dissociate, allowing activation of E2F-DP-dependent genes (Fig. 3H, model II). In either case, GCL or GCL-containing complexes can be competed by BAF (Fig. 3H, model III). Interestingly, BAF was recently shown to bind directly to tissue-specific homeodomain transcription factors and block their activity (28).

GCL appears to be concentrated at the nuclear envelope in Drosophila (25) and mammalian cells (20). Our biochemical analysis of recombinant proteins suggests that emerin can form stable complexes with lamin A and GCL, but only in the absence of BAF. Because many nuclear envelope proteins are differentially phosphorylated during interphase, including lamin A, LAP2β, and emerin (29, 30), we propose that GCL binding to emerin or displacement of BAF from BAF-emerin-lamin complexes is regulated by signal transduction in vivo. This predicted interplay between GCL and BAF for binding to emerin will be interesting to explore in living cells, where binding affinities may be further influenced by chromatin. For example, the affinity of BAF for LEM proteins appears to increase in the presence of DNA (14). Additional studies of BAF and its regulation are clearly important to understand how BAF affects chromatin attachment to the nuclear envelope.

The discovery that emerin binds the transcriptional repressor GCL strongly supports gene expression models for the EDMD disease mechanism. Further supporting this model, the disease-causing mutation R95–99 potently disrupted emerin binding to both GCL and lamin A. Nevertheless, the loss or disruption of GCL binding to emerin is insufficient to explain disease, for two reasons. First, LAP2β is theoretically available as backup to bind GCL in skeletal muscle and heart, although we do not know whether GCL-LAP2β-lamin B complexes are functionally equivalent to GCL-emerin-lamin A complexes. Secondly, and more importantly, we and our collaborators recently identified two other transcription factors that bind emerin. We therefore suggest that EDMD disease results from the combined loss of emerin binding to multiple transcription factors, some of which may be more “important” than others in maintaining the function of a specific tissue. Our analysis of GCL-emerin interactions, presented here, suggests an important testable paradigm for the binding of many different transcription factors to emerin-lamin complexes.

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