O-Linked β-N-Acetylgalcosamine (O-GlcNAc) Regulates Emerin Binding to Barrier to Autointegration Factor (BAF) in a Chromatin- and Lamin B-enriched “Niche”*

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Background: Nuclear membrane protein emerin binding to nuclear intermediate filaments (lamin) and BAF contributes to forming a nuclear “lamina” structure.

Results: Emerin is O-GlcNAc-modified at eight sites: two (Ser-53 and Ser-54) influence further O-GlcNAcylation, and one (Ser-173) regulates association with BAF in the chromatin/lamin B “niche.”

Conclusion: O-GlcNAc transferase, a nutrient-responsive enzyme, regulates emerin.

Significance: Emerin hyper-O-GlcNAcylated may contribute to cardiomyopathy and other conditions.

Emerin, a membrane component of nuclear “lamina” networks with lamin and barrier to autointegration factor (BAF), is highly O-GlcNAc-modified (“O-GlcNAcylated”) in mammalian cells. Mass spectrometry analysis revealed eight sites of O-GlcNAcylation, including Ser-53, Ser-54, Ser-87, Ser-171, and Ser-173. Emerin O-GlcNAcylate was reduced ~50% by S53A or S54A mutation in vitro and in vivo. O-GlcNAcylate was reduced ~66% by the triple S52A/S53A/S54A mutant, and S173A reduced O-GlcNAcylate of the S52A/S53A/S54A mutant by ~30%, in vivo. We separated two populations of emerin, A-type lamin and BAF; one population solubilized easily, and the other required sonication and included histones and B-type lamin. Emerin and BAF associated only in histone- and lamin-B-containing fractions. The S173D mutation specifically and selectively reduced GFP-emerin association with BAF by 58% and also increased GFP-emerin hyper-phosphorylation. We conclude that β-N-acetylgalcosaminyltransferase, an essential enzyme, controls two regions in emerin. The first region, defined by residues Ser-53 and Ser-54, flanks the L-domain. O-GlcNAc modification at Ser-173, in the second region, is proposed to promote emerin association with BAF in the chromatin/lamin B “niche.” These results reveal direct control of a conserved L-domain lamin area component by β-N-acetylgalcosaminyltransferase, a nutrient sensor that regulates cell stress response, mitosis, and epigenetics.

The nuclear envelope includes diverse membrane proteins, some of which mechanically link the cytoskeleton to the nuclear skeleton (1) or have roles in signaling or genome management (2). Among these are proteins encoded by seven human genes that share the ~40-residue LAP2 (lamin-associated polypeptide 2), emerin, MAN1 domain (LEM domain)2 (3, 4). Emerin and most other LEM domain proteins localize predominantly at the nuclear envelope inner membrane. All characterized LEM domain proteins directly bind nuclear intermediate filaments (A-type lamins encoded by LMNA and/or B-type lamins encoded by LMNB1 and LMNB2) and barrier to autointegration factor (BAF) (encoded by BANF1) (3, 4). Lamins, BAF, and LEM domain proteins are essential to reassemble nuclear structure after mitosis (5–7).

Emerin binds directly to nucleoskeletal proteins (lamin A, actin, and nuclear myosin 1c) (8–11) and to proteins that form nuclear envelope-spanning LINC (“links the nuclear skeleton and cytoskeleton”) complexes (nesprin-1α, nesprin-2β, SUN1, and SUN2) (12–14). Emerin also influences signaling and transcription by binding to partners such as GCL (15), β-catenin (16), and Lmo7, which localizes at cell adhesions (17, 18) and focal adhesions and is regulated by p130Cas-dependent focal adhesion signaling (19).

Emerin, other LEM domain proteins, and lamins organize and dynamically tether silent chromatin at the nuclear envelope (20–27). For example, emerin and LAP2β each directly bind HDAC3 (histone deacetylase 3), a chromatin-silencing enzyme, and stimulate HDAC3 activity (28, 29). At the IgH locus, the regulated tethering of lamina-associated domains of chromosomes is mediated by DNA sequence repeats bound to a transcriptional repressor (cKrox) in complex with HDAC3 and LAP2β and is established during mitosis (30). Notably, a pair of LEM domain proteins (emerin and LEM 2) plus lamin B

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2 The abbreviations used are: LEM domain, ~40-residue domain shared by LAP2, emerin, and MAN1; BAF, barrier to autointegration factor; EDMD, Emery-Dreifuss muscular dystrophy; CAD, collision-activated dissociation; CIP, calf intestinal phosphatase; ETD, electron transfer dissociation mass spectrometry; HEK293T, human embryonic kidney cell line; MEF, mouse embryonic fibroblast; GGA, β-N-acetylgalcosaminidase; OGT, UDP-N-acetylgalcosamine-peptide β-N-acetylgalcosaminyltransferase; TMG, 1,2-dideoxy-2-ethylamino-α-D-glucopyranosyl-(2,1-d)-Δ2-thiazoline; WCL, whole cell lysate; WB, Western blot; S52–54A, triple mutant S52A/S53A/S54A.
and BAF are essential for mitosis, nuclear assembly, and chromosome organization in Caenorhabditis elegans (7, 31).

Emerin is expressed ubiquitously in human cells and tissues (32, 33). Mutations in the emerin gene (EMD) cause X-linked recessive Emery-Dreifuss Muscular Dystrophy (EDMD) and related conditions (34). EDMD is also caused by mutations in five other genes (14, 35–38), most of which encode proteins that either bind emerin (nesprin-1 (SYNE1), nesprin-2 (SYNE2), and lamin A (LMNA)) or associate with emerin in vivo (LUMA (TMEM43)). Evidence supports multiple disease mechanisms, including overactive MAPK signaling (39) and loss of mechanotransduction signaling at the nuclear envelope (1, 40). Specific mutations in lamin A (LMNA) or BAF (BANFI), both key partners for emerin, cause segmental progeroid (“accelerated aging”) syndromes (41–43). These numerous partners and roles raised questions about how emerin is controlled and whether its misregulation contributes to disease.

Emerin is highly modified by Ser/Thr and Tyr phosphorylation during interphase and mitosis (44, 45). Emerin also has features (rich in Ser/Thr residues; large regions of predicted regulation) contributes to disease.

Emerin is O-GlcNAc-regulated during interphase and mitosis (44, 45). Emerin also has features (rich in Ser/Thr residues; large regions of predicted disorder) characteristic of proteins modified by O-linked β-N-acetylgalactosamine (O-GlcNAc) (46–48), a single sugar modification of Ser or Thr residues that is abundant, reversible, and dynamic. O-GlcNAcylation of proteins are found in the cytoplasm, mitochondria, and nucleus (49), especially the nuclear envelope (50, 51). O-GlcNAc modification can compete or augment phosphorylation to regulate signaling, transcription, mitosis, and stress responses (52).

Potential O-GlcNAcylation of emerin was interesting because the enzymes that add and remove O-GlcNAc (UDP-N-acetylgalactosamine-peptide β-N-acetylgalactosaminyltransferase (OGT) and β-N-acetylglucosaminidase (OGA), respectively) are each encoded by a single gene in mammals. Furthermore, OGT is essential in mice; the genetic OGT-null condition is lethal at embryonic day 4.5 and in embryonic stem cells (52, 53). OGA-null mice show developmental delay and neonatal lethality, with severe defects in mitosis (54). Overexpressing either OGA or OGT disrupts mitosis and cytokinesis (55, 56). O-GlcNAc cycling helps link energy metabolism (OGT uses UDP-GlcNAc as donor) to epigenetics, and its misregulation is implicated in heart disease, aging, cancer, and diabetes (52, 57). We report that OGT regulates emerin’s most fundamental and least understood role in cells: association with BAF, the essential chromatin component of the nuclear lamina. This discovery was facilitated by a fractionation method, reported here, that separates two functionally distinct populations of emerin.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfection**—HeLa cells (American Type Culture Collection, Manassas, VA) and HEK293T cells (from Susan Michaels, Johns Hopkins University School of Medicine) were cultured in Complete Medium (high glucose DMEM plus 10% (v/v) FBS (both from Invitrogen)) with 1% (v/v) penicillin/streptomycin (Quality Biological Inc., Gaithersburg, MD). Cells were plated at ~30–40% confluence 24 h before transfection with 100 µl of Opti-MEM (Invitrogen), 2.5 µl of Trans-IT LT1 (Mirus, Madison, WI), and 0.5 µg of DNA/ml of Complete Medium, per Mirus protocol. Cells were incubated for 24 h and then either harvested for immunoprecipitation or fixed for indirect immunofluorescence staining (see below).

Stable populations of mouse OGT(ΔY) embryonic fibroblasts expressing either GFP or mER-Cre-2A-GFP (provided by Natasha Zachara, Johns Hopkins University School of Medicine) (58) were cultured in Complete Medium. To induce OGT deletion, cells were plated (1 × 10⁶ cells/15-cm plate); 6 h later, we added ethanol with or without 4-hydroxytamoxifen (0.5 µM final; Sigma) for 24 h and then washed in sterile PBS and cultured 16 h longer in Complete Medium prior to harvest (58).

**Cell and Nuclear Fractionation**—Cells and nuclei were fractionated as described (58, 59) with some modifications. In brief, cell pellets (~80 °C) were thawed for 10 min on ice, incubated for 10 min in low salt, hypotonic lysis buffer (20 mM HEPES, pH 7.4, 50 mM GlcNAc, 1 mM DTT, 100 µM PMSF, 1 µg/ml pepstatin A, 1× Roche protease mixture inhibitor, 1× Roche PhoStop phosphatase inhibitor (Roche Applied Science)), resuspended by gentle pipetting, incubated for 10 min on ice, and then centrifuged (1 min, 17,000 × g, 4 °C) to yield a supernatant (cytoplasmic fraction) and pellet (enriched for nuclei). The nuclear pellet was washed three times in low salt extraction buffer and then centrifuged (17,000 × g, 10 s, 4 °C). Pelleted nuclei were resuspended in nuclear lysis buffer (50 mM Tris-HCl, pH 7.4, 300 mM NaCl, 0.3% (v/v) Triton X-100, 50 mM GlcNAc, 1 mM DTT, 100 µM PMSF, 1 µg/ml pepstatin A, 1× Roche protease mixture inhibitor, 1× Roche PhoStop phosphatase inhibitor), vortexed, and centrifuged (17,000 × g, 20 or 30 min, 4 °C). This supernatant comprised the “easily extracted” nuclear fraction (Nₑ). The insoluble pellet was resuspended in nuclear lysis buffer, vortexed, sonicated ~20 times (0.5-s bursts; Sonicator-M100, Fisher), and centrifuged (17,000 × g, 20 or 30 min, 4 °C). This supernatant comprised the “sonicated” nuclear fraction (Nₛ). Alternatively, to obtain the total nuclear fraction (Nₜ), the first nuclear pellet was resuspended in nuclear lysis buffer, sonicated ~20 times (0.5-s bursts), and centrifuged (17,000 × g, 20–30 min, 4 °C), and the supernatant was harvested. Easily extracted, sonicated, or total fractions from whole cell lysates (WCLₑ, WCLₛ, and WCLₜ, respectively) were prepared similarly. Equal percentages of each fraction were immunoblotted (described below).

**Immunoprecipitation**—For Figs. 2 and Fig. 5 (D and E), cells at ~80% confluence were incubated for 4 h or overnight in Complete Medium with 1 µM TMG (1,2-dideoxy-2′-ethylamino-α-D-glucopyranosyl-[2,1-d]-2′-thiazoline (Thiamet-G), from 2000× stock in 50 mM Tris-HCl, pH 7.4 (provided by G. W. Hart, The Johns Hopkins University School of Medicine)) or vehicle alone as control and then scrape-harvested and washed twice in PBS. For other experiments that lacked TMG, cells were collected similarly. Cell pellets were lysed with ice-cold lysis buffer (50 mM Tris-HCl, pH 7.4, 0.3 mM NaCl, 0.3% (v/v) Triton X-100 or 10 mM CHAPS, 2 mM EDTA, 100 µM PMSF, 1 µg/ml pepstatin A, 1× Roche protease mixture inhibitor, 1× Roche PhoStop phosphatase inhibitor, 50 mM TMG, and/or 50 mM GlcNAc); for co-immunoprecipitations, we included 1 mM 4′-thiazoline (Thiamet-G), from 2000× stock in 50 mM Tris-HCl, pH 7.4 (provided by G. W. Hart, The Johns Hopkins University School of Medicine)) or vehicle alone as control and then scrape-harvested and washed twice in PBS. For other experiments that lacked TMG, cells were collected similarly. Cell pellets were lysed with ice-cold lysis buffer (50 mM Tris-HCl, pH 7.4, 0.3 mM NaCl, 0.3% (v/v) Triton X-100 or 10 mM CHAPS, 2 mM EDTA, 100 µM PMSF, 1 µg/ml pepstatin A, 1× Roche protease mixture inhibitor, 1× Roche PhoStop phosphatase inhibitor, 50 mM TMG, and/or 50 mM GlcNAc); for co-immunoprecipitations, we included 1 mM DTT.

Where indicated, protein concentrations were measured by a Quick Start Bradford Protein assay (Bio-Rad) and adjusted with lysis buffer to achieve equivalent protein concentrations (0.5–1...
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mg/ml). For each immunoprecipitation, 0.5–1 mg of lysate protein (concentrations were identical between replicates) was incubated overnight (rotating, 4 °C) with 1 μg of nonspecific rabbit IgG (SC-2027) or 1 μg of emerin-specific rabbit antibody (SC-15378; Santa Cruz Biotechnology, Inc.) plus prewashed γ-bind bead slurry (GE Healthcare). Beads were pelleted and washed three times (50 mM Tris-HCl, pH 7.4, 0.3 mM NaCl, 0.3% (v/v) Triton X-100); proteins were recovered using SDS-sample buffer, resolved by SDS-PAGE (100% of each immunoprecipitate from HeLa cells, 20% of HEK293T immunoprecipitates, or 1% of each input lysate) on 4–12% BisTris NuPage gels (Invitrogen), and then transferred to Immobilon-P PVDF (Millipore, Billerica, MA). Endogenous emerin O-GlcNAc signals were more readily detected by immunoblotting on PVDF than nitrocellulose. GFP was immunoprecipitated using either GFP-specific agarose-conjugated llama antibodies with agarose alone as a control (gta-200 and bab-20, respectively; Chromotek, Martinsried, Germany), or rabbit GFP antibody A6455 (Invitrogen) with nonspecific rabbit IgG as a control.

Fluorescence Microscopy—HeLa cells plated on sterile, acid-washed, polyllysine-coated coverslips at 1.5 × 10^5 cells/well were transfected to express GFP or GFP-emerin for 24 h. Coverslips were rinsed three times in PBS; fixed in PBS, 3.7% (w/v) formaldehyde (15 min; VWR International, Radnor, PA); washed three times in PBS; permeabilized in PBS, 0.2% (v/v) Triton X-100 (20 min; Sigma); briefly stained with 25 μg/ml 4',6-diamidino-2-phenylindole (DAPI); and mounted on 1.0-mm glass slides using Vectashield (Vector Laboratories, Burlingame, CA), all at 22–25 °C. GFP fluorescence was visualized using a ×60 oil immersion DICH objective (1.4 numerical aperture) on an Eclipse E600 wide field fluorescent microscope (Nikon, Melville, NY) and imaged using a Retiga Exi charge-coupled device camera (Q Imaging, Surrey, Canada) and iVision-MacTM image-capturing software (BioVision Technologies, Exton, PA). Images were adjusted with Photoshop CS4 (Adobe, San Jose, CA).

Phosphatase Treatment—HeLa cell Nt/Ns lysates were prepared as above using modified hypotonic lysis buffer (20 mM HEPES, pH 7.4, 1 mM DTT) and lysis buffer (50 mM Tris-HCl, pH 7.4, 300 mM NaCl, 0.3% (v/v) Triton X-100, 1 mM DTT, 10 mM MgCl2, 1 mM MnCl2). Each fraction (18 μl) was incubated for 1 h at 37 °C with either 2 μl of reaction buffer (control), 2 μl of calf intestinal phosphatase (CIP) (20 units), 2 μl (800 units) of λ-phosphatase, or both enzymes (1 μl of CIP (10 units) plus 1 μl (400 units) of A-phosphatase; New England Biolabs, Ipswich, MA) and then quenched with SDS-PAGE sample buffer, resolved by SDS-PAGE, transferred to nitrocellulose, and immunoblotted (see below).

Immunoblotting—Proteins were resolved on NuPage Novex 4–12% BisTris gradient gels in 1× NuPage MES SDS running buffer or (for Figs. 6A and 7C) 10% BisTris gels in 1× NuPage MOPS SDS running buffer (Invitrogen). Gels were transferred to nitrocellulose (Bio-Rad); blocked for 1 h (22–25 °C) in PBS (PBS with 0.1% (v/v) Tween 20) containing 3% (w/v) BSA (Sigma); and incubated overnight in PBS, 3% (w/v) BSA with either rabbit anti-emerin serum 2999 (dilutions 1:2000–5000) (9), mouse NCL-emerin (1:2000–10,000; Novacastra, Newcastle, UK), rabbit anti-BAF serum 3273 (1:1000–2000) (5), mouse anti-GFP (1:100,000; sc-9996, Santa Cruz Biotechnology), mouse anti-actin (1:100,000–500,000; MAB1501; Millipore), mouse anti-β-catenin (1:10,000; 610154, BD Biosciences), rabbit anti-lamin A/C (1:2000; SC-20681, Santa Cruz Biotechnology), rabbit anti-lamin B serum NC-7 (1:2000; gift from N. Chaudhary), rabbit anti-histone H3 (1:2000; Poly6019, Biologend, San Diego, CA), mouse anti-α-tubulin (1:10,000; Sigma), or affinity-purified rabbit anti-OGT serum AL-28, from Gerald Hart (1:5000) (60). Blots were incubated for 1 h (22–25 °C) with horseradish peroxidase-conjugated mouse or rabbit secondary antibodies (1:10,000; NA931V and NA934V, respectively; GE Healthcare) in PBST, 3% (w/v) BSA and visualized by chemiluminescence (HyGlo, Denville) and autoradiography (HyBlot CL, Denville).

To probe for O-GlcNAc, blots were blocked for 1 h (22–25 °C) in 5% (w/v) milk in TBST (Tris-buffered saline, 0.1% (v/v) Tween 20); washed thoroughly; incubated overnight in TBST, 5% (w/v) BSA with affinity-purified O-GlcNAc-specific mouse antibody CTD110.6 (1:4000–10,000 dilution; provided by G. W. Hart) (61) and then for 1 h (22–25 °C) with secondary anti-mouse IgM horseradish peroxidase-conjugated antibody (1:10,000; SC-2064, Santa Cruz Biotechnology, Inc.) diluted in TBST, 5% (w/v) milk; and visualized by chemiluminescence using either HyGlo (Denville) or Immobilon Western (Millipore) on autoradiography film (HyBlot CL, Denville) or high performance chemiluminescence film (Amersham Biosciences Hyperfilm ECL, GE Healthcare). Film was digitally scanned with Perfection 2450 Photo (Epson), densitometry was done using Quantity One (version 4.5.2; Bio-Rad), and images were processed using Adobe Photoshop CS4 and Illustrator CS4 (Adobe).

Plasmids—Plasmids encoding pGST-emerin 1–222 (62) and pEGFP-C1 fused to wild type human emerin (63) were described. Plasmids (pET29b) expressing His-tagged emerin residues 1–220, 1–176, or 1–70 and pGEX plasmids expressing GST-tagged emerin residues 70–140, 140–176, or 170–220 were gifts from J. Ellis (14). Missense mutations in pET29b His-emerin 1–220 and pEGFP-C1 emerin were generated by QuikChange site-directed mutagenesis (Agilent Technologies, Santa Clara, CA).

Recombinant Protein Purification—Protein expression was induced for 4 h (37 °C) in E. coli (BL21 DE3) at A600 0.6–0.7 using 0.4 mM isopropyl-β-d-thio-galactoside. GST-emerin 1–222 polypeptides were purified as described (62). To purify other GST-tagged polypeptides, bacterial pellets were resuspended in ice-cold resuspension buffer (PBS containing 0.05% (v/v) Tween 20, 2 mM EDTA, pH 8.0, 0.1% (v/v) β-mercaptoethanol (BME), 0.1 mM PMSF), sonicated on ice (5 × 30 s; 10-s rests), and centrifuged (20,000 × g, 30 min). Supernatants were added to pre-equilibrated glutathione-agarose resin (GE Healthcare) in a 25-ml column (Bio-Rad); rotated (4 °C; 4 h or overnight); washed in two volumes of resuspension buffer; and eluted using 10 mM glutathione, 50 mM Tris-HCl, pH 7.4, 150 mM NaCl. Contaminant-free fractions were pooled and dialyzed overnight (4 °C) against 50 mM Tris-HCl, 150 mM NaCl; aliquots flash-frozen in liquid nitrogen were stored at −80 °C.

Soluble His-tagged polypeptides (pET29b His-emerin residues 1–70 and 1–176) were purified similarly, using Ni2+-NTA
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Subcellular fractionations were employed to assess the localization of emerin, O-GlcNAcylation of emerin in vitro, and the dependence of O-GlcNAcylation on proteasomal or lysosomal proteolysis. We found that lysates from cells or isolated nuclei typically contained only about half of the endogenous emerin, the "easily released" fraction. The other half was recovered (solubilized) only when samples were sonicated prior to centrifugation. Using the protocol depicted in Fig. 1A (see "Experimental Procedures"), we isolated the cytoplasm (C), "easy" nuclear lysate (E) and sonicated nuclear lysate (S) from HeLa cells. Alternatively, HeLa cells were directly lysed in 0.3% Triton X-100 and 300 mM NaCl, sonicated, and centrifuged to obtain "total" whole cell lysates (WCL; Fig. 1B). Similarly, "total" nuclear lysates (N1) were prepared by sonicating the low-speed nuclear fraction (nuclei/organelle) pellet (Fig. 1C). All fractions were resuspended in equal volumes. Equal percentages of each fraction (2%) were resolved by SDS-PAGE and immunoblotted with antibodies to endogenous emerin, lamin A/C, lamin B, actin, BAF, or histone H3 (Fig. 1D). Emerin was detected at very low levels in isolated cytoplasm (Fig. 1D, lane 1) and was abundant in all fractions that included nuclear proteins (Fig. 1D, lanes 2–7). We quantified the emerin distribution in each nuclear fraction (e.g. N3) as a percentage of each corresponding sum (e.g. N1 + N3; Fig. 1E). Starting with isolated HeLa cell nuclei, on average, 56% of emerin was easily extracted, and 44% was sonication-dependent (Fig. 1E, n = 4). Similarly, 65% of whole cell lysate emerin was easily extracted, and 35% was sonication-dependent (Fig. 1E, n = 4). We concluded that 35–44% of endogenous emerin is insoluble unless sonicated, potentially due to association with chromatin or insoluble nucleoskeletal structures, because histone H3 and lamin B were both found predominantly in sonicated fractions (Fig. 1D, lanes 2 and 3 and lanes 5 and 6). Two nonspecific bands recognized by the lamin B antibody provided internal loading controls (Fig. 1D). Lamin A and C (70 and 60 kDa, respectively) were present at low levels in the cytoplasm and were abundant in both nuclear fractions (Fig. 1D, lanes 2 and 3). More than half of BAF was cytoplasmic (Fig. 1D, lane 1), as expected (66–68). In HeLa cells, most (~74%) endogenous nuclear BAF was sonication-dependent (Fig. 1D, lanes 2 and 3), suggesting avid association with chromatin or other N3-specific component(s) in vivo. Actin was abundant in the cytoplasm, as...
expected (Fig. 1D, lane 1), and was highly enriched in N_s compared with N_e (Fig. 1D, lanes 2 and 3). We concluded that the N_e and N_s fractions were enriched for distinct nucleoskeletal “niches”; both niches included emerin, A-type lamins, and BAF, whereas actin was primarily easy, and two other components, chromatin and lamin B, were primarily sonication-dependent.

**GFP-Emerin and BAF Associate Only in the Sonication-dependent Fraction of HEK293T Cells**—To compare these distributions in a different cell type, we used human HEK293T cells. Equal percentages (1%) of untransfected HEK293T cell cytoplasm (C), N_e, N_s, and N_T fractions were resolved by SDS-PAGE and immunoblotted for endogenous emerin, A-type lamins, and BAF, whereas actin was primarily easy, and two other components, chromatin and lamin B, were primarily sonication-dependent.

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nuclear lysates ($N_r$). Control immunoblots of input lysates revealed GFP-emerin and endogenous BAF in both $N_E$ and $N_S$ (Fig. 1G, input lanes 1 and 2). In cells expressing GFP-emerin, endogenous BAF distributed more evenly between the $N_E$ and $N_S$ fractions (Fig. 1F (lanes 2 and 3) versus Fig. 1G (lanes 1 and 2, short exposure (short exp.))). We used agarose-conjugated llama GFP antibodies or agarose alone to immunoprecipitate an equal percentage of each fraction. Precipitates were resuspended in SDS-sample buffer, and 20% of each sample was resolved by SDS-PAGE and immunoblotted using antibodies specific for BAF or GFP (Fig. 1G, $n = 3$). The agarose controls were negative (Fig. 1G, lanes 4–6), confirming specific precipitation of GFP-emerin and BAF. Although both proteins were present in both fractions, endogenous BAF co-immunoprecipitated with GFP-emerin only from sonicated samples (Fig. 1G, lane 7 versus lane 8). This unexpected result suggested that emerin-BAF association is biologically restricted (e.g. by post-translational modification of one or both proteins) to the chromatin-containing niche. Around this time, we discovered a new posttranslational modification of emerin, O-GlcNAc, described next.

**Endogenous Emerin is O-GlcNAcylated in Mammalian Cells**—To explore whether endogenous emerin is O-GlcNAcylated, we treated HeLa cells for 4 h with or without $1\mu M$ TMG to specifically inhibit the enzyme (OGA) that removes O-GlcNAc (69). Equal protein concentrations of unsonicated whole HeLa cell lysates were then immunoprecipitated using either emerin-specific (anti-Emr) or nonspecific (IgG) rabbit antibodies (Fig. 2A). Immunoprecipitates were resolved in parallel gels (SDS-PAGE), transferred to PVDF, and immunoblotted with an antibody specific for the O-GlcNAc modification (70), with or without competing 100 mM GlcNAc (Fig. 2A). The O-GlcNAc antibody detected rabbit IgG light chain (32 kDa; Fig. 2A) as expected, and HeLa protein signals were higher in TMG-treated input lysates than in untreated lysates (Fig. 2A, input), confirming sugar-specific detection (71). The emerin antibody specifically immunoprecipitated a 34 kDa GlcNAcylated band from both the TMG-treated and untreated lysates (Fig. 2A).
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2A, boxed region; n = 3); specificity was confirmed by stripping each blot and reprobing with an emerin-specific monoclonal antibody (NCL-emerin; Fig. 2A, bottom panels). Most signals, including the 34 kDa band, were reduced or eliminated by competition with 100 mM GlcNAc (Fig. 2A, right panel; n = 3), confirming specific recognition of O-GlcNAcylated proteins by this antibody. Consistent detection of the O-GlcNAcylated 34 kDa band in cells not treated with TMG suggested that the endogenous easy emerin population is naturally O-GlcNAcylated in vivo.

To determine if the sonication-dependent population was also O-GlcNAc-modified, we immunoprecipitated endogenous emerin from equal volumes of HeLa cell NE and NS fractions (Fig. 2B; 1% input shown), resolved by SDS-PAGE, immunoblotted for O-GlcNAc, and then stripped and reprobed for emerin (Fig. 2B). Specific O-GlcNAc signals at 34 kDa (the main emerin band) were detected at similar levels in both fractions (Fig. 2B; n = 3). Thus, both populations of emerin (NE and NS) are O-GlcNAcylated in vivo.

To test emerin O-GlcNAcylation in a different species, the above experiment was repeated using easy whole cell lysates from the inducible mouse estrogen receptor-Cre-loxp OGT line of mouse embryonic fibroblasts (mER-Cre-2A-GFP-MEFs) (58). When these cells are pretreated with the estrogen receptor-activating drug 4-hydroxytamoxifen (0.5 μM), a cytoplasmic fusion protein (Cre-recombinase fused to mouse estrogen receptor; mER-Cre) translocates to the nucleus, where Cre excises the loxp-flanked OGT gene. These cells constitutively express a GFP reporter, confirming that >90% of cells contain the mER-Cre-inducible protein (58). As controls, we used OGT/3′/V′ MEFs that constitutively express GFP alone (58). Both lines were treated with 4-hydroxytamoxifen/ethanol or ethanol alone for 24 h and then washed to remove drug and cultured for 16 h in complete medium prior to immunoprecipitation and immunoblot analysis. The emerin antibody precipitated emerin specifically (no signal in the Ig control; Fig. 2B) (WB: Emr). However, in cells with reduced levels of OGT enzyme, the level of O-GlcNAcylated emerin decreased 92% (Fig. 2C, left box; Fig. 2D, p < 0.004). Control blots with competing free sugar (Fig. 2C, right box) verified that the O-GlcNAc signal on emerin was specific. Immunoblotting of input lysates with antibodies to OGT protein, O-GlcNAc, or α-tubulin (loading control) verified reduction of both OGT itself and overall O-GlcNAcylation levels (Fig. 2E). These results independently validated emerin as endogenously O-GlcNAcylated in a different species (mouse).

In Vitro Modification of Recombinant Emerin by Purified OGT—To map O-GlcNAcylated regions in emerin, we incubated purified recombinant emerin polypeptides (~1 μg) comprising either GST-tagged human residues 1–222, 70–140, 140–176, or 170–220 or S- and His-tagged human residues 1–70 or 1–176 (Fig. 3A) in reactions containing 10 mM UDP-GlcNAc and CIP with or without 1 μg of purified OGT enzyme (see “Experimental Procedures”). Reactions were incubated overnight at 4 °C and then quenched with SDS-sample buffer, resolved by SDS-PAGE, transferred to nitrocellulose, Ponceau-stained for qualitative protein detection (Fig. 3B, Pon-}

3 J. M. Berk and K. L. Wilson, unpublished observations.
His-tagged emerin 1–220 polypeptides bearing Ala substitutions in known or suspected O-GlcNAc sites. We specifically tested six single mutations (S52A, S53A, S54A, S58A, S171A, and S173A), one triple mutation (S52–54A), and two quadruple mutations (S52A/S53A/S54A/S171A and S52A/S53A/S54A/S173A). Each reaction contained 1 μg of emerin polypeptide (wild type or mutated His-tagged emerin 1–220) plus 1 μg of OGT and CIP and 1.0 μCi of [3H]UDP-GlcNAc diluted with “cold” UDP-GlcNAc to a final GlcNAc concentration of 8.33 μM (10-fold dilution of radiolabeled sugar). We chose this UDP-GlcNAc concentration to exceed the lowest reported $K_m$ for OGT (6, 35, or 217 μM) (73, 74). Reactions were incubated overnight, quenched with SDS, resolved by SDS-PAGE, Coomassie-stained, and autoradiographed (Fig. 4C). Results were quantified as the $^{3}$H/Coomassie ratio relative to wild type emerin (Fig. 4D). Single mutations S52A, S58A, S171A, and S173A had no significant effect on emerin O-GlcNAcylation in vitro (Fig. 4, C and D). Mutating one residue would not necessarily reduce overall O-GlcNAcylation if the site were rarely occupied or if OGT compensates by modifying alternative residues nearby (75).

By contrast, emerin O-GlcNAcylation was reduced significantly by the single mutations S53A (reduced by 56%; $p < 0.02, n = 5$; Mann-Whitney $U$ test) and S54A (reduced by 47%; $p < 0.02, n = 5$) and by the triple S52–54A mutation (reduced by 71%; Fig. 4, C and D; $p < 0.02, n = 5$). In these assays, the triple mutant was not significantly affected by a fourth mutation (S171A or S173A; Fig. 4, C and D).
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These in vitro findings validated the mass spectrometry results and implied that Ser-53 and Ser-54 were either abundant or influenced further O-GlcNAcylation in vitro, because loss of either Ser-53 or Ser-54 (of at least eight O-GlcNAc sites) had a disproportionately large impact, reducing emerin O-GlcNAcylation by −50% in vitro (Fig. 4, C and D). This result for Ser-54 was noteworthy because the S54F mutation is sufficient to cause EDMD, although the S54F protein is expressed and the majority localizes normally (76–78) (see below).

Ser-53, Ser-54, and Ser-173 Are Relevant to Emerin O-GlcNAcylation in Cells—Before studying O-GlcNAc-site mutations further in vivo, we did controls to determine if they affected GFP-emerin localization. HeLa cells that expressed GFP or GFP-emerin (wild type or mutant) for 24 h were visual-
O-GlcNAcylated forms of SSLDLSYYPTSSSTSFMSSSSSSSS-SSS-<br/>S173A) for 24 h were treated with (or without) 1 μM TMG<br/>during the last 4 h. Sonicated (total) whole cell lysates (WCLT)<br/>were precipitated using GFP antibodies, resolved by SDS-<br/>PAGE, and immunoblotted first for O-GlcNAC and then<br/>stripped and reprobed for GFP (Fig. 5D). Results were quanti-<br/>fied by densitometry as the O-GlcNAC/GFP-emerin ratio. GFP<br/>controls showed no O-GlcNAC signal at the position of GFP-<br/>emerin (~58 kDa; Fig. 5D, lane 3, box). As expected, TMG treat-<br/>ment boosted overall O-GlcNAC levels (inputs in Fig. 5D, lane 2<br/>versus lane 1) and increased O-GlcNACylation of wild type GFP-<br/>emerin by 48% (p < 0.03; n = 3; Fig. 5D, lane 5 versus lane 4).<br/>Under these TMG-treated conditions, O-GlcNACylation of the<br/>triple mutant (S52–54A) was reduced significantly (by 66%; p <<br/>0.04; n = 3) compared with wild type (Fig. 5D, lane 8 versus lane 5),<br/>consistent with earlier results from untreated, unsonicated sam-<br/>ples (Fig. 5B). Importantly, GFP-emerin O-GlcNACylation was<br/>further reduced (by ~30%) by S171A or S173A in the S52–54A<br/>background (Fig. 5E). This reduction was significant for S173A<br>(p < 0.03; n = 3; Fig. 5E). We concluded that residue Ser-173 is<br>important for emerin O-GlcNACylation in cells. Furthermore,<br>the S173A mutation appeared to affect the easy and sonication-de-<br>pendent emerin populations differently.<br><br>We wondered if fusion to GFP or O-GlcNac site mutations<br>disrupted the N_e/N_s distribution of emerin in HeLa cells.<br>Therefore, as controls, we tested the distribution of endoge-<br>nous emerin, GFP-emerin (wild type, S53A, S54F, S173A, or<br>S173D) and endogenous nuclear markers by immunoblotting<br>the isolated cytosol (C), N_e and N_s fractions of HeLa cells (24 h<br>posttransfection) for emerin (rabbit serum 2999), lamins A/C,<br>lamin B, BAF, actin, or histone H3 (Fig. 6A; 2% of each fraction<br>loaded, n = 3). Wild type and mutant GFP-emerins all partitioned<br>similarly to each other (evenly split between N_e and N_s;<br>Fig. 6A) and did not disrupt partitioning of other nuclear pro-<br>teins. Specifically, endogenous emerin and lamins A/C were<br>present in both N_e and N_s, actin was abundant in cytosol and<br>N_s, BAF was abundant in cytosol and N_s, and histone H3 and<br>lamin B were predominantly in N_s (Fig. 6A; compare Fig. 1A).<br><br>Ser-173 Modifications Regulate GFP-Emerin Association with<br>BAF—HEK293T cells were transfected to express GFP-emerin<br>(wild type, 53A, 54F, 173A, 173D, 175A, or 175D) for 24 h. We<br>included 175A and 175D because Ser-175 phosphorylation was<br>indirectly implicated in regulating emerin binding to BAF in<br/mitotic Xenopus extracts (80). Isolated HEK293T nuclei were<br>lysed and sonicated to generate a total nuclear supernatant<br>(N_s). We then immunoprecipitated equal protein concentra-<br>tions or cell-equivalent lysates using GFP-specific llama anti-<br>bodies conjugated to beads or beads alone. Precipitates were
resuspended in SDS-sample buffer, and 20% of each sample was resolved by SDS-PAGE and immunoblotted using antibodies specific for either lamin B or β-catenin (Fig. 6B; n = 2 each), or A-type lamins or BAF (Fig. 6B; n = 4 each). Among these various emerin mutations and known partners, we detected only one significant difference; the phosphomimetic GFP-emerin S173D polypeptide showed significantly reduced binding to endogenous BAF (58% less than wild type GFP-emerin; Fig. 6, B and C; n = 7; p < 0.0002). BAF association was not significantly affected by any other mutation tested (Fig. 6, B and C; n = 6 and 7). This suggested that GFP-emerin Ser-173 phosphorylation (mimicked by S173D) might specifically inhibit binding to
BAF. This result was initially puzzling, because the LEM domain (residues 4–44) is sufficient to bind BAF (3), and in early studies, emerin binding to BAF was disrupted only by LEM domain mutations, not by the few tested mutations elsewhere in emerin (9).

However, several results provided a clue as to the mechanism. Emerin migrates slowly in SDS gels when hyperphosphorylated (62, 81, 82), and slowly migrating bands were obvious in the endogenous easy emerin population (e.g. Figs. 1 and 7A), and detectable in the endogenous NS population (e.g. see Fig. 2B). The S173D mutation appeared to increase the slowly migrating signal, compared with wild type and S173A-mutated GFP-emerin (Fig. 7A); and affected 30% of the easy GFP-emerin population (Fig. 7B).

To test potential hyperphosphorylation of GFP-emerin in the NE and NS fractions, we tested sensitivity to α-phosphatase (preferentially dephosphorylates Ser/Thr residues) with or without CIP, which preferentially dephosphorylates Tyr residues. NE and NS fractions from isolated HeLa nuclei were treated with or without α-phosphatase or CIP. Samples were resolved by 10% BisTris SDS-PAGE to improve resolution of hyperphosphorylated bands and immunoblotted for emerin and lamins A/C (Fig. 7C). In the NE fraction, we detected two upper bands for GFP-emerin S173D (60 kDa and 58 kDa); α-phosphatase eliminated both and produced a α-resistant 56 kDa band (Fig. 7C, lane 13 versus lane 15). CIP eliminated both upper bands of

**FIGURE 6. S173D mutation disrupts GFP-emerin association with BAF.** A, the Nc versus Ns distributions of GFP or GFP-emerin (wild type, S53A, S54F, S173A, or S173D) were tested in HeLa cells 24 h posttransfection. Shown are equal percentages (2%) of each cytoplasm (C) and separated Nc and Ns fractions, resolved by SDS-PAGE and immunoblotted with antibodies to emerin, lamin B, lamins A/C, BAF, actin, or histone H3 (n = 3). B and C, sonicated total nuclear lysates (Ns) from HEK293T cells 24 h posttransfection with GFP-emerin (wild type or mutant), precipitated with bead-conjugated GFP antibodies or beads alone, SDS-PAGE-resolved, and probed with antibodies to GFP, lamins A/C, or BAF. Controls show input Ns lysate (1%) and beads-only precipitation from wild type GFP-emerin lysates. Results are quantified in C as the amount of BAF that co-immunoprecipitated with each GFP-emerin mutant, relative to wild type GFP-emerin (n = 7; **, p < 0.0002 by Student’s t test; bars, S.E.).
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GFP-emerin S173D and yielded trace amounts of a ~55 kDa “CIP-resistant” band (Fig. 7C, lane 13 versus lane 14). Treatment with both enzymes gave the same results as CIP alone (Fig. 7C, lane 16 versus lane 14), suggesting that CIP effectively dephosphorylated both Tyr and Ser/Thr residues in emerin.

The S173D mutation also increased emerin hyperphosphorylation in the Ns niche, but non-identically to its effects in the easy niche; we detected only one major S173D upper band in the Ns fraction (~58 kDa; Fig. 7C, lane 13, bracket) (not two upper bands, as seen in Ns) that was similarly sensitive to λ and CIP (Fig. 7C, lanes 14–16 versus lane 13; bracket). The slight downshift of the main GFP-emerin band in all CIP-treated samples (e.g. see Fig. 7C, lane 5 versus lane 6) was attributed to co-migration of 2 μg of CIP (~56 kDa; samples treated with both phosphatases had 1 μg each). CIP treatment also caused endogenous A-type lamins in the Ns niche, particularly lamin C, to migrate faster (Fig. 7C, lane 1 versus lane 2), consistent with many reported phosphorylation sites in A-type lamins (83). The minor (~50 kDa) band (Fig. 7, A and C) is probably a GFP-emerin breakdown product.

FIGURE 7. Posttranslational consequences of GFP-emerin S173D versus S173A mutations in HeLa cells and model. A and B, immunoblot of equal protein concentrations of easy whole cell lysates (WCL) from HeLa cells 24 h posttransfection with GFP or GFP-emerin (wild type, 173A, or 173D), probed with GFP antibodies (A) and quantified by densitometry (B) as the ratio of upper band to total GFP-emerin per lane (n = 3; **, p < 0.009, Student’s t test). C, potential Ser/Thr or Tyr phosphorylation of GFP-emerin upper bands tested by treatment with CIP (which preferentially dephosphorylates Tyr) with or without λ-phosphatase (which preferentially dephosphorylates Ser/Thr) or with neither as a control. Untransfected HeLa cells (UN) and HeLa cells 24 h posttransfection with GFP-emerin (wild type, S173A, and S173D) were fractionated; separate NE and NS fractions were treated with (or without) CIP or both and then resolved using 10% BisTris SDS-PAGE (to improve band resolution) and probed with antibodies to emerin (serum 2999) or lamins A/C (Ns fractions only). *, main GFP-emerin band. Brackets, slowly migrating GFP-emerin bands (n = 2). D, schematic summary and model of our main results. Emerin mono- and di-O-GlcNAcylation at Ser-53 and Ser-54 is proposed to control emerin conformation near the LEM domain and favor O-GlcNAcylation at additional (unidentified) sites. Ser-173 O-GlcNAcylation is proposed to promote BAF association in the sonication-dependent niche. Potential (nonexclusive) mechanisms include (a) control of emerin conformation at S173; (b) control of an unidentified partner that influences emerin-BAF association; (c) inhibition of an alternative fate, namely Ser-173 phosphorylation and consequent hyperphosphorylation at unidentified sites.
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Our estimate that the S173D mutation increased GFP-emerin hyperphosphorylation at least 5-fold in the N_e fraction (Fig. 7A) was conservative, because it did not include potentially phosphorylated molecules in the main emerin band (62). We conclude that emerin Ser-173 phosphorylation, mimicked by S173D, increases emerin phosphorylation in vivo in different ways, depending on whether emerin occupies the easy or sonication-dependent niche. Considered together with the S173A mutation, which increased O-GlcNAcylation in the easy WCL_e niche (Fig. 5C) but decreased overall emerin O-GlcNAcylation in combination with the S52–54A mutation, we propose that emerin Ser-173 (and possibly neighboring residues) functions as a molecular “switch” that influences downstream O-GlcNAcylation and phosphorylation events and controls BAF association in vivo.

DISCUSSION

These results show emerin is regulated by O-GlcNAcylation in vivo. At least eight residues in human emerin are O-GlcNAcylated in vitro, including Ser-53, Ser-54, Ser-87, Ser-171, and Ser-173. Mutations at three sites (Ser-53, Ser-54, and Ser-173) had significant posttranslational or functional consequences, discussed below. We also report a fractionation protocol that separates emerin (along with BAF and A-type lamins) into two functionally and posttranslationally distinct populations. One population included the vast majority of lamin B and chromatin; only in this context did GFP-emerin associate with endogenous BAF. We propose that this sonication-dependent fraction corresponds, in living cells, to a niche in which chromatin is fundamentally organized by association with emerin, BAF, and B-type lamins. This niche also included variable levels of A-type lamins that might, we speculate, tether silent chromatin. By contrast, the paucity of emerin-BAF association in the easy fraction, where A-type lamins were also abundant, suggests that all three proteins have alternative functions in the easy niche. Potential alternative functions include roles in signaling, proliferation, epigenetic regulation, and association with other nucleoskeletal components (1, 44, 83). These two fractions significantly extend the nuclear matrix and nucleoskeleton as operational concepts by separating the nuclear membrane protein emerin and other nuclear lamina proteins based on their association with the genome; B-type lamins and a subset of emerin, BAF, and A-type lamins partition with chromatin, whereas easy emerin, BAF, and A-type lamins are either fully chromatin-independent or associate with trace amounts of chromatin (H3) present in this fraction. The unique functions and regulation of lamins, emerin, and BAF in each niche and their relationships to chromatin are important new questions for future work. Furthermore, our results collectively show that OGT, an essential enzyme, regulates at least two regions in the emerin molecule and controls emerin association with BAF (and hence, we propose, chromatin) at the nuclear envelope.

BAF Associates with Emerin Only in the Lamin B- and Chromatin-containing Niche—Unsonicated nuclear lysate supernatants (N_s) contained roughly half the emerin, BAF, and A-type lamins and most nuclear actin. Sonication was required to release the N_s populations of emerin, BAF, A-type lamins, and ~82–96% of histone H3 and lamin B. Chromatin was no surprise as a defining component of the N_s fraction, because sonication shears chromosomal DNA. We speculate that lamin B filaments associate more avidly with chromatin or other stable nucleoskeletal component(s) (1). Differential solubility of A-type lamins has ample precedent (84–86), including FRAP evidence that “internal” GFP-lamins are more mobile than envelope-associated GFP-lamins (87, 88) and evidence that lamins “loosen” locally during egress of nascent ribonucleoprotein particles (89) and herpes simplex virus-1 particles (90–92).

We expected to find BAF predominantly in the easy fraction, because GFP-BAF is diffusively mobile in HeLa cell nuclei (FRAP recovery half-time, 80–260 ms) (93) and C. elegans nuclei (halftime, 2.24 s) (94). Instead, most (~74%) endogenous nuclear BAF was sonication-dependent in HeLa cells, whereas ~22% was sonication-dependent in HEK293 cells, suggesting that N_e/N_s partitioning is influenced by cell type or malignancy status (67).

The sonication-dependent population of endogenous BAF is, we propose, closely associated with chromatin. BAF directly cross-bridges and “loops” dsDNA in vitro (95–97), condenses chromatin (66), mediates chromatin attachment to the nuclear lamina (98, 99), associates with telomeres and “core” regions of chromatin during mitosis (5, 6, 100), and protects chromatin from DNA damage (101). Our discovery that GFP-emerin and BAF associate only in the N_e fraction, when both were also abundant in N_p, provides a vital new tool for isolating endogenous BAF and testing its proposed role in linking chromatin to other major “lamina” components (emerin and lamins) in mammalian cells.

Emerin O-GlcNAcylation at Ser-53 and Ser-54; Implications for Conformation, Mitosis, and EDMD—Mutating either Ser-53 or Ser-54 significantly (~50%) reduced emerin O-GlcNAcylation, both in cells (where other enzymes and partners might influence outcome) and by purified OGT in vitro. We conclude that residues Ser-53 and Ser-54 are each individually important for OGT regulation of emerin. Interestingly, O-GlcNAc and phosphate can have opposite effects on peptide conformation; O-GlcNAc stabilized and tightened a canonical type-II β-turn, whereas phosphate opened and extended this turn (102). We therefore hypothesize that emerin Ser-53/Ser-54 O-GlcNAcylation either (a) influences OGT activity or substrate specificity (103, 104) or (b) alters the conformation of emerin and thereby facilitates OGT access to additional sites. The hypothesis that O-GlcNAcylation alters emerin conformation will be tested in future work.

Five O-GlcNAc sites (Ser-54, Ser-57, Ser-58, Ser-171, and Ser-173) are also reportedly phosphorylated (44), suggesting that OGT competes with unknown kinases to control emerin during both mitosis (105) and interphase (e.g. EGF stimulates phosphorylation at Ser-54, Ser-58, Ser-171, and Ser-173) (106, 107).

S54F is a “special” EDMD-causing mutation that does not disrupt emerin expression or localization yet still causes EDMD (78). S54F disrupts direct binding to two (of eight tested) partners in vitro: mRNA splicing/processing factor Btf (108, 109) and HDAC3 (28). Emerin stimulates HDAC3 deacetylase activity; hence, EDMD disease may reflect insufficient HDAC3-de-
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dependent gene silencing (28). However, our results suggest the S54F mutation also profoundly misregulates emerin; it blocks both phosphorylation and O-GlcNAcylation at Ser-54, near the LEM domain, and also significantly disrupts further emerin O-GlcNAcylation at unknown sites, the molecular and functional consequences of which are unknown.

Emerin Residues 161–175, a Proposed “Nexus” for Complex Posttranslational Control of Emerin Binding to BAF—In striking contrast to Ser-53 and Ser-54 mutations, which disrupted emerin O-GlcNAcylation by purified OGT in vitro, mutations at Ser-173 had detectable consequences only in cells. Furthermore, these consequence(s) depended on whether the mutation was phosphomimetic (S173D versus S173A) and differed between the easy and sonication-dependent populations. The single S173A mutation enhanced GFP-emerin O-GlcNAcylation in the easy fraction. However, combined with the triple S52–54A mutation, S173A reduced emerin O-GlcNAcylation in the sonication-dependent fraction. The phosphomimetic S173D mutation increased hyperphosphorylation in both fractions but non-identically (one versus two slowly migrating bands). BAF association was unaffected by S173A, suggesting that BAF requires neither a Ser nor O-GlcNAcylation at residue 173. However, BAF association was reduced significantly by the phosphomimetic S173D.

These consequences of emerin Ser-173 mutations and their effects on BAF can be explained by at least three nonexclusive mechanisms, depicted in Fig. 7D. First, phosphorylation versus O-GlcNAcylation at Ser-173 might have opposite effects on emerin conformation, as discussed above. Second, Ser-173 modifications might control binding to a partner(s) that influences emerin association with BAF, for example either sterically (e.g. GCL competes with BAF) (15) or posttranscriptionally (e.g. tyrosine protein phosphatase 1B) (110). This hypothetical partner(s) is unknown. Third, Ser-173 O-GlcNAcylation might promote BAF association by blocking an alternative fate in the sonication-dependent niche: Ser-173 phosphorylation and subsequent hyperphosphorylation. Our model that Ser-173 modifications, far from the LEM domain, somehow control binding to BAF is supported by previous evidence that the Y161F and S175A mutations also influence BAF association in whole cell lysates (62, 80). These findings collectively define emerin residues 161–175 as a nexus for complex posttranslational regulation of emerin binding to BAF.

OGT Regulation of the Nuclear Lamina?—OGT responds to cell nutrient (including glucose) status, and its targets include histones, HDAC complexes, RNA polymerase II, and key transcription factors (57, 64) and now also the conserved LEM domain protein, emerin. OGT regulation of emerin provides a direct molecular mechanism for cross-talk between cell nutrient status and emerin association with BAF, an enigmatic nuclear lamina component that influences histone posttranslational modifications (111). Furthermore, OGT misregulation of emerin may be relevant to aging, heart disease, and diabetes, all of which are characterized by elevated protein O-GlcNAcylation (57). How O-GlcNAcylation influences the emerin molecule and its roles in mitosis, signaling, and gene silencing are major open questions for future work.

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