BAF-1 mobility is regulated by environmental stresses

Daniel Z. Bar a,*, Maya Davidovich a,*, Ayelet T. Lamm a, Hagit Zer b, Katherine L. Wilson b, and Yosef Gruenbaum a

a Department of Genetics, Institute of Life Sciences, Hebrew University of Jerusalem, Givat Ram Jerusalem 91904, Israel; b Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

ABSTRACT Barrier to autointegration factor (BAF) is an essential component of the nuclear lamina that binds lamins, LEM-domain proteins, histones, and DNA. Under normal conditions, BAF protein is highly mobile when assayed by fluorescence recovery after photobleaching and fluorescence loss in photobleaching. We report that Caenorhabditis elegans BAF-1 mobility is regulated by caloric restriction, food deprivation, and heat shock. This was not a general response of chromatin-associated proteins, as food deprivation did not affect the mobility of heterochromatin protein HPL-1 or HPL-2. Heat shock also increased the level of BAF-1 Ser-4 phosphorylation. By using missense mutations that affect BAF-1 binding to different partners we find that, overall, the ability of BAF-1 mutants to be immobilized by heat shock in intestinal cells correlated with normal or increased affinity for emerin in vitro. These results show BAF-1 localization and mobility at the nuclear lamina are regulated by stress and unexpectedly reveal BAF-1 immobilization as a specific response to caloric restriction in C. elegans intestinal cells.

INTRODUCTION Barrier to autointegration factor (BAF) is an essential, 10-kDa protein expressed in multicellular animals (Zheng et al., 2000; Furukawa et al., 2003; Margalit et al., 2005b). BAF was first identified as a mammalian protein required for retroviral DNA to integrate into the host chromosome (Chen and Engelman, 1998) and is required for human immunodeficiency virus 1 integration into macrophage chromosomes (Lin and Engelmann, 2003; Jacque and Stevenson, 2006; Shun et al., 2007; Van Maele et al., 2006). BAF was independently identified as a novel partner for the nuclear membrane protein lamin-associated polypeptide 2 β (LAP2β), encoded by TMPO;

Furukawa, 1999) and subsequently shown to bind the LAP2, emerin, MAN1 (LEM) domain of LAP2 (Shumaker et al., 2001), which is shared by all members of the LEM-domain family of nuclear proteins (Shumaker et al., 2001; Margalit et al., 2007a).

Homodimers of BAF bind nonspecifically to double-stranded DNA (Cai et al., 1998; Zheng et al., 2000), allowing it to “bridge” two DNA molecules in vitro (Bradley et al., 2005) or “condense” longer DNA molecules in vitro by making loops (Skoko et al., 2009). BAF also directly binds three fundamental groups of proteins: LEM-domain proteins (Cai et al., 2001, 2007; Lee et al., 2001; Wagner and Krohne, 2007), histones (H3, H4, and certain H1 isoforms; Montes de Oca et al., 2005, 2009), and nuclear intermediate filament proteins, named laminas (Lee et al., 2001). BAF, lamins, and LEM-domain proteins can bind each other directly and simultaneously (Holaska et al., 2003) and potentially synergistically (Bengtsson and Wilson, 2006). In Caenorhabditis elegans, all three components are required to assemble the nuclear “lamin” (Liu et al., 2003; Margalit et al., 2005b; Simon and Wilson, 2011), a major component of nuclear structure (Simon and Wilson, 2011).

Biochemical studies of BAF are challenged by its poorly understood ability to oligomerize as hexamers of dimers in the presence of DNA (Skoko et al., 2009). Excess BAF profoundly disrupts higher-order chromatin structure in cell extracts (Segura-Totten et al., 2002).
In living cells, BAF influences higher-order chromatin structure (Furukawa et al., 2003; Margalit et al., 2005a; Haraguchi et al., 2007), represses transcription at specific promoters (Wang et al., 2002; Margalit et al., 2007b; Huang et al., 2011), and is required for postmitotic nuclear assembly (Margalit et al., 2005a). Through mechanisms that are not understood, BAF helps tether chromatin to the nuclear envelope (Margalit et al., 2005a; Asencio et al., 2012) and functions as an epigenetic regulator (Montes de Oca et al., 2011). Depletion of BAF-1 dramatically increases susceptibility to radiation in C. elegans (Dittrich et al., 2012).

In living cells, the DNA-binding activity of BAF and its nucleocytoplasmic distribution are controlled by a conserved Ser/Thr kinase named vaccinia-related kinase 1 (VRK1; Nichols et al., 2006; Gorjánácz et al., 2007). The distribution of BAF can change dramatically during the cell cycle (Dechat et al., 2004; Haraguchi et al., 2007; Capanini et al., 2010; Asencio et al., 2012). VRK1 phosphorylates human BAF residues Thr-2, Thr-3, and Ser-4 (Nichols et al., 2006). Phosphorylation at Ser-4 reduces binding to emerin and abolishes DNA binding, whereas phosphorylation at Thr-2 or Thr-3 reduces binding to DNA (Bengtsson and Wilson, 2006; Nichols et al., 2006). In C. elegans, BAF-1 can be dephosphorylated either directly, by protein phosphatase 2A (PP2A, mediated by cobinding to LEM-4–like [LEM-4L]), or passively, via LEM-4L–dependent inhibition of VRK-1 (Asencio et al., 2012).

Previous FLIP/FRAP studies of green fluorescent protein (GFP)-fused lamin A, lamin B, emerin, LAP2β, and MAN1 showed these nuclear lamina components are predominantly immobile. The immobile fractions of lamin A and lamin B2 are ∼95%, and similar t1/2 recovery times were measured for lamin A (87 ± 25 s), lamin B1 (120 ± 40 s), emerin (62 ± 31 s), and MAN1 (97 ± 64 s) (Moir et al., 2000; Shimizu et al., 2004; Broers et al., 2005; Schutz et al., 2005). By contrast, most GFP::BAF is mobile in both interphase human cells (t1/2 of 270 ± 49 ms at the nuclear periphery, ~80 ms in the nucleoplasm, and ~47 ms in the cytoplasm; Shimizu et al., 2004) and in C. elegans embryos (2.24 ± 0.66 s; Margalit et al., 2007b). This mobility suggested BAF-1 might function as a mobile “communicator” component of the nuclear lamina. We tested this hypothesis by fluorescence loss in photobleaching (FLIP) and fluorescence recovery after photobleaching (FRAP) analysis of C. elegans lines bearing integrated wild-type or missense-mutated GFP::BAF-1. We report GFP::BAF-1 is immobilized in L1 larvae subjected to dietary restriction (eat-2 mutant), food deprivation, or brief (1 h) heat shock.

**RESULTS**

We previously showed that an integrated GFP::BAF-1 construct rescues most known baf-1 null (gk324) phenotypes (Margalit et al., 2007b). To determine whether BAF-1 mobility was affected by stress, we used FRAP to analyze GFP::BAF-1 localization and dynamics in cells of L1 and L2 larval of wild-type (N2) or eat-2 animals. The eat-2 mutation disrupts a ligand-gated ion channel, resulting in decreased pharyngeal contractions and dietary restriction (Mastick et al., 1995). Photobleached regions recovered rapidly in both the wild-type and eat-2 animals; however, a significantly (p < 0.01, two-tailed t test) higher fraction of GFP::BAF-1 was immobile in the eat-2 animals (Figure 1A). To determine whether this phenotype was specifically due to starvation or another effect of the eat-2 mutation, we studied wild-type animals that were either fed or food-deprived overnight. The next day, fed animals had reached the L1 or L2 larval stage, whereas food-deprived animals were exclusively in L1, as previously reported (Johnson et al., 1984). Consistent with the eat-2 results, FRAP analysis revealed an increase in the immobile fraction of GFP::BAF-1 in food-deprived animals (Figure 1B). This difference was significant (p < 0.00056, two-tailed t test) and did not depend on larval stage, as L1 and L2 well-fed controls gave the same results (Figure 1B).

Because intestinal cells have major roles in responding to food deprivation (Walker et al., 2005; Palgunow et al., 2012), we focused on intestinal cell nuclei of L1- and L2-stage wild-type (N2), eat-2, and food-deprived animals. As BAF-1 is highly mobile, the bleaching in FRAP must be short, which reduces bleach depth. To overcome this technical problem, we used FLIP analysis to more accurately measure the immobile fraction and obtain a better quantitative representation of the phenomena (Figure 2). We bleached a small region overlapping the nucleus for ~1 min, while observing the whole cell. In wild-type animals, this caused an almost complete disappearance of GFP::BAF-1 fluorescence at the nuclear envelope and throughout the cell (Figure 2, A–C, wild-type/fed), as expected (Margalit et al., 2007b). By contrast, in both the eat-2 and food-deprived intestinal cells, GFP::BAF-1 fluorescence outside the bleached area was essentially unaffected (FD animal cells shown in Figure 2A and GFP::BAF-1 in fed and food-deprived intestinal cells shown in Supplemental Movies S1 and S2, respectively). This suggested that nearly all GFP::BAF-1, notably including the nuclear lamina population, was immobilized in response to dietary stress. There was no correlation between GFP::BAF-1 fluorescence intensity, which can vary between different intestinal cells, and its mobility. Immobilization was only slowly reversible, as food-deprived animals that were returned to feeding plates for 2 h (“FD/fed”) showed a slight but significant increase in GFP::BAF-1 mobility (Figure 2C, p < 0.05, n = 6, two-tailed t test). Thus BAF immobilization may be a relatively
response to three different cellular stresses: dietary restriction (eat-2), food deprivation, and brief heat shock. To explore the mechanisms by which food deprivation and stress pathways regulate BAF-1 dynamics, we focused on heat shock, which triggers some of the same signaling pathways as food deprivation (Raynes et al., 2012), affects BAF-1 mobility in most or all cell types, and is readily manipulated in adult animals.

GFP-BAF-1 Ser-4 phosphorylation doubles in response to short heat shock

To determine whether BAF-1 was posttranslationally modified in response to stress, we immunoprecipitated GFP::BAF-1 from unsynchronized populations of control or heat-shocked (1 h, 37°C) mixed population C. elegans L1 larvae that were well fed (blue line), animals that were food deprived overnight (red line), or following 2-h recovery from FD (green line). FLIP analysis of emerin (D) HPL-1 (E) and HPL-2 (F) fused to GFP did not reveal any difference in mobility after food deprivation. (G) Mobility plot of the relative intensity of GFP::BAF-1 in wild-type animals with time following 1-h heat shock at 37°C. Error bars indicate SEM. For each experiment in (B), n = 7; in (C–G), n = 6.

long-term response to dietary stress in intestinal cells (see Discussion).

To determine whether other chromatin- or lamina-associated proteins were immobilized by food deprivation, we used FLIP to measure the mobility of GFP-fused heterochromatin protein 1 (HPL-1 and HPL-2; Schott et al., 2006) or emerin in intestinal cells. Food deprivation had no significant effect on the mobility of these proteins (Figure 2, D–F). Thus whole-cell immobilization is not a general phenomenon of chromatin-associated proteins.

Short heat shock is known to extend life span, potentially by activating small heat shock proteins and other stress-response genes (Olsen et al., 2006). Interestingly, a brief heat shock (1 h at 37°C) also significantly reduced GFP::BAF-1 mobility in the intestine (Figure 2G) and in epidermal and muscle cells of wild-type L1 or L2 animals (Supplemental Figure S1A and unpublished data). These results are stage specific, as adult worms showed weaker GFP::BAF-1 immobilization in response to heat shock, and early-stage embryos showed an inverse response (Figure S2). As BAF-1 is an essential protein, we knocked down baf-1 postdevelopmentally and tested heat shock survival. Despite the role of heat shock in BAF-1 mobility, we saw no effect of baf-1 RNA interference (RNAi) on animal survival (12 h at 35°C; unpublished data). However, we cannot exclude an essential role for baf-1 in other stresses or developmental stages. These results collectively showed that GFP::BAF-1 is specifically immobilized at the nuclear envelope and elsewhere in response to three different cellular stresses: dietary restriction (eat-2), food deprivation, and brief heat shock. To explore the mechanisms by which food deprivation and stress pathways regulate BAF-1 dynamics, we focused on heat shock, which triggers some of the same signaling pathways as food deprivation (Raynes et al., 2012), affects BAF-1 mobility in most or all cell types, and is readily manipulated in adult animals.

GFP-BAF-1 Ser-4 phosphorylation doubles in response to short heat shock

To determine whether BAF-1 was posttranslationally modified in response to stress, we immunoprecipitated GFP::BAF-1 from unsynchronized populations of control or heat-shocked (1 h, 37°C) mixed population C. elegans (Rothbauer et al., 2008) and used mass spectrometry to detect posttranslational modifications. GFP::BAF-1 resolved as two abundant bands (Figure S3), similar to endogenous human BAF (Puente et al., 2011), in which the slow-migrating BAF band is proposed to be hyperphosphorylated (Nichols et al., 2006).

Both bands were analyzed by liquid chromatography–tandem mass spectrometry, with tryptic peptide coverage of ~80% (Figure 3A). We detected the same modification in all four bands: phosphorylation at Ser-4 (Figure 3A). No other phosphorylated residues were detected. For each sample, the relative amount of the Ser-4–phosphorylated peptide, CAAGPGSTGMSTSVK, was estimated as a fraction of the intensity (total ion current) of its corresponding
We generated control animals (top) and heat-shocked animals (bottom). We then calculated the percentage of each population that was mobile before, and immediately after, heat shock (Figure 5A). We to determine how BAF-1 mutations affected mobility, we used FRAP to analyze the GFP::BAF-1 mutants that localized normally in L1 larval intestinal cells. With one exception, Ser-4 (Bengtsson and Wilson, 2006), the effects of BAF-1 missense mutations on binding to lamins were unstudied. To determine whether our BAF-1 mutations affected direct binding to C. elegans partners, we generated recombinant maltose-binding protein (MBP) fusions to the N-terminus of wild-type or mutant BAF-1 and tested binding to three recombinant purified C. elegans proteins: emerin residues 1–125 (nucleoplasmic domain only), the lamin tail domain (residues 388–566), and full-length lamin bearing the R55H mutation (lamin-R55H) to maintain solubility when expressed in bacteria (Wiesel et al., 2008). BLACore surface plasmon resonance was used to measure association and dissociation constants and equilibrium affinities for BAF-1 binding to emerin (Figure 4C) or lamin-R55H (Figure 4D). Wild-type BAF-1 bound with high affinity to emerin (57 nM; reported affinity of human proteins is 200 nM; Holaska et al., 2003). This variation may be species specific or may reflect differences in polypeptide constructs and assay conditions. Wild-type BAF-1 also bound with high affinity to lamin-R55H (84 nM; human BAF affinity for B-type lamins is untested). C. elegans lamin-R55H and emerin bound each other with 4–5 nM affinity (Figure 4C), 10-fold higher that than reported for human emerin and lamin A (40 nM; Holaska et al., 2003); human emerin affinity for B-type lamins is untested). Emerin bound itself tightly (14 nM affinity; Figure 4C), consistent with human emerin (Berk et al., personal communication). Full-length lamin (lamin-R55H) also showed high affinity for itself (3.7 nM) and to the isolated lamin tail domain (0.56 nM; Figure 4), suggesting that the lamin tail domain might mediate lateral association between lamin filaments in C. elegans.

All four tested BAF-1 mutations bound emerin with either wild-type affinity (~57 nM, S4E), two- to threefold higher affinity (31 nM, K6A; 20 nM, S4A), or 10-fold higher affinity (5.6 nM, F46E; Figure 4C). Note that the corresponding mutation in human BAF, L46E, abolished binding to human emerin in vitro (Segura-Totten et al., 2002); whether this reflects a species-specific difference or extends to living cells (in which association can be influenced by other factors (e.g., posttranslational modifications) remains undetermined.

All four BAF-1 mutations reduced binding to full-length lamin R55H by either two- to threefold (K6A and S4A: 198 and 281 nM, respectively), seven- to ninefold (F46E, 623 nM), or 382-fold (S4E, 32.1 μM) (Figure 4D). Thus BAF-1 Ser-4 phosphorylation in C. elegans is predicted to inhibit binding to lamin without affecting emerin (see Discussion).

Heat shock–induced immobilization of GFP::BAF-1 in larvae intestinal cells is disrupted by the S4A and S4E mutations

To determine how BAF-1 mutations affected mobility, we used FRAP to analyze the GFP::BAF-1 mutants that localized normally in L1 larvae (K6A, S4E, S4A) both before and after 1-h heat shock (Figure 5A). We then calculated the percentage of each population that was mobile before, and immediately after, heat shock (Figure 5B). In L1 intestinal cells expressing wild-type GFP::BAF-1, mobility was compared with each GFP::BAF mutant, we measured the GFP fluorescence intensity distribution across the nucleus in five to seven intestinal cells and then normalized and averaged by aligning the nuclear envelope peaks (trace below each image; Figure 4B). The S4A, S4E, and K6A polypeptides all concentrated near the nuclear envelope, like wild-type (Figure 4B). The F46E mutant also concentrated near the nuclear envelope but had higher signals in the nucleoplasm (Figure 4B). Thus these mutations did not visibly perturb GFP::BAF-1 localization in L1 larvae intestinal cells.
**FIGURE 4:** In vivo analysis of wild-type and missense-mutant GFP::BAF-1 polypeptides. (A) Aligned amino acid sequences of BAF-1 (top) and human BAF (bottom), showing the missense mutations studied in this work (yellow arrow). (B) Fluorescence localization of GFP::BAF-1 (wild-type or mutant) protein in intestinal cell nuclei of L1 larvae. Scale bar: 5 μm. Each strain expresses a different GFP::BAF-1–based construct. The graph below each image shows the average intensity distribution across six nuclei (blue) on the same baseline as wild-type GFP::BAF-1 (red). (C and D) BIAcore analysis of the kinetics and equilibrium affinities of recombinant MBP::BAF-1, Ce-emerin residues 1–125, or Ce-lamin tail domain residues 388–566, each tested for binding to immobilized recombinant Ce-emerin residues 1–125 (C) or Ce-lamin R55H (D). $\chi^2$ represents the mean square of the signal noise.
These results also support the hypothesis that BAF-1 mobility is regulated in intestinal cells by mechanisms that include (but are not limited to) posttranslational modification of Ser-4 and binding to emerin.

DISCUSSION

Under normal conditions, most nuclear BAF-1 is highly mobile during interphase, suggesting frequent but transient interactions (Shimi et al., 2004; Margalit et al., 2007b). Our FRAP and FLIP analyses of interphase cells showed GFP::BAF-1 becomes immobilized under multiple stress conditions. When larval-stage animals were calorie restricted through food deprivation or the eat-2 mutation, BAF-1 was specifically immobilized in intestinal cells, with normal or intermediate phenotypes in other tested cell types, including muscle and epidermis (Figure S1B and unpublished data). By contrast, brief heat stress immobilized BAF-1 in all cell types tested, including intestinal, muscle, hypodermis, and pharyngeal cells (unpublished data). Immobilization was not a general phenomenon of chromatin proteins, as the C. elegans heterochromatin proteins HPL-1 and HPL-2 showed no significant changes in mobility. The signaling pathway(s) responsible for intestine-specific versus ubiquitous immobilization of BAF-1, which might include signaling via specific neurons (e.g., ASI neurons for dietary restriction; AFD neurons for heat shock; Bishop and Guarente, 2007; Prahlad et al., 2008) are important questions for future work. Together these results show that BAF-1 immobilization at the nuclear lamina is a fundamental cellular response to stress. However, because dietary restriction and food deprivation are regulated by multiple pathways in C. elegans (Greer and Brunet, 2009), it will be interesting to investigate whether different dietary restriction regimes, as well as different stressors, lead to different patterns of BAF-1 immobilization.

Mechanisms of BAF-1 immobilization

Mass spectrometry analysis of GFP::BAF-1 from mixed-stage animal populations showed BAF-1 is phosphorylated on Ser-4, consistent with previous studies of human BAF (Bengtsson and Wilson, 2006; Nichols et al., 2006) and C. elegans BAF-1 (Asencio et al., 2012). We did not detect phosphorylation at two other sites reported in human BAF (Thr-2 and Thr-3 [Nichols et al., 2006], equivalent to BAF-1 Ser-2 and Thr-3) that might be less abundant in C. elegans (Asencio et al., 2012). BAF-1 Ser-4 phosphorylation appeared to increase in response to brief heat shock, but whether heat shock affects the localization or activity of its kinase (VRK-1) is unknown. Further analysis using S4A- or S4E-mutated BAF-1 strains, as well as vrk-1 knockdown, confirmed that Ser-4 is important for BAF-1 dynamics. Interestingly, the mobility data of mutations in BAF-1 Ser-4 do not support a simple phosphorylation-immobilization model. This could be the result of altered protein–protein interactions in Ser-4 mutants.

| Localization | t_1/2 (s) | Mobile (%) |
|--------------|----------|------------|
| Control      | HS       | Control    | HS         |
| BAF WT       | 2.42 ± 0.12 | 2.01 ± 0.24 | 65 ± 1.6   | 44.2 ± 4.4   |
| BAF S4A      | 2.21 ± 0.25 | 2.05 ± 0.11 | 38.7 ± 2   | 40.5 ± 5.9   |
| BAF S4E      | 2.67 ± 0.64 | 2.21 ± 0.24 | 45.4 ± 2.5 | 61.2 ± 2.6   |
| BAF K6A      | 2.57 ± 0.5  | 2.46 ± 0.47 | 60.5 ± 3   | 25.7 ± 1.64  |

HS, heat shock; NE, nuclear; NP, nucleoplasm; NE+NP, nuclear and mild nucleoplasm.

TABLE 1: Summary of the localization, t_1/2, and mobile/immobile fractions for each tested GFP::BAF-1 mutant strain in L1 larvae based on FRAP (± SEM).
that include increased affinity for emerin (and perhaps other lamin) are compensated in nondividing cells by mechanism(s) of chemical defects (e.g., significantly reduced affinity for DNA and emerin, localized normally. These results show that de facto bio-
ity for emerin. The S4A and K6A mutations, with two- to threefold stronger binding to lamin and two- to threefold weaker binding to DNA and lamin (S4A and S4E). Weaker binding cannot explain immobilization. However, for both mutations (K6A and S4A), higher immobile fractions in vivo correlated with higher affinity for emerin in vitro. The exception (S4E) had normal affinity but twofold slower rates of association and dissociation; thus bound S4E might tend to “linger” on emerin. About 55% of BAF-1 S4E was immobile under normal conditions, essentially the same as wild-type BAF-1 after heat shock (56% immobile). Immobility was increased (from 35% in wild-type to 55–61%) by mutations that weakened binding to both DNA and lamin (S4A and S4E). Weaker binding cannot explain immobilization. However, for two mutations (K6A and S4A), higher immobile fractions in vivo correlated with higher affinity for emerin in vitro. The exception (S4E) had normal affinity but twofold slower rates of association and dissociation; thus bound S4E might tend to “linger” on emerin. About 55% of BAF-1 S4E was immobile under normal conditions, essentially the same as wild-type BAF-1 after heat shock (56% immobile). Overall BAF-1 immobility in intestinal cells correlated with normal or increased affinity for emerin in vitro.

Dietary restriction and insulin-like signaling regulate the baf-1 gene: long-term effects on nuclear lamina function?
Both dietary restriction and short-term heat shock lead to transcriptional changes that can extend life span (Raynes et al., 2012). We used chromatin immunoprecipitation data from the modENCODE database to analyze the promoter region of baf-1. We found that four transcription factors that modulate life span, either by dietary restriction or insulin-like signaling, namely SKN-1, PHA-4, DAF-16, and ELT-3; and other transcription factors (ALR-1, BLMP-1, LIN-15B) bind the baf-1 promoter (see Figure S4). These data predict that stress signaling, in addition to immobilizing BAF-1 protein at the nuclear lamina, might also regulate BAF-1 protein levels. We speculate that this is a long-term effect, as we detected no significant change in GFP::BAF protein levels after overnight food deprivation (p value of 0.057, n = 6). Consistent with this idea, BAF-1 immobilization was only slightly reversed by feeding for 2 h.

Stress-induced BAF-1 immobilization and chromatin
Our most exciting and unexpected result is that caloric restriction and heat stress regulate BAF-1 dynamics in L1 larvae intestinal cells and stabilize BAF-1 localization at the nuclear lamina. To our knowledge, this is the first evidence that external stress can change the intranuclear mobility of any nuclear lamina protein. How might BAF-1 immobilization affect chromatin? A human BAF proteome

FIGURE 6: GFP::BAF-1 immobilization is regulated by vrk-1. FLIP analysis of GFP::BAF-1 in animals grown on empty vector (EV) or vrk-1 RNAi (VRK) before and after heat shock (HS). Scale bar: 10 μm. Bleaching area: 3.1 μm².
revealed 56 high-confidence targets in a single cell type, including multiple proteins that regulate histone modifications (Montes de Oca et al., 2009). Because BAF both stabilizes nuclear lamina structure and influences histone posttranslational modifications (Montes de Oca et al., 2011), stress-induced BAF-1 immobilization has the potential to stabilize chromatin structure and broadly influence gene expression.

MATERIALS AND METHODS

C. elegans strains

C. elegans strains were handled as described previously (Brenner, 1974). Strains N2 (wild-type), DP38 unc-119(ed3), and eat-2(ad1116) were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN). Strains HPL-1::GFP and FR463 (HPL-2::GFP+pRF4) were kindly provided by F. Palladino (CNRS). The independent GFP::BAF-1–expressing strains (YG1001, YG2501, YG2502), GFP::BAF-1 S4A (YG2503, YG2504), GFP::BAF-1 S4E (YG2505, YG2506), GFP::BAF-1 K6A (YG2507, YG2508), GFP::BAF-1 F46E (YG2511, YG2512), and the independent emerin::GFP–expressing strain (YG002) were generated by micro particle bombardment of DP38 animals, as described previously (Margalit et al., 2007b), and were outcrossed three times. eat-2(ad1116); GFP::BAF-1 (YG1001) was generated by crossing these two stains.

Dietary restriction, food deprivation, and heat shock conditions

L1 measurements were performed by synchronizing embryos as previously described (Motohashi et al., 2006). Control and eat-2 embryos were seeded on OP50 Escherichia coli plates overnight at 23°C, while empty plates were used for food deprivation experiments. For heat shock, plates were incubated for 1 h at 37°C.

Bacterial expression of the mutant MBP::BAF-1 proteins

Wild-type baf-1 gene and all mutated baf-1 cDNAs were cloned into the pMal C2 vector containing maltose-binding protein (MBP) parallel 1 (New England Biolabs). In this construct baf-1 is located 5′ to the multiple cloning site of the pMal plasmid. Plasmids were transformed into E. coli BL21(DE3)-(codon plus-RIL), and expressed proteins were purified on amylose resin per the manufacturer’s protocol (New England Biolabs; www.neb.com/products/e8021-amylose-resin). Recombinant His-tagged Ce-lamin-T, Ce-lamin R55H, and Ce-emerin residues 1–125 were purified as previously described (Ben-Harush et al., 2009), dialyzed into column buffer (20 mM sodium phosphate buffer, 200 mM NaCl, 1 mM EDTA) for 24 h at 4°C, and then filtered in liquid nitrogen and thawed on ice 30 min, with extensive pipetting every 10 min. Samples were then sonicated (30 s, five times) and centrifuged (20,000 × g, 10 min, 4°C). GFP::BAF-1 was purified using GFP-Trap antibodies per the manufacturer’s protocol (GFP-Trap_M; ChromoTek). Samples were boiled (100°C, 10 min), resolved by SDS–PAGE (15% acrylamide), and stained with Coomassie blue. GFP::BAF-1 bands were identified by immunoblotting an additional lane with mouse-anti GFP (1:1000; Roche) and excised for MS analysis.

Image analysis, microscopy, and live-cell imaging

Live fluorescence images were acquired with the Leica SP5 confocal microscope and a 63×/1.4 oil-immersion objective. Imaging was performed and initially analyzed with Leica Application Suite 2.3.1. Bleaching was done on a single z-plane, at 100% transmission. Imaging typically required 1–6% of laser power. For pre- and post-bleach images, four frames were extracted and averaged from corresponding movies using ImageJ. Graphs were generated from multiple regions of interest after normalization and averaging. Worms were mounted on agarose pads and paralyzed with 1–10 mM levamisole and immediately taken for imaging. Typically, animals were imaged for 5–30 min after levamisole administration. When visible, the FLIP experiments started with the most anterior intestinal cells, and we moved backward in sequential FLIPs. For FRAP analysis, all strains were imaged with a Leica SP5 confocal microscope and a 63×/1.4 oil-immersion objective. GFP::BAF-1 fluorescence was photobleached by a 488-nm laser in a defined region of each cell and was imaged with a 488-nm (Figure 1) or 496-nm laser for all other experiments. For FRAP analysis, fluorescence intensity in the bleached area, the background area, and the total cell area were measured as a function of time after bleaching and were normalized essentially as described previously (Rabut and Ellenberg, 2005). For FLIP experiments, we used 488-nm light to repetitively bleach GFP::BAF-1 in a region of interest. For determination of the half-time of FRAP, the normalized fluorescence intensity of the mobile fraction was divided by two. The corresponding time (half-time) was extracted from the plot/equation (Rabut and Ellenberg, 2005).

Protein distribution

Protein distribution was calculated using ImageJ (Schneider et al., 2012) by measuring the fluorescence intensity along a line crossing the nucleus. For each graph, five to seven nuclei were normalized and averaged by aligning peaks representing the nuclear envelope. Error bars represent SEM. p values were calculated by averaging the intensity of seven adjacent dots along the crossing line. Averages of measured nuclei from different BAF mutations were compared with wild type using a two-tailed t test.

GFP-TRAP-M

For isolation of GFP::BAF-1 protein, YG1001 asynchronous population worms were grown in 9-cm plates. Six plates were subjected to heat shock (37°C, 1 h). Worms were collected, washed twice with M9, and resuspended in 200 ml lysis buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.5% NP40, 1% Protease Inhibitor Cocktail [Roche, Indianapolis, IN]). lysates were frozen in liquid nitrogen and thawed on ice 30 min, with extensive pipetting every 10 min. Samples were then sonicated (30 s, five times) and centrifuged (20,000 × g, 10 min, 4°C). GFP::BAF-1 was purified using GFP-Trap antibodies per the manufacturer’s protocol (GFP-TRAP_M; ChromoTek). Samples were boiled (100°C, 10 min), resolved by SDS–PAGE (15% acrylamide), and stained with Coomassie blue. GFP::BAF-1 bands were identified by immunoblotting an additional lane with mouse-anti GFP (1:1000; Roche) and excised for MS analysis.

In-gel proteolysis and mass spectrometry analysis

Proteins in excised gel bands were reduced (3 mM dithiothreitol), modified (12 mM iodoacetamide), and digested with modified trypsin (Promega, Madison, WI) at a 1:10 enzyme-to-substrate ratio in 10 mm ammonium bicarbonate and 10% acetonitrile. Tryptic peptides were resolved by reverse-phase chromatography on 0.075 × 200–mm fused silica capillaries (J&W; Agilent, Santa Clara, CA), packed with Reprosl reversed-phase material (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany). The peptides were eluted for 65 min using a linear 5–45% gradient followed by 15 min in 95% acetonitrile/0.1% formic acid [vol/vol] in water at flow rates of 0.25 μl/min. Mass spectrometry was performed with an ion-trap mass spectrometer (OrbitrapXL; Thermo-Finnigan, San Jose, CA) in a positive mode using repetitively full MS scan followed by collision-induced dissociation of the seven most-dominant ions selected from the first MS scan. Multistage activation was used to analyze phosphopeptides. The mass spectrometry data were analyzed using Sequest 3.31 software, searching against the uniprot database and against a specific sequence with 5 ppm accuracy.
BIACore analysis

Binding was analyzed using a BIACore 3000 (BIACore, Uppsala, Sweden) and sensor chip CM1 (BIACore), at 25°C. The chip was activated using the BIACore EDC/NHS amine-coupling protocol (www.biacore.com). In kinetics experiments, recombinant His::lamin R550 and His::emerin 1–125 proteins at 10 μg/ml in 10 mM acetate (pH 4) were immobilized and gave ~2000 resolution units (RU) and 1200 RU, respectively. Analytes were injected at 30 μl/min in 10 mM phosphate (pH 7.4), 150 mM NaCl, and 0.005% Tween 20. The chip was regenerated using 10 mM phosphate (pH 7.4), 150 mM NaCl, and 0.005% Tween 20. Results were evaluated using BIAevaluation software version 4.1. The 1.1 Langmuir model was used to fit experimental results and calculate affinities and kinetics constants.

ACKNOWLEDGMENTS

We thank Tsafi Danieli for helping with protein expression and the Smoler Proteomics Center (Technion) for mass spectrometry analyses. We also thank Francesca Palladino for providing the strains expressing GFP-fused HPL-1 and HPL-2 and Alon Zaslaver for critical reading of the manuscript. We gratefully acknowledge funding from the Binational Israel-USA Science Foundation, the National Institutes of Health (RO1 GM048646 to K.L.W. and Y.G.), the Israel Ministry of Health (MOH 2965), the Muscular Dystrophy Association, and the COST GM048646 to K.L.W. and Y.G.), the Israel Ministry of Health (MOH 2965), the Muscular Dystrophy Association, and the COST NANOMET (BM1002).

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