Introduction

Barrier to autointegration factor (BAF) was first discovered as a host component of retroviral preintegration complexes, which is required for integrase-mediated retroviral DNA insertion into target DNA in vitro (Chen and Engelman, 1998; Lee and Craigie, 1998; Lin and Engelman, 2003). BAF is highly conserved in metazoan evolution (52% identical between human and Caenorhabditis elegans; Margalit et al., 2005) and typically localizes at the nuclear periphery, nucleoplasm, and cytoplasm (Segura-Totten and Wilson, 2004). BAF binds directly to many different partners, including double-stranded DNA, histone H3, and certain linker histones, and to all characterized LAP2–emerin–MAN1 (LEM) domain proteins plus lamins and selected homeodomain transcription activators (Segura-Totten and Wilson, 2004; Montes de Oca et al., 2005). These interactions are regulated, at least in part, by the posttranslational modification of BAF (Bengtsson and Wilson, 2006; Górríñáncz et al., 2006; Nichols et al., 2006). In C. elegans, the nuclear envelope localization of BAF-1 protein (previously termed Ce-BAF) depends on its interaction with two inner nuclear membrane LEM domain proteins encoded by emr-1 (Ce-emerin) and lem-2 (Ce-lem2; formerly known as Ce-MAN1; Liu et al., 2003). In turn, BAF-1 is required to localize both LEM domain proteins and Ce-lamin in embryonic cells, suggesting mutual structural interdependence (Margalit et al., 2005). RNAi-mediated down-regulation in C. elegans of either baf-1 or lmn-1 or double down-regulation of both emr-1 and lem-2 caused chromosome segregation defects and failure to properly assemble daughter nuclei (Liu et al., 2003). In mammalian cells, the ectopic expression of mutant BAF that cannot bind DNA or LEM domain proteins dominantly blocked the recruitment of lamin A and the LEM domain proteins emerin and LAP2β but had no effect on B-type lamins or on LBR (lamin B receptor protein; Haraguchi et al., 2001).

Structural roles were also seen in Xenopus laevis egg extracts, in which nuclei can assemble in vitro; adding recombinant BAF either inhibited or enhanced nuclear assembly, depending on the amount of BAF added (Segura-Totten et al., 2002), suggesting important roles for BAF in organizing chromatin and the nucleus. Indeed, BAF is essential in both migration, vulva formation, and the timing of seam cell fusion. In the seam cells, BAF-1 represses the expression of the EFF-1 fusogen protein, but fusion still occurs in C. elegans lacking both baf-1 and eff-1. This suggests the existence of an eff-1–independent mechanism for cell fusion. BAF-1 is also required to maintain the integrity of specific body wall muscles in adult animals, directly implicating BAF in the mechanism of human muscular dystrophies (laminopathies) caused by mutations in the BAF-binding proteins emerin and lamin A.

Barrier to autointegration factor blocks premature cell fusion and maintains adult muscle integrity in C. elegans

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Barrier to autointegration factor (BAF) binds double-stranded DNA, selected histones, transcription regulators, lamins, and LAP2–emerin–MAN1 (LEM) domain proteins. During early Caenorhabditis elegans embryogenesis, BAF-1 is required to organize chromatin, capture segregated chromosomes within the nascent nuclear envelope, and assemble lamin and LEM domain proteins in reforming nuclei. In this study, we used C. elegans with a homozygous deletion of the baf-1 gene, which survives embryogenesis and larval stages, to report that BAF-1 regulates maturation and survival of the germline, cell

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Abbreviations list: BAF, barrier to autointegration factor; ChIP, chromatin immunoprecipitation; DIC, differential interference contrast; DTC, distal tip cell; LEM, LAP2–emerin–MAN1; VPC, vulval precursor cell.

The online version of this article contains supplemental material.

Supplemental Material can be found at:
/content/suppl/2007/08/13/jcb.200704049.DC1.html

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The Journal of Cell Biology, Vol. 178, No. 4, August 13, 2007 661–673
http://www.jcb.org/cgi/doi/10.1083/jcb.200704049
C. elegans (Margalit et al., 2005) and Drosophila melanogaster
(Furukawa et al., 2003). BAF-null Drosophila die at the larval-
pupal transition, when they run out of maternally deposited
BAF, with phenotypes that include arrest at various stages of
the cell cycle, chromatin clumping, abnormal lamin distribu-
tion, aberrant nuclear morphology, small brains, and missing
imaginal discs (Furukawa et al., 2003). RNAi-mediated down-
regulation of baf-1 in C. elegans revealed that the loss of both
maternal and zygotic BAF-1 caused anaphase chromatin bridges,
abnormal chromatin morphology, and chromosome missegre-
gation as early as the two-cell stage, and embryos died at or
before the ~100-cell stage (Zheng et al., 2000; Margalit et al.,
2005). The few embryos that escaped baf-1(RNAi) lethality
grew into sterile adults with misplaced distal tip cells and gonads
(Margalit et al., 2005).

To better understand the potential cellular and develop-
mental roles of BAF, we studied a loss of function mutation in
baf-1(gk324). In animals homozygous for the gk324 allele, the
maternal supply of BAF-1 was sufficient to allow these animals
to complete embryogenesis and larval stages, bypassing BAF-1’s
mitotic roles and allowing us to focus on later stages of C. elegans
development. This genetic analysis reveals several novel tissue-
specific roles for BAF-1 and sheds new light on the disease
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**Results**

**BAF-1 is ubiquitously expressed and enriched at the nuclear envelope throughout development**

In early C. elegans embryos, BAF-1 is enriched near the nu-
clear inner membrane (Margalit et al., 2005). To follow BAF-1
expression, localization, and dynamics in larval and adult stages,
we prepared a construct in which the gfp ORF was fused to the
5′ end of the complete baf-1 ORF and driven by the baf-1 pro-
moter (Fig. 1 A). Microparticle bombardment (Praitis et al.,
2001) was used to create three independent stable transgenic
lines expressing the GFP–BAF-1 fusion protein. GFP–BAF-1
localized primarily at the nuclear envelope, with weaker sig-
als in both the nucleoplasm and cytoplasm (Fig. 1, B–E), as
seen previously for endogenous BAF-1 (Margalit et al., 2005).
GFP–BAF-1 was expressed ubiquitously throughout C. elegans
development, as seen in gonads (Fig. 1 B, arrowhead), early
embryos (Fig. 1 B, arrows), late embryos (not depicted), all
larval stages (Fig. 1 C; shows L1), and adults (Fig. 1 D; adult
vulva indicated by an arrow). The localization of GFP–BAF-1
at different stages of the cell cycle was similar to that of endog-

One possible explanation for this difference is that the mobil-
ity of human BAF was measured in the presence of endog-

GFP–BAF-1 was mislocalized in embryos with RNAi–
down-regulated Ce-lamin expression (Fig. 1 F, arrowheads) and
associated with anaphase bridged chromatin (arrow in F). The image in B was obtained with a confocal microscope,
whereas images in C–F were viewed with an Axioplan II microscope (Carl Zeiss MicroImaging, Inc.). Bars, 10 μm.

**Figure 1. GFP–BAF-1 protein expression.** (A) Schematic view of the baf-1–
gfp construct used for generating transgenic strains. It includes the baf-1
promoter driving the gfp gene fused to the baf-1 ORF followed by the baf-1 3′
untranslated region. (B–D) A stronger GFP–BAF-1 signal was detected at
the nuclear periphery, whereas weaker signals were detected in the nucle-
oplasm and cytoplasm. GFP–BAF-1 was detected in the gonad (arrowhead
in B) in young embryos (arrows in B) and in late embryos (not depicted),
in L1 larvae (C), and in adult worms (arrow in D points toward the vulva).
(E and F) DIC, left; GFP fluorescence, right. Although GFP–BAF-1 was local-
ized normally at the nuclear periphery of control embryos (L4440; E), it
was mislocalized in lmn-1(RNAi) embryos (arrowheads in F), where the
GFP–BAF-1 was also found associated with anaphase chromatin bridges
(arrow in F). The image in B was obtained with a confocal microscope,
whereas images in C–F were viewed with an Axioplan II microscope (Carl
Zeiss MicroImaging, Inc.). Bars, 10 μm.

**baf-1-null animals have diverse tissue-specific phenotypes**

The VC699 strain contains the gk324 allele in which the
baf-1 promoter and ORF are deleted (Fig. S1 A, available at
http://www.jcb.org/cgi/content/full/jcb.200704049/DC1). This
allele was outcrossed three times and balanced with the hT2
balancer, which carries an integrated pharyngeal GFP element.
BAF-1 is required to prevent the premature fusion of seam cells to the epidermis

Homozygous gk324/gk324 animals frequently exploded when touched, suggesting cuticle defects. Worms contain two syncytial rows of seam cells that interrupt the hypodermis and form alae on the cuticle surface during certain developmental stages (Fig. 3 A). During the L1–3 stages, the epithelial seam cells positioned along each side of the worm proliferate to create two cell types: epidermal daughter cells that fuse to the main body hyp7 hypodermis and ectoblastic daughter cells that remain unfused (Fig. 3 A; Sulston and Horvitz, 1977). During the L4 stage, these unfused ectoblastic seam cells, which extend from the tail to the head, fuse to each other laterally to form two syncytia on each side of the animal that eventually create cuticular structures (Fig. 3 A; Podbilewicz and White, 1994). To visualize the borders of the ectoblastic seam cells, we crossed the apical junction marker AJM-1–GFP into heterozygous gk324/hT2 worms (Shemer et al., 2000) and examined progeny that were homozygous or heterozygous for the baf-1 deletion. Until the late L3 stage, seam cells in both homozygous and heterozygous worms behaved like wild-type animals, forming one row of ~12 cells on each side of the worm (unpublished data). In heterozygous gk324/hT2 animals, the ectoblastic seam cells remained unfused at the late L3/early L4 stage (Fig. 3 B, a; arrows), which is similar to wild-type animals (Podbilewicz and White, 1994). In contrast, the ectoblastic seam cells of homozygous gk324/gk324 animals fused prematurely with the epidermis, which was visualized by loss of the GFP signal at the apical borders of the seam cells (Fig. 3 B, b; arrows). Differential interference contrast (DIC) analysis revealed that at L3, after ectopic fusion, the number of seam cells remained at ~12 (unpublished data). By late L4/early adult stage, all seam cells had fused to the epidermis instead of to each other in all tested worms (n = 20; Fig. 3 B, c; arrows). DIC analysis showed that after that stage, the seam cells disappeared. These cells did not stain for SYTO 11 (Invitrogen; Fridkin et al., 2004), indicating that their disappearance probably did not involve apoptosis (unpublished data).

In addition, DIC analysis revealed no apoptotic bodies. This phenotype was specific for BAF-1 because in gk324/hT2 animals, which apparently used maternally supplied BAF-1 to complete embryogenesis and larval stages, were ~50% thinner and ~35% shorter and arrested at late L4/early adult stage with several tissue-specific phenotypes, as detailed in the following sections. The short/thin phenotype could be the result of normal numbers of smaller cells, fewer cells, and/or abnormal gonads.

BAF-1 represses eff-1 in the seam cells

The premature fusion of the seam cells implies that BAF-1 represses the expression of key genes involved in cell fusion. EFF-1 is a cell surface protein that directly mediates most somatic cell fusion events in C. elegans (Shemer et al., 2004;
cells of L3-stage animals (Fig. 4 B, arrow).

In 2004). In contrast, the eff-1–driven GFP expression was strong in the head), which is similar to wild-type animals (Shemer et al., 2007). To test whether the premature fusion of seam cells in gk324/gk324 animals depends only on EFF-1. Therefore, we introduced an eff-1–null deletion, eff-1(ok1021) (Podbilewicz et al., 2006), into gk324/hT2 animals expressing the apical junction marker AJM-1–GFP and tested AJM-1–GFP expression in gk324/hT2;eff-1(ok1021)/eff-1(ok1021) and gk324/gk324;eff-1(ok1021)/eff-1(ok1021) offspring animals. At the L2 stage, the seam cells remained unfused in both heterozygous and homozygous worms for the gk324 allele (Fig. 4, C and D; arrows). During the L3 stage, the seam cells of gk324/gk324;eff-1(ok1021)/eff-1(ok1021) double homozygous worms did not migrate but probably fused to the hypodermis (Fig. 4 F, arrow), and, during the L4 stage, the seam cells became very small and then disappeared (Fig. 4 H, arrow). These cells were SYTO 11 negative, suggesting that they did not undergo apoptosis (unpublished data). Likewise, DIC analysis could not find apoptotic bodies. In contrast, the seam cells of the L3- or L4-stage gk324/hT2;eff-1(ok1021)/eff-1(ok1021) baf-1 heterozygous worms did not fuse and continued to migrate, forming several rows of cells (Fig. 4, E and G; arrows; Mohler et al., 2002). We concluded that BAF-1 inhibits premature fusion of the seam cells by repressing both EFF-1 fusion–dependent and -independent pathways. AFF-1 fusogen was recently found to be active in seam cell fusion independently of EFF-1 (Sapir et al., 2007). To test whether BAF-1 can bind eff-1 promoter regions, we used a GFP antibody and the ChIP assay on worms expressing GFP–BAF-1 (Yan et al., 2006; Ercan et al., 2007). We specifically analyzed the binding of GFP–BAF-1 to the eff-1 promoter (Fig. 5 A) compared with the coding and intronic regions of eff-1. A GFP antibody and the ChIP assay on wild-type worms and worms expressing AJM-1–GFP were used as controls for nonspecific binding to the same regions. The binding of BAF-1 to eff-1 promoter regions spanning from −2,165 to −2,057, −894 to −795, or −289 to −180 (Fig. 5, regions 4, 3, and 1) was 5.3–8.5-fold higher than its binding to eff-1 coding and intronic regions, 3.7–6.5-fold higher than nonspecific binding to the same region in wild-type animals, and 5.3–8.5-fold higher than its binding to the same region in worms expressing AJM-1–GFP. GFP–BAF-1 bound to two other regions in the promoter (−2,696 to −2,560 and −656 to −530; Fig. 5, regions 5 and 2) to the same extent as nonspecific antibody controls or to GFP control. Furthermore, GFP–BAF-1 did not show increased binding to two regions in the actin (act-1) promoter as compared with either coding and intronic regions of act-1 or with wild-type control (Fig. 5). We conclude that BAF-1 represses EFF-1 expression probably by binding directly to the eff-1 promoter.

BAF-1 deletion can cause the premature fusion of seam cells in an EFF-1-independent pathway

We next wanted to test whether the premature fusion of seam cells in gk324/gk324 animals depends only on EFF-1. Therefore, we introduced an eff-1–null deletion, eff-1(ok1021) (Podbilewicz et al., 2006), into gk324/hT2 animals expressing the apical junction marker AJM-1–GFP and tested AJM-1–GFP expression in gk324/hT2;eff-1(ok1021)/eff-1(ok1021) and gk324/gk324;eff-1(ok1021)/eff-1(ok1021) offspring animals. At the L2 stage, the seam cells remained unfused in both heterozygous and homozygous worms for the gk324 allele (Fig. 4, C and D; arrows). During the L3 stage, the seam cells of gk324/gk324;eff-1(ok1021)/eff-1(ok1021) double homozygous worms did not migrate but probably fused to the hypodermis (Fig. 4 F, arrow), and, during the L4 stage, the seam cells became very small and then disappeared (Fig. 4 H, arrow). These cells were SYTO 11 negative, suggesting that they did not undergo apoptosis (unpublished data). Likewise, DIC analysis could not find apoptotic bodies. In contrast, the seam cells of the L3- or L4-stage gk324/hT2;eff-1(ok1021)/eff-1(ok1021) baf-1 heterozygous worms did not fuse and continued to migrate, forming several rows of cells (Fig. 4, E and G; arrows; Mohler et al., 2002). We concluded that BAF-1 inhibits premature fusion of the seam cells by repressing both EFF-1 fusion–dependent and -independent pathways. AFF-1 fusogen was recently found to be active in seam cell fusion independently of EFF-1 (Sapir et al., 2007). To test whether BAF-1 can bind eff-1 promoter regions, we used a GFP antibody and the ChIP assay on worms expressing GFP–BAF-1 (Fig. 5). The binding of BAF-1 to eff-1 promoter regions spanning from −355 to −390 or from −329 to −209 (regions 2 and 1, respectively; Fig. 5) was 4.9–4.6-fold higher than its binding to eff-1 intronic and coding regions. We concluded that BAF-1 inhibits premature fusion of the seam cells to the hypodermis probably by regulating both EFF-1 and AFF-1 pathways (Fig. 4 I).

**Figure 3.** Baf-1 deletion (gk324/gk324) homozygous animals have premature and ectopic fusion of the seam cells. [A] An illustration of seam cell fusion during larval development adapted from Shemer and Podbilewicz (2003). The epithelial seam cells divide during each larval molt, yielding epidermal daughter cells (which fuse to the hypodermis) and ectoblastic daughter cells, which stay unfused, forming single, lengthwise rows along the left and right sides (L1). These aligned cells fuse to themselves and form two syncytia that will form specific cuticle structures (L4/A). [B, a–d] Fluorescence micrographs of animals either homozygous or heterozygous for a baf-1 deletion expressing AJM-1–GFP. In gk324/hT2 animals, the ectoblastic seam cells stayed unfused at the late L3/early L4 stage (arrows in a). In gk324/gk324 animals, the seam cells ectopically fused to the epidermis (arrows in b), and, by the late L4/A stage, all of the seam cells fused to the epidermis (arrows in c). Transgenic expression of GFP–BAF-1 in gk324/gk324 animals rescued the cell fusion phenotype, allowing L4 stage seam cells to fuse to themselves and form two parallel syncytia (arrow in d); the GFP–BAF-1 nuclear signal is not in focus. Bars, 10 μm.

Podbilewicz et al., 2006). Therefore, we tested whether the loss of BAF-1 expression causes the early expression of eff-1 in ectoblasticdaughter cells. We generated a gk324/hT2 strain expressing GFP driven by the eff-1 promoter (Podbilewicz et al., 2006) and followed GFP expression in the seam cells of gk324/hT2 and gk324/gk324 offspring animals. At the L3 stage, the epidermal daughter cells of the seam cells in gk324/hT2 worms that fused to the hyp7 hypodermis expressed eff-1 (Fig. 4 A, arrow), whereas the ectoblastic daughter seam cells that remained unfused did not show a detectable GFP signal (Fig. 4 A, arrowhead), which is similar to wild-type animals (Shemer et al., 2004). In contrast, the eff-1–driven GFP expression was strong (>3.5-fold) in the presumed prematurely fused ectoblastic seam cells of gk324/gk324 L3-stage animals (Fig. 4 B, arrow).

**GFP–BAF-1 binds to the eff-1 promoter**

The ability of BAF-1 to bind DNA and chromatin (Margalit et al., 2007) and to repress eff-1 expression (see previous section) suggested that BAF-1 regulates eff-1 expression by binding to its promoter. To test this hypothesis, we used a GFP antibody and the chromatin immunoprecipitation (ChIP) assay on worms expressing GFP–BAF-1 (Yan et al., 2006; Ercan et al., 2007). We specifically analyzed the binding of GFP–BAF-1 to the eff-1 promoter (Fig. 5 A) compared with the coding and intronic regions of eff-1. A GFP antibody and the ChIP assay on wild-type worms and worms expressing AJM-1–GFP were used as controls for nonspecific binding to the same regions. The binding of BAF-1 to eff-1 promoter regions spanning from −2,165 to −2,057, −894 to −795, or −289 to −180 (Fig. 5, regions 4, 3, and 1) was 5.3–8.5-fold higher than its binding to eff-1 coding and intronic regions, 3.7–6.5-fold higher than nonspecific binding to the same region in wild-type animals, and 5.3–8.5-fold higher than its binding to the same region in worms expressing AJM-1–GFP. GFP–BAF-1 bound to two other regions in the promoter (−2,696 to −2,560 and −656 to −530; Fig. 5, regions 5 and 2) to the same extent as nonspecific antibody controls or to GFP control. Furthermore, GFP–BAF-1 did not show increased binding to two regions in the actin (act-1) promoter as compared with either coding and intronic regions of act-1 or with wild-type control (Fig. 5). We conclude that BAF-1 represses EFF-1 expression probably by binding directly to the eff-1 promoter.
BAF-1 is required for vulva formation

During the L3 stage, the vulva begins to form in the ventral epidermis when the anchor cell induces three (P5.p, P6.p, and P7.p) of the six vulval precursor cells (VPCs) to divide and to adopt vulval fates (Sharma-Kishore et al., 1999; Sommer, 2001). Strikingly, all \( gk324/gk324 \) animals were vulvaless (\( n = 50 \); Fig. 6 D; normal vulva in the control worm is indicated by an arrow in Fig. 6 C). To determine whether the lack of vulva formation is caused by a missing anchor cell, we introduced the anchor cell–specific marker CDH-3–GFP (Pettitt et al., 1996) into \( gk324/hT2 \) worms. During the L3 and L4 stages, CDH-3–GFP expression was detected in the anchor cell in both \( baf-1 \) heterozygous and homozygous animals (Fig. 6, A–D). We next followed the VPCs in the offspring of \( AJM-1–GFP;gk324/hT2 \) animals and wild-type worms expressing \( AJM-1–GFP \). In early L3 stage, the six VPCs, P3.p to P8.p, were present in both wild-type and \( baf-1 \) homozygous \( gk324/gk324 \) worms (Fig. 6, E and F; arrows). During the L3 stage, the six VPCs, P3.p to P8.p, were present in both wild-type and \( baf-1 \) homozygous \( gk324/gk324 \) worms (Fig. 6, E and F; arrows). During the L3 stage, all VPCs degenerated but did not stain for SYTO 11 (unpublished data). The vulvaless phenotype of \( gk324/gk324 \) animals...
was specific to the loss of BAF-1 expression because vulva formation was fully rescued in strains expressing the GFP–BAF-1 transgene, as seen in L4 larvae (Fig. 6, I and J; arrows) and adults (Fig. 6 K, arrow). We conclude that BAF-1 is required for VPC divisions and vulva formation either at the level of anchor cell signaling, defects in responding cells, or both.

BAF-1 is required for germline maintenance and maturation

Homozygous *gk324/gk324* animals became sterile. The gonads of early L3 *gk324/gk324* animals were similar in size to wild-type gonads, with similar numbers of germ cells compared with *gk324/hT2* or wild-type animals (mean of 32 ± 5 germ cells in *gk324/hT2* worms [n = 12] and 30 ± 9 germ cells in *gk324/gk324* worms [n = 12]). However, during the L4 and adult stages, both gonad size and germ cell numbers were substantially reduced in *gk324/gk324* animals (102 ± 30 germ cells in *gk324/hT2* at LA [n = 12], 58 ± 4 germ cells in *gk324/gk324* worms at the L4 time stage [n = 12], 212 ± 26 germ cells in young adult *gk324/hT2* [n = 12], and 36 ± 19 germ cells in *gk324/gk324* worms at the young adult stage [n = 12]; Fig. 7, C and D; DAPI stain) compared with *gk324/hT2* animals (Fig. 7, A and B; DAPI stain). Fewer germ cells in adult *gk324/gk324* gonads suggested germline cell proliferation defects, germ cell degeneration, or both. These findings were consistent with essential roles for BAF-1 in cell proliferation, as reported in *Drosophila* (Furukawa et al., 2003), and also with potentially essential roles in meiosis. Gametes were never seen in *gk324/gk324* animals (unpublished data). The few surviving germ cells expressed the germline marker protein matefin/SUN-1 (Fig. 7 C; Fridkin et al., 2004), suggesting that BAF-1 was not essential for early germ cell differentiation. These phenotypes were specific for the loss of BAF-1 expression because *gk324/gk324* animals expressing the GFP–BAF-1 transgene had normal sized gonads and apparently normal numbers of germ cells (Fig. 7 E) plus sperm cells that appeared to be wild type but were less confined to a specific area in the gonads. There were cells at the pachytene stage, but oocytes were not formed (unpublished data). The gonad size phenotype independently supported our previous conclusion based on animals that escaped the lethal consequences of RNAi-mediated *baf-1* down-regulation that BAF-1 is required for gonad development (Margalit et al., 2005).

BAF-1 is required for the second phase of DTC migration

The position of the gonads was abnormal in *baf-1* homozygous animals (unpublished data) and closely resembled the abnormal gonad position in animals under conditions of incomplete RNAi of BAF-1 (Margalit et al., 2005). To specifically test distal tip cell (DTC) migration, we prepared a *gk324/hT2* strain expressing...
BAF-1 is required for postembryonic organization of the nuclear envelope and chromatin

BAF-1 is required to properly localize Ce-lamin at the nuclear envelope of early embryos (Margalit et al., 2005). To investigate this potential role for BAF-1 at later stages, we introduced Ce-lamin–GFP (Liu et al., 2000) into gk324/hT2 animals and localized Ce-lamin–GFP in gk324/gk324 somatic postembryonic nuclei. Until the L4 stage, Ce-lamin–GFP localization was similar to that of wild-type worms (unpublished data). However, afterward, the Ce-lamin–GFP signal coalesced into one to three strong patches at the nuclear periphery of body muscle cells (Fig. 7 F, inset), epidermal cells, and pharyngeal cells (not depicted). This redistribution was not seen in wild-type worms (Fig. 7 G, inset) and differed from the bright foci of the Ce-lamin–GFP signal that accompany normal aging in C. elegans (Haitchcock et al., 2005). In the gk324/gk324 germ cells (Fig. 7 D, inset) but not in control gk324/hT2 germ cells (Fig. 7 B), the Ce-lamin signal was weak and localized primarily in the nuclear interior. This phenotype was fully rescued in gk324/gk324 animals expressing the GFP–BAF-1 transgene (Fig. 7 E, inset). We conclude that BAF-1 is required to organize Ce-lamin in both germline cells and adult somatic nuclei, including muscle nuclei.

The disorganization of Ce-lamin in the germ cell nuclei of gk324/gk324 animals suggested potential gross defects in nuclear architecture. Therefore, we used the thin section transmission electron microscopy method to visualize the nuclear membranes and chromatin of germ cells derived from gk324/gk324 and control animals (Fig. 9). Gonads of control gk324/hT2 animals had normal-appearing nuclei (Fig. 9 A, arrows) and were indistinguishable from wild-type (N2) gonads (not depicted). In contrast, the gonads of gk324/gk324 animals had only a few large nuclei (Fig. 9 B, arrowhead), whereas most nuclei were small and lobulated (Fig. 9 C, arrows). About 65% of these small nuclei (n = 26) had gaps in their nuclear envelope (Fig. 9 C, arrowheads), and 27% had extra layers of nuclear membranes (Fig. 9 D, arrow). In some cases, the chromatin was condensed in electron-dense patches (Fig. 9 D, arrowheads). We speculate that the more drastic phenotype of gapped nuclear envelopes seen in germline cells might be caused by proliferation-linked defects in nuclear assembly. Thus, for germline cells, we conclude that BAF-1 is required not only to organize Ce-lamin but also for nuclear envelope and chromatin organization. Loss of nuclear envelope integrity could account for germline failure, but our results did not distinguish whether this integrity was lost during germ cell mitotic proliferation, meiosis, interphase, or a combination of these events.

Loss of BAF-1 causes rapid deterioration of body and tail muscles

An unexpected finding was that baf-1–null animals had an uncoordinated (unc) phenotype. When grown at 20°C, the movement of gk324/gk324 animals was similar to that of gk324/hT2 or wild-type animals up to day 4 (Video 1, available at lag-2–GFP, which expresses in DTC cells, and followed GFP expression at different stages in gk324/gk324 animals (Fig. 8). At the late L2 and early L3 stages, migration of the two DTCs away from the midbody occurred normally in both gk324/gk324 and gk324/hT2 animals (Fig. 8, A and B). At the late L3 stage, the ventral to dorsal migration of DTCs was normal in gk324/hT2 animals (Fig. 8 C) but failed to occur in gk324/gk324 animals (Fig. 8 D). The DTCs migrated back toward the midbody in gk324/hT2 animals but not in gk324/gk324 animals (unpublished data). The DTCs in gk324/gk324 animals had normal morphology (Fig. 9 B, arrow). DTCs migrated normally in gk324/gk324 animals expressing the GFP–BAF-1 transgene (unpublished data). These results confirmed our previous suggestion that BAF-1 is required for the second and third steps of DTC migration (Margalit et al., 2005) and further demonstrated that this phenotype is specific to BAF-1.
the control worms could still move their tail and were classified as class C aging animals, in which worms do not move even at 20°C on days 4, 8, and 12. In both control $gk324/hT2$ and homozygous $gk324/gk324$ animals, the head muscle tissues were similar to wild-type animals in each age group (Fig. 10, A–F). Muscle cells in the tail region of $gk324/hT2$ animals had normal morphology in all examined days, including day 12 (Fig. 10, G–I). In striking contrast, muscle cell morphology in $gk324/gk324$ animals was normal only at day 4 (Fig. 10 J) and deteriorated considerably by days 8 and 12 (Fig. 10, K and L). By day 12, the thin and thick filaments in the tail muscles became misorganized and contained dark aggregates (Fig. 10 L, arrows), which appeared already in day 8 (Fig. 10 K, arrow). Tail muscle cells in homozygous worms also had deteriorated nuclei (unpublished data). A previous study had shown that bodywall muscle cells are necessary for the normal distribution of myotactin, a protein that maintains the association between the muscle contractile apparatus and hypodermal fibrous organelles (Hresko et al., 1999). To further study the muscle deterioration, we immunostained $gk324/hT2$ and $gk324/gk324$ worms at day 12 with MH46, an antibody against myotactin. Myotactin distribution was normal at the tail and head of $gk324/hT2$ (Fig. S2 A and C) or $gk324/gk324$ (B and D), which express GFP driven by lag-2. DIC and GFP fluorescence microscopy images were merged. The DTCs in $gk324/hT2$ animals migrated normally at phases 1 (A), 2 (C), and 3 (not depicted; Lehmann, 2001). In $gk324/gk324$ animals, DTCs migrated normally at phase 1 (B) but failed to migrate at phases 2 (D) and 3 (not depicted). Arrows illustrate the direction of DTC migration. Bars, 10 μm.

http://www.jcb.org/cgi/content/full/jcb.200704049/DC1). From day 5 onwards, all homozygous $gk324/gk324$ animals ($n = 146$) developed an uncoordinated movement (Video 2 shows homozygous animals at day 5). In addition, a gradually increasing fraction of animals became paralyzed in the mid- and tail regions but not the head (Video 3 shows homozygous animals at day 12). The fraction of $gk324/gk324$ animals that could only move their head was 2.4% on day 7, increasing to 21.7% on day 11 and 88.5% on day 18 ($n = 130, n = 104$, and $n = 76$, respectively). Control $gk324/hT2$ animals did not develop uncoordinated movement, and the fraction of paralyzed animals was 0%, 0%, and 32.6% on days 7, 11, and 18, respectively ($n = 144, n = 108$, and $n = 49$, respectively). The paralysis phenotype of the $gk324/hT2$ controls differed from the $gk324/gk324$ animals because the control worms could still move their tail and were classified as class C aging animals, in which worms do not move even after prodding and can only move their head and/or tail or twitch in response to touch (Herndon et al., 2002). Both the uncoordinated and paralysis phenotypes of the $gk324/gk324$ animals were completely rescued by the GFP–BAF-1 transgene (unpublished data).

The Unc and paralyzed phenotypes could be caused either by nerve degeneration or muscle cell–intrinsic deterioration. To test the latter possibility, we used the thin section transmission electron microscopy method to examine the morphology of muscle cells in the head and tail regions of wild-type (N2), heterozygous $gk324/hT2$, and homozygous $gk324/gk324$ animals grown at 20°C on days 7, 11, and 18, respectively ($n = 49, n = 49$, and $n = 49$, respectively). The paralysis phenotype of the $gk324/hT2$ deletion had normal gonads and normal-looking nuclei (arrows in A). (B–D) Worms homozygous for the $baf-1$ deletion had one or two large-sized nuclei (arrowhead in B), but most gonadal nuclei were small and tabulated (arrows in C). Many of these small nuclei also had gaps in their nuclear envelope (arrowheads in C), additional nuclear membranes (arrow in D), and condensed chromatin (arrowheads in D). The DTC is indicated (arrow in B).

Discussion

**BAF-1 is required for multiple developmental pathways**

All phenotypes seen in homozygous $baf-1$–null ($gk324/gk324$) animals appeared after the larval L2 stage. These phenotypes
affected specific cell types, including seam cells, vulva precursor cells, germ cells, DTCs, and selected muscle cells in the midbody and tail regions. These phenotypes were specific for BAF-1 because a transgene expressing GFP–BAF-1 fully rescued all phenotypes but one: transgenic BAF-1 did not restore the production of oocytes. We speculate that this failure might be caused by (1) insufficient expression of transgenic BAF-1 in germline cells, (2) incomplete activity of the GFP fusion protein, or (3) a second mutation in a gene located near baf-1, which was not removed by three to five outcrosses of the gk324/gk324 strain. Interestingly, C. elegans heterozygotes with a single copy of baf-1, which expressed ~60% of the normal level of BAF-1 protein, were apparently normal with brood sizes similar to wild-type animals (unpublished data). Furthermore, two extra copies of the GFP–BAF-1 transgene (seen in the YG1001-3 strains) also had no apparent effects, although protein levels were not measured. Thus, in contrast to nuclear assembly extracts derived from Xenopus eggs, C. elegans may tolerate a wider range of BAF-1 protein levels.

The mitotic and chromatin phenotypes seen in baf-1(RNAi) C. elegans embryos (Margalit et al., 2005) were seen to a limited extent at later stages of development, specifically in germ cells and in the VPCs, which might reflect a failure to assemble nuclei after mitosis. However, the successful embryonic development of homozygous gk324/gk324 embryos indicates that maternally contributed BAF-1 is sufficient for early development, when most somatic nuclear divisions occur. In this respect, our findings are consistent with findings in Drosophila, wherein maternal D-BAF was sufficient to complete all larval stages in flies homozygous for a baf deletion (Furukawa et al., 2003). At later stages of Drosophila development, the D-BAF deletion caused defects in chromatin organization, including clumped heterochromatin, which is similar to our findings for germ cell nuclei of baf-1-null C. elegans.

Perhaps there were defects in anaphase at postembryonic cells, but we could not see anaphase bridges in any cells examined either by DAPI staining, thin section EM, or DIC microscopy. The lack of anaphase chromatin bridges in somatic nuclei of post-L2 gk324/gk324 animals can be explained by the fact that most somatic cells are not dividing or by the activity of checkpoints that block entry into mitosis. Consistent with the first possibility, we saw severe nuclear morphology defects in proliferating germline cells after the L3 stage. Furthermore, the lack of mitotic figures in germline cells was consistent with the activity (in germline cells) of one or more checkpoints that are inactive in embryos (Encalada et al., 2005). We cannot rule out that many of the phenotypes of baf-1 mutants, including the lack of proliferation of germ cells and VPCs and the thin and uncoordinated phenotype, could be the results of defects in overall postembryonic cell divisions. On the other hand, it is likely that most phenotypes are probably caused by the aberrant regulation of BAF-1–regulated genes because these phenotypes were either absent or different in worms containing mutations in the cell cycle genes (Boxem et al., 1999; Fay and Han, 2000). In addition, the specific binding of BAF-1 to the eff-1 promoter confirms that BAF-1 is directly involved in gene regulation, as suggested previously in mammalian retinal cells (Wang et al., 2002). Given the differences in the timing of various developmental stages in C. elegans and Drosophila and the uncertain rate of loss of maternally provided BAF-1 protein, we are struck by the general similarities between the null phenotypes of these two organisms. In both organisms, BAF is required for efficient mitosis, chromatin organization, and nuclear envelope formation and also has partners involved in regulating tissue-specific roles during...
development. These results strongly support the hypothesis that many of BAF’s roles are conserved in evolution. Our results suggest that BAF has additional partners involving the regulation of tissue-specific functions that remain to be discovered.

BAF-1 blocks the premature fusion of seam cells

In baf-1–null animals, the seam cells fused prematurely at the L3 stage. Most known genes involved in fusion and patterning of the epidermis are transcription factors (Podbilewicz, 2006). Ectopic and premature fusion of seam cells is also seen in worms deleted for ceh-16/engrailed (Cassata et al., 2005), which represses the transcription of a key gene, eff-1, encoding a cell surface protein that directly mediates most somatic cell fusion events in C. elegans (Shemer et al., 2004), including the ventral cell fusions required for vulva formation. The EFF-1 protein must be expressed in both cells for fusion to occur (Podbilewicz et al., 2006). Mutations in Ceh-16/engrailed derepress eff-1 and lead to abnormal fusion events during embryogenesis (Cassata et al., 2005). Ceh-16/engrailed normally blocks seam cell fusion to the syncytial hypodermis during embryogenesis. BAF is also involved in gene regulation. In mouse retinal cells, BAF inhibits gene expression by binding directly to Otx2, Crx, and other paired-like homeodomain transcription activators and blocks Crx-dependent gene expression in vivo (Wang et al., 2002). Our findings demonstrate that BAF-1 has more extensive roles in tissue-specific gene regulation because BAF-1 was required to prevent the premature fusion of seam cells. Our results suggest that BAF-1 normally represses eff-1 during embryogenesis and later stages of development by binding to the eff-1 promoter. It is also worth noting that BAF-1 is currently the only factor known to bind the eff-1 promoter directly.

Although eff-1 expression was altered in gk324/gk324 worms, premature seam cell fusion still occurred in worms homozygous for mutations in both eff-1 and baf-1. The seam cells in these worms are smaller and disappear later. Thus, the eff-1 mutant background may cause additional deleterious effects. We speculate that there are other proteins mediating cell fusion, which are repressed by BAF-1. One such protein is AFF-1, which has fusogenic activities that do not involve EFF-1 activity, especially in anchor cell fusion and fusion between the lateral seam cells (Sapir et al., 2007). Our ChIP analysis suggests that BAF-1 also binds the promoter of aff-1 and, therefore, prevents seam cell fusion by regulating both the aff-1 and eff-1 promoters (Fig. 4 I).

baf-1–null adults develop dystrophic muscles

One of the most intriguing phenotypes of animals homozygous for a baf-1 deletion was the accelerated deterioration of specific muscles in aging animals. This suggests a role for BAF-1 in adult muscle integrity. This finding is consistent with the idea that BAF is required to efficiently localize emerin at the nuclear envelope of mammalian cells (Bengtsson and Wilson, 2006) and Ce-lamin in C. elegans cells (this study). The observed deterioration of muscle cells in C. elegans differed from normal muscle aging (Herndon et al., 2002) in several ways. First, the baf-1–null muscular dystrophy–like phenotype appeared only in midbody and tail muscles (which are innervated by the ventral or dorsal nerve cords), whereas head muscles (which synapse to the nerve ring) remained functional. Consequently, the head muscles often behave differently. During normal aging, C. elegans suffers from loss of muscle mass and the function of muscles in the body wall and pharynx (Herndon et al., 2002). We cannot rule out the possibility that the midbody and tail muscle phenotypes result from problems with the ventral or dorsal nerve cords. However, we disfavor this model because the morphology of the affected muscles was visibly defective on day 8, when the gk324/gk324 animals could still move, and, therefore, nerve cells were still functional. Furthermore, similar phenotypes of the disorganization of muscle cell filaments and gradual paralysis were previously reported in worms with mutations in muscle-related genes such as unc-54 (myosin heavy chain; Tajsharghi et al., 2005), unc-27 (Troponin I; Burkeen et al., 2004), or unc-52 (perlecan; Mackenzie et al., 1978), which also influence DTC migration (Merz et al., 2003). These genes might be regulated directly or indirectly by BAF-1. In addition, muscle attachment to hypodermal cells was also aberrant in the tail of the gk324/gk324 animals. Therefore, we hypothesize that loss of baf-1 function directly disrupts the function of selected muscle cells in C. elegans.

Possible involvement of BAF in laminopathies

In humans, mutations in A-type lamins cause several forms of muscular dystrophy, including autosomal dominant Emery-Dreifuss muscular dystrophy, Limb-Girdle muscular dystrophy, and dilated cardiomyopathy with conduction system defects (Decostre et al., 2005). Mutations in emerin, an inner nuclear membrane LEM domain protein that directly binds to lamin A, cause X-linked recessive Emery-Dreifuss muscular dystrophy, which is clinically indistinguishable from autosomal dominant Emery-Dreifuss muscular dystrophy (Decostre et al., 2005). Both proteins (lamin A and emerin) directly interact with BAF (Holaska et al., 2003), and these interactions are conserved in C. elegans: Ce-emerin and Ce-lamin (B type) each bind BAF-1 directly (Lee et al., 2001; Liu et al., 2003; unpublished data). The selective muscular dystrophy–like phenotype of baf-1–null animals strongly suggests a novel role for BAF in muscle cell integrity, potentially at the level of muscle-specific gene regulation. Future work will aim to identify putative BAF-regulated genes in muscles to shed new light on the mechanisms of Emery-Dreifuss muscular dystrophy and other laminopathies.

Materials and methods

C. elegans strains

C. elegans strains were handled as described previously (Brenner, 1974). Strains N2 (wild type), JK2049, PS3352, PS3729, eff-1(ok1021), and FC121 were obtained from the C. elegans genome center. Strain VC699 containing the gk324 deletion allele of baf-1 [baf-1[gk324] III/hT2[S94]] was prepared by the C. elegans Reverse Genetics Core Facility at the University of British Columbia. It was outcrossed three times before balancing with hT2. Strain VC699 was crossed with the following strains: PS3352, JK2049, jcl, PS3729, PD4810, YG1001, YG1002, FC121, and...
Microscopy and live cell imaging

Transmission electron microscopy analysis of C. elegans was performed as described previously (Haitzcock et al., 2005). DIC and immunofluorescence images were taken either with a CCD camera (Axioim; Carl Zeiss Microimaging, Inc.) mounted on a microscope (Axioplan II; Carl Zeiss Microimaging, Inc.) equipped for fluorescence and DIC or with a confocal scanhead (MRC-1024, Bio-Rad Laboratories) coupled to an inverted microscope (Axiovert 135M; Carl Zeiss Microimaging, Inc.) equipped with a 63× NA 1.3 oil immersion objective (Carl Zeiss Microimaging, Inc.). For FRAP analysis, gk324/gk324 worms expressing GFP–Baf–1 were imaged using a confocal microscope (FV-1000; Olympus) equipped with an inverted microscope (IX81; Olympus) and a 60× NA 1.4 oil immersion objective (Olympus). GFP–Baf–1 fluorescence was photobleached by a 405-nm laser in a defined region of each cell and was imaged with a 488-nm laser line for excitation and a 505–525-nm filter for emission before, during, and after the photobleach. 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).

Online supplemental material

Fig. S1 describes the gk324 allele and provides evidence that baf–1 is not expressed in L4 worms homozygous for the gk324 allele. Fig. S2 shows that muscle attachment to hypodermal cells is aberrant in the tail region of gk324/gk324 animals by staining gk324/gk324 animals with anti-MH46 antibody. The supplemental text gives further details on primers that were used in this study. Videos 1–3 show movement of heterozygous and homozygous worms for the gk324 allele at days 5 and 12. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200704049/DC1.

We thank Yuval Nevo for assistance with RT-PCR, Orit German-Gruenbaum for helping with the movement assays, Amir Sapir, Gidi Shemer, and Meital Sussia for assistance with the seam cells and FPC fusion; Verena Jantsch for assistance with analysis of the baf–1 deletion rescue strain, Naomi Melamed-Bock for assistance with FRAP experiments, Timor Brodsky for antibodies and helpful discussions, and Shai Mostov for critical comments. We are grateful to the C. elegans Gene Knockout Consortium and C. elegans Reverse Genetics Core Facility at the University of British Columbia for providing the baf–1 deletion allele (gk324). We also thank the C. elegans Genetic Center (which is funded by the National Institutes of Health [NIH] National Center for Research Resources) for strains.


References


