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Barrier to Autointegration Factor (BANF1): interwoven roles in nuclear structure, genome integrity, innate immunity, stress responses and progeria

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Abstract

The Barrier to Autointegration Factor (BAF or BANF1) is an abundant, highly conserved DNA binding protein. BAF is involved in multiple pathways including mitosis, nuclear assembly, viral infection, chromatin and gene regulation and the DNA damage response. BAF is also essential for early development in metazoans and relevant to human physiology; *BANF1* mutations cause a progeroid syndrome, placing BAF within the laminopathy disease spectrum. This review summarizes previous knowledge about BAF in the context of recent discoveries about its protein partners, posttranslational regulation, dynamic subcellular localizations and roles in disease, innate immunity, transposable elements and genome integrity.

Introduction

The barrier to autointegration factor (BAF/BANF1) is a small (10 kDa), conserved and abundant DNA-binding protein. BAF is involved in multiple pathways including mitosis, post-mitotic nuclear assembly, intrinsic immunity against foreign DNA, transcription regulation, and the DNA damage response. BAF is a vital protein; complete loss of BAF is lethal during embryogenesis in *Caenorhabditis elegans* and *Drosophila melanogaster* [1,2]. However BAF also has intriguing roles in physiology, since BAF missense mutations appear sufficient to cause a human progeroid syndrome [3]. These and other new findings about BAF function and regulation are discussed below in the context of its known roles as an essential DNA-binding protein that also interacts with nuclear intermediate filament proteins (lamins), nuclear membrane proteins (`LEM-domain' proteins) and transcription regulators.

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DNA binding properties of BAF

The unique DNA-binding properties of BAF are likely fundamental to its roles. Early studies showed that BAF forms homodimers, each subunit of which binds double-stranded DNA in a sequence-independent manner [1,4]. BAF can compact or loop DNA *in vitro* [1,5,6]. Rigorous biochemical and mutational studies combined with insights from atomic structures revealed an elegantly straightforward mechanism by which BAF interacts with DNA. Specifically, each BAF monomer has a helix-hairpin-helix DNA-binding domain, allowing BAF dimers to bind and `bridge' two strands of DNA [5,7] either intra-molecularly or inter-molecularly. BAF-DNA complexes formed *in vitro* are incredibly stable, with estimated dissociation constants in the low femtomolar range [6]. This poses the first conundrum, since BAF is not `glued' to DNA in living cells. Instead BAF is regulated by its partners in specific subcellular locations and by dynamic phosphorylation and dephosphorylation.

BAF-associated proteins

BAF is regulated at least in part by specific protein partners. Heterodimerization of BAF with BAF-L, a protein 40% identical to BAF but incapable of binding to DNA, is speculated to impair its DNA-bridging activity and potentially also its binding to other partners [8]. The best-characterized BAF partners are a family of proteins that share the ~40-residue LEM-domain fold, including LAP2, EMERIN, and MAN1 [9–12]. BAF homodimerization creates a binding cleft for one LEM domain, which contacts both BAF monomers [13]. BAF association with LEM-domain proteins can be enhanced by DNA or influenced by regions outside the LEM-domain [11,14–17]. Many LEM-domain proteins are anchored at the inner nuclear membrane and function with BAF and lamins as components of nuclear lamina structure ([18,19]; see Barton et al.; this issue). LEM-domain proteins and other BAF partners discussed here are summarized in Table I.

Dynamic subcellular localization

BAF can concentrate near the inner nuclear membrane but is also detected in the cytoplasm; its subcellular localization can vary in different cell types or at different stages of the cell cycle [20]. Seminal fluorescence photobleaching studies revealed separate nucleoplasmic and cytoplasmic pools of BAF, each of which had high diffusional mobility [21]. This dynamic mobility can be explained by several mechanisms including phosphorylation, which has a major role in regulating its localization and activity [22–25]. For example a phospho-mimetic mutation causes BAF to localize in the cytosol; BAF also localizes in the cytosol when transiently co-overexpressed with its kinase, VRK1 [23,26]. BAF partners also influence its distribution. For example BAF accumulates in the cytoplasm of cells that overexpress Lap2ζ (a cytosolic LEM-domain protein; [27]), but accumulates in the nucleus of cells that overexpress the lamin A precursor [28]. Viral infections can lead to interesting changes in the subcellular distribution of BAF. For example, infection with a B1 kinase-deficient vaccinia virus (discussed in more detail below) caused BAF to relocalize at sites of viral DNA accumulation in the cytoplasm, while no change in localization was found during infection with wild-type vaccinia [29]. By contrast in cells infected with herpes simplex

virus type-1 (HSV-1) BAF localizes to the nucleus, where HSV-1 viral DNA replicates [30]. Interestingly, stresses including heat shock, caloric restriction and food deprivation reduce BAF-1 mobility in *C. elegans*, apparently stabilizing its association with the nuclear lamina [31]. Finally, cells from certain laminopathy patients show increased BAF accumulation in the nucleus [32]. These findings suggest BAF localization and mobility are controlled by multiple pathways that directly target either BAF, or its partners.

Posttranslational modification, especially phosphorylation, has key roles in regulating BAF function. BAF is phosphorylated by a conserved family of Ser/Thr kinases, named vaccinia-related kinases (VRKs), and by a closely-related vaccinia virus kinase, named B1 [23,24,33,34]. BAF is specifically phosphorylated on residues Ser-4 (the major VRK phosphorylation site), Thr-2 and Thr-3 [22,23]. Phosphorylation inhibits BAF binding to DNA, and can reduce its ability to homodimerize or bind LEM-domain proteins [22–24,26]. Phosphorylation also favors BAF localization in the cytoplasm [22–24,26], consistent with disrupted binding to DNA and LEM-domain proteins. BAF is dephosphorylated by at least two Ser/Thr phosphatases, named PP2A and PP4 [35,36], whose roles are best understood in the context of mitosis, discussed below.

BAF and nuclear reassembly during mitosis

BAF has essential roles during mitosis. Loss of BAF in either *C. elegans* or *D. melanogaster* leads to embryonic lethality with mitotic phenotypes that include anaphase chromosome bridges and aberrant nuclear envelope morphology [1,2,24,37]. Later in mitosis, BAF associations with DNA and nuclear membrane proteins are critical to recruit nuclear membranes to chromosomes [24,37,38]. Similar roles for BAF are reported during karyosome formation (the clustering of meiotic chromosomes after recombination) in oocytes of *D. melanogaster* [39]. Proper control of BAF association with other proteins, and with DNA, is critical at multiple stages of mitosis [40]. For example, phosphorylation by VRK1 early in mitosis triggers BAF release from chromatin and from LEM-domain proteins [24,25]. If BAF is not phosphorylated (e.g., due to loss of VRK1, or expression of an unphosphorylatable BAF mutant), nuclear disassembly is disrupted [24,25]. Thus BAF, like other nuclear proteins, is controlled by the wave of phosphorylation that drives nuclear disassembly.

Conversely, BAF is dephosphorylated in late mitosis by two phosphatases, PP2A and PP4 [35,36]. Studies in both *C. elegans* and HeLa cells indicate that PP2A is targeted to BAF by a LEM-domain protein named Ankle2 (also known as LEM4) [35]. Ankle2/LEM4 is required for BAF dephosphorylation; in its absence, BAF remains hyperphosphorylated throughout mitosis [35]. Ankle2/LEM4 also further enhances BAF dephosphorylation by associating with VRK1 and inhibiting its catalytic activity [35]. During interphase, Ankle2/LEM4 is detectable at the nuclear envelope, but the bulk of this protein is ER-localized [35], reducing its access to VRK1, which localizes predominantly in the nucleus [33]. Thus Ankle2/LEM4 may only encounter VRK1 (and thereby promote BAF dephosphorylation) later in mitosis when cytoplasmic and nuclear components are fully mixed, and provide a cue for nuclear reassembly as proposed by Asencio et al. [35]. PP4 is also a major regulator of BAF dephosphorylation and BAF localization during late mitosis in HEK293 cells [36].

Specifically, depletion of either the catalytic or regulatory subunit of PP4 led to an increase in BAF phosphorylation and a nuclear envelope invagination phenotype [36]. This phenotype might be only partially attributed to BAF, but raises the possibility that dynamic phosphorylation and dephosphorylation of BAF maintains nuclear envelope integrity.

BAF as an effector of intrinsic immunity

BAF actively protects the genome by intercepting foreign DNA. Ironically this protective function is exploited by retroviruses. BAF was first identified as a cellular factor that associates with the Moloney murine leukemia virus preintegration complex (PIC), and promotes integration into exogenous target DNA by blocking `suicidal' auto-integration within salt-stripped PICs [41,42]. BAF is also an essential host component of the HIV-1 PIC [43,44]. Elegant structural and functional analysis revealed that BAF helps compact retroviral DNA within the PIC [4,45]. Phosphorylation of BAF interferes with these activities, disrupting the PIC [46]. The big question— why BAF helps retroviruses— was recently answered by Izsvak and colleagues [47], who discovered that BAF assembles on transposon DNA in the nucleus and thereby protects at least two endogenous mobile genetic elements, *Sleeping Beauty* and *piggyBac*, from suicidal autointegration, much like it functions on retroviral PICs. Since mobile elements have profoundly influenced genome evolution (reviewed in [48–50]), we speculate that BAF has also contributed to metazoan development by modulating DNA transposition.

However BAF is a potent weapon against other viruses, including the poxvirus vaccinia. Vaccinia expresses its own replication and transcriptional machinery, and therefore completes its entire lifecycle in the cytoplasm of infected cells [51,52]. Vaccinia encodes a Ser/Thr kinase named B1; this kinase is essential for viral DNA replication and gene expression [53–57], and also phosphorylates BAF to block its DNA binding activity [23,29]. Without this inhibition, BAF co-localizes with viral DNA and interferes with both genome replication [29,58] and transcription [59], sharply reducing the number of virus progeny [58].

Antipoxvirus defense by BAF may be augmented by the protein phosphatase PP2A, which appears to actively counteract B1-induced BAF phosphorylation in the cytoplasm of vaccinia-infected cells [26]. The subcellular localizations and timing of these competing enzymes, and BAF, are interesting questions. Is this defensive role performed by BAF already in the cytoplasm, or do cells phosphorylate nuclear BAF to drive it into the cytoplasm, where the poxvirus DNA lurks? In either case, PP2A-mediated BAF dephosphorylation, potentially near sites of accumulating viral DNA, might be required to activate its antipoxvirus activity.

In addition to poxviruses, BAF may also defend against Herpes Simplex Virus type 1 (HSV-1), a DNA virus that replicates and transcribes its genes within the nucleus of infected cells. In cells that express an unphosphorylatable BAF mutant (normal DNA-binding activity; restricted to the nucleus), viral DNA replication and viral protein expression are both reduced [30]. Further studies of the BAF defense against HSV-1 may provide new insights into its roles in innate immunity on its `home turf': the nucleus.

The antiviral effector function of BAF requires its ability to bind DNA and homodimerize [26,58], strongly suggesting BAF must crossbridge DNA to impair viral replication. Bacterial proteins such as H-NS, with well-established roles in silencing foreign nucleic acid and regulating transposition events in prokaryotes [60], also crossbridge DNA. BAF and H-NS share no sequence homology, emphasizing the importance of DNA bridging proteins as intrinsic immune effectors against invading DNA or endogenous transposons in both prokaryotes and eukaryotes [30].

BAF in gene expression and epigenetic regulation

BAF can influence gene expression either positively or negatively, but its mechanisms of action remain unknown. For example, depletion of BAF in mouse embryonic stem cells (ESC) reduces the mRNA levels of ESC markers (Sox2, Oct4, Nanog), while increasing that of mesoderm and trophectoderm markers [61], suggesting BAF helps maintain the pluripotent state. In the L-HaCaT psoriasis cell model, BAF depletion up-regulated the expression of S100A9 and c-Jun, suggesting BAF normally suppresses inflammation [62]. In *C. elegans*, BAF is known to repress a gene (*eff-1*) involved in somatic cell fusion [63]. Interestingly BAF also represses viral (vaccinia) gene expression in the cytoplasm via mechanisms that remain to be explored [59].

BAF's roles in transcriptional regulation are major open questions, and might involve interactions with a variety of players including transcription factors, chromatin modifiers and nuclear lamina components (Table 1). For example, BAF associates with Sox2 in ESCs [64] and is proposed to help form or regulate Sox2 complexes, which maintain pluripotency [61]. BAF associates indirectly (via DNA) with transcription factor Crx in the mouse retina, and with other paired rule homeodomain proteins *in vitro* [65,66]. The transcriptional repressor, germ cell-less (GCL) competes with BAF for binding to the LEM-domain protein emerin [67], whereas the apoptotic regulator Requiem co-immunoprecipitates with BAF and emerin [68]. Clearly we are missing important pieces of these puzzles.

Direct BAF binding to histones H1.1, H3 and H4 has many implications, including potential roles in chromatin remodeling during transcription [69,70]. Indeed, BAF overexpression influences histone posttranslational modifications, globally reducing histone acetylation and increasing or decreasing many specific histone marks [69]. BAF depletion affects heterochromatin and transcriptional repression in *C. elegans*, highlighting its importance for gene expression across species [71]. However, much remains to be learned about these epigenetic roles. BAF does not appear to directly interact with HATs or HDAC1 [69]; instead it might block regulators, or recruit specific chromatin modifiers such as G9A, the DNA methyltransferase Dnmt3 [69,72] or RBBP4, all of which can interact with BAF and are components of chromatin remodeling complexes [68]. These interactions may be key to understanding how BAF relates to its generally repressive nuclear lamina partners at the nuclear periphery, and may provide novel insights into genome regulation.

BAF and the DNA damage response

Proteomic analysis of the BAF interactome identified over 70 potential partners, many of which are involved in DNA damage responses, such as PARP1, DDB1, DDB2 and CUL4

[68]. UV exposure altered BAF or emerin association with DDB1, DDB2 and CUL4A; this suggested BAF interactions are differentially regulated in response to UV damage [68], but did not illuminate the nature of its role. Further evidence linking BAF to the DNA damage response comes from the study of a previously uncharacterized LEM-domain protein, Ankle1 (*C. elegans* LEM-3), which is unique among LEM-domain proteins in possessing endonuclease activity conferred by a conserved GIY-YIG nuclease domain [73,74]. Its proposed role in the DNA damage response is based on its identification as a *C. elegans* mutation that increased sensitivity to ionizing radiation [74]. BAF appears to be required for LEM-3 functions, as the introduction of a *baf-1* mutation in a *lem-3* null background increased embryonic lethality even in the absence of DNA damage [74]. Interestingly Ankle1 is a soluble protein that localizes predominantly in the cytoplasm of mammalian cells, but cycles in and out of the nucleus [73]. These dynamics suggest subcellular localization may regulate Ankle1 interactions with BAF and DNA, raising new questions about how these proteins contribute to the DNA damage response [73].

BAF and Néstor-Guillermo Progeria Syndrome (NGPS)

NGPS is a rare `accelerated aging' syndrome with many clinical features similar to Hutchinson-Gilford Progeria Syndrome (HGPS), caused by dominant mutations in the lamin A precursor [3,75]. However, NGPS patients lack the cardiovascular pathology characteristic of HGPS, and have lifespans well beyond their 20s [3,75]. Whole genome and exome sequencing of two NGPS patients revealed both were homozygous for an Ala12-to-Thr12 (A12T) missense mutation in *BANF1* [3]. Fibroblasts from these individuals showed aberrant nuclear morphology that could be rescued by transient expression of *BANF1*, arguing that NGPS is caused by recessive loss of functional BAF [3]. Further work suggested the BAF A12T mutant protein is stable, folds correctly and co-immunoprecipitates with lamin, emerin and histone H3 [76]. However the A12T mutation weakened BAF binding to DNA and, when overexpressed, caused aberrant nuclear morphology [76]. Thus, weakened DNA binding by BAF is likely a major determinant of NGPS. Future studies of this mutation may offer crucial biological insight into the many roles of BAF, potentially including roles that are DNA-independent, and its interactions with lamin A and other proteins disrupted in progeria.

Conclusions and Future Perspectives

We are only beginning to scratch the surface of BAF's involvement in mitosis, nuclear structure, chromatin regulation and myriad other roles including the interception of foreign DNA and the protection of endogenous transposable elements. The discovery of a BAF mutation in NGPS patients highlights its relevance to human physiology and disease. Further characterization of BAF will continue to yield novel insights into events governing nuclear structure and organization, gene expression, and genome integrity.

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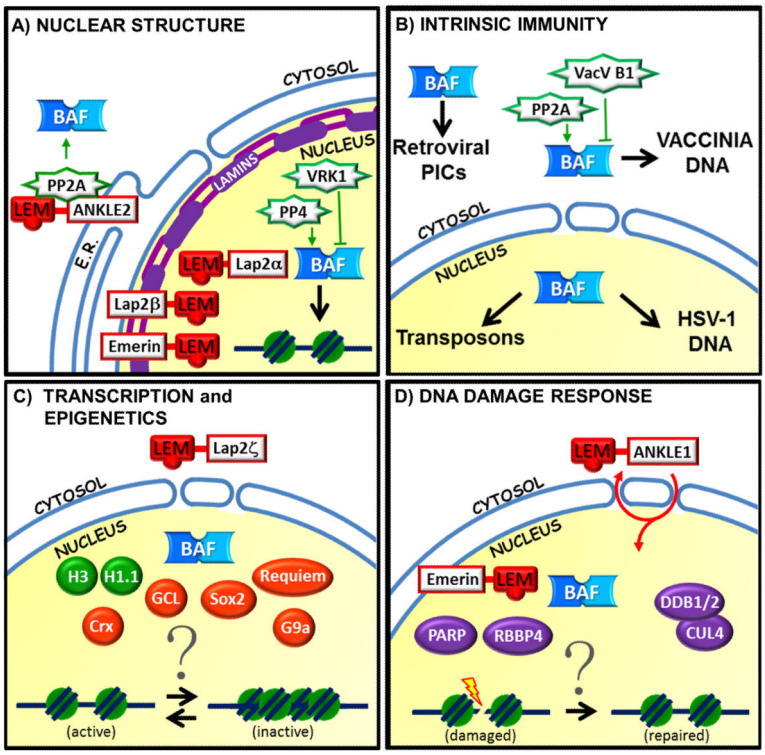

Figure 1.

Overview of BAF-associated proteins in different functional pathways. (A) **Nuclear structure**. BAF interacts dynamically with LEM-domain proteins, lamins and chromatin at the inner nuclear membrane and in the nucleoplasm. BAF also functions dynamically in the cytoplasm. BAF binding to key partners including DNA and LEM-domain proteins is inhibited by phosphorylation (by VRK1) and promoted by dephosphorylation (by PP2A and PP4). (B) **Intrinsic Immunity**: In the cytoplasm BAF intercepts and fights certain viruses by binding their DNA (the poxvirus vaccinia), but is acquired and exploited to protect retroviral DNA in preintegration complexes. BAF defense against vaccinia infection may be supported by PP2A-dependent dephosphorylation of BAF, competing against vaccinia B1 kinase-dependent inhibitory phosphorylation of BAF. Within the nucleus, BAF inhibits the replication and transcription of the DNA virus HSV-1 as part of the host innate immune defense. Remarkably BAF binds and protects endogenous transposons (mobile genetic elements), a role with implications for the evolution of eukaryotic genomes. (C) **Transcription and Epigenetics**: BAF influences transcription, and associates with proteins known to regulate transcription including LEM-domain proteins (emerin, MAN1, Lap2β not shown; Lap2ζ shown), transcription factors (Sox2, Crx, GCL, Requiem), specific histones (H3, H1.1) and histone modifiers (G9a). However the mechanisms of BAF involvement in chromatin regulation remain unknown. (D) **DNA Damage Response**. BAF is needed for a robust DNA damage response, and interacts with proteins that respond to or mediate the DNA damage response including Ankle1/LEM3, emerin, RBBP4, DDR1/2, CUL4 and PARP. However the mechanisms of BAF involvement are open questions.

Table 1

Summary of BAF protein partners

BAF binding partners	Functions	Assays to analyze interaction with BAF[1,2]	References
LEM-domain proteins			
Emerin	Mitosis, integral component of nuclear inner membrane	**Blot overlay assay, co-IP, microtiter binding assay**, FRET, **NMR**, co-localization by IF	[11,14,21,38,40,67]
LAP2α	Mitosis, transcriptional regulator	**Native gel shift assay, NMR, *in vitro* binding assay**, co-IP, co-localization by IF	[13,15,77]
LAP2β	Mitosis, integral component of nuclear inner membrane, transcriptional repressor	Y2H, **native gel shift assay, *in vitro* binding assay, NMR**	[9,13,15]
MAN1	Integral component of nuclear inner membrane	**Microtiter binding assay**	[10]
Lamin A	Mitosis, structural component of the nuclear envelope, signaling	**Microtiter binding assay**, FRET, AP-MS**	[40,67,68]
Prelamin A	Precursor form of lamin A	Co-IP, co-localization by IF	[28,32]
Progerin	Truncated form of farnesylated prelamin A	Co-IP, co-localization by IF	[28]
Lamin B	Structural component of the nuclear envelope	Subcellular co-fractionation	[78]
LEM2	Integral component of nuclear inner membrane	Co-localization by IF	[79]
Ankle1/Lem3	DNA damage response	***In vitro* pulldown assay**	[73]
Nemp1	Inner membrane nuclear protein in Xenopus, neural development	GST-pulldown assay, co-localization by IF	[80]
Transcriptional regulators			
Crx *	Homeodomain transcription activator, organ morphogenesis	Y2H, ***in vitro* pulldown**, co-IP, IF, **NMR**	[65,66]
LAP2ζ	Regulator of LAP2β-mediated transcriptional repressor	Co-IP, co-localization by IF	[27]
Requiem	Transcription factor in myeloid cells, apoptosis	Co-IP, AP-MS	[68]
Sox2	Embryonic stem cell differentiation	AP-MS (MudPIT)	[64]
Histones and histone regulators			
H1.1	Nucleosome	**Blot overlay assay, microtiter binding assay**, GST-pulldown	[70]
H3	Nucleosome	**Blot overlay assay, microtiter binding assay**, GST-pulldown, AP-MS	[68,70]
H4	Nucleosome	***In vitro* pulldown assay**	[69]
RBBP4	Histone chaperone	Co-IP, AP-MS	[68]
SET/I2PP2A	Mitosis, nucleosome assembly, gene expression	Co-IP, AP-MS	[68,69]
G9A	Histone methylation	Co-IP, AP-MS	[69]
DNA damage repair proteins			
PARP1	DNA damage response, gene expression	Co-IP, AP-MS	[68]
DDB1, DDB2	DNA damage response, protein degradation	Co-IP, AP-MS	[68]
CUL4	Protein ubiquitination	Co-IP, AP-MS	[68]
Kinases			

BAF binding partners	Functions	Assays to analyze interaction with BAF[1,2]	References
VRK1	Mitosis, histone phosphorylation, protein phosphorylation	***In vitro* kinase assay**	[23,24,39,46]
VRK2	Protein phosphorylation, signaling	***In vitro* kinase assay**	[23,46]
B1	Vaccinia kinase required for viral DNA replication and gene expression	***In vitro* kinase assay**	[23,29]
Phosphatases			
PP2A	Mitosis, protein dephosphorylation, gene expression	***In vitro* phosphatase assay**	[35]
PP4	Protein dephosphorylation, DNA damage response	siRNA depletion of PP4	[36]
Others			
BAF-L	Potential regulator of BAF DNA binding	***In vitro* pulldown assay**	[8]

Abbreviations: Co-IP (co-immunoprecipitation), Y2H (yeast two hybrid), IF (immunofluorescence), NMR (nuclear magnetic resonance), FRET (fluorescence resonance energy transfer), AP-MS (affinity purification followed by mass spectrometry).

*Interaction with BAF is mediated by DNA

1Bold letters indicate assays performed using purified proteins suggesting direct interaction with BAF

2Underlined letters indicate assays performed using sequence common to all isoforms of LAP2