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Opportunity Knocks for Uncovering New Function of an Understudied Nucleosome Remodeling Complex Member, the Bromodomain PHD Finger Transcription Factor, BPTF

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Abstract

Nucleosome remodeling provides access to genomic DNA for recruitment of the transcriptional machinery to mediate gene expression. Aberrant function of nucleosome remodeling complexes has been correlated to human cancer, making them emerging therapeutic targets. The bromodomain PHD finger transcription factor, BPTF, is the largest member of the human nucleosome remodeling factor NURF. Over the last five years, BPTF has become increasingly identified as a pro-tumorigenic factor, prompting investigations into the molecular mechanisms associated with BPTF function. Despite a druggable bromodomain, small molecule discovery is at an early stage. Here we highlight recent investigations into the biology being discovered for BPTF, chemical biology approaches used to study its function, and small molecule inhibitors being designed as future chemical probes and therapeutics.

Graphical Abstract

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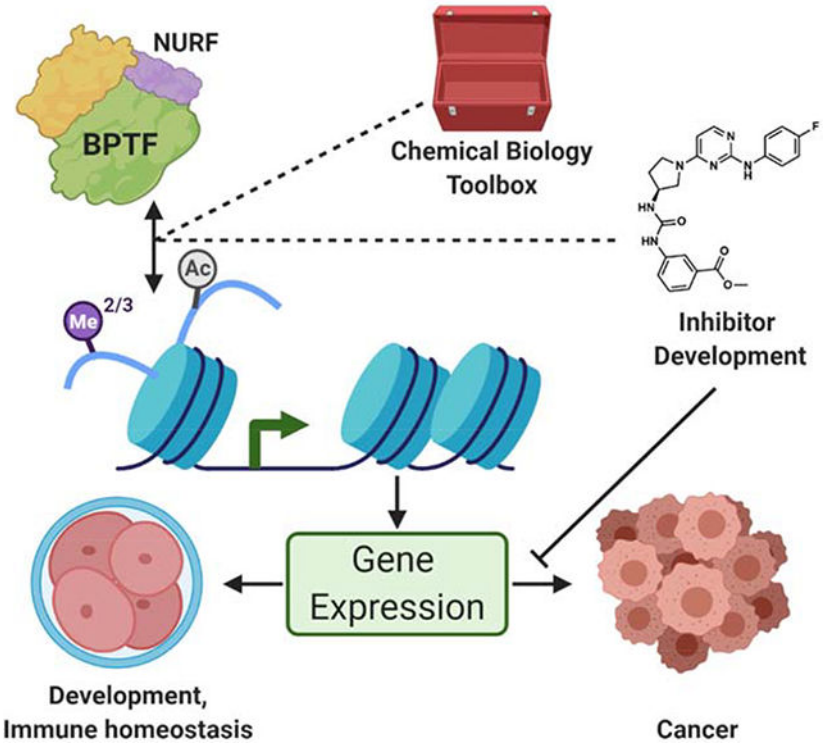
Declaration of Competing Interest

The authors declare that they have no competing interests influencing the work reported here.



Keywords

BPTF; NURF; Bromodomain; Chemical Probe; Chemical Epigenetics

Introduction

Epigenetics research focuses on the molecular mechanisms associated with the heritability of genetic information [1]. Chemical epigenetics is a subdiscipline of this field with a chief aim of developing chemical tools, probe molecules, and technological advances to illuminate these mechanisms at the molecular level, and in some cases with atomic-level precision [2]. Through this lens, we will provide an update on the current biology and chemical epigenetics approaches used to study an emerging epigenetic regulatory protein, the bromodomain and plant homeodomain (PHD) finger transcription factor, BPTF, while highlighting opportunities for innovation.

Bromodomain and PHD finger-containing proteins, such as BPTF, are two classes of epigenetic effector proteins, or “readers”. The PHD finger family is one of the largest classes of readers found in 291 human proteins [3]; however, drug discovery efforts targeting this domain have proven difficult with no chemical probes yet reported. Conversely, there are 46 human bromodomain-containing proteins. These bromodomains can be classified into eight structural families. Despite early efforts, significant chemical probes/drug candidates were not reported in the primary literature until 2010 when the first submicromolar inhibitors were reported for class II bromodomain and extraterminal (BET) proteins [4,5]. Chemical biologists and medicinal chemists have since made significant progress studying the

molecular mechanisms of BET bromodomains and advancing therapeutic agents to clinical trials. Comparatively, translational studies of inhibitors for many non-BET bromodomain-containing proteins have lagged.

In this *Current Opinion*, we review the recent literature concerning the emerging biology and chemical biology of a class I non-BET bromodomain-containing protein, BPTF, the largest member of the nucleosome remodeling factor, NURF. We describe the role of BPTF in normal and pathophysiology, elaborate on new chemical biology tools, and discuss the first set of chemical inhibitors as important steps towards chemical probe development and novel therapeutics (Figure 1).

Discovery of BPTF

BPTF is expressed in several isoforms, including the full-length protein, a shorter N-terminal isoform, fetal Alzheimer's clone 1 (FAC1), and oncogenic BPTF fusion proteins. FAC1 was identified first from amyloid plaques of Alzheimer's patients [6] and later shown to regulate gene transcription through interaction with specific DNA sequences and the Myc-associated zinc finger protein ZF87/MAZ [7,8]. FAC1 shares the N-terminal 801 amino acids of BPTF and likely arises from an alternative splicing event [9]. BPTF is homologous to NURF301, the largest subunit of the Drosophila NURF complex which was isolated from embryo extracts the same year as the identification of FAC1.[10] The full length *BPTF* gene, also named *FALZ*, was identified through a cDNA database search for bromodomain motifs. BPTF contains a DDT DNA-association domain [11], two PHD fingers, a bromodomain, three LXXLL nuclear receptor binding motifs, and a glutamine-rich acidic region (Figure 1D) [9]. Apart from the C-terminal PHD and bromodomain, the functions of the additional domains remain largely uncharacterized. Additionally, an oncogenic fusion protein containing the c-terminal chromatin binding domains of BPTF, NUP98-BPTF, was recently identified from patient samples with acute megakaryoblastic leukemia (Figure 1D). BPTF-NUTM1 was also identified in acute lymphoblastic leukemia samples but is less well-characterized. [12,13].

BPTF Interactome and Functionality

BPTF serves as the largest subunit of NURF, the founding member of the ISWI family of ATP-dependent chromatin remodeling complexes [15]. Human NURF contains an ISWI ATPase domain, SNF2L, and WD-50 repeat containing protein RbAp46/48 (Figure 1C) [16]. In Drosophila, reconstitution of the ATPase and NURF301 was shown to be both sufficient and necessary for maintaining nucleosome remodeling activity. Deletion of the N-terminal 121 amino acids of NURF301 preserved complex formation but reduced activity [10]. A truncated NURF301 isoform lacking the PHD and Bromodomain also forms a NURF complex [17]. Given their difference in size, it remains unclear if either FAC1 or the BPTF fusion proteins form stable NURF complexes.

Once recruited to chromatin through interactions with modified histones or gene-specific transcription factors, NURF is responsible for sliding nucleosomes in *cis* to alter nucleosome position. The resulting changes in DNA accessibility allows for transcription factors to bind

and regulate gene expression [15,18–20]. In mice, BPTF regulates genes and signaling pathways for early tissue development [21], and BPTF loss-of-function is embryonic lethal [22]. BPTF is essential for thymocyte maturation [23], immune homeostasis [24], melanocyte stem cell differentiation [25], and mammary gland development [26]. In flies., a NURF complex containing a BPTF isoform without the PHD and bromodomain leads to impaired spermatogenesis [17].

Chemical biologists have contributed to the understanding of BPTF PHD- and bromodomain-chromatin interactions using synthetic peptides and nucleosomes with post-translational modifications (PTMs) (Figure 1B). The C-terminal PHD domain was shown to directly interact with H3 K4me2/3 using synthetic H3 tail peptides [27] and *in cells* via co-immunoprecipitation [28]. Trimethylation of K4 on H3 is closely associated with the 5' end of actively transcribed genes [29], whereas mono- and dimethylation are more broadly distributed across active genes [30,31] and are found at enhancers [32]. Although the PHD domain binds the H3 K4me3 (1–15) peptide robustly (K_d = 2.7 μM) [28], Morrison et al. found that BPTF binding was prevented when the histone was incorporated in nucleosomes. Further investigations revealed the basic H3 tail associates with nucleosomal DNA which precludes binding. Additional modifications to H3 tails that change the electrostatic environment (e.g. lysine acetylation) are necessary to weaken the association with DNA and allow PHD domain binding [33]. These studies underscore the importance of characterizing histone interactions in their native environment.

In the context of BPTF bromodomain interactions, Ruthenburg et al. found that the bromodomain promiscuously interacts with several monoacetylated H4 peptides, including K12ac, K16ac, and K20ac. However, when these modifications were incorporated into nucleosomes containing an H3 K4me3 histone, the BPTF bromodomain showed enhanced specificity for the H4 K16ac mark. The interaction of BPTF's C-terminal PHD domain with H3 K4me3 is ~100 fold stronger than the interaction between the bromodomain and acetylated H4. Tethering the PHD domain to H3 directs bromodomain binding to H4 K16ac to achieve specificity [34]. Both the PHD domain and bromodomain were shown to colocalize with H3 K4me3 and H4 K16ac near transcription start sites in chromatin immunoprecipitation experiments [27,34,35]. Mutations in either domain abrogated binding [34]. Furthermore, treatment of cells with bromodomain inhibitors reduces chromatin residence time [36] and BPTF-mediated transcription in reporter assays [37**].

Although H4 acetylation is considered the canonical interaction with BPTF, recent experiments showed the BPTF bromodomain can engage both isoforms of histone variant H2A.Z. Acetylation at K7 and K13 on synthetic peptides was the highest affinity modification pattern [38,39*] with similar affinity for BPTF as H4 K16ac. Although BPTF co-immunoprecipitates with nucleosomes containing H2A.Z, a direct interaction in a cellular context needs to be verified [35]. Beyond histone interactions, BPTF/FAC1 interacts with various transcription factors and nucleosome remodeling subunits which can be targeted for modulating BPTF function (Figure 2A) [23,40–45]. Searching for novel BPTF interactions continues to be an active area of research [46].

Emerging Oncogenic Roles of BPTF

BPTF was first implicated in disease by Buganim et al. through characterizing a translocation breakpoint in the *BPTF* gene at the chromosome 17q24.3 locus. BPTF was overexpressed in cells showing this translocation, and knockdown of BPTF slowed proliferation, indicating its role in driving malignancy. Further investigation showed amplification of BPTF in human tumors, most significantly in lung cancer and neuroblastomas [47*], the latter of which is closely correlated to 17q aberrations [48]. BPTF overexpression has now been verified in several cancers including non-small-cell lung cancer [49], hepatocellular carcinoma [50], and colorectal cancers [51]. BPTF's most characterized protumorigenic role has been in melanoma, where BPTF overexpression predicts poor survival outcomes. *BCL2*, *BCL-XL*, and *CCND2* were identified as key genes regulated by BPTF to have proliferative and antiapoptotic effects on cancer cells [52**]. In melanoma, BPTF expression is activated by microphthalmia-associated transcription factor (MITF), which binds at the BPTF promoter to facilitate transcription (Figure 2B) [53]. In a downstream signaling event, MITF associates with NURF affecting MAPK signaling to regulate cell cycle and survival genes [25].

A second emerging mechanism for BPTF and cancer is through an interaction with c-Myc, a transcription factor that is overexpressed in many human cancers [54*]. BPTF silencing impairs c-Myc recruitment to chromatin and reduces DNA accessibility at c-Myc target genes while also regulating *Myc* expression (Figure 2C) [55]. Through these mechanisms, BPTF silencing leads to reduced cell proliferation and replication stress [54*,56]. Therefore, disruption of the c-Myc-BPTF interaction is a potential strategy for the treatment of c-Myc driven tumors. In high grade gliomas, knockdown of the additional BPTF subunits did not have a significant effect on proliferation, suggesting additional function of BPTF outside of NURF [55,57]. Given the emerging role of BPTF in cancer, there is a significant unmet need for new synthetic inhibitors.

Chemical Biology Approaches to Study BPTF Function

To enhance our understanding of BPTF function, a number of chemical biology approaches have been developed (Figure 3). In the context of deciphering native histone interactions, synthetic peptide arrays (SPOT blots) have been used with BPTF to panel diverse PTMs. This method involves synthesis of peptide arrays with PTMs on a cellulose membrane, followed by incubation with the protein of interest, and detection by western blotting or fluorescence imaging. Ruthenburg et al. used SPOT blotting to identify the BPTF bromodomain-H4 K16ac interaction [34]**. Filippakopolous et al. also used SPOT arrays to look at combinations of histone PTMs, and discovered that the BPTF bromodomain bound strongly with H3 pT3, K4ac, K9ac [58]. To profile the binding of multidomain proteins with modified histones, Mauser et al. developed a mixed peptide array screening tool [59]. An exhaustive set of modified histone peptides were mixed pairwise and incubated with a tandem PHD-bromodomain construct. This method confirmed the synergistic binding of BPTF with H4 K16ac and H3 K4me3. However, the weak affinity of histone peptides for bromodomains poses a challenge for SPOT array-based methods and highlights the need for orthogonal biophysical assays to validate these interactions.

NMR is one of serval biophysical techniques employed for quantifying bromodomain and PHD domain-ligand interactions *in vitro* [28,60*]. To increase the speed of analysis, a protein-observed ^{19}F NMR (PrOF NMR) method has been developed [61]. ^{19}F-labelling of W2950 in the BPTF bromodomain binding site provides a reporter resonance which is responsive to changes in ligand binding. The change in chemical shift as a result of increasing ligand concentration is monitored to measure affinity. The fluorine substitution has been shown to induce minimal effects on the bromodomain structure and function [62*]. PrOF NMR is particularly suited for quantifying moderate-to-weak affinity binders of BPTF, such as acetylated histones [38,39*]. The resonances in these experiments are sufficiently resolved that two different proteins have been studied in the same NMR test tube [37**], as well as fluorinated multidomain proteins [63]. PrOF NMR may therefore be useful to study the tandem BPTF PHD-bromodomain interactions with native or synthetic ligands.

Affinity-based photocrosslinking has also been used to discover new binding partners for bromodomains [46]. Sudhamalla et al. used amber suppression to incorporate *p*-azido-L-phenylalanine (pAzF) in the binding site of bromodomains. This photosensitive amino acid can form a covalent bond with binding partners to capture transient interactions. Preliminary work with BPTF W2950AzF showed that the mutant underwent photocrosslinking with a tetraacetylated H4 peptide. This protein was subsequently used to validate a protein-protein interaction with acetylated H2A.Z [38]. Recent work optimized 4-(trifluoromethyldiazirinyl)-phenylalanine (tmdF) incorporation into BPTF and demonstrated that the W2950tmdF variant can crosslink with H4 proteins and endogenous histones [64*]. Further proteomic analysis has yet to be described.

While SPOT blotting, NMR, and photocrosslinking have played a key role in validating BPTF binding to PTMs, nucleosome-based approaches provide a more biologically relevant system to study protein-protein interactions. Ruthenburg et al. used synthetic mononucleosomes containing modified histones, prepared via expressed protein ligation [34**]. Using the tandem PHD-bromodomain, they found that the bivalent interaction conferred selectivity to the bromodomain binding with H4 K16ac which was not discernible through the SPOT arrays. To distinguish between intra- vs internucleosomal binding mechanisms, they constructed dinucleosomes using heteromeric DNA ligation of mononucleosomes. In this model, simultaneous engagement of histones across different nucleosomes was not observed, indicating that BPTF binds to chromatin via an intranucleosomal mode. To improve the throughput and sensitivity of nucleosome-based studies, Nguyen et al. designed DNA-barcoded nucleosome libraries [65]. This study validated previous findings of bivalent binding with H3 K4me3 and H4 K16ac and found that the previously unstudied pentaacetylated H4 Kac5 marks enhanced affinity by seven-fold. A high-throughput nucleosome remodeling assay profiled the nucleosome sliding activity of ISWI remodelers [66*]. This assay revealed that nucleosomes with H2A.Z have enhanced remodeling rates and that the nucleosome's acidic patch is required for remodeling.

A number of other biophysical assays have been reported to study BPTF-ligand interactions such as isothermal calorimetry (ITC) [37**], surface plasmon resonance (SPR), AlphaScreen [68*], and fluorescence polarization [34**,69]. Further insight into

the structural biology of the protein has been enabled by X-ray crystallography with histones and small-molecule inhibitors. Recently, Ycas et al. reported five of the first BPTF bromodomain-small molecule cocrystal structures, which provide key information for future structure-based drug design [68*]. BPTF-specific cell-based experiments such as a luciferase reporter assay [37**], ATAC-seq, and ChIP-seq [26] have also been optimized, providing a framework for studying the biological effects from BPTF inhibition.

Small Molecule Inhibitors of BPTF

Given the biological relevance of BPTF and its role in disease, there is a need to develop small-molecule inhibitors. The BPTF bromodomain remains the most extensively investigated. Vidler et al. used the computational program SiteMap to assess the druggability of human bromodomains [70]. BPTF was predicted to be highly druggable (Dscore 0.95 vs 0.93 for BRD4), along with other class I family members PCAF, GCN5L2, and CECR2. However, unlike BET bromodomains and other family I bromodomains, chemical probe development for BPTF is still in its infancy. Table 1 shows the small-molecule inhibitors reported for the BPTF bromodomain.

In the first report of a BPTF-small molecule ligand, bromosporine was identified as a pan-bromodomain inhibitor with nanomolar potency for BET bromodomains and a moderate affinity for BPTF (1.8 – 9 μM) [68*,71]. Subsequently, Urick et al. reported AU1 as a BPTF inhibitor (K_d = 2.8 μM), discovered from a library of 229 small molecules via a PrOF NMR screen simultaneously screening against fluorinated BPTF and BRD4 bromodomains [37**]. Although comparable in affinity to bromosporine, AU1 was the first selective inhibitor for BPTF over BET bromodomains. Using a BPTF-dependent luciferase reporter assay, AU1 was used to demonstrate the importance of bromodomain function on transcriptional activity. In Eph4 cells, AU1 treatment resulted in decreased proliferative capacity and G1 arrest of the cell cycle [26]. AU1 treatment also reduced c-Myc-DNA occupancy. Further SAR analysis led to the active (*S*)-enantiomer which was used in cell-based experiments, along with CRISPR/Cas9 BPTF depleted cells, to identify a BPTF-sensitive chronic myelogenous leukemia cell line, K562 [62*]. Despite the early use of AU1, limitations such as off-target kinase activity, low ligand efficiency, and stability, made AU1 a challenging inhibitor to develop and signified the need for new and more potent BPTF inhibitors.

Several new inhibitors have been recently identified. TP-238 was reported as a CECR2/BPTF chemical probe by Takeda and the Structural Genomics Consortium (SGC), with 12-fold higher selectivity for CECR2 (K_d = 10 nM) [36]. TP-238 was used to validate the engagement of the BPTF bromodomain binding site by acetylated H2A.Z [39*]. Novartis and SGC reported NVS-BPTF-1 with *in vitro* binding affinity of 3–71 nM [72]. However, NVS-BPTF-1 is described as poorly soluble with inadequate ADME (absorption, distribution, metabolism, and excretion) properties for *in vivo* applications. Two moderate affinity BPTF binders, DCB29 [73] and C620–0696 [74] have also been reported (K_d = 17.9 and 35.5 μM, respectively). While an extensive analysis was not conducted, C620–0696 suppressed Myc protein levels in non-small-cell lung cancer cells.

Although most inhibitor development efforts are still in preliminary stages, they have enabled the cross-validation of a number of BPTF-specific biophysical methods. Ycas et al. used several of these compounds to optimize SPR and AlphaScreen assays and cross-validate PrOF NMR binding studies [68*]. A tetrahydroquinoline, discovered from a fragment screen [75], was investigated as a starting point for inhibitor development. Deconstructed fragments derived from GSK4027, a PCAF/GCN5 inhibitor [76], were also tested. Pyridazinone-based fragment **8** had a K_d of 6 μM and ligand efficiency of 0.45. The high ligand efficiency, compared to 0.22 for AU1, makes this scaffold a suitable lead for further inhibitor development.

Summary

New molecular mechanisms associated with BPTF function in human development and disease are emerging. Chemical biology has played a significant role in deciphering mechanisms through new tools and perturbing function through inhibitor development. While significant progress has been made for inhibiting the bromodomain as the most druggable domain, selectivity and ADME properties need to be improved to develop useful tool compounds for validating BPTF functional inhibition. In vivo studies using inhibitors have yet to be reported. One approach recently applied for class I bromodomains PCAF/GCN5, are proteolytically targeting chimeric molecules (PROTACs), which efficiently degrade these proteins [77*]. Importantly, PROTACs can recapitulate knock-down phenotypes in the absence of therapeutic effects from bromodomain inhibition. BPTF PROTACs are envisioned to be reported soon and may also lead to improved selectivity due to the formation of distinct ternary complexes. Relative to the bromodomain, the BPTF PHD domain remains a more challenging drug target. Despite the absence of inhibitors, mutagenesis of the PHD domain and use of a nonselective compound disulfiram have been used to support the role of the PHD domain of NUP98 fusions in transformed leukemia cells [60*]. More selective inhibitors would be a significant contribution to further elucidate the functional significance of the role of BPTF in oncogenic fusions and potential NURF-dependent and independent mechanisms. Given the increased number of reports associating BPTF in disease, and the growing number of BPTF small molecule inhibitors, we predict the next five years will produce exciting advances in our understanding of BPTF biology and new classes of BPTF chemical probes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

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Abbreviations

ADME	absorption, distribution, metabolism, and excretion

BET	bromodomain and extraterminal
BPTF	bromodomain- and PHD finger-containing transcription factor
BRD	bromodomain
CECR2	cat eye syndrome chromosome region candidate 2
DNL	DNA-barcoded nucleosome libraries
FAC1	fetal Alzheimer's clone 1
ITC	isothermal calorimetry
ISWI	imitation switch
MAPK	mitogen-activated protein kinase
MITF	microphthalmia-associated transcription factor
NURF	nucleosome remodeling factor
PHD	plant homeodomain
PROTAC	proteolysis targeting chimera
PTM	post translational modification
pAzF	*p*-azido-L-phenylalanine
SGC	Structural Genomics Consortium
SNF2L	SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A, Member 1
SPR	surface plasmon resonance
tmdF	4-(trifluoromethyldiazirinyl)-phenylalanine

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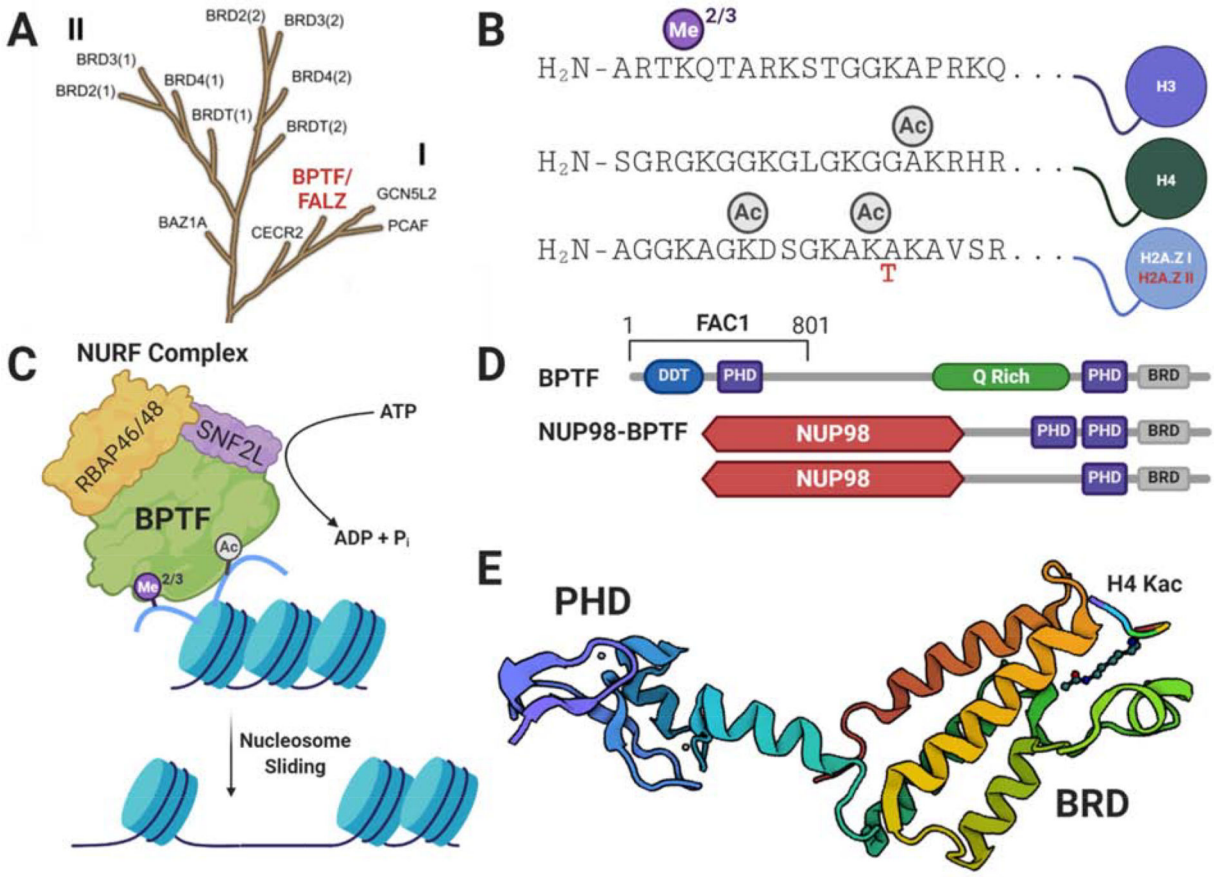


Figure 1: BPTF structure and function as an epigenetic reader protein.

A) Branch of the bromodomain phylogenetic tree representing the BET family (II) and family I, to which BPTF/FALZ belongs. (adapted with permission from Filippakopoulos et al. [14]) B) Modified Histone binding partners of BPTF's PHD domain (H3 K4me2/3) and BRD (H4 K16ac and H2A.Z I/II K7ac, K13ac). C) Recruitment of NURF to chromatin through modified histone interactions facilitates *cis* nucleosome sliding. D) Domain diagram of BPTF with DNA binding homeobox and different transcription factor (DDT) domain, PHD domains, glutamine rich region (Q rich), and bromodomain (BRD) shown. The residues corresponding to the FAC1 truncation are outlined above (top). Also shown are the two domain diagrams of two reported NUP98-BPTF fusion proteins (middle, bottom). E) X-ray crystal structure of BPTF PHD-BRD in complex with acetylated H4 peptide shown in chainbow. PHD and bromodomains are annotated. PDB: 3QZV.

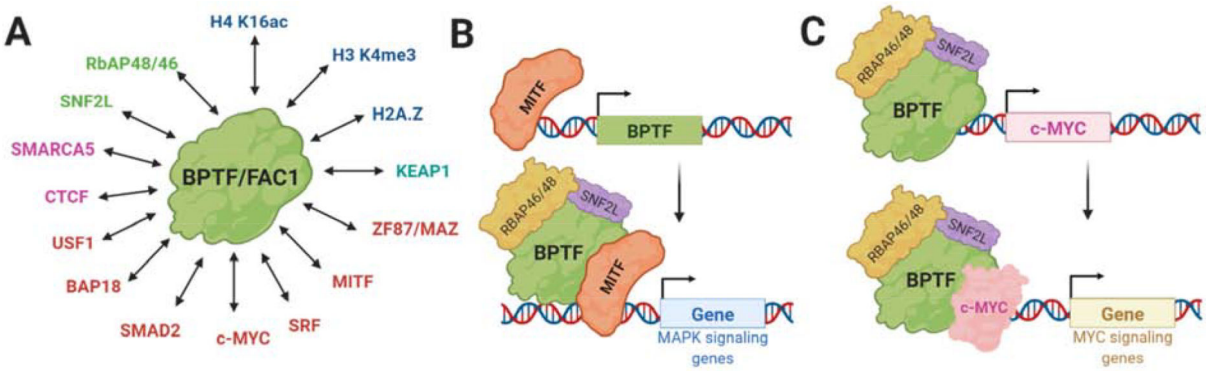


Figure 2: Molecular mechanisms associated with BPTF function.

A) Reported interactions of BPTF/FAC1. Histone proteins shown in blue, transcriptional regulators in red, antioxidant response proteins in cyan, chromatin regulators in magenta, and NURF subunits in green. B) Mechanistic role of MITF and BPTF in melanoma. MITF regulates the expression of BPTF and associates with NURF to co-regulate the expression of cell-cycle-regulating genes. C) BPTF drives c-Myc expression and binds to c-Myc to facilitate its transcriptional activity. Supplementary Table 1 is provided as a resource for additional direct BPTF genes in various disease states.

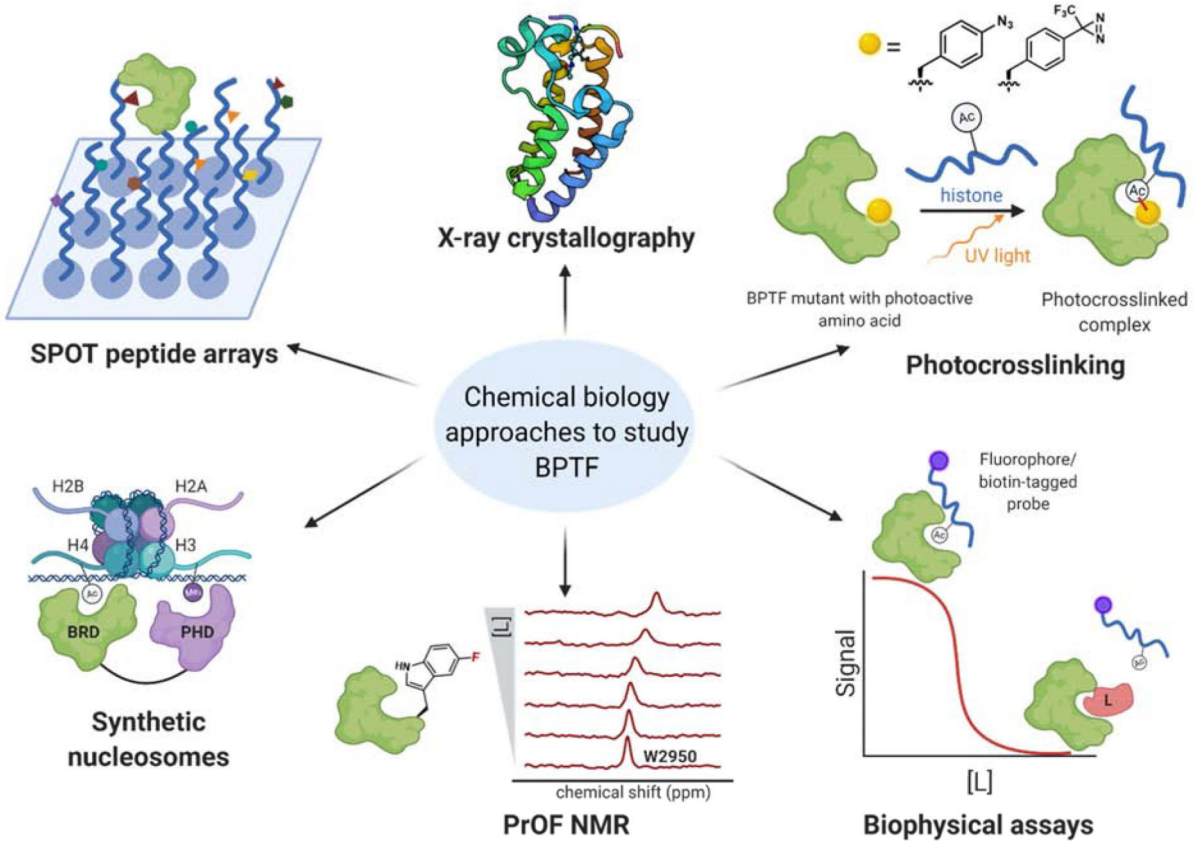
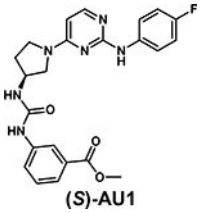
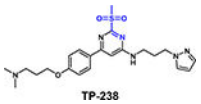
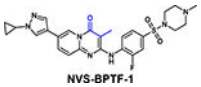
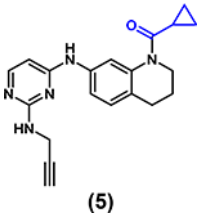
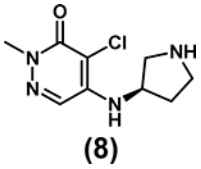


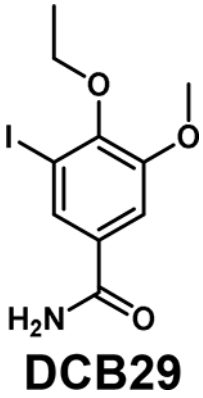
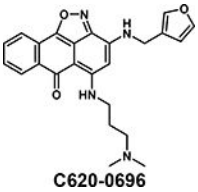
Figure 3: Chemical biology tools developed to study BPTF interactions with peptides and small-molecule inhibitors.

Methods include SPOT peptide arrays, photocrosslinking, direct binding assays such as NMR and SPR, and competitive inhibition-based biophysical tools including AlphaScreen and fluorescence polarization. Synthetic nucleosomes provide a more biologically relevant platform and structural characterization has been enabled by X-ray crystallography (PDB 3QZT).

Table 1:

BPTF bromodomain inhibitors with *in vitro* affinity values and reported off-target effects. Functional groups acting as acetyl lysine mimics are shown in blue based on reported cocrystal structures.

Inhibitor	BPTF *in vitro* affinity	Reported off-targets	References, PDB ID
 Bromosporine	K_d = 1.8 μM (ITC)	pan-bromodomain inhibitor	[71] 5IGK (with BRD4)
 (*S*)-AU1	*rac*-AU1 K_d = 2.8 μM (ITC)	Kinases: TRKC K_d = 200 nM CDKL2 K_d = 260 nM (KINOMEscan)	[26,37**,62*]
 TP-238	K_d = 120 nM (ITC)	CECR2 K_d = 10 nM (ITC)	[36,39*] 7KDZ
 NVS-BPTF-1	K_d = 3–71 nM (BROMOscan)	BRPF K_d = 37 nM CECR2 K_d = 66 nM GCN5L2 K_d = 62 nM PCAF K_d = 74 nM (BROMOscan)	[72]
 (5)	IC_{50} = 36 μM (AlphaScreen)	Not determined	[68*] 7KDW
 GSK4027	K_d = 1.7 μM (SPR)	PCAF/GCN5 K_i = 1.4 nM (BROMOscan)	[68*,76] 7K6R
 (8)	K_d = 6 μM (SPR)	Not determined	[68*]

Inhibitor	BPTF *in vitro* affinity	Reported off-targets	References, PDB ID
 DCB29	K_d = 17.9 μM (SPR)	Not reported	[73]
 C620-0696	K_d = 35.5 μM (Bio-layer Interferometry)	Not determined	[74]