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Bromodomain-containing proteins in the unicellular eukaryote *Tetrahymena thermophila*

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ABSTRACT

Bromodomain (BRD)-containing proteins are central mediators of gene regulation, serving as key components of chromatin remodeling complexes and histone recognition scaffolds. By specifically recognizing acetylated lysine residues on histones (Kac) via their conserved BRD, these proteins influence chromatin structure and gene expression. Although their overarching role is well-established, the precise molecular functions and mechanisms of individual BRD proteins remain incompletely characterized. The ciliate *Tetrahymena thermophila*, a unicellular eukaryote with a transcriptionally active macronucleus enriched in histone acetylation, is an excellent model for exploring the significance of BRD-containing proteins. In this comprehensive review, all BRD-containing proteins encoded in the *T. thermophila* genome are systematically examined, including their expression profiles, histone acetylation targets, interacting proteins, and potential roles. This review lays the groundwork for future investigations into the complex roles of BRD proteins in chromatin remodeling and transcription regulation, offering insights into basic eukaryotic biology and the molecular mechanisms underlying BRD-linked diseases.

Keywords: Bromodomain-containing proteins; Histone acetylation; *Tetrahymena*; Chromatin remodeling

INTRODUCTION

Bromodomains (BRDs) are evolutionarily conserved protein interaction modules first characterized in the *Drosophila melanogaster brahma* gene (Haynes et al., 1992). These domains function as selective readers of lysine acetylation (Kac), with particular affinity for acetylated histone residues (Fujisawa & Filippakopoulos, 2017). Kac is a well-studied post-translational modification (PTM) that modulates chromatin architecture and gene expression by neutralizing the positive charge of lysine residues, thereby weakening histone-DNA interactions and promoting euchromatin formation (Bannister & Kouzarides, 2011; Verdin & Ott, 2015). Through this mechanism, Kac facilitates the dynamic regulation of transcription and metabolic homeostasis.

BRD-containing proteins (hereafter referred to as BRD proteins) contribute to gene regulation through a variety of mechanisms. First, they serve as integral components of chromatin remodeling complexes, such as Brahma-related gene 1 (BRG1) in the switch/sucrose non-fermentable (SWI/SNF) complex (Singh et al., 2007), polybromo-1 (PB1) in the polybromo Brg1-associated factors (PBAF) complex (Huang et al., 2008), and cat eye syndrome chromosome region candidate 2 (CECR2) in the imitation switch (ISWI) complex, which modulate chromatin compaction or decompaction, thereby influencing chromatin remodeling (Fairbridge et al., 2010). Second, certain BRD proteins, such as p300/CBP-associated factor (PCAF) and general control nonderepressible 5 (GCN5), act as multidomain enzymes, which possess a distinct PCAF N-terminal domain, catalytic acetyltransferase domain, and BRD for protein interactions, collectively conferring histone acetyltransferase (HAT) activity essential for chromatin regulation (Linares et al., 2007). Third, BRD proteins function as scaffolds that recognize histone acetylation to regulate the recruitment and segregation of transcriptional complexes, as exemplified by BET family proteins containing N-terminal BRD and extra-terminal (ET) protein-protein interaction domains that dock onto acetylated chromatin to recruit transcriptional machinery (Fujisawa &

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Filippakopoulos, 2017). Finally, BRD proteins can act as transcriptional co-regulators, such as tripartite motif-containing 66 (TRIM66), which cooperates with heterochromatin protein 1 (HP1) to modulate transcriptional machinery (Khetchoumian et al., 2004).

Dysregulation of BRD proteins has been increasingly linked to the pathogenesis of diverse diseases, reflecting their central roles in chromatin-mediated gene regulation. Several BRD proteins have emerged as promising therapeutic targets, particularly in cancer biology. For example, bromodomain-containing protein 7 (BRD7), a component of the SWI/SNF complex, exhibits tumor-suppressing activity across multiple cancer types (Park et al., 2014), whereas overexpression of TRIM66 has been implicated in osteosarcoma progression, correlating with poor patient prognosis, with its knockdown shown to reduce tumorigenicity in preclinical models (Chen et al., 2015). The relevance of BRD proteins extends beyond cancer. For example, Gcn5 regulates multiple disease-related pathways involved in diabetes, osteoporosis, and systemic lupus erythematosus (Xiao et al., 2023). Furthermore, BET family proteins play critical roles in immunity-related gene transcription, with their dysregulation implicated in various inflammatory and immune-related disorders, highlighting their suitability for pharmacological intervention (Wang et al., 2021). Collectively, this evidence underscores the multifaceted roles of BRD proteins in disease etiology and their potential as therapeutic targets across multiple pathological conditions.

The unicellular ciliate *Tetrahymena thermophila* (hereafter referred to as *Tetrahymena*) provides a powerful model for studying the molecular functions of BRD proteins in chromatin regulation. Distinguished by nuclear dimorphism, *Tetrahymena* harbors two structurally and functionally distinct nuclei within a single cell: a transcriptionally active macronucleus (MAC) and a transcriptionally silenced micronucleus (MIC) (Gao et al., 2024; Jiang et al., 2025; Jin et al., 2023; Lyu et al., 2024; Orias et al., 2011). The MAC features extensive transcription-related histone acetylation, which contributed to the identification of the first nuclear histone acetyltransferase (HAT), GCN5 (Brownell & Allis, 1995; Brownell et al., 1996; Rojas et al., 1999). In contrast, histone acetylation linked to histone deposition is transiently observed during the assembly of histones H3 and H4 into the MIC, and is rapidly removed following incorporation (Smith et al., 2008). These features make *Tetrahymena* an ideal model for studying the functions of BRD proteins and distinguishing their roles between transcription-related and deposition-related acetylation.

In this review, all BRD proteins encoded in the *Tetrahymena* genome are systematically characterized, integrating prior findings with functional analyses to elucidate their expression profiles, recognition and catalytic targets, protein interaction networks, and potential biological functions (Saettone et al., 2018; Wahab et al., 2020). These insights provide a foundational framework for advancing mechanistic studies of BRD protein-related chromatin remodeling and transcriptional regulation, contributing to fundamental eukaryotic biology and BRD-related disease mechanisms.

BRD PROTEINS IN THE *TETRAHYMENA* GENOME

BRD proteins in *Tetrahymena* were initially classified into groups by Saettone et al. (2018). We reanalyzed these BRD proteins based on structural alignments and domain architecture, resulting in the identification of 14 BRD proteins (Saettone et al., 2018; Wahab et al., 2020) (Table 1). Each of these proteins possesses a canonical BRD module composed of four α-helices (αZ, αA, αB, and αC) linked by three loops (ZA, AB, and BC loops) (Figure 1), consistent with the conserved features of this domain (Dhalluin et al., 1999; Fujisawa & Filippakopoulos, 2017; Meslamani et al., 2016). Conservation analysis revealed the presence of several key residues across all 14 BRDs, including a conserved tyrosine (Y) residue in the AB loop, an aspartic acid (D) residue in αB,

Table 1 Detailed information on 14 BRD proteins in *Tetrahymena*

Group	Protein	TTHERM No.	GenBank Acc. No.	Gene ID	Protein length (aa)	# of BRDs	Other domains†	Localization	Expression‡
I	MLL1	01309130	XP_001029767.2	7834785	2445	1	LIM (1) PHD (5) SET (1) POST-SET (1)	MAC	C2–C8
	BroP-3	00146040	XP_001011243.3	7839556	307	1	–	–	C6–C14
II	CHD1	00193800	XP_001017133.2	7844606	2196	1	Chromo (2) SNF2-like ATP-dependent helicase domain (1) PHD (3)	–	C6–C12
	BroW-1	00142380	XP_001011076.2	7827504	2061	1	WD40 (5)	–	C4–C12
	BrAn-1	00073170	XP_001015556.2	7842806	851	1	ANK (5)	–	LI–Lh, C8–C14
	BroP-1	00715810	XP_001031940.2	7831852	1749	1	–	–	C2–C12
	BroP-2	00449140	XP_001013314.2	7845795	418	1	–	–	C2–C6、C10–C14
	BroP-4	00529810	XP_001032692.1	7837842	292	1	–	–	C4–C12
	IBD1/ BroP-5	00729230	XP_001022708.3	7829420	270	1	NET (1)	MAC	LI–Lh, C4–C14
	IBD2/ BroP-6	00483490	XP_001017462.2	7840802	231	1	NET (1)	MAC	LI–Lh, C4-C6, C14
III	BrEt-1/ NRK27	00219200	XP_001020592.2	7837696	1867	1	NET (1)	–	C6–C12
	BrEt-2	00499530	XP_001022211.3	7838178	883	1	NET (2)	–	C4–C6, C10–C14
	BrEt-3	000446029	XP_012655125.1	24439022	672	1	NET (1)	–	C4–C10
	GCN5	00248390	XP_001023869.1	7824056	418	1	Acetyltransferase (1)	MAC	C2–C12

–: Not available. †: Numbers in parentheses represent quantity of corresponding domain. ‡: Summary of Figure 4: C represents conjugation stage; LI, Lm, and Lh correspond to vegetative stage.

and an asparagine (N) residue at the amino terminus of the BC loop (Figure 1). These residues serve critical structural and functional roles, with the Y residue forming a salt bridge with D to stabilize the BRD fold and N facilitating Kac docking (Fujisawa & Filippakopoulos, 2017). Additionally, five of the 14 BRD proteins (MLL1, GCN5, BrEt1, BrEt2, and BrEt3) contain a conserved PxY motif in the ZA loop, primarily observed as PDY (Figure 1). The first residue of the αC helix, known as the 'gatekeeper residue' (Meslamani et al., 2016), is typically hydrophobic, a feature conserved in *Tetrahymena* BRDs. Together, the BRD module features a hydrophobic pocket formed by four α-helices, flanked by the ZA and BC loops, which can accommodate the neutralized side chain of Kac (Filippakopoulos & Knapp, 2012).

Based on phylogenetic analysis, the 14 BRD proteins can be classified into three distinct groups (Groups I, II, and III) (Figure 2), as reported in previous research (Saettone et al., 2018; Wahab et al., 2020). Group I includes MLL1 and BroP-3. Group II includes BroW-1, CHD1, BrAn-1, BroP-1, BroP-2, BroP-4, IBD1/BroP-5, and IBD2/BroP-6. Group III includes GCN5, BrEt-1, BrEt-2, and BrEt-3. Charge distribution analysis of BRD module surfaces (Figure 2) revealed predominantly negatively charged surfaces, consistent with their capacity to bind acetylated histones enriched in positively charged lysine residues.

This review primarily focuses on the domain architecture of BRD proteins in *Tetrahymena*, leading to their classification into two major categories: multidomain BRD proteins and BRD-only proteins (BroP-1, BroP-2, BroP-3, and BroP-4) (Table 1, Figure 3). The multidomain group comprises several functional subtypes, including those with catalytic activity for histone modification and chromatin remodeling (MLL1, GCN5, and CHD1), BET family proteins containing an extra-terminal (ET) domain for protein recruitment (IBD1/BroP-5, IBD2/BroP-6, BrEt-1, BrEt-2, and BrEt-3), and those that facilitate binding capabilities (BroW-1 and BrAn-1).

Multidomain BRD proteins

Subtype 1: Proteins with catalytic domains for histone modification and chromatin remodeling

MLL1, with a SET domain: Mixed-lineage leukemia 1 (MLL1), a member of the mixed-lineage leukemia/lysine methyltransferase 2 (MLL/KMT2) family of histone

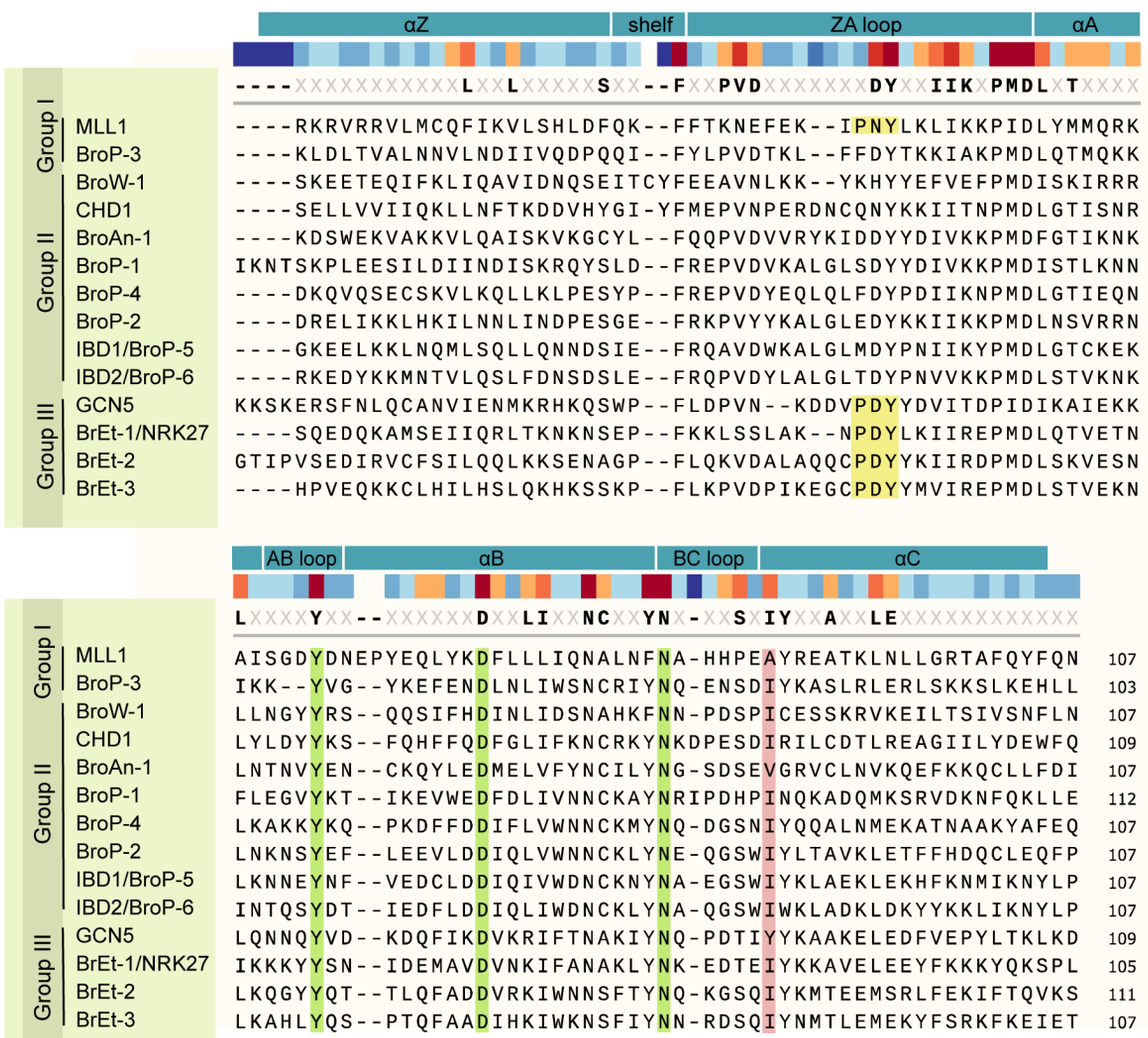

Figure 1 Sequence alignment of 14 BRD proteins in *Tetrahymena*

MAFFT v.7.526 (Katoh & Standley, 2013) was used for sequence alignment. Secondary structure, displayed above the alignment, was generated using ESPript v.3.0 (Robert & Gouet, 2014). Key features are highlighted: PxY motif in yellow, conserved residues in green, and gatekeeper residue in red.

methyltransferases (MTases), is a multidomain BRD protein that catalyzes trimethylation of histone H3 at lysine 4 (H3K4me3) (Dou et al., 2005) (Table 2; Figure 3A). This methyltransferase activity is mediated by a conserved su(var)3–9, enhancer of zeste, and trithorax (SET) domain, which defines a broad class of histone-modifying enzymes (Figure 3A) (Milne et al., 2002). Although yeast lacks direct homologs of MLL1, its complex of proteins associated with Set 1 (COMPASS), methylates H3K4me3 (Briggs et al., 2001; Roguev et al., 2001). Notably, Set1 itself lacks a BRD (Zhang et al., 2010).

In *Tetrahymena*, H3K4me3, a classical epigenetic marker associated with active transcription, is correlated with the histone variant H2A.Z and DNA N^6-methyladenine (6mA) (Sheng et al., 2024; Wang et al., 2025). Functional studies have demonstrated that loss of *MLL1* leads to a marked reduction in H3K4me3, accompanied by a substantial increase in 6mA at genes with initially low 6mA enrichment (low-6mA genes), resulting in increased transcriptional activity. Notably, these 6mA-induced genes also display elevated H2A.Z levels compared to other low-6mA genes (Wang et al., 2025). This suggests that MLL1-dependent H3K4me3 plays a key role in limiting off-target 6mA, potentially through the guidance of H2A.Z, with all three factors collaboratively regulating gene transcription.

GCN5, with a HAT domain: General control nonderepressible 5 (GCN5, also known as Kat2a) represents the first transcription-related histone acetyltransferase (HAT) to be functionally characterized and is well known for its role in histone acetylation and gene activation (Brownell et al., 1996). In yeast, GCN5 functions as a core component of the Spt-Ada-GCN5 acetyltransferase (SAGA) complex, which targets histones H3 and H4 within nucleosomes to promote chromatin accessibility (Grant et al., 1997). In metazoans, GCN5 is functionally and structurally homologous to p300/CBP-associated factor (PCAF, also known as Kat2b), with both proteins harboring conserved HAT and BRD domains across yeast, metazoans, and *Tetrahymena*. The HAT domain confers histone acetyltransferase activity, while the PCAF N-terminal domain, which displays ubiquitination activity and plays a crucial role in nucleosome binding *in vitro*, is a unique metazoan feature (Figure 3B) (Toma-Fukai et al., 2020). Interestingly, metazoans also contain a GCN5 isoform that lacks the PCAF N-terminal domain, a feature similarly

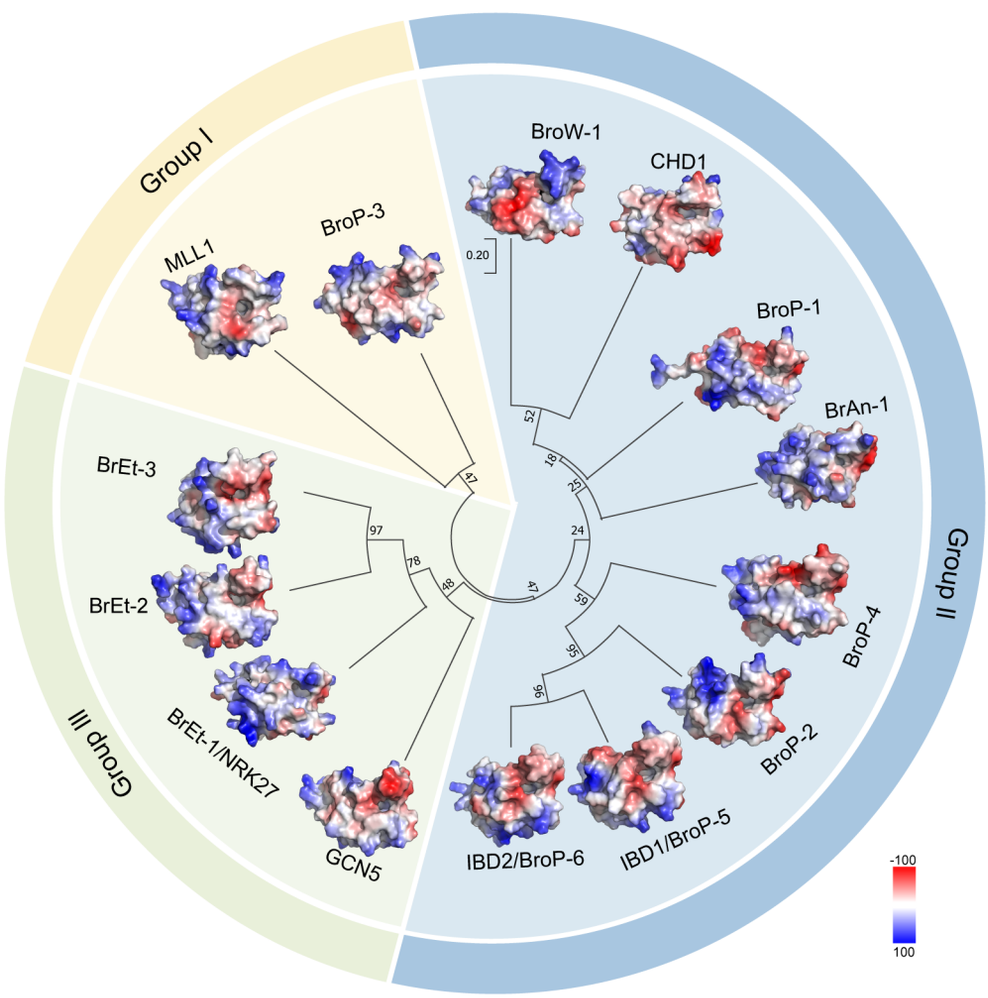

Figure 2 Structural classification of *Tetrahymena* BRD proteins

BRD modules were aligned using MUSCLE v.3.2 (Edgar, 2004) and classified into three distinct groups (Groups I, II, and III). Phylogenetic analysis was conducted using the neighbor-joining method with 1 000 bootstrap replicates, as described by Saettone et al. (2018), and the structures were predicted using AlphaFold v.3.0 (Jumper et al., 2021; Varadi et al., 2024). Surface charge was calculated using PyMOL Molecular Graphics System v.2.6.0 (Schrödinger LLC, 2015). All BRD modules were uniformly oriented with the Kac-recognition site positioned upward.

observed in *Tetrahymena* (Figure 3B). This variant is likely a product of alternative splicing, potentially regulating the production of full-length GCN5 (Smith et al., 1998).

Given the prevalence of transcription-associated histone acetylation in the MAC of *Tetrahymena*, this organism provided the first structural insight into histone acetyltransferase activity through the crystallographic resolution of GCN5 bound to coenzyme A and a histone H3 peptide (Brownell & Allis, 1995; Brownell et al., 1996; Rojas et al., 1999). Recent studies have also identified cooperation between GCN5 and the BRD protein IBD1 (reviewed in the "Proteins with extra terminal domains" section) as a component of the SAGA complex in *Tetrahymena* (Saettone et al., 2018; Wahab et al., 2020). Despite these advances, the precise molecular interplay between GCN5 and IBD1 within this chromatin-modifying complex remains incompletely

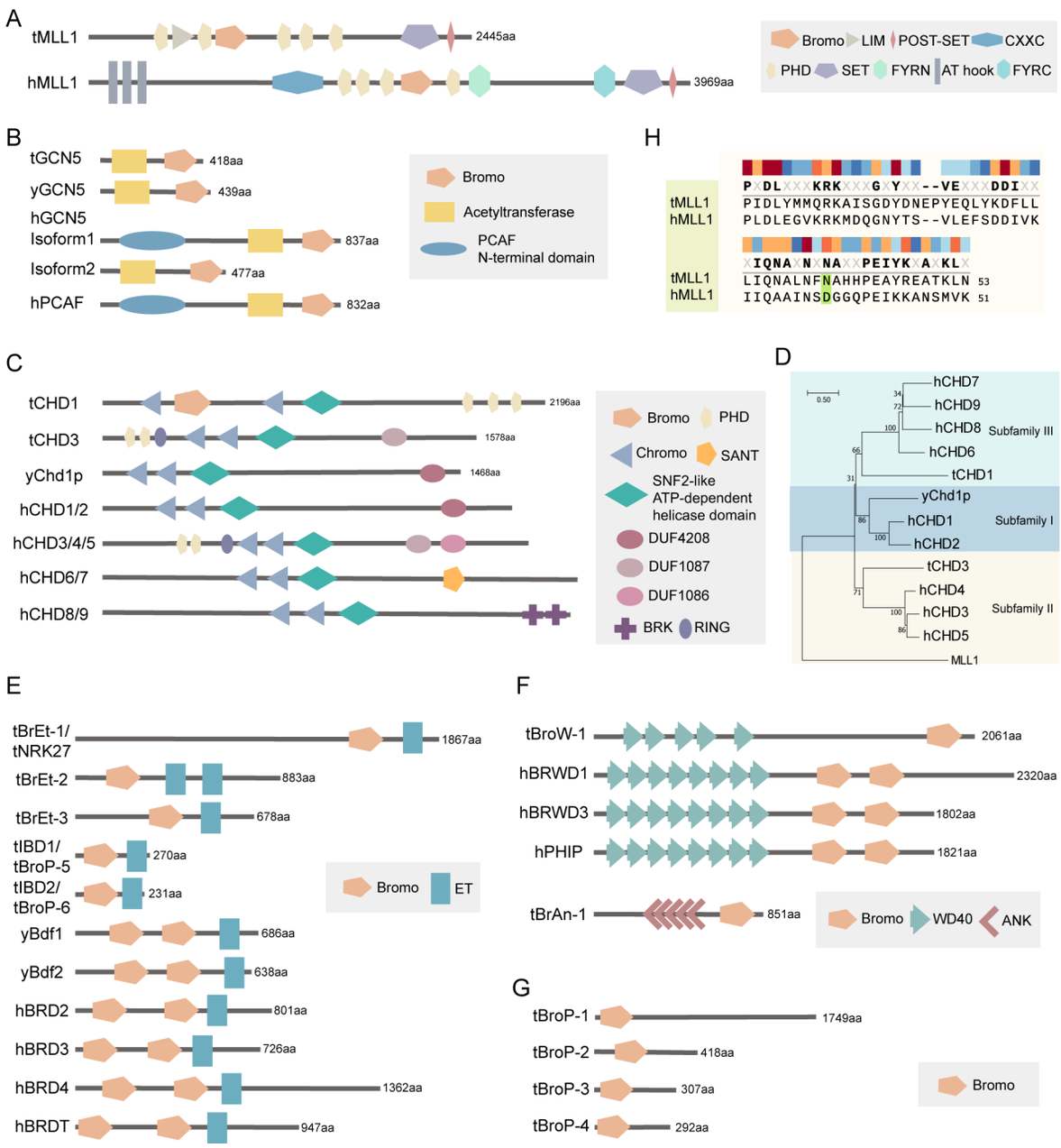

Figure 3 Domain architecture and sequence analysis of BRD proteins

A: Domain architecture of MLL1 homologs in humans (hMLL1) and *Tetrahymena* (tMLL1). Predicted domains were identified using SMART (Schultz et al., 1998), InterPro (Blum et al., 2025), and UniProt (Consortium, 2025) online tools. Yeast lacks MLL1 homologs. B: Domain architecture of GCN5 homologs in humans (hGCN5 and hPCAF), yeast (yGCN5), and *Tetrahymena* (tGCN5). C: Domain architecture of CHD homologs in humans (hCHD1-9), yeast (yChd1p), and *Tetrahymena* (tCHD1 and tCHD3). D: Phylogenetic analysis of human, yeast, and *Tetrahymena* CHD proteins. Sequences were aligned using MUSCLE, and phylogenetic analysis was performed using the neighbor-joining method with 1 000 bootstrap replicates. E: Domain architecture of BET proteins in humans (hBRD2, hBRD3, hBRD4, and hBRDT), yeast (yBdf1 and yBdf2), and *Tetrahymena* (tBrEt-1, tBrEt-2, tBrEt-3, tIBD1, and tIBD2). F: Domain architecture of BRD proteins with WD40 or ANK domains in humans (hBRWD1, hBRWD3, and hPHIP) and *Tetrahymena* (tBroW-1 and tBrAn-1). Human BRD proteins lack the ANK domain, whereas yeast BRD proteins lack both. G: Domain architecture of BroP1–4 in *Tetrahymena*. Both humans and yeast systems lack corresponding homologs. H: Sequence alignment of hMLL1 and tMLL1, with Kac-recognizing asparagine (N) in tMLL1 (D in hMLL1) highlighted in green.

defined and warrants further investigation.

CHD1, with tandem chromodomains and SNF2-like ATP-dependent helicase domain: The chromodomain helicase DNA-binding (CHD) family comprises a distinct class of ATP-dependent chromatin remodelers that orchestrate gene expression by modulating nucleosome positioning and chromatin accessibility (Clapier et al., 2017; Lusser et al., 2005). Canonical CHD proteins are defined by two conserved structural features: tandem chromodomains and SNF2-like ATP-dependent helicase domain (Figure 3C) (Delmas et al., 1993; Li et al., 2023). Remarkably, *Tetrahymena* CHD1 (tCHD1) harbors an additional BRD between its two chromodomains (Saettone et al., 2018; Wahab et al., 2020) (Figure 3C), a domain architecture not observed in CHD homologs from other eukaryotic lineages.

Human CHD1 utilizes its tandem chromodomains to selectively bind histone H3 tails bearing lysine 4 methylation (H3K4me), a modification associated with transcriptionally active chromatin (Table 2). This interaction facilitates CHD1 recruitment to gene promoters, where it promotes chromatin decompaction and transcription activation (Flanagan et al., 2005; Sims III et al., 2007). The SNF2-like ATP-dependent helicase domain, conserved across the CHD family, serves as the enzymatic core responsible for remodeling nucleosomes and altering chromatin conformation (Marfella & Imbalzano, 2007).

In humans, CHD proteins are divided into three subfamilies based on the presence or absence of additional domains (Liu et al., 2021) (Figure 3C, D). Subfamily I, which includes CHD1 and CHD2, possesses well-characterized DNA-binding domains and exhibits high sequence homology, suggesting similar DNA-binding properties. Subfamily II, consisting of CHD3, CHD4, and CHD5, features two plant homeodomains (PHDs) that enhance binding to methylated histone residues and protein cofactors. Subfamily III, which includes CHD6, CHD7, CHD8, and CHD9, is characterized by the presence of SWI3, ADA2, N-CoR, and TFIIIB (SANT) domains that facilitate non-specific DNA binding, as well as Brahma and Kismet (BRK) domains. In contrast, budding yeast contains a single CHD protein (Chd1p), which closely resembles members of subfamily I in humans (Woodage et al., 1997) (Table 2).

In *Tetrahymena*, two CHD paralogs, CHD1 and CHD3, have been identified. Phylogenetic analysis has revealed that *Tetrahymena* CHD3 (tCHD3) clusters with human subfamily II, while tCHD1 aligns with human subfamily III (Figure 3D). These results suggest that tCHD1 may possess a distinct ability to recognize both histone methylation and acetylation. However, further functional studies on tCHD1 are required to provide deeper insights into its dual recognition capabilities.

Subtype 2: Proteins with extra terminal domains

The BET family of proteins is defined by a conserved architecture comprising two tandem N-terminal BRDs and an extra terminal (ET) domain. While the BRD modules bind to chromatin regions enriched in Kac, the ET domain facilitates protein-protein interactions and recruits components for transcriptional machinery (Fujisawa & Filippakopoulos, 2017). Together, these domains enable BET proteins to function as dynamic scaffolds at transcriptionally active loci.

Yeast expresses two BET family proteins, bromodomain-containing factor 1 and 2 (Bdf1 and Bdf2), while humans possess four BET family proteins (bromodomain-containing protein 2 (BRD2), BRD3, BRD4, and bromodomain testis-specific factor (BRDT)) (Wang et al., 2023; Zhang et al., 2010) (Figure 3E). These proteins share common structural features, including two tandem N-terminal BRDs and an ET domain (Wang et al., 2023; Zhang et al., 2010) (Figure 3E). In yeast, Bdf1 interacts with transcription factor II D (TFIID) and is recruited to TATA-containing promoters to regulate gene expression (Matangkasombut et al., 2000). Beyond its role in gene regulation, Bdf1 is essential for proper sporulation and mediates cellular responses to environmental stress (Chua & Roeder, 1995; Fu et al., 2013; Garabedian et al., 2012). Bdf2, while less well-characterized, displays partial functional

Table 2 Substrates and catalytic activities for histone modifications of BRD proteins in humans, yeast, and *Tetrahymena*

Complex	protein	Human			Yeast			*Tetrahymena*		
		# of BRDs†	Substrate		# of BRDs	Substrate		# of BRDs	Substrate	
			Recognition	Catalytic target		Recognition	Catalytic target		Recognition	Catalytic target
MLL1	MLL1	MLL1 (1)	–	H3K4me3	–	–	–	MLL1 (1)	–	H3K4me3
CHD	CHD	CHD1–9 (0)	CHD1: H3K4me CHD6: H3K4me3, H3K9me3, H3K27me3 CHD7/8: H3K4me	–	Chd1p (0)	–	–	CHD1 (1) CHD3 (0)	–	–
SAGA	GCN5	GCN5 (1) /PCAF(1)	PCAF: H3 (K9ac, K14ac, K36ac); H4 (K8ac, K16ac, K20ac);	GCN5: H3 (K9ac, K14ac, K18ac, K23ac, K56ac) H4K8ac PCAF: H3 (K9ac, K14ac) H4K8ac	GCN5 (1)	H3 (K9ac, K14ac, K18ac) H4 (K5ac/K8ac/K12ac /K16ac‡)	H3 (K9ac, K14ac, K18ac, K23ac, K27ac, K36ac) H4 (K8ac, K16ac)	GCN5 (1)	H3K18ac	H3 (K14ac, K18ac)
SWI/ SNF	BRG1	BRG1 (1)	H3 (K14ac, K9ac/K14ac‡) H4(K8ac, K12ac, K16ac)	–	Snf2 (1) Sth1 (1)	Snf2: H3K14ac Sth1: H3K14ac, H2AK21ac	–	BRG1 (0) IBD1 (1)	H3 (K9ac/ 14ac‡) H4K8ac	–

†: Corresponding proteins, with number of BRDs indicated in parentheses. ‡: Specifically recognizes substrates with multiple simultaneous acetylation modifications. –: Not available.

redundancy with Bdf1 and can compensate for its loss under certain genetic conditions (Matangkasombut et al., 2000). In humans, BET proteins retain the conserved domain architecture and perform distinct functions. BRD2 is involved in chromatin remodeling by recruiting transcription factors and repressors, as well as histone deacetylases (Wang et al., 2023). BRD3 plays a role in adaptive immunity and binds with acetylated residues on the GATA binding protein 1 (GATA1) transcription factor, a crucial interaction for regulating the expression of genes specific to the red lineage and megakaryocytes (Lamonica et al., 2011). BRD4 binds to acetylated histones at the transcription initiation site, releasing RNA Pol II and acting as a regulator to recruit tumor-associated target genes (Wang et al., 2023). BRDT is specifically expressed in the testis and plays a critical role in spermatogenesis, with its dysfunction linked to male infertility (Shang et al., 2007).

In *Tetrahymena*, members of the BET family feature a single N-terminal BRD and one or two C-terminal ET domains, including IBD1/BroP-5, IBD2/BroP-6, BrEt-1, BrEt-2, and BrEt-3 (Figure 3E). Among these, IBD1 and IBD2 have been the most extensively characterized, while the BrEt-1, BrEt-2, and BrEt-3 remain largely unexplored (Saettone et al., 2018; Wahab et al., 2020; Wang et al., 2025).

IBD1 has emerged as a versatile chromatin-associated factor, participating in multiple protein complexes, including SWI/SNF, SWI2/SNF2-related (SWR), SAGA and H3K4 methyltransferase trithorax-related protein 3 (TXR3) (Saettone et al., 2018; Wahab et al., 2020). Analogous to Bdf1 in yeast—which contributes to the SWR complex by facilitating *in vivo* deposition of the histone variant H2A.Z without directly influencing catalytic activity (Altaf et al., 2010) —IBD1 is believed to perform a similar chromatin-targeting function in *Tetrahymena*. Notably, while human SWI/SNF complexes include BRD-containing subunits such as BRG1, the *Tetrahymena* homologs lack this domain entirely (Fillingham et al., 2006) (Table 2). Instead, IBD1 appears to compensate for this absence by serving as the BRD-bearing component of the SWI/SNF complex. Consistent with this role, IBD1 is enriched at highly expressed genes, indicating a potential role in gene transcription (Saettone et al., 2018; Wahab et al., 2020).

IBD2 interacts with several key players in transcription-associated epigenetic pathways, including RuvB-like protein 1 (RVB1), RVB2, Ino eighty subunit 2 (IES2), and H2A.Z (Wang et al., 2025). These proteins are evolutionarily conserved subunits of the inositol-requiring mutant 80 (INO80) complex, which regulates nucleosome sliding and H2A.Z distribution (Ashraf et al., 2019; Papamichos-Chronakis et al., 2011). In *Tetrahymena*, INO80 associates with H2A.Z (also known as Hv1), as demonstrated by H2A.Z affinity purification coupled with mass spectrometry (AP-MS) analysis (Ashraf et al., 2019). These interactions suggest that IBD2 may function as a component of the INO80 complex. Furthermore, IBD2 plays a critical role in the recruitment of the 6mA MTase adenine methyltransferase 7 (AMT7). Loss of IBD2 impairs the 5'-localized AMT7 recruitment and delays the initiation of 6mA maintenance methylation (Wang et al., 2025).

Based on current studies of IBD1 and IBD2 in chromatin remodeling complexes, these factors likely exhibit distinct functional roles; however, the potential for partial functional redundancy cannot be excluded and warrants further investigation. BrEt-1, BrEt-2, and BrEt-3 likely act as scaffolds for the recruitment and segregation of transcriptional machinery components, recognizing histone acetylation in a manner similar to other BET family proteins. However, their specific functions remain to be explored.

Subtype 3: Proteins with domains potentially facilitating binding capabilities

BroW-1 and BrAn-1: WD40 repeats are composed of approximately 40 amino acids, typically flanked by glycine-histidine (GH) and tryptophan-aspartic acid (WD) residues (Smith et al., 1999). These repeats serve as versatile interaction modules, facilitating the assembly of heterotrimeric or multiprotein complexes (Philipps et al., 2008). WD40 repeats are widespread in various proteins, including G proteins, phosphatases, and transcription regulators (Schapira et al., 2017). Three human BRD proteins (bromodomain and WD repeat-containing protein 1 (BRWD1), BRWD3, and pleckstrin homology domain-interacting protein (PHIP)) contain eight WD40 repeats, a structural motif entirely absent from BRD-containing proteins in yeast (Wang et al., 2023; Zhang et al., 2010; Zou et al., 2016) (Figure 3F). BRWD1 associates with BRG1, regulating chromatin remodeling and transcription (Zaware & Zhou, 2019). BRWD3 regulates H3K4 methylation by interacting with lysine-specific demethylase 5 (KDM5), promoting its polyubiquitination and degradation to maintain H3K4me homeostasis, which is crucial for chromatin regulation (Han et al., 2023). PHIP has been implicated in melanoma progression and serves as both a biomarker and functional mediator of metastatic potential (De Semir et al., 2012). In contrast, *Tetrahymena* BroW-1 contains five N-terminal tryptophan-aspartic acid 40 (WD40) repeats, in addition to a C-terminal BRD domain (Figure 3F, top panel).

Tetrahymena BrAn-1 features five N-terminal ankyrin (ANK) repeats, alongside a C-terminal BRD domain, a domain combination not found in either yeast or human BRD proteins (Wang et al., 2023; Zhang et al., 2010) (Figure 3F, bottom panel). However, in human euchromatic histone lysine methyltransferase 2 (EHMT2, also known as G9a) and G9a-like protein (GLP), ankyrin repeats function as methyllysine-binding modules, interacting with K9-methylated peptides to repress transcription through H3K9me1/2 (Collins et al., 2008). Similarly, ankyrin repeats in BrAn-1 act as protein-chromatin interaction domains, potentially recognizing both H3K9 methylation and acetylation and modulating chromatin dynamics and transcription. However, the specific functions of BroW-1 and BrAn-1 in *Tetrahymena* remain unexplored.

BRD-only proteins (BroP)

BroP-1, BroP-2, BroP-3, and BroP-4 constitute a distinct subclass of BRD proteins in *Tetrahymena*, characterized by the presence of a solitary BRD and the absence of any additional recognizable functional domains (Figure 3G). While BroP-2, BroP-3, and BroP-4 share similar protein lengths with IBD1 and IBD2, they lack ET domains, distinguishing them from other BRD proteins. This structural simplicity contrasts with BRD proteins in yeast and humans, which typically contain additional functional domains, such as ET or chromodomains, in addition to their BRD modules (Wang et al., 2023; Zhang et al., 2010). Although the specific functions of BroP proteins remain to be elucidated, their structural features suggest potential involvement in chromatin remodeling complexes, possibly exhibiting functional redundancy or compensatory mechanisms among family members.

EXPRESSION AND LOCALIZATION

Transcriptomic data from the *Tetrahymena* Genome Database (TGD, https://tet.ciliate.org/) and the *Tetrahymena* Functional Genomics Database (TetraFGD, http://tfgd.ihb.ac.cn/) (Miao et al., 2009; Xiong et al., 2011) reveal dynamic expression patterns of BRD-containing proteins across developmental stages (Figure 4). Most BRD proteins are strongly up-regulated during the conjugation stage (C4–C10), likely reflecting transcriptional activity linked to MAC formation. Consistent with this, IBD1 is initially detected in maternal MAC and subsequently localizes to the developing new MAC during conjugation (Saettone et al., 2018; Wahab et al., 2020). During the vegetative stage, several BRD proteins, particularly BrAn-1, IBD1, and IBD2, display elevated expression, suggesting crucial roles during this phase. BrAn-1 shows especially high transcript abundance, consistent with its predicted role in chromatin regulation via ankyrin-mediated recognition of histone modifications, as observed in homologous metazoan proteins. IBD1, which specifically interacts with the SWI/SNF complex, facilitates Kac recognition and chromatin remodeling during vegetative transcription (Saettone et al., 2018; Wahab et al., 2020). Concurrently, IBD2 interacts with the 6mA MTase AMT7 complex, with its expression closely mirroring the functional activity of this complex (Wang et al., 2025). Both IBD1 and IBD2 are localized to the MAC during the vegetative stage (Saettone et al., 2018; Wahab et al., 2020; Wang et al., 2025), reinforcing their involvement in chromatin-associated processes. Similarly, *Tetrahymena* GCN5, a histone acetyltransferase required for transcriptional activation, is nuclear-localized in vegetatively growing cells (Brownell & Allis, 1995; Brownell et al., 1996; Rojas et al., 1999) (Table 1).

RECOGNITION OF ACETYLATED LYSINE RESIDUES

A defining feature of BRD proteins is their ability to selectively bind Kac residues, primarily on histone tails (Mujtaba et al., 2007). Comparative analysis of *Tetrahymena* BRD proteins with their yeast and human counterparts reveals both conserved and lineage-specific features, offering insight into evolutionary divergence and potential functional specialization in this ciliate model.

Unlike its human homolog, the BRD of *Tetrahymena* MLL1 retains the conserved asparagine (N) in the BC loop —a residue essential for Kac recognition in canonical BRDs (Figure 3H) (Wang et al., 2010). In contrast, human MLL1 lacks this critical residue and is unable to bind Kac. Whether *Tetrahymena* MLL1 can engage acetylated lysines remains untested, but its preserved binding pocket architecture raises the possibility of a functional Kac reader activity that warrants further biochemical validation.

Another striking example of domain innovation in *Tetrahymena* is CHD1, which features a BRD between two

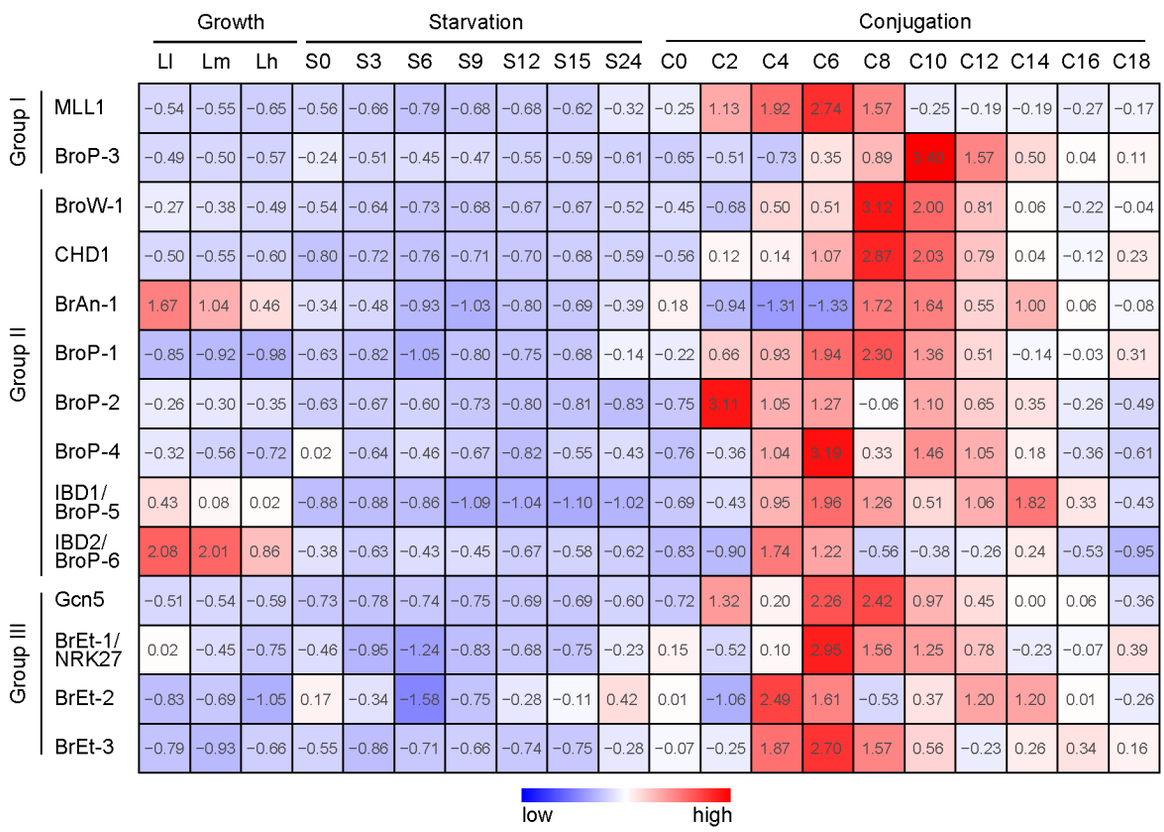

Figure 4 Expression profile of *Tetrahymena* BRD-coding genes during growth, starvation, and conjugation

Expression levels of BRD-coding genes across different cell phases were obtained from the TetraFGD database (http://tfgd.ihb.ac.cn/). For growth, LI, Lm, and Lh corresponded to approximately ~1×10^5 cells/mL, ~3.5×10^5 cells/mL, and ~1×10^6 cells/mL, respectively. For starvation, cells were collected after 0, 3, 6, 9, 12, 15, and 24 h of starvation, referred to as S0, S3, S6, S9, S12, S15, and S24, respectively. For conjugation, cells were mixed and collected at 0, 2, 4, 6, 8, 10, 12, 14, 16, and 18 h post-mixing, referred to as C0, C2, C4, C6, C8, C10, C12, C14, C16, and C18, respectively. Color gradient, ranging from blue (low expression) to red (high expression), indicated variation of expression levels. All BRD-coding genes showed high expression during conjugation (C4–C10). BrAn-1, IBD1, and IBD2 exhibited notable expression during vegetative growth.

chromodomains (Figure 3C) and may facilitate Kac recognition, a configuration absent in CHD homologs in other eukaryotes (Figure 3C) (Li et al., 2023). Phylogenetically, tCHD1 clusters with human subfamily III (CHD6–9) (Figure 3C, D). In humans, CHD6 lacks detectable binding to histone marks under normal conditions *in vitro* but shows enhanced affinity for H3K9me3 and H3K27me3 upon viral infection and colocalizes with H3K4me3 *in vivo* (Alfonso et al., 2011). CHD7 and CHD8, in turn, can recognize H3K4me-enriched regions (Schnetz et al., 2009; Yuan et al., 2007) (Table 2). These findings suggest that *Tetrahymena* CHD1 may recognize both histone methylation and acetylation, thereby integrating diverse epigenetic signals to achieve precise regulation of chromatin remodeling and gene expression, a hypothesis that warrants further functional investigation.

The BRD of GCN5 plays a pivotal role in chromatin remodeling by recognizing site-specific Kac (Cieniewicz et al., 2014; Dhalluin et al., 1999). In humans, PCAF preferentially recognizes H3K14ac but also engages other acetyl marks, including H3K36ac, H3K9ac, H4K20ac, H4K16ac (Zeng et al., 2008), and H4K8ac (Dhalluin et al., 1999; Filippakopoulos & Knapp, 2012). Yeast GCN5, as a core component of the SAGA complex, recognizes multiple acetylated residues on histone H3 (K9ac, K14ac, K18ac) and combinatorial acetylation patterns on H4 (K5/K8/K12/K16) (Grant et al., 1999; Hassan et al., 2007) (Table 2). Similarly, in *Tetrahymena*, GCN5 also forms part of the SAGA complex (Saettone et al., 2018) and exhibits specificity for H3K18ac (Garg et al., 2013). Mechanistically, GCN5 acetylates the nucleosome at the H3 tail via its HAT domain, after which its BRD binds the newly modified lysine to facilitate subsequent acetylation of adjacent tails, thus promoting efficient propagation of histone acetylation (Li & Shogren-Knaak, 2009).

In humans, BRG1 binds multiple acetylated residues on H3 and H4, including H3K14ac and dual H3K9/14ac (Shen et al., 2007), as well as H4K8ac, H4K12ac, and H4K16ac (Filippakopoulos & Knapp, 2012; Singh et al., 2007) (Table 2). In yeast, which lacks BRG1 homologs, BRD activity is provided by Snf2 and Sth1. Snf2 selectively recognizes H3K14ac, while Sth1 recognizes H3K14ac, H2AK21ac, and other Kac (Zhang et al., 2010) (Table 2). In *Tetrahymena*, IBD1 exhibits a strong binding affinity to H3K9/14 double acetylation, H3K14ac, and H4K8ac (Table 2) (Saettone et al., 2018). However, the substrate preferences of other BRD proteins in *Tetrahymena* remain unexplored.

INTERACTING PROTEINS AND POTENTIAL FUNCTIONS

Catalytic activity for histone modifications: MLL1, GCN5, and IBD1

MLL1 exhibits conserved H3K4me3 catalytic activity in both *Tetrahymena* and other eukaryotes (Malik & Bhaumik, 2010; Wang et al., 2025) (Table 2). In *Tetrahymena*, MLL1 cooperates with the histone variant H2A.Z and 6mA to regulate gene expression, forming a tripartite epigenetic module that stabilizes transcriptionally permissive chromatin environments (Wang et al., 2025).

GCN5 also functions as a histone acetyltransferase and is highly conserved across eukaryotes. In humans, GCN5 preferentially acetylates H3K14 and H4K8 (Xu et al., 1998), but also targets H3K9, K18, K23 (Lee et al., 2010), and K56 (Tjeertes et al., 2009) (Table 2). PCAF catalyzes acetylation at H3K9 (Kebede et al., 2017), H3K14 (Kornacki et al., 2015; Lau et al., 2000), and H4K8 (Schiltz et al., 1999) (Table 2). In yeast, GCN5 also exhibits broad substrate specificity, acetylating H3 at K14, K9, K18, K23, K27, and K36 (Cieniewicz et al., 2014; Morris et al., 2007), as well as H4 at K8 and K16 (Kuo et al., 1996; Tsukiyama & Wu, 1997) (Table 2). In *Tetrahymena*, the SAGA complex comprises 10 proteins, including GCN5, ADA2, and ADA2-associated proteins (AAP1–8) (Saettone et al., 2018) (Figure 5A). Recombinant *Tetrahymena* GCN5 has been shown to acetylate free histone H3 at K14 (Rojas et al., 1999) and K18, but lacks the ability to acetylate histone H3K56, a modification catalyzed by its human ortholog GCN5 (Garg et al., 2013) (Table 2).

Chromatin remodeling: IBD1, IBD2, and CHD1

Several chromatin remodelers are present in eukaryotes, including those in the SWI/SNF, INO80, and CHD subfamilies, which enable the translation of genetic and epigenetic information into specific transcriptional outcomes (Eustermann et al., 2024). SWI/SNF remodelers facilitate transcriptional activation by displacing nucleosomes from promoter and enhancer regions (Eustermann et al., 2024). The INO80 subfamily includes Ino80 and Swr1 ATPases, which serve as the catalytic cores of the SWR-C and INO80 complexes, respectively —two evolutionarily conserved, multi-subunit assemblies that share several core components (Morrison & Shen, 2009). The INO80 complex facilitates nucleosome sliding (Shen et al., 2000), while the SWR complex promotes the incorporation of H2A.Z into nucleosomes through a dimer-exchange reaction (Mizuguchi et al., 2004).

In *Tetrahymena*, IBD1 interacts with several components of the SAGA complex, such as Aap1, GCN5, and ADA2 (Saettone et al., 2018) (Figure 5B), indicating its potential function as either a component or cofactor of the SAGA complex. IBD1 also interacts with the SWI/SNF and SWR complexes (Saettone et al., 2018), indicating possible involvement in nucleosome removal and H2A.Z replacement (Figure 5B). Furthermore, its physical association with the H3K4 methyltransferase SET1 implies a broader regulatory role in H3K4 methylation (Saettone et al., 2018) (Figure 5B).

IBD2, another BRD-containing factor, is implicated in the function of the INO80 complex through interactions with RVB1, RVB2, and the INO80 complex-specific subunit IES2 (Wang et al., 2025) (Figure 5C). This suggests a possible role in chromatin remodeling via nucleosome sliding. In addition, IBD2 serves as a critical scaffold for the recruitment of the AMT7 complex. Loss of IBD2 results in reduced AMT7 complex enrichment at chromatin targets (Wang et al., 2025) (Figure 5C), supporting a model in which IBD2 binds acetylated histones and facilitates the locus-specific deposition of 6mA on adjacent DNA.

CHD1 belongs to a family of chromatin remodelers defined by their SNF2-like ATPase domain and tandem chromodomains. It functions as a DNA translocase that remodels nucleosomes by altering histone-DNA contacts in an ATP-dependent manner (Alendar & Berns, 2021). CHD1 is widely conserved across eukaryotes and contributes to diverse epigenetic processes, including chromatin organization, histone variant incorporation, and transcription reprogramming (Li et al., 2023). Its chromatin-binding specificity is regulated by various factors, including histone

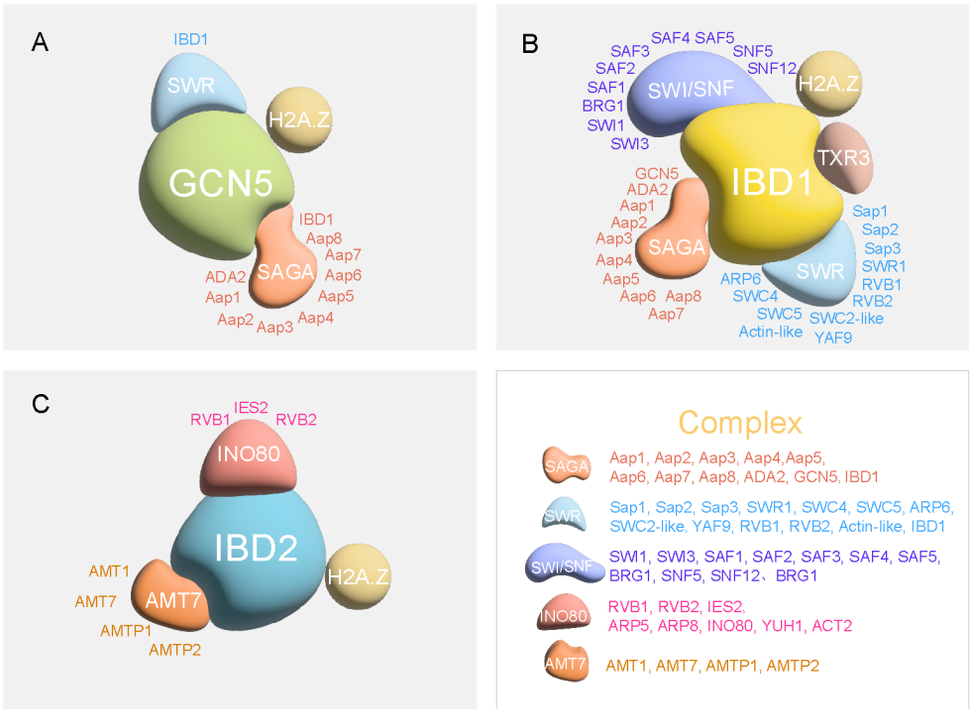

Figure 5 Interacting proteins of GCN5 (A), IBD1 (B), and IBD2 (C)

Central pattern represents BRD proteins, while surrounding smaller patterns correspond to interacting proteins. Complex components that directly interact with BRD proteins are organized around their respective complexes. All reported complex components are enclosed within dashed boxes.

modifications (particularly methylated H3K4), transcription factors, and methylated DNA and RNA (Alendar & Berns, 2021). *Tetrahymena* CHD1 likely plays a vital role in chromatin remodeling, possibly regulated by both histone methylation and acetylation.

Other potential functions

The ET domain found in BET family proteins is known to mediate protein-protein interactions by serving as a recruitment platform for transcriptional coactivators and chromatin regulators (Fujisawa & Filippakopoulos, 2017). Given this, *Tetrahymena* BET homologs (BrEt-1, BrEt-2, BrEt-3) may act as potential scaffolds that recognize histone acetylation and recruit additional factors. The specific roles of BrAn-1, BroW-1, and BRD-only proteins (BroP-1, BroP-2, BroP-3, and BroP-4) remain unexplored. However, their predicted affinity for acetylated lysine residues suggests that they may serve as transcriptional co-regulators or epigenetic readers that modulate gene expression through histone mark recognition.

CONCLUSIONS AND PERSPECTIVES

The *Tetrahymena* proteome encodes 14 bromodomain-containing proteins, each harboring a single BRD module. This streamlined configuration contrasts with yeast, which expresses 9 BRD proteins comprising a total of 14 BRD modules (Zhang et al., 2010), and with humans, where functional diversity is markedly expanded, with 61 BRDs distributed among 42 distinct proteins (Fujisawa & Filippakopoulos, 2017). Notably, the increased complexity in higher eukaryotes is exemplified by the presence of multiple tandem BRD modules within single proteins, a feature entirely absent in *Tetrahymena*. While the BRG1 subunit of the human SWI/SNF complex includes a BRD domain, its *Tetrahymena* homolog lacks this feature (Table 2). Instead, IBD1 serves as a separate BRD-containing component of the *Tetrahymena* SWI/SNF complex. Moreover, *Tetrahymena* CHD1 uniquely incorporates a BRD, a structural feature absent in CHD1 orthologs from other eukaryotes, while its MLL1 retains a conserved asparagine residue critical for acetyl-lysine recognition. Collectively, these distinctions underscore the lineage-specific reconfiguration of BRD protein architecture in *Tetrahymena*, providing a valuable model for investigating the evolutionary diversification of BRD-mediated chromatin regulation.

In *Tetrahymena*, transcription-associated Kac marks are restricted to the MAC, while the MIC primarily exhibits acetylation marks associated with chromatin deposition. This nuclear dimorphism adds complexity to the spatial regulation of BRD protein function. It remains unclear how these proteins partition between the MAC and MIC, and whether distinct subpopulations exhibit specialized roles in transcriptional regulation or chromatin assembly. Furthermore, the apparent structural redundancy among certain BRD proteins raises questions about their contributions to gene regulation. Future research should aim to unravel the precise functions and localization patterns of these proteins, thus providing insights into how they regulate transcription and chromatin dynamics.

COMPETING INTERESTS

The authors declare that they have no competing interest.

AUTHORS' CONTRIBUTION

Conceptualization: Y.Y.W., Z.Z., and A.L.J.; Supervision: Y.Y.W.; Writing - Original draft preparation: Y.Y.W., Z.Z., and A.L.J.; Writing - Review & Editing: Y.Y.W. and S.G.; Visualization: Z.Z., A.L.J., Y.Q.L., and H.Z.J. All authors read and approved the final version of the manuscript.

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