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BET domain co-regulators in obesity, inflammation and cancer

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Abstract

The bromodomain is a highly conserved motif of 110 amino acids that is bundled into four anti-parallel α-helices and found in proteins that interact with chromatin, such as transcription factors, histone acetylases and nucleosome remodelling complexes. Bromodomain proteins are chromatin 'readers'; they recruit chromatin-regulating enzymes, including 'writers' and 'erasers' of histone modification, to target promoters and to regulate gene expression. Conventional wisdom held that complexes involved in chromatin dynamics are not 'druggable' targets. However, small molecules that inhibit bromodomain and extraterminal (BET) proteins have been described. We examine these developments and discuss the implications for small molecule epigenetic targeting of chromatin networks in cancer.

This Review begins with the recollection of a 1991 report of an unusual chromosome translocation. Investigators identified a novel t(15;19)(q15;p13) translocation that was associated with a case of poorly differentiated thymic carcinoma (known as NUT midline carcinoma (NMC)) that proved fatal for a young Japanese woman[1]. This type of carcinoma is rare (affecting only 20–40 patients annually in the United States), is refractory to all treatment, uniquely aggressive and is almost uniformly lethal. The tumours involve balanced translocations of the nuclear protein in testis (*NUT*) gene on chromosome 15q14 and the translocation of the bromodomain-containing protein 4 (*BRD4*) gene[2] on chromosome 19p13.1, or sometimes the closely related[3] *BRD3* at 9q34. These genes encode transcriptional regulators that contain a double, mutually related motif that comprises 110 amino acids called a bromodomain in the amino-terminal region (FIG. 1) and an extraterminal (ET) protein–protein interaction domain in the carboxy-terminal region. The BRD2, BRD3 and BRD4 proteins share these structural features[4,5] and hence are known as BET family proteins[6]. Studies to define the structure and function of the bromodomain motif have been the object of considerable research interest over the past few years, and data from recent noteworthy studies of BET protein phenotypes that have relevance to cancer are the focus of this Review.

The characteristics of BET proteins

Bromodomains[6–9] were first identified in connection with components of the SWI/SNF nucleosome remodelling complex and the Mediator transcription complex[10–12]. The bromodomain comprises a highly conserved, four-helix, left-twisted bundle with a characteristic hydrophobic cleft between two conserved loops. The so-called ZA and BC loops in the bromodomain bind to the ε-aminoacetyl groups of nucleosomal histone

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lysines[13] (FIG. 1a). In humans, there are estimated to be 56 bromodomains encoded in 42 proteins[14]. Bromodomains are found in the closely related DNA helicase superfamily members SWI/SNF-related matrix-associated, actin-dependent, regulator of chromatin, subfamily A, member 2 (SMARCA2; also known as brahma, BRM and SNF2α) and SMARCA4 (also known as BRG1 and SNF2β)[15]. SMARCA2 and SMARCA4 are the mutually exclusive core catalytic ATPase subunits of a SWI/SNF complex[16] that regulates chromatin status[9,17]. The brahma subunit (its name conferred by *Drosophila melanogaster* biologists who were searching the Hindu pantheon for evocative descriptors) gives bromodomain its etymology. The bromodomain is also found in many transcriptional and developmental regulators that function through histone modification and nucleosome remodelling[6–8] (FIG. 1b). These regulators include authentic histone acetyltransferases (HATs), such as CREB-binding protein (CBP)[18,19] and TBP-associated factor 1 (TAF1; also known as CCG1 and $TAF_{II}250$)[6,20].

In proteins that contain two bromodomains, such as BET proteins, these domains are mutually related and arranged in tandem. However, in homologous proteins, first bromodomains are more closely related to each other than they are to second bromodomains in the same protein. In yeast and plants, double bromodomains do not exist and instead are found on separate genes that encode single bromodomain proteins[4,5]. Such proteins combine to give the functionality seen in a single protein in humans. For example, yeast Bdf1 and Bdf2 proteins are encoded by different genes but are closely related to each other, as well as to TAF1. Bdf1 and Bdf2 combine with yeast TAFII130/145 to execute the analogous functions of human TAF1 (REF. 21).

The *BRD2* gene, which Trowsdale and colleagues[6,20] originally named *RING3*, is situated within the human class II major histocompatibility complex (MHC) on chromosome 6 at p21.3, and in syntenic regions of other organisms[20,22–24]. The RING etymology was unfortunate because BRD2 is completely unrelated to the RING domain family of zinc finger proteins that are involved in protein ubiquitylation and degradation[25], a confusion that persists to this day. The *BRD2* gene was also identified as part of a major effort by the Sanger Centre to sequence and publish all the open reading frames on human chromosome 6. *BRD2* is flanked by genes involved in antigen presentation, inflammation and other immune functions, although structurally *BRD2* is highly dissimilar to these nearby genes[26]. BRD2 was the first mammalian BET protein to be functionally characterized[27] as a nuclear-localized[28] non-canonical protein kinase[29,30] and effector of mitogenic signal transduction. BRD2 binds to ε-aminoacetyl groups of nucleosomal histone lysines[31], particularly acetyl-histone H4 (REFS 32,33), recruits transcription factors, transcriptional co-activators and transcriptional co-repressors[29,34] and regulates transcription[35]. BRD2 and related bromodomain proteins provide a scaffold on chromatin[36] to recruit E2F proteins[29,37], histone deacetylases (HDACs)[34], histone H4-specific acetyltransferase (HAT)[38] and proteins involved in chromatin remodelling, including SWI/SNF subunits and elements of the Mediator complex[10–12,34], thereby coupling histone acetylation to transcription[35,38]. The ATP-dependence of association of these complex components[34] suggests that other BET proteins such as BRD4 also participate in multiprotein complexes that are conserved in composition or that may be shared among BET proteins.

The close similarity between the bromodomains of BRD2 and BRD4 (about 80% identity at the amino acid level in humans and mice) implies that the substantial functional divergence between BRD2- and BRD4-manipulated phenotypes *in vivo* probably does not lie in the relative specificity of the dual bromodomains for their target promoters per se, so much as in the enzymes recruited through interaction with the ET protein–protein interaction domain[4,5] and C-terminal domain (CTD) (FIG. 2). All mammalian BET family members possess the ET domain[39] in some form, along with putative nuclear localization signals[28]. The ET

domain is also about 80% identical among BET family members[4,5], thus, factors that are recruited through this domain might be expected to be shared among the family and to possibly contribute to functional redundancy across certain promoters. In addition, the ET domain of BRD2, BRD3 and BRD4 independently recruits transcription-modifying factors, including glioma tumour suppressor candidate region gene 1 (GLTSCR1); NSD3, a SET domain-containing histone methyltransferase; JMJD6, a histone arginine demethylase; and CHD4, a catalytic component of the NuRD nucleosome remodelling complex[40]. Depletion of GLTSCR1, JMJD6 and NSD3 through small interfering RNA (siRNA) establishes the importance of these factors for BRD4 transactivation of a model viral promoter, the bovine papilloma virus 1 locus control region[40], as well as important cellular genes, including cyclin D1 (*CCND1*). These results suggest potential new targets to treat cancers for which cyclin D1 is particularly important, such as breast cancer[41–43]. Small-molecule inhibitors of BET proteins would also be expected to displace these activities from chromatin, by virtue of their association with the ET domain; the functional consequences of such a loss should be resolved from the loss of the CTD-associated activities and further investigated.

Several isoforms of each BET protein have been reported, but most fall into long or short isoform categories owing to alternative splicing (FIG. 2), an organizational pattern first described in *D. melanogaster* (BOX 1). It is not understood how short and long forms, if co-expressed in the same cell, compete for binding to chromatin at the same promoter, or whether BRD2, BRD3 and BRD4 exhibit unique or partially overlapping functions on crucial promoters. Different protein–protein interaction motifs in each isoform or related BET proteins are likely to define important differences in the functional interactions. The CTD of BRD4 is several hundred amino acids longer than the CTD of BRD2 and includes polyserine stretches interspersed with glutamate and aspartate (SEED) motifs and a proline- and glutamine-rich unstructured region of about 500 amino acids in length that is not found in other BET proteins[4,5]. This unstructured region is similar to the highly phosphorylatable CTD of RNA polymerase II and, like this region in RNA polymerase II, the BRD4 CTD interacts with the positive transcription elongation factor b (P-TEFb; a heterodimer of cyclin-dependent kinase 9 (CDK9) and cyclin T)[44,45]. P-TEFb has a crucial role in transcription elongation, and phosphorylates the CTD of RNA polymerase[46]. The M phase to G1 phase transition is likely to be partly controlled by BRD4 recruitment of P-TEFb to target postmitotic genes for transcription immediately on the resumption of G1 phase[47].

Box 1

BET proteins and *Drosophila melanogaster* homeosis

An interesting insight into BET protein function in cancer originated in studies on the *Drosophila melanogaster* homologue of human BET genes, called *female sterile homeotic* (*fs(1)h*), which was the first BET gene to be functionally characterized. This developmental gene activates *Ultrabithorax* and exhibits maternal effects: mutational analysis has shown that maternally supplied mRNA controls developmental programming before a zygotic mRNA takes over. Maternal effect has also been demonstrated in zebrafish[133], raising the possibility that BET genes might have maternal effects in humans, but no reports are yet available to address this question. The FS(1)H protein exerts chromatin-modification functions during fly development[121,122]. Both FS(1)H and BRD2 are highly homologous to TAF1 (REF. 20). Like TAF1 and BRD2, FS(1)H exhibits protein kinase activity[121], a property that has also recently been reported to be shared with BRD4 (REF. 147), although the physiological importance of phosphorylation events that BET proteins catalyse directly has not been explored mechanistically. Mutation of *fs(1)h* causes severe defects in differentiation and cell fate; *fs(1)h*-null is lethal[148–150]. The *fs(1)h* locus is an upstream activator of *trithorax* in *D. melanogaster*[151,152], an important, homeotic control gene that in mice positively

regulates *Hox*-controlled differentiation, countering repression by the Polycomb group (PcG) proteins. The *trithorax* gene (formerly called *ALL1*, *HRX* or *HTRX1* and now known as *MLL)* encodes a bromodomain-containing transcription factor that is disrupted in human 11q23 mixed lineage leukaemias[153–156]. Several recurring chromosomal translocations associated with human acute leukaemias are characterized by breakpoints that interrupt genes that encode transcription factors of importance for *D. melanogaster* development[138], suggesting that the multiprotein interactions that regulate *D. melanogaster* development may be conserved in human haematopoiesis. Fly developmental systems remain an underexploited resource for the formulation of mechanistic hypothesis to investigate human malignant transformation. The above facts led to a proposal in 1996 that these functional relationships were conserved among *D. melanogaster* and humans, linking BET proteins to MLL[27], a functional connection that has since been supported by studies of small-molecule BET protein inhibitors that exhibit potent antineoplastic activity in human and murine MLL cell lines[92].

BET proteins in cell cycle control

BRD4 (REFS 48,49) and BRD2 (REFS 28,29,38) have crucial roles in cell cycle control of normal mammalian cells[32]. Initial reports showed that E2F1 and E2F2 (REFS 34,37), which are key transcriptional regulators of S phase genes, are associated with BRD2 multiprotein complexes[29,34,38]. BRD3-dependent functional relationships with the cell cycle control machinery in normal cells are poorly understood, although there is some evidence that forced expression of *Brd3* downregulates the RB–E2F pathway in nasopharyngeal carcinoma cells[50]. BRD4 seems to be required for the G2 to M phase transition of the cell cycle because microinjection of BRD4-specific antibodies leads to cell cycle arrest[51]. Interestingly, forced expression of BRD4 opposes the function of replication factor C and also results in G1 to S phase arrest[48]. More recent evidence shows that, unlike non-BET bromodomain proteins, BRD2 and BRD4 remain bound to mitotic chromatin[35,51,52], a property that has been postulated to be important for the maintenance of epigenetic memory[47,53–55]. This mechanism primes a set of M phase to G1 phase postmitotic genes for transcription at the outset of the next cell cycle after cytokinesis is complete[56], which is consistent with the BRD4 dependence of P-TEFb recruitment. BRD2 also associates with postmitotic chromatin, but does not recruit P-TEFb — the functional importance of which is still unclear. Association with mitotic chromatin is a highly conserved property of BET proteins in yeast[57], thale cress (*Arabidopsis thaliana*) [58,59], roundworm[60], zebrafish[55], murine[49,51,53,56] and human cells[61,62].

In view of the elemental role of BET proteins in the normal cell cycle and their ubiquitous expression, it is not surprising that the study of BET protein function through genetic deletion experiments has been difficult: *Brd2* and *Brd4* are essential for cell growth (TABLE 1). Knockout of *Brd2* is lethal in mice[63–65], *Brd4*$^{+/-}$ mice have severe defects in differentiation and organogenesis, and *Brd4*-null animals die *in utero*[66] owing to mitotic defects[48,51]. Such defects may occur owing to the failure of the Aurora B spindle checkpoint during mitosis, as Aurora B expression is BRD4 dependent[67]. Similarly, yeast requires a functional copy of either of the transcription factors *BDF1* or *BDF2*, and double mutants are lethal[57,68]. The *BDF1*-null phenotype is also lethal if combined with a block in histone H4 acetylation[69]. There are no *Brd3*-knockout model systems available.

It is interesting that these studies indicate that, although each of the BET proteins is closely related to the others, whatever functional redundancy might exist among them is insufficient to rescue the null phenotypes. Moreover, the TAF1 transcription factor — which also has a double bromodomain, binds acetylated histone H4 (REF. 70) and is crucial for cell cycle

progression[71,72] — also does not compensate for BET protein loss, despite the fact that it shares some sequence homology and functional similarities with the BET proteins. Indeed, small-molecule inhibitors of BET proteins bind relatively poorly to other bromodomain-containing factors (FIG. 1b).

BET protein interactions, inhibitors and cancer

The data discussed above indicate that BET proteins have a crucial role in regulating gene transcription through the recruitment of proteins to form complexes that modify chromatin. Histone-binding, chromatin-regulatory proteins have long been implicated in cancer; a considerable amount of literature concerning their carcinogenic mechanisms has grown up alongside literature that addresses how dysfunctions of sequence-specific DNA-binding transcription factors are linked to cancer. Indeed, epigenetic deregulation of transcription is now appreciated to be as important for carcinogenesis as genetic mutation[73]. For example, chromosomal translocations can mistarget bromodomain-containing histone-modification enzymes, such as CBP and p300, or chromatin remodelling machines to incorrect promoters, accounting for a substantial number of haematological malignancies[74]. Specifically, the t(8;16)(p11;p13) translocation that is associated with the M4/M5 subtype of acute myeloid leukaemias (AMLs) was the first report of a translocation involving CBP[75]. The t(11:16)(q23;p13.3) translocation, which arises in treatment-related myelodysplasias and AML, fuses mixed lineage leukaemia (MLL) to CBP[76]. Full oncogenicity of MLL–CBP is retained only if both the histone acetylase activity and the bromodomain of CBP are present in the transforming fusion gene[77]. In acute promyelocytic leukaemia, a histone acetylase complex replaces the NCoR–SIN3–HDAC repression complex, resulting in inappropriate transactivation of target genes[78]. Similarly, the recruitment of a transcriptional co-activator instead of a co-repressor is the mechanism by which the oncoprotein AML1–ETO alters gene expression and accounts for >10% of AML[74]. Mistargeted BET protein fusions probably work in a similar manner, inappropriately recruiting chromatin-modifying enzymes to the promoters of cell cycle control genes to corrupt proliferation programmes. Recent work with BET protein inhibitors supports this proposition.

The NMC tumours that arise from the reciprocally translocated *BRD4* or *BRD3* genes came to the attention of James Bradner, a medical chemist who had been developing targeted, small-molecule inhibitors of histone deacetylases. Bradner led a group that built on the known interaction of thienodiazepines with BRD4 (for which these compounds had been patented by the Mitsubishi Tanabe Pharma Corporation) to develop a novel, small-molecule inhibitor of the binding interface between acetylated histone H4 in chromatin and a bromodomain. The resulting first-generation BET-specific inhibitor, JQ1, contains a bulky hydrophobic substituent at a chiral centre in the molecule that prevents its binding to the central benzodiazepine receptor (FIG. 3); thus, such compounds should have no psychotropic activity. JQ1 proved to be highly effective against NMC xenografts in mice and promoted both growth arrest and differentiation of NMC cells *in vitro*[79]. No drug regimen had been shown to be effective against these tumours before, much less from the surprising angle of inhibiting the binding of proteins to acetylated histones[80], rather than inhibiting the HAT or HDAC enzymes themselves. Thus, the discovery received substantial publicity. There is now a small but growing number of structural variations on the JQ1 theme (FIG. 3) that might have clinical value for orphan cancers such as NMC, as well as for other applications.

Other investigations of the consequences of BET protein inhibition have shown that the transcription of the proto-oncogene *MYC* is downregulated[81]. This could account for much of the anti-proliferative effect of JQ1 in human cancer cell lines. BRD3 and BRD4 seem to be involved in chromatin control at the *MYC* promoter, and MYC in turn represses *CDKN2A*

transcription (which encodes the cell cycle regulator p21) in response to DNA damage and p53 activation[82]. Thus, ablation of MYC would be expected to promote cell cycle arrest in part through the derepression of p21 (REF. 81). Therefore, there is an emerging rationale to test whether BET protein inhibitors might be clinically useful to target specific human cancers that are strongly dependent on MYC-regulated transcriptional networks, such as Burkitt's lymphoma[83] and certain types of AML[81,84]. Coincidentally, the single bromodomain protein ATAD2 co-activates *MYC* and is implicated in breast and prostate cancer[85,86]. However, the bromodomain of ATAD2 is not closely related to BET bromodomains and none of the small molecules reported to date significantly inhibits its binding to acetylated histones[79]. Epigenetic inhibitors for non-BET bromodomain proteins await additional design and screening in cancer model systems, particularly breast and prostate cancer. It will be interesting to learn whether any of these small molecules also ablate MYC function.

Independent studies have shown somewhat surprisingly that mice can be treated with chemotherapeutic doses of JQ1 (50 mg per kg per day daily for 1 month) apparently without catastrophic weight loss, metabolic collapse, lethal bone marrow suppression or other systemic toxicity[79,81,84]. These findings are not in agreement with the *Brd2*- and *Brd4*-haploinsufficient or null phenotypes described above (TABLE 1), nor do they indicate that MYC is the primary target of JQ1, because inhibition of MYC activity has substantial effects on constantly renewing tissues, such as intestinal epithelium and skin[87,88]. Further investigation is clearly needed to understand the mechanisms of JQ1 activity in cancers that express MYC and to study potential, as yet unappreciated or longer-term, toxicities of BET inhibitors in general.

The full range of involvement of BET proteins in cancer is still being established. The interaction of BRD4 with P-TEFb and of TAF1 with the E2 protein of human papillomaviruses (HPVs) that are associated with cervical cancer risk[89] (BOX 2) focus attention on relationships between host BRD4 and virus-encoded proteins that are important for transcriptional control and cancer. Importantly, in cells infected with HIV, the HIV transcription factor Tat competes with host BRD4 for binding to host P-TEFb that is present at the HIV long terminal repeat[90] to control transcription together with recruited HAT and SWI/SNF activities (BOX 3). Thus, small molecule inhibition of BRD4 might be predicted to release BRD4 from chromatin and remove it from the P-TEFb–Tat equilibrium, which could potentially promote HIV transcription and virus reactivation from latency. This process could also redistribute BRD4 to other multiprotein complexes that associate with chromatin through interactions other than BRD4 bromodomains, or that are unbound to chromatin, with unpredictable results. However, because mice seem to tolerate JQ1 well[79,81,84], targeting BRD4 could be preferable to targeting P-TEFb in HIV therapeutics or in malignancies, such as MLL, in which P-TEFb is important[91]. The potential efficacy of inhibiting BET proteins in MLL has recently been published[92]. The BET protein inhibitor GSK1210151A (I-BET151) induced apoptosis in both mouse and human leukaemic cell lines with different *MLL*-fusion genes. Displacement of BRD3 and BRD4 from the chromatin and the polymerase association factor complex (PAFC) and super elongation complex (SEC) that interact with BET proteins resulted in reduced transcription of *BCL2*, *MYC* and cyclin-dependent kinase 6 (*CDK6*)[92]. These data indicate that the displacement of BET proteins from chromatin, and the factors that associate with them, may have therapeutic efficacy. Incidentally, a number of investigators continue to refer to JQ1 or I-BET as BRD4 inhibitors. This usage is incorrect: JQ1 and I-BET are not highly selective for BRD4 over BRD2, BRD3 or BRDT. Until more specific compounds are available, mechanistic studies that use genetic ablation or overexpression of each BET protein member are required to rule out the participation of the other BET proteins in each case before conclusions can confidently be drawn.

Box 2

Virus replication, latency and transcription

In certain virus infections, host-encoded BET proteins have been shown to be crucial for both transcriptional activation and transcriptional repression of virus promoters. Certain animal and human papilloma viruses (HPVs) use BET proteins as cellular adaptors to anchor viral genomes to mitotic chromosomes[157]. The E2 protein of HPV, which is required for virus episome maintenance and virus transcription[158], interacts with the carboxy-terminal domain (CTD) of BRD4 (REF. 159) to enable both E2 transcriptional activation of E2-target genes[160–162] and E2 repression of the oncogenic E6 and E7 genes[163,164]. Recombinant truncated CTD of BRD4 exerts dominant-negative effects on E2 transcriptional activation[165], possibly through redistribution of multiprotein complexes. Similarly, BRD2 (REFS 166,167), BRD3 (REF. 168) and BRD4 (REFS 61,168) interact with Kaposi's sarcoma-associated herpesvirus (KSHV; also known as HHV8)-encoded latent nuclear antigen 1 (LANA1), a functional analogue of HPV E2 protein. These BRD proteins contribute to LANA1-regulated transcription[61], promoting cell cycle progression. KSHV is a common co-infection among patients infected with HIV and has additionally been implicated in two lymphoid tumours: primary effusion lymphoma and multicentric Castleman's disease. Reinforcing the theme of context-dependent co-activation or co-repression by the same factor, LANA1 transcriptionally activates some genes, such as E2F-dependent cell cycle genes[169], whereas it represses others, such as p53-dependent pro-apoptotic genes[170], to promote the proliferation of KSHV-infected cells.

It has been proposed that in Epstein–Barr virus (EBV) episomal maintenance, transformation and latency, the EBV nuclear antigen (EBNA) proteins, particularly EBNA2, provide a functional analogue of LANA1. EBV is the causative agent of lymphoproliferative diseases, lymphomas and certain other malignancies that develop on EBV-driven immortalization. BRD4 recruits the transcription elongation complex P-TEFb to the viral C promoter, and JQ1 (50 nM for 48 hours *in vitro*) reduces BRD4 association with the promoter, as demonstrated by chromatin immunoprepitation[171]. This result introduces the possibility that JQ1 or newer small molecules that are more specific for BRD4 might have therapeutic value for EBV-associated malignancies.

A serious safety concern arises on consideration of these small-molecule inhibitors that alleviate BET protein co-repression: human genomes carry diverse, asymptomatic, cell type-specific DNA viruses in a latent form, including HIV. In the case of HIV, this reactivation might offer a solution for virus eradication, but only in the context of intensive anti-retroviral therapy (M. Montano, personal communication). Inadvertent re-activation of some of these latent viruses, of uncertain provenance, could promote viraemia with highly undesirable oncogenic or immunotoxic effects.

Unlike for EBV, inhibition of BRD4 function with JQ1 treatment has not yet been reported for HPV- or KSHV-associated tumours, but represents an obvious, immediately available approach for additional experiments. Targeted delivery of JQ1 or next-generation small molecules is likely to perturb viral transcription and latency in possibly useful therapeutic ways. In each case, the net outcome will probably be determined primarily by re-association and redistribution of the enzymatic and nucleosome remodelling activities that are separately recruited by the BRD4 CTD and ET domains[40], which depend on promoter context and respond to signal transduction demands, not by ablation of BRD4 itself.

Box 3

Parallels with the SWI/SNF nucleosome remodelling complex

Bromodomain-containing transcription complexes functionally resemble SWI/SNF chromatin remodelling complexes (with which BRD2 proteins associate) and exert opposing effects in cell cycle control[172,173]. Chromatin remodelling machines, such as the SWI/SNF complex, alter the structure of the nucleosome in an ATP-dependent manner by modifying the histone–DNA interface and causing nucleosome sliding[174]; they are capable of activating or repressing genes. Mammalian SWI/SNF comprises a 2 MDa subunit complex that possess SMARCA4 (also known as BRG1) or SMARCA2 (also known as BRM), and an additional 9–12 proteins known as BRM/BRG1-associated factors (BAFs)[16]. The majority of genes frequently classified as targets of SWI/SNF enzymes are dependent on either SMARCA4 or SMARCA2, but not on both. Although the different SWI/SNF complexes share many if not most of the same subunits, they are distinguished by the presence of either SMARCA4 or SMARCA2, and unique subunits or tissue-specific variants[16,175,176]. *Smarca4*$^{-/-}$ mice are embryonic lethal[177], whereas *Smarca2*$^{-/-}$ mice show a relatively mild phenotype[178]. The involvement of bromodomain-containing factors in both modes of chromatin remodelling, establishment of transcriptionally active euchromatin or transcriptionally silent heterochromatin, was first appreciated in yeast, in which *swi/snf* mutations were observed to turn on as many genes as were turned off[179,180]. Thus, SWI/SNF and BET complexes both co-activate and co-repress genes, depending on the context.

BRD2 activity is also increased in some human leukaemias[27], but there is only limited genetic evidence linking *BRD2* disruption to human cancer. The Mitelman database of recurrent chromosomal abnormalities associated with cancer (see the Mitelman database; see Further information) identifies >500 patient haematological malignancies involving breakpoints at 6p21 that could potentially affect *BRD2*, but only a small minority have been mapped with high resolution. The extraordinary amount of polymorphism in this region makes accurate mapping onerous and has slowed progress on this investigation. However, data from mice indicate that BRD2 could potentially be oncogenic in humans. Constitutive expression of a *Brd2* transgene in the lymphoid lineage of mice (under Eμ promoter or enhancer control, resulting in B cell-restricted expression), transcriptionally co-activates cyclin A (*Ccna2*)[38] in resting B cells, eventually causing B cell malignancy[93]. Transcriptional profiling of this aggressive tumour reveals a transcriptional signature that is most similar to the activated B cell (ABC) type of diffuse large B cell lymphoma, in which several genes with developmental functions are re-activated[93,94]. ABC lymphomas are characterized by the activation of nuclear factor-κB (NF-κB)-regulated genes, which is consistent with the phenotype of *Brd2*-hypomorphic mice described below and the effect of I-BET on NF-κB-regulated cytokines. Evidence for the involvement of *BRD3* in cancer remains preliminary. *BRD3* is involved in certain NMC translocations[3,95], and it potentially associates with MLL fusion oncoproteins in leukemogenesis[92] and with MYC in multiple myeloma[96]. *Brd3*-transgenic or knockout animals have not been reported, nor have *Brd4*-transgenic animals been developed. New mouse models of BET protein-driven malignancy are clearly needed; these models could be of great translational importance for the mechanistic study of haematological malignancy, as well as for the testing of next-generation, small-molecule BET protein inhibitors as cancer chemotherapeutic agents.

BET proteins, inflammation and obesity

Inhibitors of BET proteins also have anti-inflammatory properties. An I-BET compound that is structurally similar[97] to JQ1 demonstrates anti-inflammatory properties; however, I-BET

does not discriminate among BET family members (FIG. 1b). Nicodeme and colleagues have shown, in a well-established model system of pro-inflammatory cytokine production from bone marrowderived macrophages challenged by bacterial endotoxin, that I-BET suppresses the expression of several crucial pro-inflammatory cytokines and chemokines (such as interleukin-1β (IL-1β), IL-6, IL-12α, CXCL9 and CCL12). Chromatin immunoprecipitation experiments show that I-BET displaces BRD2, BRD3 and BRD4 from the *IL6* promoter, which was used as a model[97]. Most dramatically, I-BET injections (30 mg per kg by the retro-orbital or the tail-vein route) rescued mice from endotoxin-induced death, and caecal ligation and puncture-induced death. An important observation noted in that report[97] is that I-BET upregulates hexamethylene bis-acetamide inducible protein 1 (HEXIM1), and this has also been reported for JQ1 in other model systems[81,96]. HEXIM1 inhibits P-TEFb function, suggesting that I-BET and JQ1 can also block proliferation through MYC-independent mechanisms. HEXIM1 also inhibits NF-κB-dependent target genes and so inhibits the transcription of pro-inflammatory cytokine genes[98]. The investigators report that the expression of key inflammatory molecules, for example, tumour necrosis factor (TNF), monocyte chemotactic protein 1 and a number of other chemokines, was not ablated by I-BET treatment, which does not make sense in view of the coordinate regulation of NF-κB-dependent transcriptional programmes that are known to be mobilized in macrophages in response to endotoxin challenge. In addition, a new class of dimethyl isoxazole derivatives that inhibit BET bromodomains[99,100] has also been shown to have anti-inflammatory properties in cellular assays, in which they block IL-6 and TNF production from bacterial endotoxin-challenged human peripheral blood mononuclear cells[101].

Consistent with the ability of small-molecule BET protein inhibitors to ablate inflammation, gene disruption of *Brd2* in mice ablates a broad range of inflammatory responses and protects animals from the inflammatory complications of obesity-induced insulin resistance[65]. Gene disruption was accomplished by *lacZ* insertion into a region of the gene that is 5′ to the translational start site. This insertion only reduces rather than eliminates whole-body expression of BRD2 (*Brd2 lo*) to produce a hypomorphic phenotype, thus making these mice viable. The resultant low-inflammatory characteristics of *Brd2 lo* mice include uncoupling of Toll-like receptor (TLR) and TNF signalling[65,102] from NF-κB-directed transcription of diverse pro-inflammatory cytokine genes[103]. The mouse phenotype provides a model for a population of obese humans for whom obesity is not entirely bad[104]; population studies have not unfailingly identified an inviolable association between obesity and cardiometabolic risk. These metabolically healthy obese (MHO) subjects[105] comprise about 25% of the adult obese population in the United States. Importantly, along with preserved insulin sensitivity and excellent glucose tolerance[106], MHO subjects exhibit a reduced inflammatory profile, including less severe elevations of C-reactive protein, TNF and other pro-inflammatory cytokines in the context of their obesity[107,108]. Compared with insulin-resistant obese subjects, MHO subjects have fewer metabolic complications associated with obesity, including insulin resistance, metabolic syndrome, hypertension, type 2 diabetes and cardiovascular disease, a protection that has been attributed in no small part to their low-inflammatory responses[109,110]. These subjects are also protected from all-cause cancer mortality compared with insulin-resistant obese subjects[111]. It is intriguing to speculate that MHO individuals may harbour alleles of BET genes, particularly *BRD2*, that confer protection from metabolic dysfunction or cancer in obesity.

Beyond haematological malignancy, it remains to be tested whether single nucleotide polymorphisms of human BET genes or alternatively spliced forms of BET mRNAs influence body composition of adults, including adiposity and fat distribution, insulin resistance, inflammatory risks and associated co-morbidities, such as the obesity-associated cancers[103]. There is recent evidence that, in addition to BRD2, BRD4 can also co-activate

pro-inflammatory genes that depend on NF-κB transcription, through interaction with acetylated RELA[112]. A full account of the interplay of BRD2, BRD3 and BRD4, and how they co-activate NF-κB and cooperate with SWI/SNF complexes[113] to regulate the transcription responses of genes that encode important pro-inflammatory cytokines, such as TNF and IL-6, awaits exposition. These data are potentially relevant to the links between unresolved chronic inflammation or irritation and increased cancer risk[114], a long-established association[115]. For example, the bowel inflammation that is characteristic of Crohn's disease and related conditions[116] is strongly linked to colorectal cancer[117]. It is possible that inflammation promotes certain obesity-associated cancers that are resident in or near to inflamed white adipose tissue in insulin-resistant obese subjects[103]. The role of unresolved, chronic inflammation and metabolic dysfunction in obesity-associated cancers is a considerable public health problem, and new epigenetically acting drugs such as the BET protein inhibitors might provide a novel pathway for treating or preventing obesity-associated cancer. Additional preclinical studies are required to more firmly establish the mechanisms underlying hypotheses that the anticancer and anti-inflammatory properties of BET protein inhibitors usefully combine in a chemopreventive strategy for the obesity-associated cancers.

BET proteins and transcriptional repression

The obese phenotype of *Brd2*-hypomorphic mice is partly due to the activation of peroxisome proliferator-activated receptor-γ (PPARγ)-directed transcription in adipocytes[65,103]. Independent support for this mechanism comes from experiments in which short hairpin RNA (shRNA) knockdown of *Brd2* in 3T3-L1 pre-adipocytes strongly promotes adipogenic differentiation. BRD2 directly interacts with PPARγ and opposes its transcriptional function[65,118]. The discovery that a BET protein can also function to repress transcription in differentiated adult cells raises some interesting questions. This mechanism suggests that a balance exists on PPARγ-controlled promoters between BRD2-associated repressive factors, including N-CoR, SMRT and repressive SWI/SNF complexes, and co-activating factors, such as PPARγ–RXR-ligand complexes, HATs and activating SWI/SNF complexes (FIG. 4). Interestingly, the Nicodeme group[119] has described a new class of benzodiazepine-based small-molecule inhibitors with anti-inflammatory effects. These molecules were identified in a screen for compounds that upregulate the transcription of the atheroprotective gene apolipoprotein A1 (REF. 119). Although these authors do not explicitly define the molecular mechanism for this upregulation, the screen almost certainly depends on the alleviation of BET protein repression complex that is present at the human apolipoprotein A1 promoter in hepatocyte HepG2 cells.

In retrospect, it is not surprising that BET protein knockdown or inhibition should derepress the transcription of certain genes, as this was first suggested for BRD2 more than 10 years ago[29], and transcriptional repression mechanisms have long been apparent in BET homologues in other model organisms. The gene product of *BDF1* (REF. 68), is a 'reader' of histone acetylation[120] in *Saccharomyces cerevisiae* and is important for chromatin restructuring[69]. In *D. melanogaster*, the BET homologue *female sterile (1) homeotic* (*fs(1)h*) has important transcriptional repression functions that are essential for proper differentiation in the early embryo[121,122]. Like the SWI/SNF complexes, BET protein complexes can function as both co-activators and co-repressors[103,123]. They recruit either HATs or HDACs depending on the requirements of signal transduction and the promoter to which they are bound[36]. This behaviour is enacted with other bromodomain proteins, such as SMARCA2, which binds to the tumour suppressor protein RB[124,125] and facilitates co-repression of cell cycle genes through the recruitment of histone deacetylases[126–129] (BOX 3).

It has recently been suggested that small-molecule inhibitors of BET proteins, such as JQ1, exert their anti-proliferative effects in AML simply as anti-proliferative agents, with more rapidly growing cells being more sensitive[130], much the same way that antimetabolite therapies work in diverse malignancies by targeting cells with high mitotic indices. Although downregulation of MYC in several haematological malignancies undoubtedly contributes to the anti-proliferative phenotype, because of the dual nature of bromodomain-containing multiprotein complexes and their cousins the SWI/SNF complexes, this view is incomplete. Rather, we propose that inhibition of BET proteins works through transcriptional reprogramming of a network of target genes, ablating co-activation of cell cycle genes while simultaneously ablating co-repression of other specific, differentiation-associated genes (FIG. 5). Thus, the altered balance of co-activation and co-repression reprogrammes cell fate. The long-appreciated antagonism between haematopoietic proliferation and differentiation[131,132] might be explained in part by these dual, opposing functions[123] of BET protein-containing co-regulator complexes.

The requirement for BET protein homologues in proper development, pattern formation and stable cell fate in Metazoans, particularly BET-1 in the roundworm[60], FS(1)H in the fruitfly[121] and Brd2a and Brd2b in the zebrafish[133] indicates that this co-regulator system is functionally well conserved. The high degree of relatedness at the amino acid level among bromodomains of diverse species[4,5] attests to evolutionary conservation. It follows from this observation and their trithorax-like functions (BOX 1) that BET proteins are also likely to engage an antagonistic relationship with the Polycomb (PcG) group of co-repressors. The balance of trithorax group and PcG genes in the silencing and derepression of transcriptional networks of genes during the development of model organisms is well studied, but BET gene interactions in different tissues during human development are totally unexplored. Interestingly, the single bromodomain protein human BRD7 (REF. 134) tightly interacts with protein arginine methyltransferase 5-containing SWI/SNF complexes, as well as with three core subunits of the PcG repressor complex (PRC)2, that differentially regulate transcriptional silencing and derepression[135]. In yeast, there is evidence that Bdf1 opposes silencing by restricting the heterochromatic spreading of silent information regulator (SIR) sirtuin proteins[136]. In addition, *BDF1*, but not *BDF2*, was recently identified as a suppressor of mutation in the variant histone H2A.Z pathway, which regulates heterochromatin silencing in *S. cerevisiae*[137], which is consistent with a crucial, fundamental role for BET proteins in chromatin states. It seems likely that additional bromodomain proteins will be found to interact as co-regulators with different families of trithorax and PcG proteins to direct transcriptional programmes that influence development and pattern formation.

It is not coincidental that when developmentally crucial circuits of homeotic genes are disrupted by reciprocal chromosomal translocations in humans, malignancies, particularly acute leukaemias, are a common outcome[138,139]. For example, perturbation of BET protein expression can lead not only to increased proliferation in malignancy, but also to the reactivation of developmentally silenced genes[94], supporting a long-held view that a class of malignancies derived from corrupted transcription factors bear the morphological and transcriptional signatures of poorly differentiated tissues[2,3,95] or progenitors of the cell lineage in which the tumour arises. Similar patterns of poorly differentiated sarcomas have been reported for certain tumours arising from mutations in SWI/SNF subunits[140]. Mutations in SWI/SNF subunits likewise reveal crucial roles for chromatin regulation in adipogenesis, osteogenesis and haematopoiesis. Thus, both SWI/SNF complexes and BET protein complexes are likely to contribute to cell fate in the adult by associating with lineage-specific transcription factors to regulate exit from the highly proliferative state of progenitors and entry into the terminally differentiated state of specialized tissues.

Conclusions and implications

Consideration of BET protein functions discussed above informs the hypothesis that networks of apparently orthogonal transcriptional programmes — adipogenesis, inflammatory cytokine production, cell cycle control and developmental programmes — are actually deeply interrelated because they share this limited set of epigenetic actors. It is true that dedicated adipogenic transcription factors (such as PPARγ), inflammatory transcription factors (such as NF-κB), cell cycle transcription factors (such as E2Fs) and lineage-specific transcription factors (such as PU.1 and TBET) are required for cell-specific responses to signal transduction in each case. However, the phenotypes observed in *Brd2*-manipulated mice or cells cannot be explained by the alteration of a single pathway, nor do they lead to genome-wide transcriptional confusion, senescence, apoptosis or other catastrophic outcomes. Thus, the extent of BET protein-dependent transcriptional programmes is limited. A possible complication to small-molecule inhibition of BET family bromodomain interactions is that other histone-binding proteins might become available to assume transcriptional co-regulator functions once the BET protein is displaced from chromatin. Similarly, changes in histone acetylation patterns can redistribute important catalytic activities such as DNA topoisomerase II (REF. 141) and mutation of chromatin-regulatory enzymes can redistribute histone variant proteins[142] with major consequences for DNA replication and the cell cycle. Redistribution of co-activator and co-repressor complexes on ablation of a BET protein might in some contexts cause unexpected transcriptional activation or repression of transcriptional networks. However, small-molecule BET protein inhibitors have now been shown to be well-tolerated, potent, epigenetically acting, potential anti-neoplastic agents for BRD4-driven NUT midline carcinoma[79], multiple myeloma[96], AML[84] and MLL[92]. Mice seem to tolerate high doses of JQ1 without major, acute systemic side effects[79,81,84]. The discovery that BRD3 associates with acetylated GATA1 (REF. 143), a transcription factor that is crucial for normal erythroid and megakaryocytic development, raises concerns that BET protein inhibition might be associated with haematopoietic toxicity. Both JQ1 and an I-BET-like derivative GW841819X inhibit this interaction[144], but apparently do not show obvious myelotoxic side effects in mice. Animal models chronically treated with these inhibitors should be more fully evaluated for the suppression of specific haematopoietic lineages. Alternatively, the apparently minimal side effect profile may indicate that BET protein complexes are mobilized in response to specific signal transduction in specialized, differentiated cells of the adult and do not constitutively regulate ordinary metabolic processes or housekeeping functions.

It follows from the above discussion that ablation of BET co-regulator complexes is likely to be simultaneously anti-neoplastic and anti-inflammatory. Pro-adipogenic transcriptional networks that are controlled by PPARγ are also expected to be activated by BET protein inhibition, based on the phenotype of *Brd2*-hypomorphic mice. It will be interesting to determine whether MHO humans have a lower risk for obesity-associated cancers, attributable to reduced inflammatory responses[103]. The mechanistic links between inflammation and cancer[114], and between inflammation and insulin-resistant obesity[106–110], ground an overarching hypothesis that, at the transcriptional level, chronic inflammation in obesity exacerbates risk for both metabolic complications and cancer. The ongoing and anticipated dire consequences of the world wide epidemic of obesity[145] highlight the translational importance of this discovery, because about 90% of type 2 diabetes is attributable to obesity[146]. Chemoprevention of obesity-associated cancers by uncoupling NF-κB-driven cytokine gene expression with small-molecule BET protein inhibitors would represent an innovative, epigenetically based approach to protect obese subjects who are at risk of both diabetes and cancer. Targeting one set of processes with a BET protein inhibitor might confer benefit by targeting other, apparently orthogonal transcriptional networks that

are actually fundamentally related. An extraordinary interconnectedness of chromatin-dependent transcription programmes is thus revealed.

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Glossary

Mediator transcription complex	Mediator is a large (1.2 MDa) multiprotein complex of up to 30 subunits that responds to signal transduction and that regulates transcription from a diverse set of RNA polymerase II-controlled promoters
Thienodiazepines	A chemical structural class built on a seven-membered 1,4-diazepine ring fused to a thiophene ring that provides a scaffold for derivatives of great current interest to medicinal chemistry because they target several pharmacologically important proteins, such as certain benzodiazepine derivatives that have neurological activity
Insulin resistance	A complex phenotype arising from reduced insulin responsiveness in tissues that transport glucose in response to insulin action, such as adipose tissue and skeletal muscle

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Biographies

Anna C. Belkina is a doctoral student in the Program in Molecular Medicine at Boston University School of Medicine, USA. Along with Fangnian Wang and others, she contributed the first report of functional involvement of a BET protein in mammalian inflammation, adipogenesis and energy balance.

Gerald V. Denis is a faculty member in the Cancer Research Center and Department of Pharmacology and Experimental Therapeutics of Boston University School of Medicine. He discovered mammalian BET protein function. Building on the knowledge base of BET protein mechanisms in *Drosophila melanogaster* homeosis, he was the first to show that constitutive expression of a BET protein in mice is oncogenic and that reduced expression reveals a co-repression mechanism of major importance for obesity and inflammation. These observations underpin the new hypothesis that reduced inflammation protects against obesity-associated cancers, in part through BET protein co-regulation of transcriptional networks.

At a glance

- Mammalian BET proteins, a class of transcriptional co-regulators that contain dual, mutually related bromodomain motifs and an extraterminal domain, are important in the control of networks of genes; these proteins bind to acetylated lysines in the histones of nucleosomal chromatin, recruit chromatin-modification enzymes to target promoters and function as co-activators or co-repressors of gene expression, depending on the context.
- New small-molecule inhibitors have recently been developed that disrupt the binding interface between the bromodomain and the acetylated lysine groups; the inhibitors have remarkable potency, selectivity and are well tolerated. They have recently been used as anticancer and anti-inflammatory agents.
- These developments are important because chromatin was not considered to be a druggable target; as a result of these new drugs, a whole field of new epigenetically targeted therapeutics has become available for investigation.
- As this field of therapeutics rapidly expands, several features of BET protein function will need to be considered, including possible redundancy among the closely related family members, the selectivity of next-generation agents for specific BET proteins, and possible undesirable consequences of systemic administration without cellular targeting. These side effects might include uncontrolled transcriptional derepression of genes, altered haematopoiesis, immunosuppression or reactivation of latent viruses.

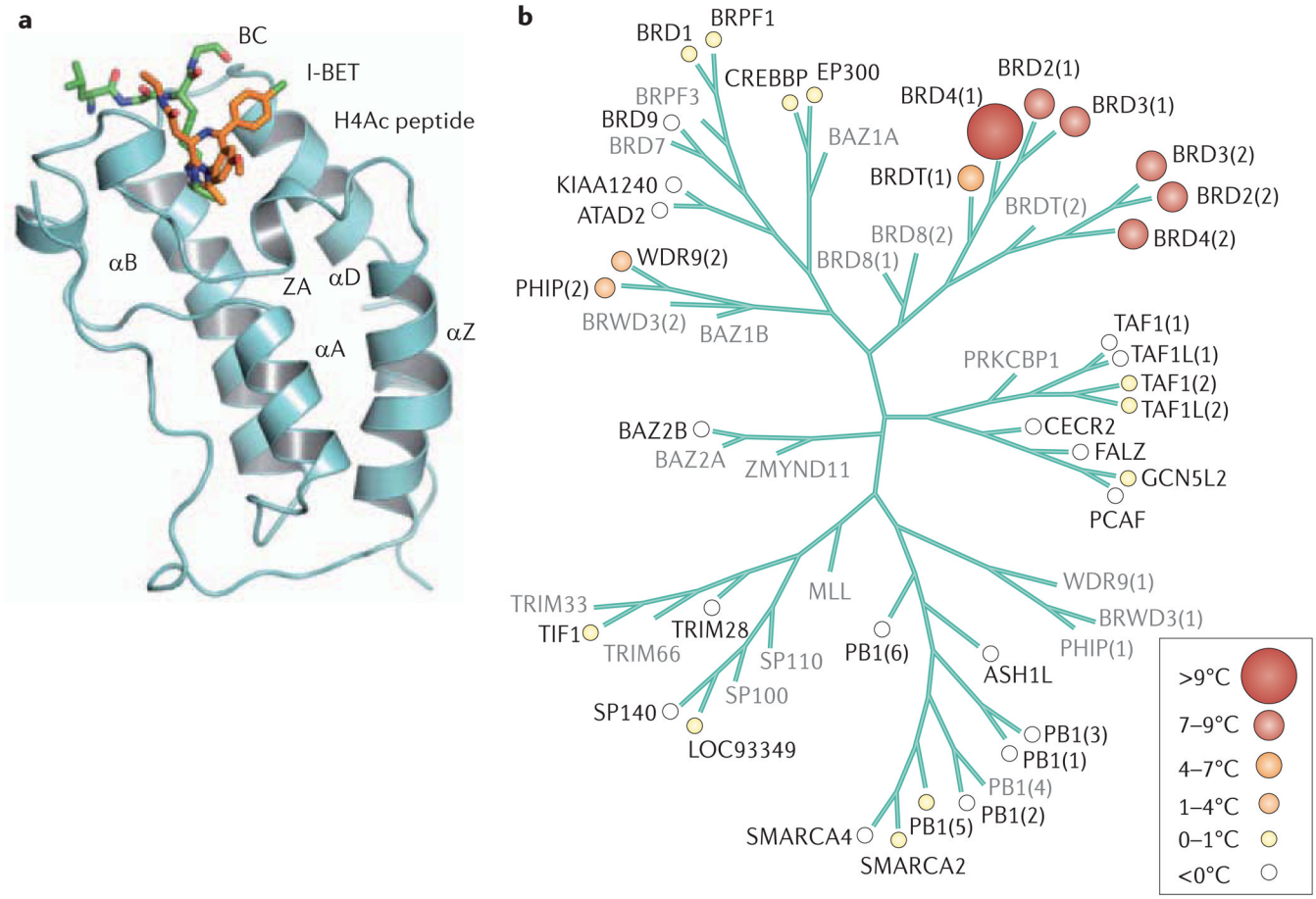

Figure 1. Structure and relationships among bromodomain-containing proteins

a. The anti-parallel α-helices of the bromodomain bundle are shown in association with the small-molecule inhibitor I-BET and a histone H4 lysine peptide acetylated at position 12 (REF. 97). The BC and ZA loops form the binding pocket for the ε-acetyl-lysine groups of nucleosomal histones in the structure, which the Zhou group first described in detail[13]. **b.** Relatedness among bromodomain families, as defined by selectivity for JQ1, is measured by differential scanning fluorimetry[79]. The BET proteins BRD2, BRD3 and BRD4 are shown to be closely related, with respect to both the first and second bromodomains, as well as the first bromodomain of BRDT. The second bromodomain of BRDT was not tested (shown in grey). Part **a** is reproduced, with permission, from REF. 97 © (2010) Macmillan Publishers Ltd. All rights reserved. Part **b** reproduced, with permission, from REF. 79 © (2010) Macmillan Publishers Ltd. All rights reserved.

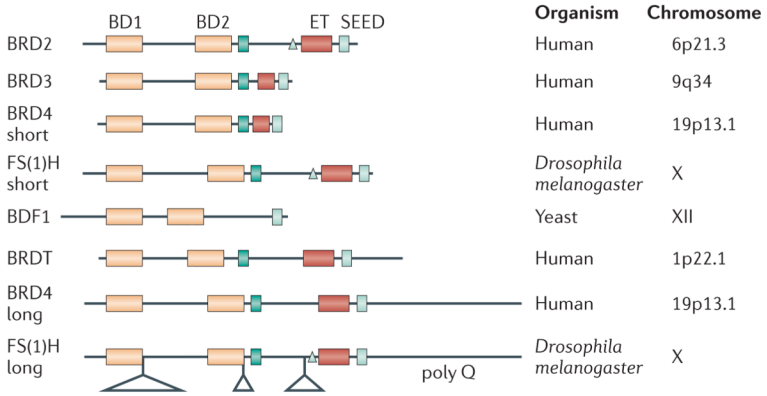

Figure 2. Motif alignment of double bromodomain-containing proteins

The dual, tandem bromodomains (BDs) are mutually related and always positioned at the amino terminus, where anchoring to nucleosomal histones is encoded, and the carboxy-terminal end of each polypeptide is available for interaction with chromatin-modifying factors, transcription factors, histone-modification enzymes and other proteins. This recruitment takes place either through poorly understood protein–protein interaction ET domains or through SEED domains rich in acidic, phosphorylatable amino acids that resemble the C-terminal domain of RNA polymerase II. The human, fruitfly and yeast proteins contain putative or verified nuclear localization signals (shown in blue) or ATP binding, kinase catalytic sites (shown by grey triangles). Additional features of the *Drosophila melanogaster* protein include large insertions (represented by triangles) and stretches of polyglutamine (poly Q), a motif that is frequently associated with transcriptional activation. The C-terminal domain of the long isoform of BRD4 is unstructured and does not contain well-established protein–protein interaction or transcriptional-activation motifs, but is nevertheless partly responsible for functional differences between this isoform of BRD4 and other, shorter BET proteins. The chromosome on which each gene is located is identified.

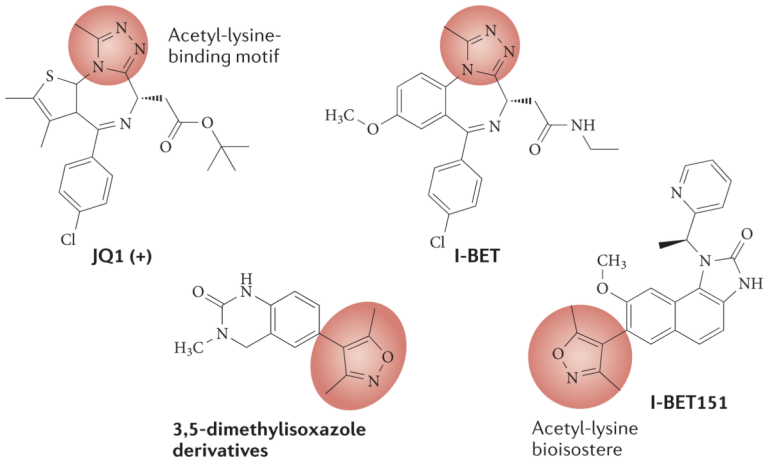

Figure 3. Small-molecule inhibitors of BET proteins

Recently reported chemical structures and measurements of inhibition constants (IC_{50}) or dissociation constants (K_d) for JQ1 (REF. 79), I-BET (REF. 97), I-BET151 (REF. 92) and other structures that incorporate acetyl-lysine bioisoteres[99] are shown. The parts of each molecule that displace the ε-acetyl-lysine group of the histone are circled in red.

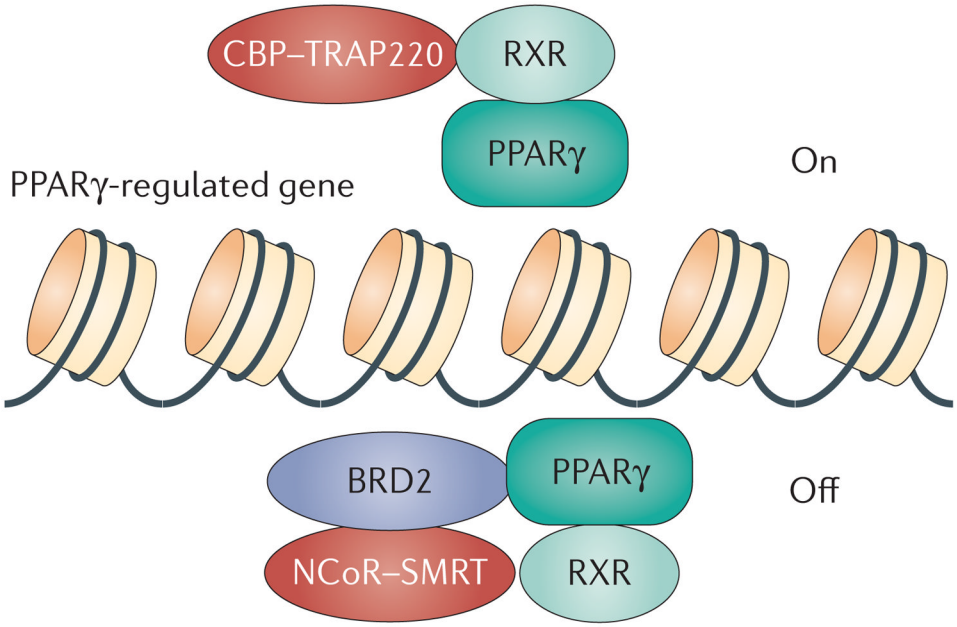

Figure 4. Model for BET protein co-repression of PPARγ-responsive genes

Transcriptional co-repression of specific loci is an active process that requires the recruitment of repressor complexes. In the case of peroxisome proliferator-activated receptor-γ (PPARγ), co-repression is enabled through BRD2 association with RXR, which is known to heterodimerize with PPARγ. Removal of BRD2 by genetic ablation promotes the transcription of adipogenic networks[118], analogous to thiazolidinedione drug treatment. Small-molecule inhibitors of BET proteins would be expected to produce a similar result.

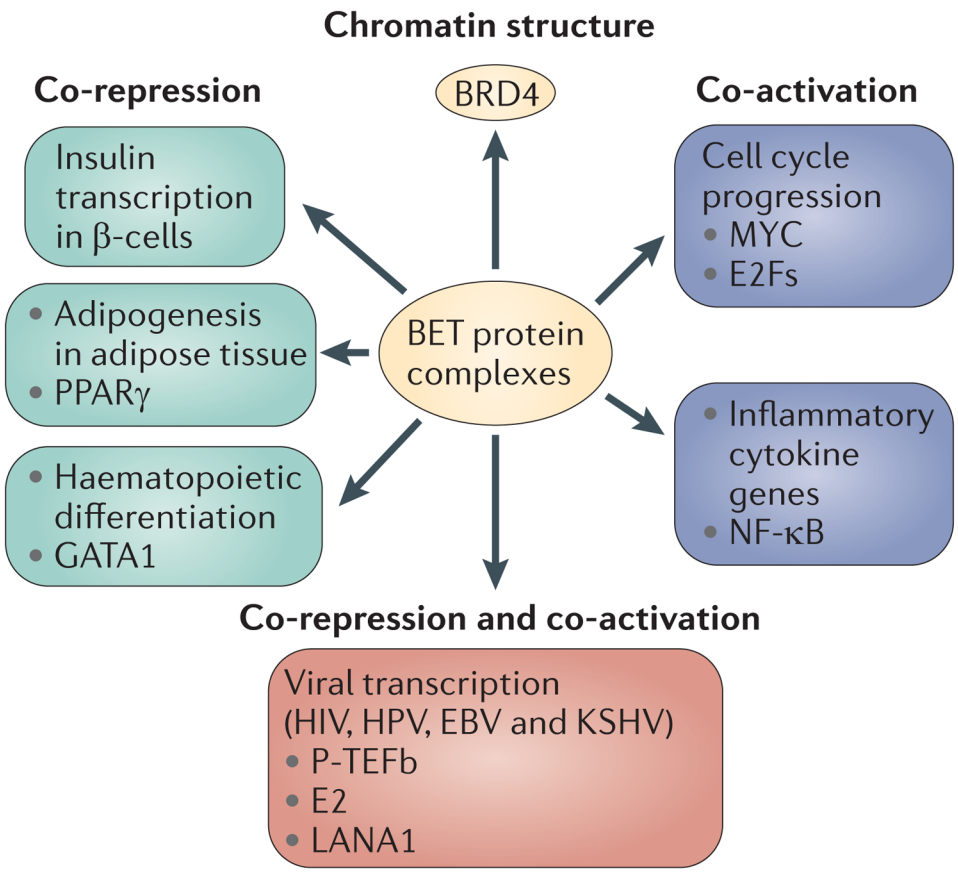

Figure 5. BET proteins co-regulate transcriptional networks of transcriptional activation and repression

Several functional networks are co-regulated by BET protein interactions. Some interactions involve transcriptional co-repression, such as insulin transcription[65], peroxisome proliferator-activated receptor-γ (PPARγ)-controlled adipogenic differentiation in adipose tissue[65,118] and GATA1-controlled haematopoietic differentiation[143,144]. Other interactions involve transcriptional co-activation, such as the activation of genes that promote cell cycle progression controlled by MYC[81,84,92,96] and E2F proteins[28,29,34,37,38]; nuclear factor-κB (NF-κB)-controlled synthesis of pro-inflammatory cytokines[65,97,101,102]; and cellular genes regulated by P-TEFb. The transcription and replication of latent viruses seem to exploit BET protein capacity for either co-repression or co-activation, depending on the demands of the virus, through P-TEFb[40,44,45,47,171], human papilloma virus (HPV) E2 protein[157–165] or Kaposi's sarcoma-associated herpesvirus (KSHV) LANA1 protein[61,166–170]. Recent data also implicate BRD4 in the maintenance of higher order chromatin structure. EBV, Epstein–Barr virus.

Table 1

Selected reported BET protein phenotypes*

Organism	Gene	Mutant or forced expression phenotype	Null	Refs
Saccharomyces cerevisiae	*BDF1*	Sporulation defect and chromatin	Lethal	57,68,69,120
Arabidopsis thaliana	*GTE4* and *GTE6*	Mitosis, cell cycle, cell fate pattern formation and histone acetylation	Unknown	58,59,181
Drosophila melanogaster	*fs(1)h*	Chromatin, maternal effect and homeotic transformation	Lethal	121,122,148–150
Danio rerio	*brd2a*, *brd2b* and *brd4*	Pattern formation, mitosis maternal effect and mitotic chromatin	Unknown	55,133
Caenorhabditis elegans	*bet-1*	Unstable cell fate	Lethal	60
Mus musculus	*Brd2*	Cancer, mitosis and cell cycle	Lethal	63–65,93
M. musculus	*Brd4*	Organ defects, mitosis and cell cycle	Lethal	48,49,51,66
Homo sapiens	*BRD2*	Cancer, virus transcription and inflammation	Unknown	27,92,96,101,166–168
H. sapien	*BRD4*	Cancer, episomal maintenance, virus transcription and higher order chromatin structure	Unknown	1–3,40,45,47,61, 159–165,182

* Published reports of phenotypes arising from mutation or expression of BET proteins under the control of a heterologous promoter in different species: yeast (*S. cerevisiae*), plants (*Arabidopsis*), flies (*D. melanogaster*), zebrafish (*D. rerio*), roundworms (*C. elegans*), mice (*M. musculus*) and humans (*H. sapiens*). Genes are indicated along with phenotypes. Comprehensive information for null phenotypes is not available for all species.