[Frontiers in Bioscience 15, 537-549, January 1, 2010]

WHAT do viruses BET on?

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. BET proteins
3.1. Brd2/RING3
3.2. Brd4/HUNK-1/MCAP
4. BET proteins and viruses
4.1. Brd4 and papillomavirus
4.2. BET proteins and KSHV
4.3. BET proteins and other herpesviruses
4.4. BET proteins and retroviruses
5. Concluding remarks
6. Acknowledgements
7. References

1. ABSTRACT

The bromodomain and ET domain (BET) proteins belong to a group of bromodomain proteins and bind acetylated histones. Two of the currently known members of this protein family were implicated in transcriptional regulation. The two most studied BET proteins Brd2 and Brd4 have been shown to bind to viral proteins of herpesviruses and papillomaviruses. These pathogens often take advantage of the cellular function of the BET proteins and exploit it for their own purposes. In some cases though, viral proteins were shown to adapt BET proteins to new virus specific functions. Additionally some retroviruses seem to encode proteins that mimic Brd4 functions and hijack Brd4-associated protein complexes to use them for their own transcription.

2. INTRODUCTION

BET proteins have been found in plants, fungi and animals. They are defined by the presence of one (plants) or two (yeast and animals) bromodains followed by the conserved extra terminal (ET) domain. The function of the ET domain is not yet known, but some studies show the ET domain to be involved in protein-protein interactions (1-6). The bromodomain is a 110 amino acids long domain, whose central 60 amino acid motif is conserved from yeast to mammals (7). Its name is derived from the Brahma protein in *Drosophila*, the first protein in which it was identified (8). The characteristic feature of a bromodomain is its ability to bind acetylated lysine residues on histones. Therefore it is not surprising that this domain is often found in chromatin associated factors. There are three major

groups of bromodomain containing proteins: (i) histone acetyltransferases (HATs) eg. Gcn5, P/CAF, TAF1, CBP, (ii) ATP-dependent chromatin remodeling complexes eg. Brahma, Swi2, Snf2, Brg1 and (iii) bromodomain and extra terminal domain (BET) proteins (7). Currently known BET proteins in mammals are: Brd2/RING3 (really interesting new gene 3), Brd3/ORFX, Brd6/BRDT (bromodomain testis specific) and two splice variants of Brd4: MCAP (mitotic chromosome associated protein) and HUNK-1. Of these the most studied and therefore also the focus of this review, are Brd2 and Brd4.

In recent years an increasing number of viral proteins was reported to interact with members of the BET family of proteins or interfere with their function. Here we discuss examples of viral interference with, or exploitation of, BET protein function.

3. BET PROTEINS

3.1. Brd2/RING3

Brd2/RING3 was originally identified as a mitogen-stimulated nuclear kinase (9). In serum starved cells, Brd2/RING3 localizes throughout the cell, but shows an exclusively nulear pattern after mitogenic stimulation and in exponentially growing cells (9, 10). The nuclear translocation of Brd2/RING3 was required for its transcriptional activation function (10). Brd2/RING3 transactivated promoters of the cell cycle genes: cyclin D1, cyclin A, cyclin E, and dihydrofolate reductase (dhfr). This activation appeared to be dependent on *ras* signalling and on the interaction with E2F transcription factors (1). Brd2 has been shown to bind specifically to acetylated Lys12 on histone H4 through both of its bromodomains (11-13). Sinha and coworkers have demonstrated that histone H4-specific HAT (histone acetyl transferase) activity was present in the Brd2 complex, and postulated that Brd2/RING3 could be involved in spreading the acetylation through the chromatin and hence transforming it into a transcriptionally active state (14). It has been observed that Brd2/RING3 mediates histone acetylation at the cyclin A promoter as well as recruitment of E2F transcription factors to this promoter (14). Induction of cyclin A by ectopically expressed Brd2 accelerates G1 to S transition (15). Since Brd2 was found to be associated with the cyclin A promoter both in the G1 phase, when cyclin A expression is low, as well as in the S phase, when cyclin A expression is high, a model was proposed, according to which Brd2 could associate with both HATs and HDACs (histone deacetylases) and thereby mediate both transcriptional activation as well as repression (14). Indeed, HDAC was subsequently found to be associated with Brd2 (16).

The role of Brd2/RING3 in transcriptional regulation is also supported by the observation that it associates with the RNA polymerase II Mediator complex, which is a part of the polymerase holoenzyme (17). The minimal polymerase II (pol II) transcription initiation complex consists of: TFIID (containing TATA binding protein (TBP) and TBP associated factors (TAFs)), TFIIB and RNA pol II, although only TBP, TFIIB and polII are necessary for basal transcription level (18). Activated transcription requires the Mediator complex in addition to TAFs (19). Transcriptional activators carry out their function by interacting with basal transcriptional machinery as well as with TAFs and the Mediator. Brd2 seems to be able to interact with RNA pol II, TBP, TAFs, Mediator components as well as members of the SWI/SNF chromatin remodeling complex, hence regulating transcription at multiple levels (16, 20). This suggests that Brd2 acts as a scaffold mediating the access of proteins regulating transcription to chromatin (21).

Recently, Brd2/RING3 has been observed to participate actively in transcription of cyclin D1 *in vivo*, allowing transcriptional elongation through acetylated nucleosomes. This activity was dependent on bromodomains and their ability to bind to acetylated histone tails. Brd2 appeared to remove nucleosomal barriers to transcription elongation by RNA polymerase II. Exactly how Brd2 could assemble and disassemble nucleosomes is not known (22).

Brd2/RING3 has oncogenic potential since it was shown to transform NIH 3T3 cells in the presence of activated *ras* (1). Transgenic mice with expression of Brd2 restricted to B-cells have elevated levels of cyclin A and develop lymphoma and upon transplantation leukemia (15). Some subtypes of human lymphoma, in which Brd2 is underexpressed, show similarities to the Brd2-mediated murine tumours (23). Using genome wide transcriptional expression profiles, Lenburg *et al.* have compared mouse derived tumours with proliferating and resting normal B cells. They have identified many genes, previously shown to participate in human lymphomagenesis, to be differentially expressed in Brd2 transgenic murine lymphomas. Taken together, it appears that Brd2 levels are critical for normal cell cycle progression and proliferation.

3.2. Brd4/MCAP/HUNK-1

There are two splice variants of the BRD4 gene in mammals: a short variant – HUNK-1 and a long one – MCAP. Brd4 binds to acetylated Lys14 on histone H3 and Lys5 and Lys12 on histone H4 (24, 25). In contrast to Brd2 bromodomain I, which forms homodimers, and TAFII250 whose bromodomains form heterodimers, Brd4 bromodomains do not form homo- or heterodimers (13, 25, 26). Brd4/MCAP is bound to acetylated chromatin during interphase and mitosis (24, 27). During mitosis transcription by all three polymerases shuts down, with the exception of a small subset of genes (28, 29). At the same time many transcription factors diffuse into the cytoplasm or are inactivated by phosphorylation (30, 31). Also, the SWI/SNF chromatin remodeling complexes are released into the cytoplasm and become inactive in mitosis (32, 33). In the light of these observations, the presence of MCAP on chromosomes during mitosis is suggested not only to play a role in recognition of the acetylated histone code, but also to ensure passing this code onto the next cell generation and its maintenance (24, 34).

Brd4/MCAP was also observed to play a role in cell cycle regulation. Injection of anti-MCAP antibodies inhibited entry into mitosis suggesting a role of MCAP in

G2/M transition (27). This G2/M arrest could potentially be explained by the fact that Brd4/MCAP interacts with Rap GTPase-activating protein (GAP), signal-induced proliferation-associated protein 1 (Sipa1) and the finding that the proper balance between these two proteins in G2 is required for cell division (35). Furthermore, Brd4/MCAP expression was induced by growth stimulation and repressed by growth arrest signals (27). BRD4 -/- mouse embryos died shortly after implantation, suggesting a role for BRD4 in fundamental cellular processes (36). The mouse BRD4 heterozygotes showed a reduced proliferation rate *in vitro* and *in vivo*(36). A recent study also supports the role of Brd4/MCAP in cell proliferation. Nishiyama *et al.* showed that introduction of PTD (protein transduction domain) fused acetyl-H4 peptides inhibited the Brd4-chromatin interaction and resulted in reduced cell proliferation (37). Brd4/MCAP was also shown to interact with replication factor C (RFC), a protein responsible for loading PCNA (proliferating cell nuclear antigen) onto DNA (38-40). Ectopic expression of Brd4/MCAP led to arrest at G1/S transition (38). Both the interaction with RFC and the G1/S arrest were dependent on the second bromodomain of Brd4/MCAP. The interaction between Brd4/MCAP and RFC was also suggested to prevent the premature onset of DNA replication (38).

Brd4 is also involved in transcriptional regulation. Similarly to Brd2/RING3, it associates with the polII Mediator complex (41). Brd4 involvement in transcription extends to the interaction with P-TEFb components (42, 43). P-TEFb consists of cyclin T1 and cdk9 in its active form, additionally about half of the P-TEFb complexes are also associated with 7SK snRNA and the HEXIM1 protein and are therefore inactive (43) (and references cited therein). The cdk9 component of P-TEFb is responsible for hyperphosphorylation of the C-terminal domain (CTD) of the largest subunit of polII and consequently for the stimulation of the processivity of RNA elongation. Brd4 associates with the active form of P-TEFb, recruits it to the promoter and enables contact between P-TEFb and the Mediator, thereby promoting transcriptional elongation by PolII (42, 43). The Brd4 bromodomains are the regions required for binding P-TEFb (42). Brd4 increases polII CTD phosphorylation and promotes transcription (42). These results would suggest that Brd4 is not only interacting with the active form of P-TEFb, but it is also stimulating its activity. Recently, it was shown that the Brd4 interaction with P-TEFb increases at the M/G1 transition (44). P-TEFb is recruited to chromosomes before nuclear envelope formation and therefore before the import of other transcriptional factors (44). The P-TEFb recruitment to chromosomes depends on Brd4 and results in the activation of transcription from G1 promoters e.g. c-myc, c-jun, cycD1 (44, 45). These results support the notion that Brd4 dependent recruitment of P-TEFb and polII complex to G1 promoters stimulates cell cycle progression (44, 45). At the same time, since histone tail lysines bound by Brd4 are unusual in that they are acetylated throughout mitosis and also bound by Brd4 throughout mitosis, this could also be a mechanism to mark the genes that need to be transcriptionally active in the daughter cells (44). Ectopic expression of Brd4 was suggested to lead to G1/S arrest due to interaction between Brd4 and RFC, but as shown by Yang *et al.*(44) and Mochizuki *et al.*(45) knocking down Brd4 also leads to G1 cell cycle block due to inhibition of Brd4 stimulated G1 gene expression required for cell cycle progression. Hence, one can speculate that a fine balance of Brd4 levels needs to be maintained in the G1 phase in order for the cell to proceed through the cell cycle in an orderly manner.

The fusion between the BRD4 gene (*nota bene* this is also true for BRD3) and the nuclear protein in testis (NUT) gives rise to a protein contributing to carcinogenesis by binding to chromatin and preventing epithelial differentiation (46-50). BRD-NUT fusions have been detected in the NUT midline carcinomas (46-50). Recent studies performed with a highly metastatic mouse mammary tumor cell line ectopically expressing Brd4 demonstrated significant reduction of invasiveness and of the ability to form metastasis upon implantation into mice (51). Moreover, in a microarray study Brd4 influences expression of extracellular matrix genes, which are important cancer metastasis markers. Finally, these exciting findings were confirmed by analysis of breast cancer data sets, where it was observed that Brd4 activation predicted cancer patient survival. These findings suggest that Brd4 may have an influence on the metastatic potential of cancer (51).

4. BET PROTEINS AND VIRUSES

4.1. Brd4 and papillomavirus

Many lytic viruses capture the host cell to achieve massive virion production which results in the destruction or lysis of the infected cell. In contrast, other viruses, among them papillomaviruses (PVs) and herpesviruses, have developed strategies to persistently infect host cells at relatively low viral copy numbers without the destruction of the infected cell. The papillomaviruses (PVs) are a large group of host-restricted animal viruses with more than 100 known types infecting humans (52). Papillomavirus infections are generally long-term and can be persistent. PVs maintain their small DNA genomes of approximately 8 kb as extrachromosomal or episomal circular elements and have evolved functions to ensure that the viral genomes are partitioned into dividing daughter cells. The viral E2 protein tethers the viral genome to mitotic chromosomes by binding directly to viral DNA *via* E2 binding sites in the viral genome on the one hand and to mitotic chromosomes on the other. This mechanism has been best studied for the bovine papillomavirus 1 (BPV-1). E2 is a modular protein with a conserved aminoterminal transactivation domain that is required for transcriptional activation and repression as well as for DNA replication in concert with the viral E1 protein. Further, E2 has a carboxyl terminal dimerization and DNA binding domain. The two domains are linked *via* a less conserved hinge region.

In 2004, You and coworkers identified Brd4 as the first host mitotic chromatin tether for a papillomavirus. Using a tandem affinity purification approach in combination with mass spectrometry, Brd4 was found to be a major interaction partner of the BPV-1 E2 protein (53).

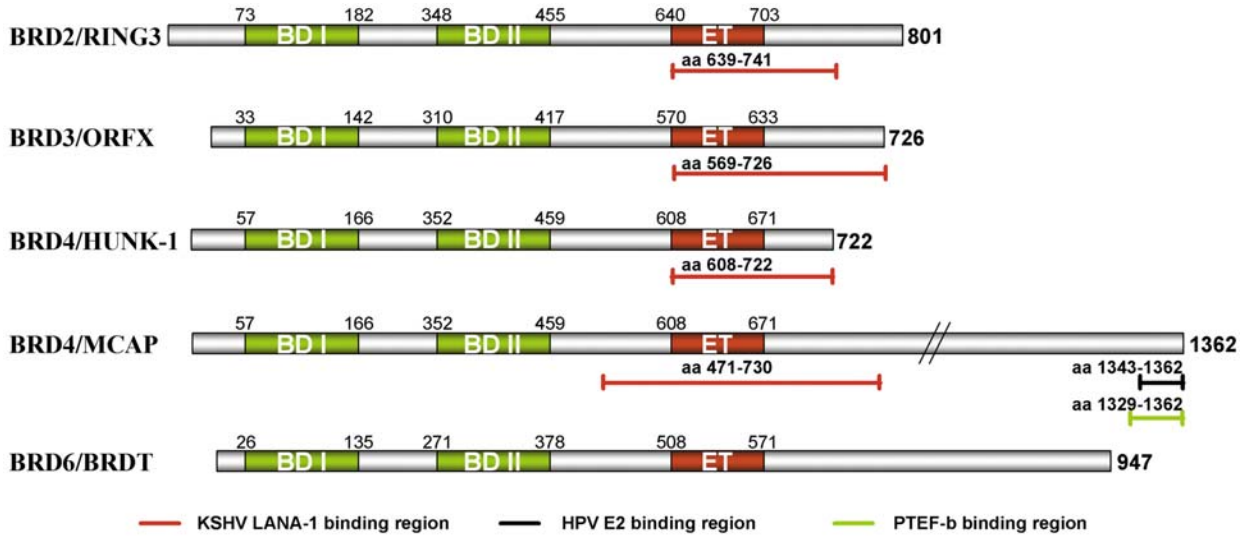

Figure 1. The BET family of proteins and their interaction regions with KSHV LANA-1, HPV E2 and P-TEFb. No published data available for BRD6/BRD-T. BD I, II: bromodomain I, II; ET: extraterminal domain

E2 interacts *via* its aminoterminal transactivation domain with the carboxyl terminal domain (CTD) of the long Brd4 isoform (MCAP) (Figure 1). BPV-1 E2 and Brd4 colocalize on mitotic chromosomes and the expression of the Brd4 CTD abrogates this colocalization in a dominant negative manner. Furthermore, the expression of the Brd4 CTD blocks the association of the viral episomes with Brd4 and inhibits the BPV-1 E2 mediated transformation of murine cells and enhances BPV-1 genome loss from transformed cells accompanied by a phenotypic reversion of transformation (53, 54). In line with these experiments, engineered BPV-1 E2 mutant proteins with an impaired E2-Brd4 interaction also show an impaired localization to mitotic chromosomes (55). All E2 proteins from a variety of different papillomaviruses interact with Brd4, albeit with various affinities (56). Based on the X-ray crystal structure of E2 in complex with a peptide representing the E2 binding site in Brd4 (Fig.1), Abbate and coworkers developed a Brd4-Tat fusion protein that was taken up by cells and displaced HPV-16 or HPV-31 plasmids from mitotic chromosomes (57). In a study using BPV-1 E2, Cardenas-Mora *et al.* found recently that the dimerization of E2 is required for efficient mitotic association and that a mutant with impaired dimerization bound Brd4 with greatly reduced efficiency even though the dimerization occurs *via* the carboxyterminus of E2 and Brd4 directly binds to the aminoterminal domain of E2 (58). This suggests that Brd4 and E2 may form larger protein complexes involving two or more molecules of each binding partner. The McBride group also demonstrated that the carboxyl terminal half of human Brd4 fused to the yeast BET protein Bdf1 could reconstitute the ability of the papillomavirus E2 protein to mediate the plasmid maintenance of E2 binding site containing plasmids in yeast cells (59). The two yeast BET proteins Bdf1 and Bdf2 lack the carboxyterminal E2 interaction region present in the long form of Brd4.

It is becoming apparent that E2 proteins of different papillomaviruses differ in their ability to associate with mitotic chromosomes (60). Even though E2 proteins from diverse papillomaviruses all interact with Brd4, apparently with differences in efficiency, a number of papillomavirus E2 proteins associate with mitotic chromosomes independently of Brd4 (56). In these, E2 protein mutations that abrogate Brd4 binding do not affect their ability to associate with chromosomes (60). Interestingly, HPV-16 E2 has been shown to interact with TopBP1, a mitotic chromatin bound cellular protein essential for cellular DNA replication, and the two proteins colocalize on chromatin at late stages of mitosis (61). ChlR1, a mitotic chromatin bound helicase with a role in sister chromatid cohesion, has been shown to interact with BPV-1 and HPV-11 E2 proteins (62). HPV-11 E2 has then been shown to colocalize with ChlR1 at early stages of mitosis (prophase), but not in metaphase, when ChlR1 localized to the spindle apparatus whereas E2 stayed on mitotic chromosomes. Interestingly, a mutant E2 that did not interact with ChlR1, but still interacted with Brd4 lost the ability to associate with mitotic chromosomes (62). These data suggest a critical role of ChlR1 for the loading of E2 onto mitotic chromosomes. Once there, E2 apparently depends on the interaction with Brd4 to stay bound to the mitotic chromosomes and at the later stages of mitosis, TopBP1 may play an additional role in regulating the E2-chromatin interaction. This model may be true for BPV-1 and possibly other papillomaviruses such as HPV-11. However other papillomavirus E2 proteins, e.g of HPV-8 or HPV-31, exhibit a different mitotic chromatin-associated staining pattern than BPV-1 and mutations that abrogate Brd4 binding in these proteins do not affect their mitotic chromosomal localization (63). Therefore, E2 proteins of various groups of papillomaviruses apparently evolved differences in the molecular details of chromatin attachment (63). The chromatin tethering function of E2 may indeed be a more dynamic process than just binding to a chromatin-associated host protein during cell division. As it seems to be the case at least for BPV-1, E2 may change

the chromatin anchor in the course of a single cell division and possibly these interactions serve additional purposes such as transcriptional regulation or the manipulation of specific cell cycle events. There is evidence from overexpression studies that some E2 proteins can indeed affect the host cell cycle by causing a mitotic block (64).

In contrast to the apparent differences in chromatin tethering mechanisms, a critical role of Brd4 in the transcriptional activation function of all E2 proteins studied so far has been well established using three different approaches (56, 65-69). Firstly, the ectopic expression of the Brd4-CTD has been shown to abrogate E2 mediated transcriptional activation in a dominant negative manner (56, 65, 68). Secondly, knock-down of endogenous Brd4 abolishes the ability of E2 to act as a transcriptional activator (67, 68). Lastly, utilizing a number of E2 mutants, the inability to bind to Brd4 exquisitely correlates with the inability of these E2 mutant proteins to activate transcription (68, 69). The E2 residues arginine 37 (R37) and isoleucine 73 (I73) direcly interact with Brd4 and the mutantion R37A (arginine 37 to alanine) and I73A abrogate Brd4 binding and E2's transcription activity (57, 68, 69). Hence, Brd4 is a critical player to mediate E2's transcriptional activation function. In contrast, even though there is good *in vitro* evidence that Brd4 is also involved in E2 mediated transcriptional silencing (70), knock-down of Brd4 or the use of the Brd4 CTD in cell based assays did not affect E2 mediated transcriptional repression as it did abolish E2 mediated activation (67). Therefore, Brd4 may well be involved in E2 mediated repression, however either in a different complex or in different complexes and/or bound at different affinities than in the activation complex. Furthermore, other cellular factors besides Brd4 seem to play a role in E2's transcriptional repression function (67). Future molecular and genetic work will reveal the different cellular factors involved in E2's ability to act as transcriptional repressor. Lee and Chiang recently showed that Brd4 enhances the binding of E2 proteins to the E2 binding sites of chromatinized HPV DNA as well as to naked HPV DNA providing a possible mechanism for Brd4's role in the transcriptional activities of E2 (66). E2 is an unstable protein with an estimated protein half life around 1h. Brd4 appears to increase E2 steady-state levels in cells through the direct Brd4-E2 interaction (66).

4.2. BET proteins and KSHV

Kaposi's sarcoma associated herpesvirus (KSHV) or human herpesvirus 8 (HHV-8) is classified as a gamma-2- herpesvirus and associated with Kaposi's sarcoma (KS), primary effusion lymphoma (PEL) and with some forms of Castleman's disease (71-73). In the tumour cells, KSHV persists in a form of a latent episome from which only a limited number of genes are expressed (74-76). Among these genes is the latency associated nuclear antigen 1 (LANA-1), a nuclear protein encoded by orf73 of KSHV (77-79). LANA plays an important role in KSHV latent replication, as it binds to the latent origin of replication – the terminal repeat (TR) region and mediates the replication of plasmids containing this origin (80-84). It also tethers viral genomes to cellular chromosomes, hence promoting viral persistence (85-91). Additionally it is also involved in transcriptional regulation of viral as well as cellular genes (6, 80, 81, 92-97).

LANA was first observed to interact with Brd2/RING3 in KSHV infected cells (5). The major interaction regions were mapped to the C-terminus on LANA and to the ET domain of Brd2/RING3 (Fig.1), although an additional contact region within Brd2 was noted (5, 6). *In vitro* LANA was found to be phosphorylated in the presence of Brd2 (5). Brd2/RING3 was found to colocalize with LANA in speckles in virally infected cells, both in mitotic as well as in interphase cells (98). LANA speckles in virally infected cells colocalize with KSHV genomes and are found on the border of heterochromatin (99). Brd2 was associated with euchromatin in interphase LANA-negative cells, while it relocalized to heterochromatic regions in the presence of LANA. LANA was also observed to induce Brd2 expression (98). The same group observed dissolution of heterochromatin in the presence of high expression levels of LANA-1 (98). The authors speculated that presence of LANA-1 and/or Brd2 in LANA-1 specles creates a microenvironment, which inhibits heterochromatization. Taking into account the suggested role of Brd2 in chromatin remodeling and spreading of histone acetylation, LANA-mediated recruitment of Brd2 to chromosomes and LANA speckles could mediate local euchromatin formation. Considering that Brd2 was found to bind to the KSHV TR, and that in contrast to the rest of the TR the LANA binding sites (LBS) are enriched in acetylated histone H3 and H4 and are devoid of nucleosomes (100), one could imagine that Brd2 is recruited to TR in order to participate in nucleosome rearrangments, although no direct proof of this hypothesis is currently available.

The minimal region in LANA responsible for the interaction with Brd2, i.e. aa 973-1143, is also required for binding to chromatin, binding to KSHV TR and hence latent replication of the virus, as well as the activation of transcription from the cyclin E promoter and the repression of transcription from the KSHV TR (6, 97). These findings suggest that the interaction between LANA and Brd2 could play a role in one or more of the above mentioned LANA functions. When expressed ectopically, the C-terminal domain of LANA was found to form speckles and localize to pericentromeric and peritelomeric regions of a subset of mitotic chromosomes (99, 101, 102). These speckles were found to associate with euchromatin (99). Combining this information with the observation that the C-terminal LANA domain contains a second chromatin binding domain, which partially overlaps with the binding region for Brd2, it has been suggested that Brd2, through its bromodomains, may participate in the attachment of the LANA C-terminus to chromatin (4, 6).

In addition to Brd2, KSHV LANA was also shown to interact with Brd3/OrfX and both splice variants of Brd4: MCAP and HUNK-1 (4, 103) (see Fig.1). Similarly to Brd2, the Brd4/HUNK-1 ET domain was sufficient to interact with the C-terminal domain of LANA, but additional LANA binding sequences in Brd4/HUNK were also identified (4). The ectopic expression of Brd2 as well as Brd4/HUNK-1 prevented entry into the S phase of

the cell cycle and the co-expression of KSHV LANA released cells from this block (4). Brd2, Brd4/HUNK-1 and LANA activate the cycE promoter (1, 4, 6). Whether the role of LANA as a transcriptional activator involves Brd2 or Brd4 (as shown for HPV E2 - see above) is currently still unclear: extensive truncation mutants of LANA that no longer bind to Brd2 or Brd4 have lost the ability to activate heterologous promoters, but have also lost other properties (dimerisation, binding to KSHV TR DNA) (4, 6).

For Brd4/MCAP and LANA the interaction domains were shown to lie in the ET domain of MCAP and the C-terminal domain of LANA, although the internal repeat region of LANA contributes an additional binding side for Brd4/MCAP (103). Brd4/MCAP, in contrast to Brd2, remains on the chromosomes throughout mitosis and was found to colocalize with LANA and the KSHV genomes on the host mitotic chromosomes (103). Brd4/MCAP could thus contibute to the association of LANA with mitotic chromosomes in addition to the direct association of the N-terminal damain of LANA with core histones or other cellular chromatin bound proteins such as MeCP2 (85, 104).

4.3. BET proteins and other herpesviruses

Like KSHV murine gammaherpesvirus 68 (MHV-68) is a gamma-2-herpesvirus. Mice infected with MHV-68 serve as a model for gamma-herpesvirus infection and pathogenesis. The LANA-1 homologue of MHV-68, orf73 protein, is expressed both in latency and during the lytic replication and has been shown to be crucial for establishment and maintenance of latency in mice (105-110). MHV-68 orf 73 has been shown to interact with Brd2/RING3, Brd3/ORFX and Brd4/HUNK-1 (111). The binding site in the orf73 protein for Brd 2/4 was identified (111). Mutation of this binding site resulted in a weakened chromatin association and decreased the ability of MHV-68 orf73 to activate cyclin D1, D2 and E promoters (111). These results suggest that MHV-68 orf73, similarly to the papilloma E2 protein interacts with BET proteins in order to be able to activate cellular transcription.

Epstein-Barr virus (EBV) a gamma-herpesvirus, is distantly related to KSHV. EBV is the causative agent of infectious mononucleosis, endemic Burkitt's B-cell lymphoma, lymphoma in immunosupressed patients (transplant recipients, AIDS patients), nasopharyngeal carcinoma and about 50% of cases of Hodgkin's Disease (112). About 95% of the world adult population is infected with EBV. The Epstein-Barr virus nuclear antigen 1 (EBNA 1) is a functional homologue of LANA and plays a role in viral persistence, replication and transcription regulation (113). EBNA 1 binds the family of repeats (FR) enhancer element on the EBV genome and consequently activates EBV gene transcription. Recently EBNA 1 has been shown to bind Brd4 (114). This interaction was observed to be important for EBNA 1-mediated transcriptional activation from the FR element (114). Silencing of Brd4 decreased transcriptional activity of EBNA 1. Additionally, Brd4 was found to preferentially bind to the FR region of the EBV genome during latency. Therefore Brd4 seems to play an important role in EBNA1 mediated transcriptional activation from the FR element.

Human cytomegalovirus (HCMV) is a beta-herpesvirus whose gene expression, as is the case with other herpesviruses, is temporally regulated. At first the immediate early (IE) genes are transcribed and for their expression no de novo synthesis of viral proteins is required (115). The IE proteins stimulate expression of viral delayed early genes, which control viral DNA synthesis. The IE2 protein is localised in punctate viral transcription sites, referred to as transcriptosome. One of the components of this transcriptosome was shown to be the Brd4 protein (116). The role of Brd4 in this complex is not known yet, but based on its interaction with P-TEFb, it could be recruting this complex to the transcriptosome.

4.4. BET proteins and retroviruses

Human T-lymphotropic virus type 1 (HTLV-1) is a retrovirus and the causative agent of adult T cell leukemia (ATL) (117-119). The Tax protein of HTLV-1 plays an important role in viral replication, transformation and transcriptional activation (120-126). The Tax protein binds to Tax responsive elements (TRE), which are higly conserved 21 bp repeat elements present within the long terminal repeat (LTR) of the HTLV-1 genome. TREs are important for Tax-mediated viral transcription activation (118, 127-130). It was shown that Tax interacts with cdk9, a component of P-TEFb and therefore recruits P-TEFb to LTR (131). This interaction was observed to be essential for transactivation of the LTR promoter by Tax. Subsequently is was shown that Brd4 and Tax compete for binding to P-TEFb (132). Both of them bind to cyclin T1, a component of P-TEFb. Tax overexpression, similarly to Brd4 overexpression, leads to increased phosphorylation of CTD of polII, suggesting that it is a positive regulator of P-TEFb, the protein directly responsible for polII CTD phosphorylation. Overexpression of Brd4 inhibits Tax transactivation. These results suggest that Tax facilitates binding of the Tax/P-TEFb complex to the promoter replacing the Brd4/P-TEFb complex, hence promoting the transcription of Tax regulated genes. The HTLV-1 Tax protein seems to mimic functions of Brd4 and compete with Brd4 for P-TEFb binding (132).

The human immunodeficiency virus (HIV) encodes the Tat protein, which is essential for activation of transcriptional elongation from the long terminal repeat (LTR) promoter and therefore also for producing full length transcripts (133-135). Tat has been shown to activate transcription by recruiting P-TEFb to the trans-activating response (TAR) RNA element found at the 5' end of the nascent viral transcript (133-135). Both Tat as well as cyclin T1 of P-TEFb bind to TAR RNA. Additionally Tat, similarly to HTLV-1 Tax binds directly to cyclin T1. These interactions form a ternary complex, which brings P-TEFb in close proximity to PolII, where P-TEFb can phosphorylate CTD of polII and hence stimulate transcription elongation from the HIV LTR (133-135). As mentioned above, Brd4 interacts with P-TEFb and positively regulates its activity. Overexpression of Brd4 leads to inhibition of Tat transcriptional activation function,

due to competition between Brd4 and Tat for binding to P-TEFb (43). Also the expression of a short peptide spanning the Brd4 domain responsible for interaction with P-TEFb (see Fig.1) inhibits interaction between Tat and P-TEFb and leads to inhibition of Tat mediated transcription (136). Consequently, it seems that the HIV Tat protein, similarly to HTLV-1 Tax, mimics Brd4 function in transcriptional activation. Additionally, Urano *et al.* have described a T cell line based screen for proteins rendering cells resistant to HIV-1 replication (137). One of the proteins identified in this assay was Brd4 and more specifically its C-terminal domain. Therefore it seems possible that Brd4 has influence not only on HIV trascription, but through the transcriptional repression also on HIV replication.

5. CONCLUDING REMARKS

A common trend that emerges among the interactions between viral proteins and BET family members is the role of BET proteins in regulation of viral transcription. HPV E2 and EBV EBNA-1 proteins interact with Brd4/MCAP and MHV-68 orf73 with Brd4/HUNK-1 and Brd2/RING3, to activate viral transcription. These viral proteins exploit the fact that Brd4 and Brd2 normally regulate expression of cellular genes. KSHV LANA might therefore also regulate viral transcription with the help of Brd4, although no direct evidence for this has so far been reported. A variant strategy is represented by the retroviruses, which do not interact with Brd4, but instead mimic its function by hijacking the Brd4 interacting complex and increasing viral transcription. Additionally, some viruses seem to usurp BET proteins for “unusual” functions, like tethering of their episomal genomes to the mitotic chromosomes (e.g. BPV E2 and KSHV LANA).

6. ACKNOWLEGEMENTS

M.W-G. is supported by the EU Integrated Project “INCA” (LSHC-CT-2005-018704) and M.O. by the national priority program SPP1130 of the German Research Council (DFG).

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Key Words: virus, BET proteins, Brd2/RING3, Brd4, KSHV, EBV, HIV, HTLV, HCMV, Review

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