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YOU BET-CHA: A NOVEL FAMILY OF TRANSCRIPTIONAL REGULATORS

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TABLE OF CONTENTS

1. ABSTRACT

The BET proteins are a novel class of transcriptional regulators whose members can be found in animals, plants and fungi. Founding members are Human RING3, Drosophila Fsh and yeast Bdf1p. BET proteins are distinguished by an N-terminal bromodomain or bromodomains and an ET domain. As predicted by the presence of the bromodomain(s), these proteins have been found to be associated with chromatin. The poorly characterized ET domain functions as a protein-protein interaction motif and may be part of a serine-kinase activity. Other regions ("modular domains"), which are conserved only in certain BET proteins, are likely to provide sub-family specific functions. Genetic, biochemical and molecular techniques have implicated BET proteins in functions as diverse as meiosis, cell cycle control and homeosis. The data suggest that BET proteins may modulate chromatin structure and affect transcription via a sequence-independent mechanism. This review will attempt to summarize current research on BET proteins and envision where future research is likely to lead.

2. INTRODUCTION

The BET proteins are a small family of proteins that probably exist in all multi-cellular eukaryotes. Members are defined as having one (plants) or two (animals/yeast) bromodomains and an ET domain. The original members are Human RING3, Drosophila Fsh and yeast Bdf1p. All three of these proteins have been shown to affect the transcription of an eclectic collection of promoters, suggesting that BET proteins are transcription factors with widespread specificity.

Eukaryotic DNA is typically wound tightly in chromatin, making it transcriptionally inert (although there are exceptions (1)). Only in the past decade have researchers begun to unravel the mechanisms by which a cell reverses the inhibitory effects of chromatin on transcription. The opening of chromatin involves phosphorylation, methylation and acetylation of nucleosomes: acetylated nucleosomes are common substrates for many bromodomain-containing transcription factors (for example 2) and are likely to be substrates for BET proteins as well.

Acetylation, however, may also affect later events in transcription; *e.g.*, the TFIID member $TAF_{II}250$ contains an acetylase activity and two bromodomains (3). By virtue of binding acetylated nucleosomes, BET proteins might function either in opening chromatin (via interactions with Histone Acetyltransferase complexes, Chromatin Remodeling Complexes and/or sequence-specific factors) or in transcriptional initiation (analogous to $TAF_{II}250$). The current data do not discriminate between these as the most likely place for BET function and, in fact, BET proteins may carry out both activities.

The data discussed below suggest a simple hypothesis for BET function: BET proteins associate with acetylated chromatin via their two bromodomains. The ET

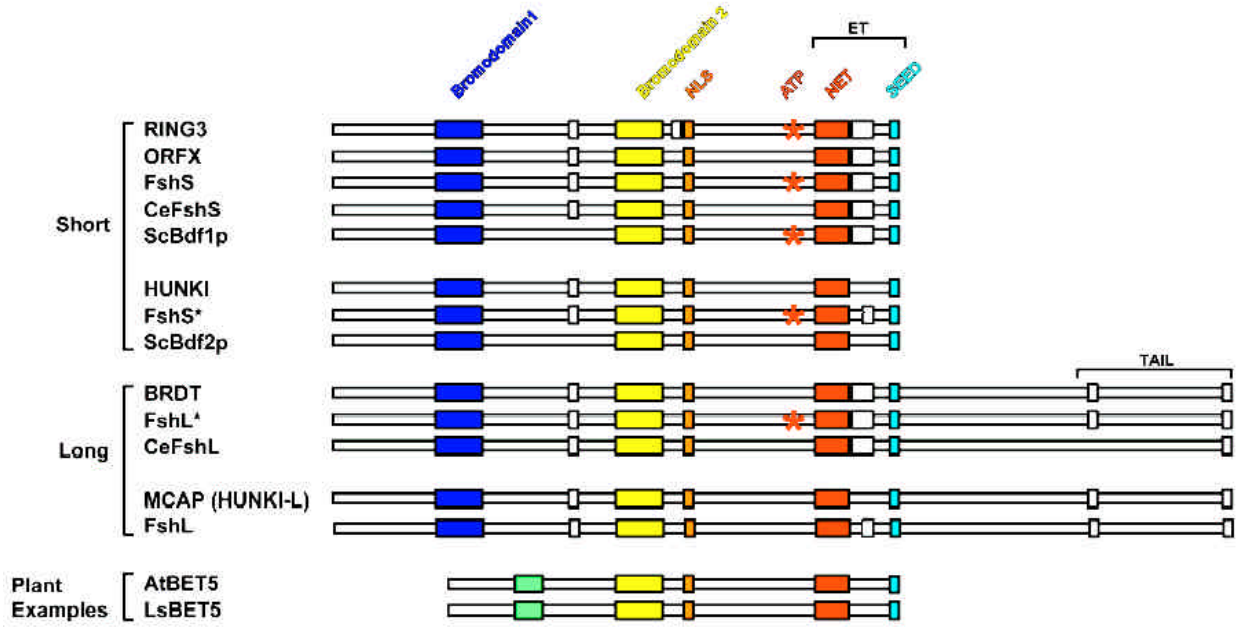

Figure 1. Published and putative BET proteins. BET proteins can be found in mammals (MCAP/HUNKI-L, BRDT, RING3, ORFX, HUNKI), Drosophila (FshS and FshL are published, the asterisk marks putative forms – see text), *C. elegans* (CeFshS, CeFshL), yeast (ScBdf1p, ScBdf2p) and plants (representatives: Arabidopsis AtBET5, and Lycopersicum LsBET5). All BET proteins from animals and yeast have two bromodomains, an extended NLS, a NET domain which is followed by a poorly conserved region and then a SEED motif. Plant BET proteins appear to have only one bromodomain (the "second") and often lack the SEED motif.

domain then serves as an interface to localize different complexes or proteins to chromatin. The identity of these associated factors may depend upon which modular domains are present. A reasonable extension is that the ET domain may also interact with sequence-specific factors in order to help localize the BET protein to the active chromatin. Although the following data are supportive of this hypothesis, determining its validity or fallibility will require further experimentation, potentially along the lines proposed below.

3. STRUCTURE AND EVOLUTION

Sequence analyses identify a family of BET proteins with multiple members in each species (where data exist) that have one or two bromodomains and an ET domain (Figures 1 and 2). With the caveat that most BET proteins are putative, the BET family appears to be ancient: at least ~1,000 million years old (Figure 3), as members can be found in plants, animals, yeast, and, possibly, *Dictyostelium.*

An obvious dichotomy between kingdoms is that plant BET proteins appear to have only a single bromodomain in contrast to animals and yeast, which have two. Tree analyses (B.F., unpublished) and "sequence-gazing" indicate that the plant bromodomain may be more closely related to the second bromodomain of yeast and animals (Figure 4). Current analyses are not sufficient to determine if the primordial BET protein had one or two bromodomains, but the simplest explanation is that the first BET protein had a single bromodomain, while the BET protein(s) of the most recent common ancestor of animals and yeast had two.

The strongest conservation amongst the BET proteins is seen in the bromodomains (Figure 4). The bromodomain is an acetylated-lysine-binding motif, consisting of four or five bundled alpha-helices, with a large loop (Z_A) protruding into the binding pocket at one end of the bundle (5, 6). The BET/CBP subfamily of bromodomains has a unique Z_A loop that has an insertion of two amino acids (the two asterisks in Figure 4). According to the NMR and crystal structures of bromodomains, these residues are in the most distal portion of the Z_A loop. This would position the insert in or near the binding pocket for acetylated lysine, possibly altering pocket structure or accessibility, suggesting this change might alter either the regulation or specificity of binding of the BET (and CBP) bromodomains to their substrate.

The affinity of a single bromodomain for acetylated-lysine is weak, only ~0.3 mM, suggesting that other interactions must be required for a meaningful biological association with chromatin (6). Since animal and yeast BET proteins have two bromodomains, their affinity for acetylated substrates should be sufficient to allow independent association with acetylated chromatin. Alternatively, the two bromodomains might provide broader substrate recognition. If two BET bromodomains

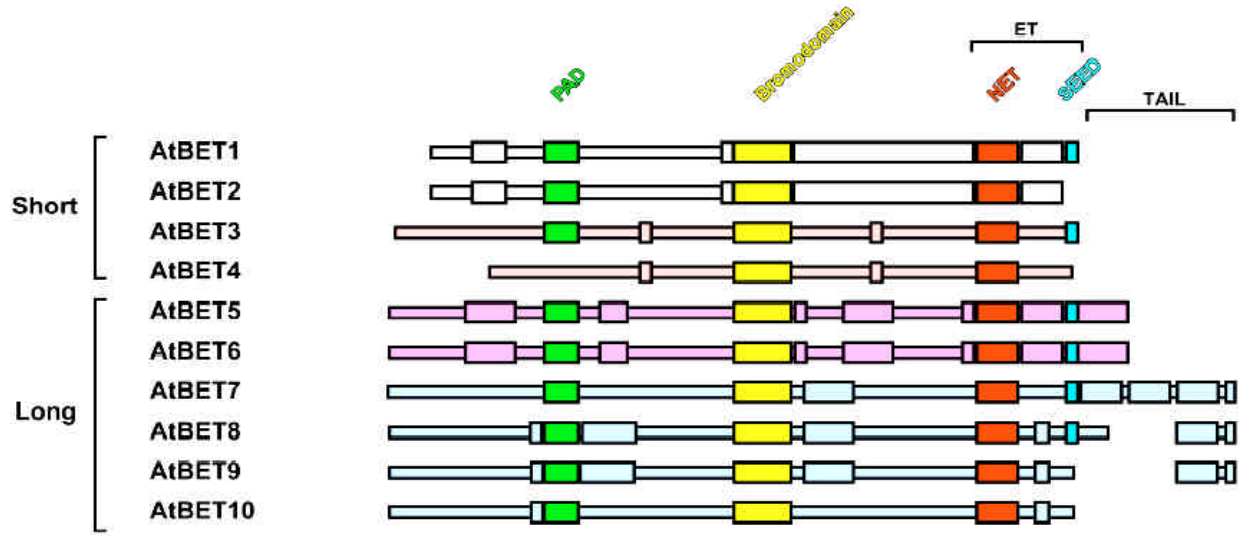

Figure 2. BET proteins from Arabidopsis. Colored backbones indicate more closely related forms based on homology outside the bromodomain and ET domain. Neighbor-joining tree analysis using either the bromodomains or the NET domains confirms this grouping, with the exception of AtBET3/4 (data not shown). The bromodomain, NET domain and SEED motif are colored as in Figure 1. Notice that each group has members lacking the SEED domain, with the exception of AtBET5/6. All of these are conceptual translations as provided by their respective databases. Accession numbers: AtBET1: GenBank AC008017, AtBET2: GenBank AC034106, AtBET3: GenBank AC025290, AtBET4: GenBank AAC12830, AtBET5: DBJ BAA98182, AtBET6: PIR T49984, AtBET7: DBJ BAB02121, AtBET8: DBJ BAB10737, AtBET9: PIR T48600, AtBET10: GenBank AC009325.

are required to bind independently to chromatin, then one must somehow rectify this hypothesis with the fact that plant BET proteins have only a single bromodomain. One possibility is that the plant BET proteins dimerize (potentially via the PAD domain in Figure 6). Another possibility is that these proteins have an affinity for histones independent of acetylation as has been suggested for yeast Bdf1p (7). Therefore acetylation may serve to enhance binding to nucleosomes allowing single bromodomain BET proteins to bind acetylated chromatin. This is consistent with the observation that some mutant BET proteins with a single functional bromodomain retain some function (see below).

Two reports show that BET proteins do, in fact, bind chromatin (8, 9). Importantly, the murine BET protein MCAP is excluded from heterochromatic regions, which typically lacks acetylated histones. Consistent with the view that BET proteins bind chromatin independently of other factors, MCAP remains associated with chromatin during M phase when many transcription factors dissociate from the DNA (9). While it has yet to be demonstrated that BET proteins directly bind acetylated nucleosomes unaided, it seems likely that this will be the case.

The ET domain (10) has been shown to be a protein-protein interaction motif (11, 12, 13) and to function as part of non-canonical serine kinase (14). All BET proteins contain at least a portion of an ET domain, which is unique to BET proteins (and a few BET-related proteins such as DmCG17692). Sequence conservation suggests the ET domain consists of three separate regions: the NET domain (for N-terminal ET), an intervening sequence and the C-terminal SEED motif. Only the NET domain is conserved in all BET proteins and all NET domain proteins have a BET/CBP-class bromodomain, suggesting these characteristics are truly descriptive of BET proteins (*N.B.*, a related, but distinct, NET-like motif can be found in the AF-9/ENL family - Figure 5). The sequence of the NET domain suggests it is bipartite: *i.e.*, two conserved motifs separated by a spacer of variable length (Figure 5). The N-terminal portion is predicted to form two alpha helices and the C-terminal region an extended beta sheet followed by an alpha helix. The spacer separates these two regions and is likely to form a loop. The predicted, overall structure is H-H-L-E-H (H for helix, L for loop, E for extended beta-sheet). The conservation of the NET domain and bromodomain(s) suggests that all BET proteins have some function or functions in common and so at least partially redundant within individual species.

Lastly, there are other regions conserved only in some family members, which, for convenience sake, I have collectively termed “modular domains” (Figure 6). Among these are the putative ATP-binding motif and the SEED motif. The SEED motif contains poly-serine residues interspersed with glutamic and aspartic acid (Figure 6A). The sequence of the SEED motif may not be noteworthy in itself, but the conservation of its location at the C-terminus of the ET domain adds to its significance. A group of modular domains found at the C-terminus of some family members - the “TAIL” region - may be used to classify BET proteins as either “long” or “short” (Figure1).

Although the placement of these domains is similar, there is no obvious homology across kingdoms or between the AtBET5/6 and AtBET7-10 subfamilies (yeast proteins do not have these extensions). Together, these

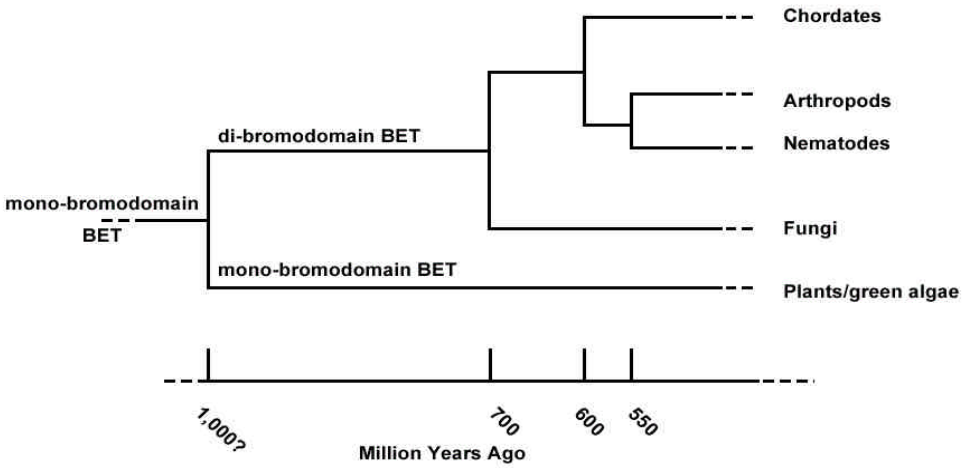

Figure 3. Currently accepted phylogeny. Dates of divergence are approximate. After Morris, 1998 (4)

observations suggest that the "TAIL" sequences arose independently and may represent convergent evolution. Genetic studies of yeast *BDF1* and Drosophila *fsh* suggest that the SEED motif and the TAIL region, respectively, are important for some but not all functions of these proteins.

4. MOLECULAR GENETICS AND BIOCHEMISTRY OF BET PROTEINS

4.1. Mammalian BET Proteins

There are no known mutations of BET genes in mammals, however molecular experiments suggest a common thread amongst these proteins: the possibility that each may be involved in some aspect of oncogenesis. This is most evident for RING3, which shows elevated kinase activity in leukemic B-cell blasts and for MCAP, which appears to play a role in cell-cycle progression. Consistent with a role for BET proteins in oncogenesis, preliminary reports for the other two mammalian proteins – BRDT and ORFX – suggest they are over-expressed in certain cancers (15) or involved in DNA-damage response (16), respectively.

4.1.1. RING3

The RING3 protein was originally purified as a nuclear kinase with mitogen-stimulated autophosphorylation activity (13). The regions of RING3 required for kinase activity show little homology to canonical kinases (non-canonical kinase activity has been reported for two other bromodomain proteins – see 17, 18). The NET domain and putative catalytic lysine of RING3 are required for this activity while the remainder of the ET domain is not. RING3 kinase activity is still controversial as other authors either could not detect intrinsic activity (19) or identified only an associated kinase activity (20). Potentially, the failure to detect intrinsic kinase activity may have resulted from a failure to appropriately activate the kinase (14, 21). Alternatively, RING3 may not have intrinsic kinase activity, but may require the NET domain and putative catalytic lysine for association with a kinase. Kinase activity (intrinsic or associated) has been reported for endogenous Bdf1p and Bdf2p and recombinant Bdf1p, as well (10). As Bdf2p does not have an obvious putative ATP-binding domain or a catalytic lysine, this would seem to argue for an *associated* activity. However, $TAF_{II}250$'s non-canonical kinase regions do not have an obvious ATP-binding motif/catalytic lysine either, so the controversy remains unresolved for the time being. Regardless of whether or not the kinase activity is intrinsic or associated, the activity appears to have been conserved from yeast to man, making it reasonable to assume that it has an imperative function *in vivo*. Currently, it is unclear whether the important kinase target is the BET protein itself or some other protein.

The ET domain has also been the focus of protein-protein interactions with RING3, which associates with the LANA from Kaposi's sarcoma herpesvirus (requires NET domain - 12) and an E2F-containing complex (requires sequences C-terminal to NET domain - 15). These interactions suggest the ET domain functions as a molecular "hook" to tether different complexes (or BET proteins) to active chromatin. Distinct sequence variations in the ET domain among the different isoforms (*e.g.*, compare HUNKI with RING3 in Figure 1) may provide variation in the associated complexes and so diversity in function.

Both the kinase activity and protein-protein interactions of the ET domain appear to be have function *in vivo*. In quiescent cells in culture, RING3 is predominantly cytoplasmic and has low levels of kinase activity (22). However, when tissue culture cells are induced by mitogen, RING3 becomes exclusively nuclear and shows associated kinase activity. The activation of RING3 activity *in vivo* is most striking in lymphoid and myeloid leukemias. In normal blasts, the associated kinase activity is undetectable, even though the protein is present. However, in transformed blasts nuclear RING3 kinase activity is readily detectable. Importantly, in the only patient in the study that went into remission, RING3 kinase activity was concomitantly lowered. Consistent with a role for RING in stimulation of cell proliferation, over-expression of RING3 in tissue culture cells leads to a slight acceleration of early cell-cycle events (G_1/S) and premature activation of cyclin A (G. Denis, personal communication).

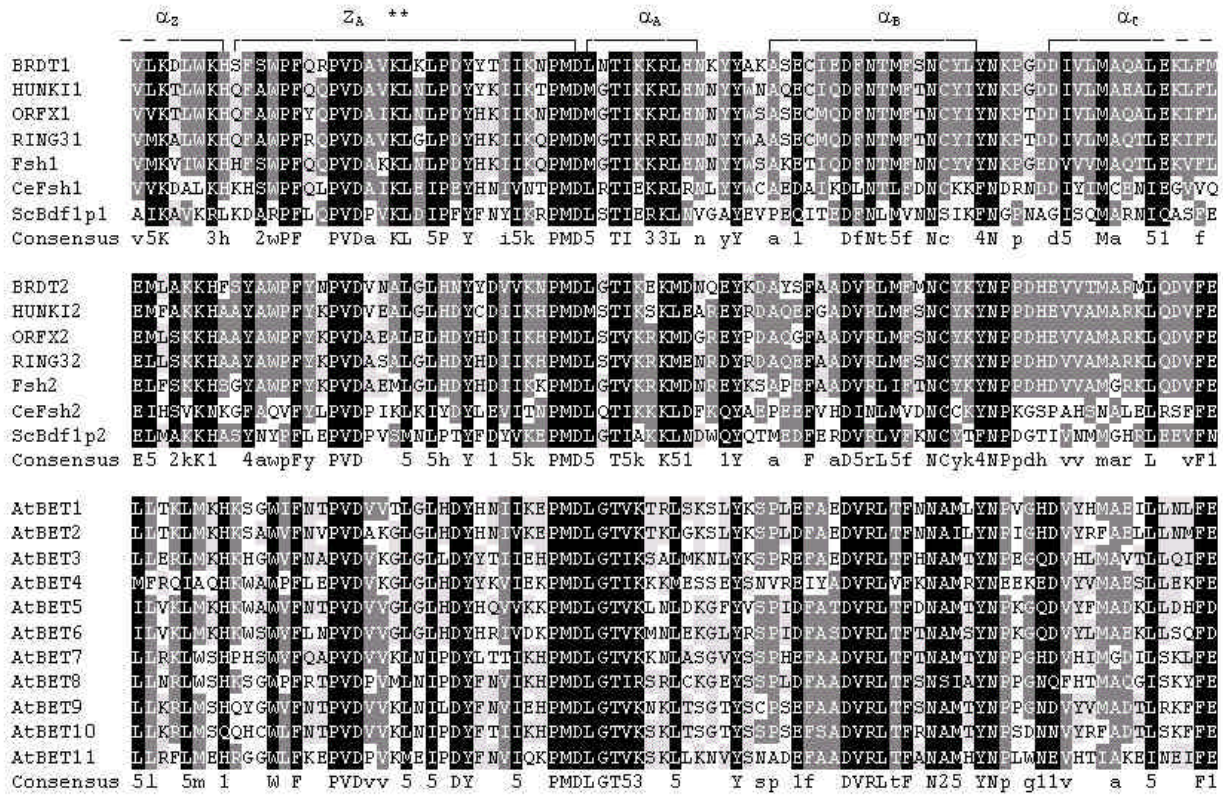

Figure 4. Bromodomain alignments. Alignments are grouped by first, second and plant bromodomains. The plant bromodomain may be more closely related to bromodomain 2 than bromodomain 1. Dayhoff PAM 250, 1: DENQH, 2: SAT, 3: KR, 4: FY, 5: LIVM.

Transient co-transfections of RING3 expression vectors and E2F-responsive reporter constructs (cycA/D/E, DHFR, *etc.*, - 15) show that RING3 can synergistically activate reporter expression in combination with Ras or Ras-pathway effectors. The synergism of RING3 with activated MEKK required the putative catalytic lysine and was not seen when using reporter constructs containing mutant E2F binding sites, suggesting the activation requires both association with E2F and kinase activity (although the former was not explicitly tested). The current data do not distinguish if the phosphorylation of RING3, E2F and/or another factor is important for this response.

4.1.2. MCAP and HUNKI

Murine MCAP is most closely related to human HUNKI, except that it also has the C-terminal TAIL present in BRDT and Drosophila FshL. The genomic sequence 3' of the HUNKI gene contains putative coding sequences that closely match that of the C-terminal TAIL of MCAP. This suggests that not only are MCAP and HUNKI true homologs, but that HUNKI (and perhaps MCAP) are alternatively spliced to give forms roughly analogous to FshS and FshL from Drosophila. Aside from the TAIL region, HUNKI/MCAP differs from RING3 in three major ways. First, both HUNKI and MCAP lack a recognizable ATP-binding motif. Second, they lack a conserved region between the ET and SEED domains. Third, a poly-acidic-residue region near the NLS (which is unique to RING3) is absent from HUNKI and MCAP. Even though RING3 and HUNKI/MCAP are likely to have overlapping function, these differences are indicative of distinct biological roles. Indeed HUNKI, in contrast to RING3, does not trans-activate E2F-responsive cyclin promoters in transient co-transfections (C. Vaziri, personal communication). Potentially, domain-swapping experiments using RING3/HUNKI chimeras could be performed to identify the region(s) necessary for the E2F response.

Recent work on MCAP has brought to light an interesting connection between cell-cycle control and BET proteins. Dey *et al*. (9) found that cells injected with an anti-MCAP antibody (but not with control antibodies) stalled in G_2, prompting the hypothesis that MCAP is required for the $G_2 \rightarrow M$ transition. It is interesting to compare this result with the observation that RING3 may regulate G_1/S progression. Are these control mechanisms similar? Might these proteins be at least partially redundant? Or do differences between the proteins dictate which portion of the cell cycle they can regulate? Again, experiments with chimeric proteins might serve to answer these questions and help us better understand their roles in cell-cycle control.

An interesting point made by the authors is that MCAP remains associated with chromatin even during mitosis when many transcription factors dissociate. If, as seems likely, MCAP is associated with actively transcribed genes, one possibility is that it may serve as a molecular tag that allows faster re-association of activating factors and/or RNA polymerase after mitosis (9). Not inconsistent with

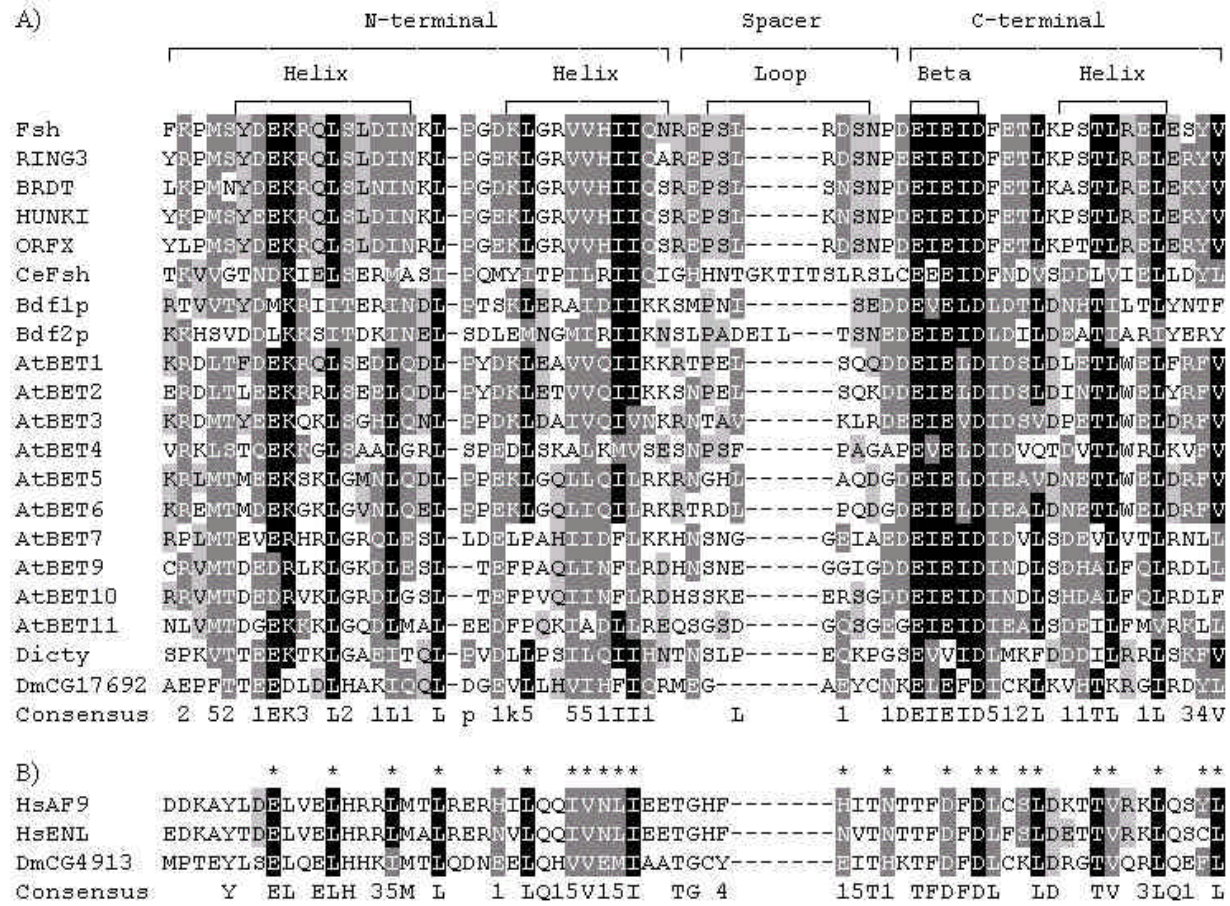

Figure 5. NET domain homology. A) NET domains from BET proteins. Predicted secondary structure is indicated above the sequences. Dicty is from Dictyostelium clone JC1b189b09. White on black indicates identity in 12 or more proteins, black on gray indicates similarity in 12 or more proteins. B) Alignment of a NET-like domain found in three homologous proteins (two from humans, the third from Drosophila). The asterisks and shading mark residues that are similar to the NET domain consensus.

this proposal is the finding that MCAP has been found associated with the murine Mediator HAT complex, which also associates with RNA Pol II (23).

4.2. Yeast *BDF1* and *BDF2*

Yeast contain two BET-encoding genes: *BDF1* and *BDF2*. Genetic analyses of *BDF1* and *BDF2* indicate these BET proteins function as general transcription factors (10, 11). The phenotypes of *bdf1* mutant cells include defects in snRNA transcription (10), failure to metabolize many sugars, temperature-sensitive lethality and defective meiosis (8); conversely, *bdf2* mutants appear normal. However, a *bdf1 bdf2* double-mutation is lethal, indicating *BDF2* is redundant to *BDF1* for some, but not all, functions (8).

All *bdf1* mutants show the defects listed above, with the exception that some mutants do *not* have defects in meiosis I (8). The deletion of the first bromodomain or the very C-terminal tail (exclusive of the NET domain) has noeffect on meiosis, although these proteins are mutant for the other functions. Deletion of most of the C-terminus (including the NET domain) results in defective meiosis (these mutants may be analogous to Drosophila's *fsh*[4] mutation – see below). The variability in the meiotic phenotype demonstrates that, while the ET domain is required for normal meiosis, the SEED domain is dispensable; suggesting the SEED domain is modular. The modularity the SEED domain is consistent with observation that several putative plant BET proteins lack a SEED domain.

The most complete molecular work on a BET protein comes from the elegant dissection of yeast Bdf1p and Bdf2p by Matangkasombut *et al.*, (10). As with RING3, both Bdf1p and Bdf2p show associated or intrinsic kinase activity. The authors also show that both Bdf1p and Bdf2p can associate with Taf67p, a member of yeast TFIID. They convincingly argue that by associating with Taf67p the Bdf1/2p proteins supply some functions present in animal $TAF_{II}250$ but absent in its yeast homolog Taf145p. Taf145p is homologous with the N-terminal two-thirds of $TAF_{II}250$, but lacks the C-terminal third. This region of $TAF_{II}250$ contains two bromodomains and kinase activity; hence the speculation that association of Bdf1pand/or Bdf2p with TFIID via Taf67p might substitute for these "deleted" $TAF_{II}250$ functions. Although the authors' hypothesis is compelling, it is not clear if this BET function is conserved across kingdoms for two reasons.

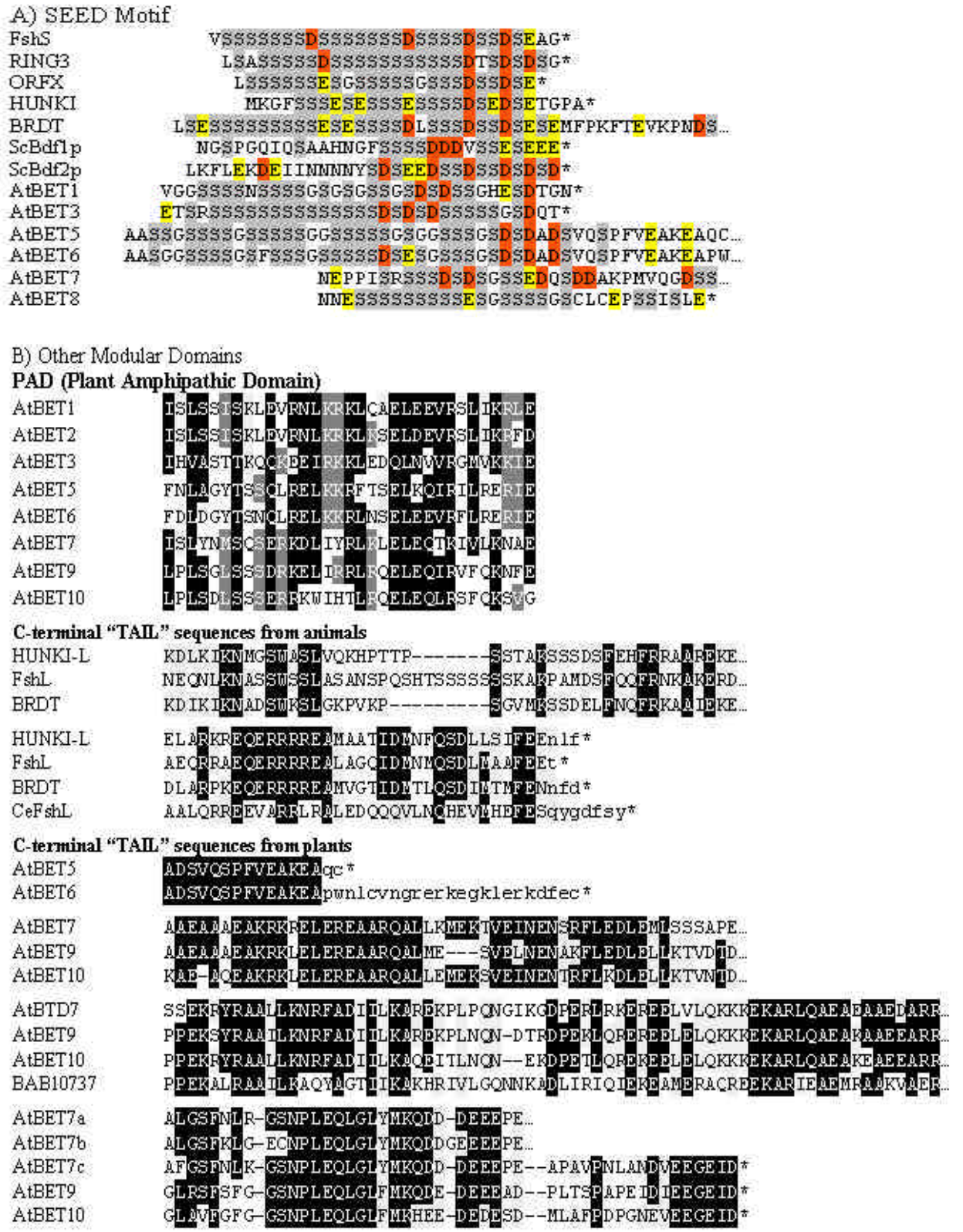

Figure 6. A Selection of Modular Domains of BET proteins. A Selection of Modular Domains of BET proteins. A) For the SEED motif, serines are in gray, aspartates in red and glutamates in yellow. B) A sampling of the modular domains present in subfamilies. For both A and B, an ellipsis indicates more residues to follow and an asterisk indicates the end of the protein. AtBET7a, b and c are repeats found in the TAIL of AtBET7.

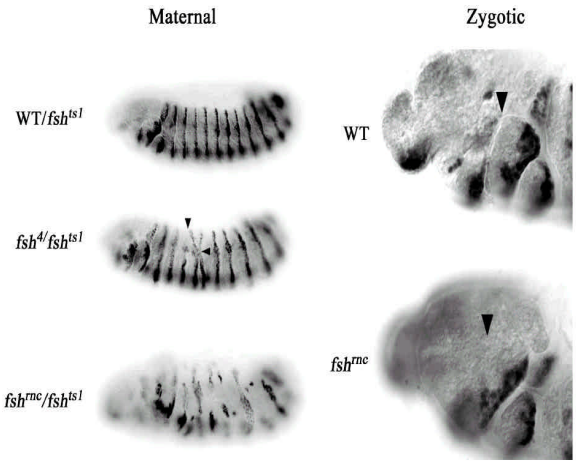

Figure 7. *Expression of en RNA in fsh mutants. Maternal*: Maternal: Embryos were raised at semi-permissive temperature (25°C). Notice the fusion of segments in the third thoracic to second abdominal segments (small arrow heads) in the hypomorph; these are similarly positioned in adult survivors. Segmentation is often absent in both null and *fsh*^{rnc} mutants, which die as embryos. *Zygotic:* Ectodermal *en* expression in the trunk is normal (B.F., unpublished). Lack of gnathal *en* expression is often seen in null and *fsh*^{rnc} mutants and is coincident with a fusion of segments (large arrow head).

First, the animal homologs for Taf67p do not have the N-terminal sequence required for binding of Bdf1/2p (although a similar sequence can be found in the *Schizosaccharomyces pombe* homolog). Second, since $TAF_{II}250$ has BET-like functions, there is no reason to propose *a priori* the animal BET proteins fulfill this role. Plant $TAF_{II}250$ homologs (GenBank accession numbers AC017118 and AP000735) may represent intermediates between $TAF_{II}250$ and Taf145p as they have a single C-terminal bromodomain, but no other obvious C-terminal conservation, suggesting they lack kinase activity.

The biological relevance of the molecular interactions between Bdf1/2p and Taf67p was supported by genetic analyses. Mutations in *TAF67* produce mutant phenotypes very similar to *bdf1* mutants and, as with *bdf1 bdf2* double mutants, *bdf1 taf67* double-mutants are lethal. This is also true for synthetic *taf67* mutants whose resulting Taf67p protein is lacking the Bdf1/2p interaction domain. This result suggests that the lethality seen in *bdf1 bdf2* double mutants is likely due to a failure of one of the BET proteins to interact with Taf67p. The fact that Taf67 mutant lacking the Bdf1/2p interaction domain does not completely mimic a *bdf1p bdf2p* double mutant suggests the situation is more complicated and may indicate there are additional Bdf1/2p contacts with TFIID.

4.3. Drosophila Female sterile homeotic

The *female sterile homeotic* (*fsh*) gene of Drosophila encodes multiple BET isoforms. The two forms that have been reported are FshS (short) and FshL (long; 25). FshS is roughly equivalent Bdf1p and RING3, while FshL also has a ~1000 amino acid TAIL region. The sequences for FshS are entirely contained within FshL, except that FshL uses an alternative splice site that removes 4 amino acids near the C-terminus of the FshS-contiguous sequence. Over 30 ESTs and cDNAs correspond to *fsh* in the database, but only two cover this splice site, leaving the possibility that two additional forms may exist which might correspond to human HUNKI and BRDT, respectively (Figure 1). Both Fsh isoforms contain a putative ATP-binding site and catalytic lysine as is seen in RING3 and Bdf1p; and Fsh has been suggested to have intrinsic kinase activity (G. Denis, personal communication).

The first *fsh* mutations were uncovered in a genetic screen for mutations that result in female sterility (26, 27). Although null mutations cause embryonic lethality (see *rnc* in 28), the original alleles are temperature-sensitive (*fsh*^{ts}), which allowed researchers to remove *fsh* function at different times of development. Mutant adult females shifted to non-permissive temperatures lay only a few, non-productive haploid eggs suggesting a defect in oogenesis (26, 27, 29). Although this phenotype has not been investigated further, it may be related to a meiotic defect seen in yeast *bdf1* mutants. When *fsh*^{ts} mothers are kept at permissive temperatures, but their progeny are raised at non-permissive temperatures, one observes different mutant phenotypes depending on when the temperature shift is done. The earliest shifts result in lethality and a halt in development, while later shifts give rise to adults with segmental deletions (likely due to misregulation of *engrailed* - Figure 7) and a homeotic, *Ultrabithorax*-like (*Ubx*) phenotype (27, 28, 30). Maternal *fsh* does regulate *Ubx* gene expression, but it also affects the expression of segmentation genes that regulate *Ubx* themselves (31). These results make it unclear if *fsh* regulates *Ubx* expression directly or indirectly. A strong genetic interaction with *trx*, a direct regulator of *Ubx*, suggests the former. It is important to point out that the *fsh*^{ts} alleles used for the maternal effect experiments still retain significant activity even at elevated temperatures, indicating the phenotypes would probably be much more severe if all *fsh* function were removed. Indeed, null mutations have been reported to be cell lethal (see *l(1)VA75* 32); that is, at least one of the Fsh isoforms is required for a cell to survive.

Only some *fsh* alleles have been molecularly characterized and can be divided into four groups: mutations in the first bromodomain (*fsh*^{ts} and *fsh*^{rnc}, B.F., unpublished), mutations affecting the ET domain (*fsh*^{4} - 25), mutations affecting only the long form of Fsh (*fsh*^{L}- 25) and null mutations (*Df(1)C128* - 26, 27, 28, 30). With the exception of the only reported null allele (*Df(1)C128*), all mutations are partial loss of function and produce subsets of the *fsh* phenotypes, which suggests that at least some of Fsh protein function is modular.

The severity of these alleles in regards to zygotic embryonic function can be described in Mullerian fashion (from normal to most severe) as wild type>*fsh*^{L}=*fsh*^{4}>*fsh*^{rnc}=*fsh*^{null}. Both *fsh*^{L} and *fsh*^{4} zygotic mutants survive embryogenesis but die as larvae (30), while *fsh*^{rnc} and *fsh*^{null} animals die as embryos (28, B.F. unpublished). When examining the maternal homeotic and segmentation defects, the order of allelic severity is different: wild type=*fsh*^{L}>*fsh*^{rnc}>>*fsh*^{4}=*fsh*^{null}. Adult progeny of *fsh*^{L}/*fsh*^{ts} mothers have no homeotic or

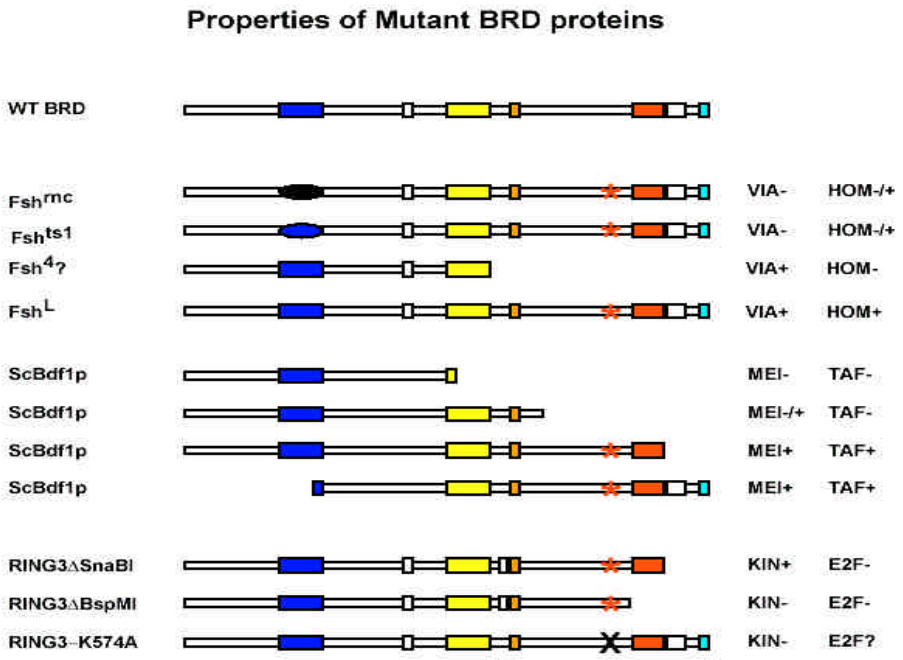

Figure 8. Properties of mutant BET proteins. A schematic of a "typical" wild type BET protein is at top. For Fsh, VIA indicates zygotic, embryonic viability and HOM indicates maternal effect homeosis. For Fsh^{ts1}, the oval bromodomain is to signify it is only partially functional and for Fsh^{rnc} the first bromodomain is probably non-functional. The schematic for Fsh^4 is approximate as the exact nature of the *fsh*^{4} mutation is not known. For Bdf1p, MEI signifies meiosis I, while TAF indicates the presence of the Taf67p binding region. For RING3, KIN- means loss of kinase activity and E2F- shows that the mutant protein does not associate with an E2F containing complex.

segmentation defects (30) and only rarely do progeny of *fsh*^{rnc}/*fsh*^{ts} mothers have a homeotic phenotype (B.F., unpublished). These alleles are distinctly different from *fsh*^{4} or *fsh*^{null}, which cause up to 40% of their progeny to show a phenotype in these experiments (30).

To summarize, the *fsh*^{L} mutations, although lethal, are the least severe. The *fsh*^{rnc} allele appears to be a null for zygotic function but only weakly affects maternal regulation of homeosis. The *fsh*^{4} mutation has the opposite pattern than *fsh*^{rnc}: it does not affect embryonic zygotic function, but strongly affects maternal regulation of homeosis and segmentation. By correlating the molecular lesions with the phenotypic effects, one can make the following three proposals:

The "TAIL" region, which is affected in *fsh*^{L} mutants, has no obvious maternal or embryonic function, but is required for larval viability.

As indicated by *fsh*^{rnc}, the first bromodomain is required for zygotic functions, but not for maternal effect homeosis. The other first-bromodomain mutation, *fsh*^{ts1}, also shows a weak maternal homeotic phenotype (27, 30).

The C-terminus (ET domain?) is not required for embryogenesis, but is required for maternal regulation of *Ubx* and *engrailed* as is indicated by *fsh*^{4}. For many reasons, these proposals are overly simple - not the least of which is that by necessity mothers have both the mutant allele of interest *and* a *fsh*^{ts} allele. Regardless of the caveats, the changes of allelic severity seen between *fsh*^{rnc} and *fsh*^{4} are important to understanding the function of the Fsh protein. It will be interesting to see if other *fsh* alleles follow the same pattern as *fsh*^{rnc} or *fsh*^{4} or have novel phenotypic effects.

5. CONCLUSIONS

The BET proteins constitute an ancient, conserved class of proteins with either one (Plants) or two (yeast/animals) bromodomains and an ET domain. Yeast Bdf1/2p, RING3 and Fsh are known to affect the transcription of various target genes, either *in vivo* or *in vitro*. Combined with the chromatin-binding ability of the bromodomain, it is likely the BET proteins act as *bona fide* transcription factors. For animals and yeast, the presence of two bromodomains suggest they can remain associated with acetylated chromatin in the absence of contacts with other chromatin or DNA binding factors, although this has yet to be demonstrated.

The genetic and molecular analyses indicate that the ET domain is required for many BET protein functions. A schematic of some mutant phenotypes described above is summarized in Figure 8, which emphasizes the importance of the ET domain for normal protein function. These data also suggest that the functions of the ET domain can be subdivided into those requiring the NET domain and the SEED motif, as was proposed above in the description of ET domain conservation.

Due to the disparate assays of gene function used, the genetic analysis in yeast and flies is not sufficiently similar to allow any concrete conclusions. Until congruent genetic studies are performed, real parallels cannot be drawn. Some simple experiments might address these issues. For example, one bdf1 phenotype that has not been discussed is a two-fold reduction in recombination. This could be simply and quickly tested in flies. Secondly, it should be relatively straightforward to determine if any of the fsh mutations show defects in cell-cycle progression. This last test might be done with available mutations specific for FshL isoforms (i.e., possibly analogous to a mutation in MCAP).

The results of parallel genetic analyses will be intriguing, especially given the unique features of each BET protein. Although there is undoubtedly some redundancy among the proteins, the presence of modular domains suggests unique functions. Analysis of mutant phenotypes specific to each form will be important to clarify the functions of each protein. Because of the complexity of the *fsh* gene in Drosophila, transgenic animals will be required to separate functions. For mammals, knockouts of all four genes will be extremely informative. For example, does RING3 have a role in hematopoesis, as is suggested by elevated kinase levels in leukemic blasts? Do any of the mutants have homeotic transformations? Can one observe misregulation of the *engrailed* homologs *En1* and *En2?* These and other analyses designed to reproduce experiments done in other species will be necessary to determine if conservation of structure results in conservation of function.

The yeast data demonstrating Bdf1/2p can function as part of TFIID shows that, at least in yeast, BET proteins function to promote initiation of transcription. However, in mammals, MCAP can be found associated with the Mediator HAT complex and RING3 with an E2F-containing complex, suggesting BET proteins may play a role in opening chromatin as well. A possibility that also is consistent with the data is that sequence-specific factors (*e.g.*, E2F) and/or HAT complexes (*e.g.*, Mediator) serve to localize BET proteins to open chromatin, which, in turn, facilitate the binding of TFIID or other members of the initiation complex.

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[1] In a single study, analysis of five microsatellites sequences from 33 families of patients with leukemia showed no linkage of the RING3 gene and the disease (24). At best, these results suggest that oncogenic mutations in RING3 are rare or that they are not a primary factor in the initiation of the disease.

Keywords: Bromodomain, Fsh, RING3, Bdf1p, ET Domain, NET Domain, SEED Motif, Review

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