The Bromodomain Mediates Transcriptional Intermediary Factor 1α-Nucleosome Interactions*

Eumorphia Remboutsika**, Ken Yamamoto†, Matthias Harber‡, and Marc Schmutz‡

From the Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/Université Louis Pasteur, BP 163, F-67404 Illkirch cedex, France and the Division of Developmental Genetics, MRC-National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, United Kingdom

Received for publication, April 18, 2002, and in revised form, September 30, 2002
Published, JBC Papers in Press, October 15, 2002, DOI 10.1074/jbc.M203759200

Nuclear histone acetyltransferases, DNA-dependent ATPases, and transcriptional intermediary factors (TIFs) all harbor a distinct structural module known as the bromodomain (BrD). Although the BrD can interact with histones H3 and H4 and their acetylated N-terminal tails in vitro, its function in a chromosomal environment remains elusive. We used the nuclear receptor coregulator TIF1α, a protein kinase that associates tightly with euchromatin, to analyze the properties of the BrD in a nucleosomal environment in vitro. Here, we report that TIF1α-chromatin association is direct and involves DNA and nucleosome interactions mediated by the BrD. Mutation of the BrD signature peptide, PMDL, abolishes DNA binding and disrupts BrD-nucleosome interactions. Based on our results, we propose that the BrD plays a critical role in vivo by directing transregulators to their cognate location on nucleosomal DNA.

Ligand-dependent transcriptional regulation by nuclear receptors involves transcriptional intermediary factors (TIFs) acting to remodel chromatin templates and/or to modulate the activity of the basal transcriptional machinery (1, 2). Among the members of the TIF1 family, including TIF1β (2–4), a kinase that interacts (5–7) and phosphorylates heterochromatin proteins HP1α, β, and γ (8, 9) and TIF1γ (10, 11), TIF1α is the only member known to interact with liganded nuclear receptors (12–16). The Drosophila homologue of TIF1, Bonus, also interacts with Drosophila nuclear receptors and inhibits betaFTZ-F1-dependent transcription, but it plays no role in position effect variegation (17). TIF1α undergoes SUMO-lation (18), hyperphosphorylation upon ligand-dependent interaction with nuclear receptors and has an intrinsic kinase activity (19); its substrates range from TIF1α itself to the basal transcription factor TFIIε and the TATA-binding protein-associated factors TAFp28 and TAFp55 (19) and also HP1α, β, and γ (8) that are found to interact with TIF1α (5).

TIF1α is an abundant nuclear protein present in embryonic stem cells and mouse embryos (20). Upon cell differentiation at the onset of organogenesis, the expression levels of TIF1α decrease dramatically (20, 21); TIF1α then becomes restricted to the developing central nervous system and to selected cell populations in the adult brain and germ-line tissues (21). In the nucleus, TIF1α is tightly bound to euchromatin, preferentially on sites of RNA polymerase II transcription and at the borders between euchromatin and heterochromatin where TIF1α may act as a docking molecule for liganded nuclear receptors (20), such as the androgen receptor (22). Thus, TIF1α defines a novel class of chromatin-associated TIFs that facilitate access of transregulators to target genes during development and differentiation (“chromatin access model”) (20).

TIF1α harbors several evolutionarily conserved domains; at the N terminus, a RING finger-B box-Coiled-Coil (RBCC/TRIM) motif (23–25), an oligomerization interphase for TIF1 proteins (11), and at the C terminus, a PHD/TTC finger (26) followed by a bromodomain (BrD) (27, 28). Both the PHD/TTCT finger and the BrD are prevalent in regulators acting at the chromatin level (26, 29, 30). Recent evidence suggests that the BrD represents an important structural module for regulators that participate in the control of gene expression (27–29, 31–35), acting as an acetyl-lysine binding domain (36–40). Although the BrD of acetyltransferases can interact with purified H3 and H4 histones (41) and their acetylated tails in vitro (42) and may be required for association with chromatin (43), its action on the chromosomal environment has been obscure.

Here, we report the molecular properties of the BrD in a nucleosomal environment in vitro. We demonstrate that the BrD is sufficient to mediate direct DNA and nucleosome interactions. Four residues, PMDL, that represent a signature present in helix A of several BrDs, are crucial both for DNA and for nucleosomal binding. Our results reveal a novel DNA-binding activity for the BrD and suggest a new role for this domain as a nucleosomal DNA anchor.

Experimental Procedures

Chromatin Assembly—Drosophila post-blastoderm extracts (S-190) were prepared and chromatin was assembled essentially as presented in Ref. 44 with minor modifications also described in Ref. 45. Chromatin was assembled with 15 mg of S-190 extract, 5 μg of calf thymus core histones (Roche), 5 μg of mRARp2-LacZ (10.2 kb) plasmid (46), and an
ATP-regenerating system (30 mM creatine phosphate, 3 mM ATP, 4.2 mM MgCl₂, and 6 μM of kinase kinase) in 500 μl of final volume supplemented with buffer R (45). The assembly factors in the extract were first charged with core histones on ice for 30 min, and the plasmid and the ATP regenerating system were added and the samples were further incubated for 5 h at 27°C. At the end of the incubation period, chromatin was either analyzed by micrococcal nuclease (MNase) (Sigma, digestion at 60°C) or purified for further analysis by sucrose gradient sedimentation as described previously (20, 44).

Sucrose Gradient Sedimentation—10.2 ml of 30–50% linear sucrose gradients were prepared in 10 mM Hepes-KOH buffer, pH 7.5, 1 mM MgCl₂, 1 mM β-mercaptoethanol, and 500 mM NaCl in 100 mM Tris HCl buffer, pH 8.0, 1 mM EDTA, 1 mM sucrose gradient sedimentation and dialyzed in 5 mM triethanolamine, 0.5M NaCl concentration was adjusted to 0.35 M. To deplete histone H1, an aliquot of the sample was centrifuged in a SW41 rotor (Beckman) at 26,000 rpm for 1 h at 4°C. At the end of the centrifugation, the cleared supernatant was diluted 10-fold with immunoprecipitation buffer N for 30 min at 37°C as described (20, 49). After centrifugation at 6,000 rpm for 10 min at 4°C, the pellet was resuspended in 100 μl of 2 mM EDTA, 1 mM dithiothreitol, 1% P1, and 1 mM PMSF solution and incubated for 10 min on ice (20). The supernatant (52 fraction, heterochromatin) was collected by centrifugation for 15 min at 4°C, and the NaCl concentration was adjusted to 0.35 M. To deplete histone H1, an equal volume of 50% slurry of CM Sephadex C-25 (in 0.5 mM NaCl) was added and incubated for 2 h at 4°C with rocking. The supernatant was recovered by centrifugation at 10,000 rpm for 5 min at 4°C. H1 depletion was confirmed by immunoblotting using a monoclonal antibody specific for mouse histone H1, which was not shown.

Nucleosome Reconstitution by Octamer Transfer—M2 and M4 DNA fragments were end-labeled with [α-32P]dCTP using Klenow DNA polymerase and incubated in a 1:1 ratio with histone H1-depleted donor nucleosomes in 10 mM Tris-HCl buffer, pH 7.5, 1 mM NaCl, 1 mM EDTA, and 1 mM β-mercaptoethanol for 1 h. TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) was added to adjust the NaCl concentration to 0.8 M. After 1 h of incubation, the NaCl concentration was adjusted to 0.66 M, followed by a 1-h incubation before NaCl was subsequently diluted to 0.2, 0.1, and 0.05 M with successive additions of TE every 10 min. All incubations were performed at room temperature. Nucleosome particles were purified by sedimentation in sucrose as described (20) in 10 mM Tris-HCl buffer, pH 7.5, 1 mM EDTA, and 1 mM β-mercaptoethanol for 16 h at 4°C in a Beckman SW60 rotor. Fractons were collected and analyzed by 4% (29:1) native polyacrylamide gel electrophoresis in 0.5X TBE (45 mM Tris base/45 mM boric acid/125 mM EDTA) for 1 h at 10V/cm. Gels were dried and subjected to autoradiography.

Nucleosome Electrophoretic Mobility-Shift Assay (EMSAs)—Unless otherwise noted, all conditions for gel electrophoresis and autoradiography were performed as described previously. Gel electrophoresis and autoradiography were performed with native polyacrylamide gel electrophoresis. 18 ng of labeled DNA was added to each reaction. Gels were dried and exposed to autoradiography.
RESULTS

The fact that TIF1α associates with euchromatin in vivo (20) prompted us to analyze its molecular properties on reconstituted chromatin in vitro. For our study, we used an in vitro chromatin assembly system prepared from Drosophila postblastoderm embryos (S-190 extract) (44, 45, 47) (Fig. 1A). Micrococcal nuclease (MNase) digestion analysis (Fig. 1E), supercoiling assays (data not shown), and electron microscopy (Fig. 1, A and F) demonstrated that the extract efficiently assembled regularly spaced nucleosomal arrays (50) on various length templates in the presence of exogenously added core histones as previously described (44). Because the S-190 extract appeared to be devoid of any detectable TIF1α cross-reacting activity (Fig. 1C, Western Blot), purified recombinant mouse TIF1α (Fig. 1C, Coomassie Staining) was included in the assembly reaction to determine whether TIF1α can be incorporated efficiently onto chromatin (Fig. 1B). At the end of the chromatin assembly reaction, chromatin (assembled on mRARβ2-LacZ, Ref. 46), 10.2 kb) was purified by sucrose gradient sedimentation and the DNA was isolated and analyzed by agarose gel electrophoresis and ethidium bromide staining (44, 45, 47) (Fig. 1D). From immunoblotting analysis of gradient fractions and by comparison to the sedimentation profile of the recombinant protein, it was evident that TIF1α was readily incorporated onto chromatin assembled in vitro (Fig. 1B). A ratio of approximately one TIF1α molecule per 3 nucleosomes was estimated. When chromatin generated with or without TIF1α was subjected to time-dependent MNase digestion (45), we found that TIF1α incorporation had no apparent effect on the regularity of nucleosomal spacing (Fig. 1E). Moreover, analysis of purified chromatin by electron microscopy, where fully assembled chromatin was indeed observed both with and without TIF1α (Fig. 1, A and F), indicated that TIF1α does not affect the efficiency of the chromatin assembly (Fig. 1, A, E, and F). However, chromatin prepared with TIF1α consistently presented a statistically significant enlargement in core nucleosome dimensions (Fig. 1F). Nucleosomes assembled in the presence of TIF1α presented on average an increase of 16–19 A when compared with core nucleosomes (50). Similar results were obtained when chromatin was packaged either using postblastoderm extracts (Fig. 1, A and D) or by salt dialysis using purified core histones (Fig. 1D; data not shown), suggesting an interaction between TIF1α and core nucleosomes. These results demonstrate that TIF1α associates with chromatin in vitro and moreover indicate that no additional factors are required to mediate TIF1α-chromatin interactions.

In the nucleus, TIF1α is tightly bound to euchromatin (20); however, the chromosomal sites that bind TIF1α in vivo are still unknown. Thus, to investigate whether TIF1α binds to nucleosomal DNA directly, genomic DNA fragments from the euchromatic fraction of the genome, fractionated as described in Refs. 20 and 49, were isolated and tested. Fractions enriched in DNA and proteins from either euchromatin or heterochromatin can be prepared from intact nuclei subjected to mild MNase digestion (20, 49). This leads to the initial release of primarily mononucleosomes that contain a subset of the chromosomal DNA (S1 fraction), which is enriched in TIF1α (20) and other non-histone chromosomal proteins (49) but is devoid of histone H1 (49) and HP1α (45), contains actively transcribed genes (49), and therefore represents euchromatin (20, 49). Subsequent hypotonic lysis of the nuclei pellet releases a soluble fraction (S2) that contains transcriptionally inactive poly nucleosomes that are enriched in histone H1 (49) and HP1α (20) and therefore represent heterochromatin (20, 49). Two different genomic DNA fragments (M2 and M4) were isolated from the S1 fraction of P19 nuclei (20), cloned, and sequenced. To verify whether TIF1α binds to these sequences in vivo, chromatin immunoprecipitations were performed (Fig. 2A). Cross-linked chromatin from P19 EC cells was precipitated with TIF1α (Fig. 2B, a-TIF1α) and acetylated histone H3 (Fig. 2B, a-AcH3) antibodies or without an antibody (Fig. 2B, no Ab) (negative control). Crosslinking was then reversed, and the DNA was purified and subjected to PCR analysis using oligonucleotide primers that generated a 130-bp fragment specific for M2 and a 160-bp fragment specific for M4 sequences (Fig. 2B). The length of the PCR products was compared with DNA fragments obtained when plasmids bearing the M2 and M4 fragments (Fig. 2B, lane 1) and chromatin DNA before immunoprecipitation (Fig. 2B, lane 2) were used as templates. No specific binding of either of the two analyzed genomic sequences was revealed in chromatin immunoprecipitates in the absence of a primary antibody (Fig. 2B, lane 3). However, TIF1α was recruited either alone on M2 chromatin (Fig. 2B, lane 5) or together with acetylated histone H3 (Fig. 2B, lane 4) on M4 chromatin (Fig. 2B, lane 5). These results indicate that TIF1α is recruited onto M2 and M4 chromatin in vivo.

To investigate whether TIF1α binds directly to DNA and nucleosomes, probes from M2 and M4 fragments were generated. Nucleosomes isolated from the S2 fraction of P19 nuclei (20) were stripped of histone H1 (data not shown) and used as donor nucleosomes in octamer transfer experiments to prepare the corresponding nucleosomal probes. Then, electrophoretic mobility shift assay (EMSA) was performed with naked DNA and nucleosomes (51, 52). EMSA demonstrated that TIF1α (0.8 μM) binds both to naked M2 (145 bp) and M4 (189 bp) DNA fragments and to their corresponding nucleosomes (Fig. 2C). Similar to histone H1 and its variants (52–54), TIF1α binds directly to core nucleosomes (Fig. 2C). When increasing amounts of protein (0.1, 0.2, 0.4, and 0.8 μM) were incubated with an equal ratio of nucleosome to naked DNA, a preference for naked DNA at low TIF1α levels was revealed (0.2–0.4 μM for naked DNA compared with 0.8 μM for nucleosomes) (Fig. 2D). TIF1α binding to nucleosomes and naked DNA in vitro appears to be sequence-independent as it can be competed out by nonspecific DNA competitor (poly(dI-dC)) (TIF1α at 0.8 μM; poly(dI-dC) at 25 and 100 μg/ml) (Fig. 2E; data not shown). These results demonstrate that TIF1α binds directly to DNA and nucleosomes.

RING/TRIM fingers have been found in proteins of diverse functions (23, 24), whereas PHD/TTC fingers (26) and BRDs (27, 28) were identified in a number of transcriptional mediators, several of which act at the chromatin level (27–29, 31–34, 38, 55–61). To identify the domain involved in TIF1α-DNA and TIF1α-nucleosome interactions, a series of His-tagged TIF1α mutants was generated, and the proteins were expressed either in baculovirus-infected SF9 cells or in E. coli. No full-length TIF1α molecules bearing mutations or deletions only in the BrD region could be identified in baculovirus-infected SF9 cells by immunoblotting analysis using an anti-His-tag monoclonal antibody (data not shown). However, other deletion mutants, including TIF1α(1–791), TIF1α(734–1017), TIF1α(734–853), and TIF1α(854–1017) shown in Fig. 3A, were identified as His-tag overexpressed proteins and affinity-purified to homogeneity (Fig. 3B; data not shown). Chromatin was assembled as described in Fig. 1A and purified by sucrose gradient sedimentation (Fig. 1D). Purified chromatin was then analyzed by immunoblotting using an anti-His-tag monoclonal antibody (Fig. 3B). The TIF1α(1–791) mutant that comprises the TIF1 oligomerization RBCC/TRIM motif (11), the nuclear receptor interaction box (NR box, LXXLL motif, amino acids 726–738),
**Fig. 1.** TIF1α stably associates with chromatin assembled *in vitro*. **A**, outline of chromatin assembly assay *in vitro*. Chromatin was assembled using *Drosophila* post-blastoderm extracts, core histones, and an ATP-regenerating system on the mRARβ2-lacZ plasmid (10.2 kbp) (46), purified by sucrose gradient sedimentation (44), and analyzed by electron microscopy (bar = 100 nm). **B**, calf thymus histones (Roche), core histones (4 μg), and individual histones (1 μg) were analyzed on a 15% SDS-polyacrylamide gel and stained with Coomassie Brilliant Blue R-250. Molecular mass markers (in kilodaltons) are indicated. **C**, recombinant TIF1α (5 μg) was purified from baculovirus-infected SF9 cells, analyzed on a 8% SDS-polyacrylamide gel and stained with Coomassie Brilliant Blue R-250. S190 chromatin, assembled with or without TIF1α and purified by sucrose gradient sedimentation (44), was subjected to 8% SDS-polyacrylamide gel electrophoresis and immunoblotting using a specific anti-TIF1α monoclonal antibody (mAb5T1E8) (20) (see Fig. 1D). Molecular mass markers (in kilodaltons) are indicated. **D**, TIF1α co-sediments with S-190-assembled chromatin on sucrose gradients. Sedimentation of recombinant TIF1α and TIF1α-incorporated chromatin (mRARβ2-lacZ, 10.5 kbp). Purified recombinant TIF1α protein (D, TIF1α alone) and TIF1α incorporated chromatin (D, TIF1α-Chr) were fractionated by sucrose gradient sedimentation in 20, 44). Fractions from the sucrose gradients were analyzed for TIF1α by immunoblotting (mAB5T1E8) and for purified DNA by 0.8% agarose gel followed by ethidium bromide staining (EtBr). P, mRARβ2-lacZ plasmid. 5–10% of each fraction was regularly analyzed. **E**, time-dependent MNase digestion of chromatin assembled with or without TIF1α. S-190 chromatin was assembled without (-) or with TIF1α, core histones, plasmid DNA (mRARβ2-lacZ plasmid), and an ATP regenerating system and subjected to microccocal nuclease (MNase) digestion analysis. The DNA mass markers indicated in the middle of the panel (M) are the 123-bp ladder (Invitrogen). The samples on the left side of the panel without TIF1α (-) and on the right, with TIF1α (TIF1α) were obtained by digestion of assembled chromatin with 0.2 units of MNase (Sigma) for 0.5, 1, and 3 min, respectively. F, electron microscopy was performed on sucrose gradient-purified chromatin assembled either using the S-190 extract (mRARβ2-lacZ plasmid, top panels) as described above or by salt dialysis (pBluescript, 2.9 kbp) (bar = 100 nm). Size measurements were performed using NIH Image program (fitted as an ellipse). Nucleosome dimensions (top panels) were 122 ± 17 Å × 143 ± 16 Å (98 particles) (core histones) and 129 ± 19 Å × 161 ± 15 Å (134 particles) (core histones + TIF1α). The probability that the difference in values is not statistically significant is *p* < 0.003 for the short axis and *p* < 10⁻⁶ for the long axis of the fitted ellipse.
(5, 62), the HP1 box (amino acids 701–750) (5), and the TIF1 signature sequence (TSS, amino acids 403–426) (10) did not associate with chromatin (Fig. 3B). However, TIF1/H9251 (734–1017) efficiently and stably associated with chromatin in vitro (Fig. 3B). Therefore, the C-terminal region of TIF1α, comprising the PHD/TTC finger and the BrD, is both necessary and sufficient for stable TIF1/H9251-chromatin interactions.

We then used EMSA to find out whether the PHD/TTC finger and/or the BrD were involved in DNA and nucleosome interactions (Fig. 3C). The PHD/TTC finger (TIF1α(734–853) (0.8, 1.6, 3.2, and 6.3 μM)) binds to naked DNA—resulting in the formation of large nucleoprotein complexes—but not to the nucleosomes (Fig. 3C). Only the BrD (TIF1α(854–1017) (0.6, 1.2, 2.3, and 4.6 μM)) associated both with DNA and nucleosomes, resulting in a single stable BrD-DNA and BrD-nucleosome complex, respectively (Fig. 3C). Moreover, multimeric complexes appear to be formed as the mobility of BrD-DNA and BrD-nucleosome complexes is dramatically reduced when the concentration of the BrD is increased (Fig. 3D). Thus, the BrD represents both a stable DNA binding domain and a nucleosome interaction motif.

A stretch of four residues, namely PMDL, is highly conserved in helix A of BrDs (spanning from the end of the ZA loop to the first residue of the αA), in the majority of BrD-containing factors (28, 38), and it represents a signature for this domain (Fig. 4A). Hence, these residues were selectively mutated to TAQA (mtBrD) (Fig. 4A), the recombinant protein was expressed as a His-tag protein in E. coli and purified by affinity chromatography. Its ability to interact with DNA and nucleosomes was then analyzed by EMSA and compared with the wild-type BrD (Fig.
4B, 4.6 μM). The disruption of both BrD-DNA and BrD-nucleosome interactions was evident (Fig. 4B). Because abolition of DNA binding correlates with the disruption of BrD-nucleosome interactions, we believe that association of BrD with nucleosomes is mediated primarily via BrD-DNA interactions.

**DISCUSSION**

We have used in vitro chromatin assembly assays and nucleosomal bandshifts to analyze the role of the bromodomain (BrD) on TIF1α-chromatin interactions. In our experiments, we found that the BrD alone is sufficient to bind to nucleosomes;
mouse embryos, where TIF1 target and/or stabilize TIF1 matin (20, 21), the extensive TIF1 receptors to bind in order to enhance the efficiency of transcription in vivo. Events may well be required for efficient euchromatin targeting in vivo and forms a stable complex with chromatin –1, scGCN5. Other factors include hsCBP, CeYNJ1, hsCCG1–2, maCCG1–2, and dmTAFlH50–2 (28). B, the PMLD motif mediates BrD-DNA and BrD-nucleosome interactions. Mutation in BrD signature, PMLD to TAQA. EMSA was performed at room temperature with 4.5 μl either wild-type BrD or PMLD signature mutant (mtBrD) and 32P-labeled naked M4 DNA or nucleosomes. The samples were subjected to electrophoresis at room temperature on a 5% native polyacrylamide gel in 0.5× TBE. The gels were dried and exposed to autoradiography. Moreover, the BrD binds directly to nucleosomal DNA.

TIF1α Binds to DNA and Nucleosomes—We have examined the functional association of nuclear receptor coactivator TIF1α with chromatin templates. We find that TIF1α binds directly and forms a stable complex with chromatin in vivo and in vitro and therefore acts as an integral component of the protein-DNA complex in euchromatin. This is achieved through TIF1α-nucleosome interactions and depends on the ability of TIF1α to interact with DNA in a way that does not necessitate the presence of additional factors. The mode of TIF1α selection of genomic sites in vivo is unknown. Although binding of TIF1α to nucleosomal DNA in vivo appears to be sequence-independent, the exclusive residence of TIF1α in euchromatin suggests that a selection mechanism for TIF1α binding to chromosomal sites must exist in vivo. Presumably, there are TIF1α-interacting factors that confer specificity on TIF1α-binding, functioning to target and/or stabilize TIF1α association with euchromatin, in a similar way proposed for the association of HP1 with heterochromatin (63). Because TIF1α is a phosphoprotein (19) and can undergo SUMO-lation (18), post-translational modification events may well be required for efficient euchromatin targeting in vivo.

A role for TIF1α as a docking platform, for liganded nuclear receptors to bind in order to enhance the efficiency of transcription by selectively scanning the chromatin (20, 22) would require the constant presence of TIF1α on chromatin. Indeed, in mouse embryos, where TIF1α associates strongly with euchromatin (20, 21), the extensive TIF1α surface on chromosomal DNA is consistent with an activation-competent environment where all TIF1α molecules are associated with chromosomal sites. TIF1α may act over some distance and with a degree of flexibility, binding to various sites within a genomic region that could consistently keep a promoter or enhancer region competent to respond; consequently, the degree of accessibility to transcription factors experienced by a given genomic region would depend on the concentration of TIF1α within a given cell (20). To assess the role of TIF1α as a docking molecule, further analyses of TIF1α chromatin targets and their potential to associate with various TIF1α deletion mutants in vivo are currently in progress.

BrD Is a DNA-binding Motif—TIF1α association with chromatin is mediated by BrD-DNA and BrD-nucleosome interactions. This is consistent with the BrD acting as a targeting module (29) for TIF1α on selected chromosomal sites. Current models of transcriptional activation via the BrD have involved the sequence-specific binding of transcription factors to DNA that recruit complexes of BrD-containing factors to promoters via protein-protein interactions. However, we have already demonstrated that in vivo interactions between TIF1α and chromatin prefigure the recruitment of transcription factors such as liganded nuclear receptors and/or other coactivators or chromatin proteins such as HP1α (20). This result argues for a role for TIF1α as a chromatin-recruiting molecule rather than a recruited one. Presumably, to be able to tether itself tightly to chromatin, a DNA-binding mechanism has been employed and interactions between the BrD and nucleosomal DNA have evolved. Whether this is a property of the TIF1α BrD alone or is shared by other BrD-containing factors remains to be determined. Nevertheless, it is noteworthy that the BrD signature motif, PMLD, which plays a crucial role both in BrD-DNA and BrD-nucleosome interactions, is highly conserved throughout the BrD family (28, 42), indicating that DNA recognition may be a more general feature of BrDs, than has previously been realized. The importance of this motif is underlined by the fact that the first two residues, P, are essential for GCN5-dependent transcriptional activation in yeast (34, 38). In fact, our observation that the BrD is a DNA- and nucleosome-binding motif explains why the BrD is indispensable for GCN5-mediated nucleosomal acetylation, (31, 55) and for subsequent activation events on selected promoters in yeast (34).

It has been proposed that the BrD is a protein-protein interaction domain that selectively recognizes amino acid sequences in which lysines are acetylated, as for histone H3 and H4 (29) and for MyoD (36) and Tat (39, 40) transactivators, suggesting a more general role for the BrD as an acetyl-lysine binding motif (36–38). Also, it has been recently shown that the BrD is necessary for p300 association with chromatin in vitro (43), thus accounting for why it is critical for efficient acetylation of nucleosomal histones and transcriptional activation (64). However, the BrD alone did not appear to be sufficient to mediate p300-chromatin interactions (43).

Here, we provide the first experimental evidence that the BrD binds to nucleosomes and DNA, adding BrD-DNA interactions to the protein-protein interactions that are known to be involved in selecting the sites that enable chromatin opening to a wide range of transcription factors. The DNA-binding activity of the BrD could be critical for tumorigenesis because, in acute myeloid leukemia, fusions exist between genes such as MORF and MOZ with CBP regions that include the BrD, thus creating chimeric proteins that could now be recruited to chromosomal DNA (65–67). Whether DNA binding or a histone-BrD interaction is the first step in the cascade of events that determine the accessibility of a specific chromosomal site remains to be investigated.
Our results suggest that the BrD targets TIF1α, and possibly other transcriptional regulators, to nucleosomal DNA at those chromatin sites where they exert their function in vivo. Remarkably, many different protein domains can be linked to BrD, including histone acetyltransferase, kinase, ATPase, and helicase activities (27, 28), emphasizing its role as a multipurpose chromatin adaptor in vivo.

Acknowledgments—We are grateful to P. Chambon for outstanding support and powerful discussions; A. Gould and Tim Jinks for advice and thorough review of the manuscript; R. Lovell-Badge and V. Episkopou for comments on the manuscript; J. T. Kadonaga and P. Becker for advice on chromatin assembly; E. Ktistaki for reagents and advice on chromatin immunoprecipitation; C. Pernet for technical assistance; J.-M. Garnier for the mBrD construct; N. Cramphorn and F. Johnson for illustrations (NIMR).

REFERENCES

1. Chambon, P. (1996) FEBS J. 10, 940–954
2. Friedman, J. R., Fredericks, W. J., Jensen, D. E., Speicher, D. W., Huang, X.-P., Nielsen, E. G., and Rauscher, F. J., III (1996) Genes Dev. 10, 2067–2078
3. Kim, S. S., Chen, Y. M., O’Leary, E., Witzigall, R., Vidal, M., and Bonventre, J. V. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 15299–15304
4. Mossman, P., Georgiev, O., Le Douarin, B., Boursqui, J. P., and Schaffner, W. (1996) Nucleic Acids Res. 24, 4859–4867
5. Le Douarin, B., Nielsen, A. L., Garnier, J. M., Ichinose, H., Jeanmougin, F., Losson, R., and Chambon, P. (1996) EMBO J. 15, 6701–6715
6. Murzina, N., Verveult, A., Laze, E., and Stillman, B. (1999) Mol. Cell 4, 529–539
7. Cammas, F., Ould-Abelhadj, M., Vonesch, J. L., Huse-Garcia, Y., Chambon, P., and Losson, R. (2002) J. Cell Sci. 115, 3439–3448
8. Nielsen, A. L., Ortiz, J. A., You, J., Ould-Abelhadj, M., Kcheuchman, R., Gansmuller, A., Chambon, P., and Losson, R. (1999) EMBO J. 18, 6358–6365
9. Nielsen, A., Ould-Abelhadj, M., Ortiz, J. A., Remboutsika, E., Chambon, P., and Losson, R. (2001) Mol. Cell 7, 739–751
10. Venturini, L., You, J., Stadler, M., Lallemand, V., Koken, M. H., Mattei, M. G., Ganser, A., Chambon, P., Losson, R., and de The, H. (1999) Oncogene 18, 1209–1217
11. Peng, H., Feldman, I., and Rauscher, F. J., III (2002) J. Biol. Chem. 277, 28338–28348
12. Le Douarin, B., Le Douarin, B., Portier, H., Bellon, H., Losson, R., and Barron, J. T. (1999) EMBO J. 18, 75–85
13. von Baur, E., Zach, C., Heine, D., Leurer, M., Garnier, M. J., Feldman, I., and Rauscher, F. J., III (2002) J. Biol. Chem. 277, 12062–12068
14. Thenot, S., Bonnet, S., Boulahtouf, A., Margeot, E., Ray, C., Borgna, J. L., and Cavadore, J. N. (1999) Mol. Endocrinol. 13, 2137–2150
15. Zong, S., Duval, L., Becskei, A., Ghandi, D., Zhang, H., Kallastray, S., Freedman, L. P., and Pandolfi, P. P. (1999) Nat. Genet. 25, 287–295
16. Beckstead, B., Ortiz, J. A., Sanchez, C., Prokopenko, S. N., Chambon, P., Losson, R., and Bellen, H. J. (2001) Mol. Cell 7, 735–765
17. Seeler, J. S., Marchio, A., Losson, R., Desterro, J. M., Hay, R. T., Chambon, P., and Dejean, A. (2001) Mol. Cell. Biol. 21, 3314–3324
18. Fraser, R. A., Heard, D. J., Adam, S., Lavigne, A. C., Le Douarin, B., Tora, L., Losson, R., Rochette-Egly, C., and Chambon, P. (1998) J. Biol Chem. 273, 16199–16204
19. Niedeerreither, K., Remboutsika, E., Gansmuller, A., Losson, R., and Dolle, P. (1999) Mech. Dev. 88, 111–117
20. Tomura, A., Goto, K., Morinaga, H., Nomura, M., Okabe, T., Yanase, T., Takayanagi, R., and Nawata, H. (2001) J. Biol. Chem. 276, 28385–28401
21. Borden, K. L., and Freemont, P. S. (1996) Curr. Opin. Struct. Biol. 6, 385–401
22. Borden, K. L. (1996) Biochem. Cell Biol. 75, 351–358
23. Borden, K. L. B. (2000) J. Mol. Biol. 285, 1103–1112
24. Aslând, R., Gibson, T. J., and Stewart, A. F. (1995) Trends Biochem. Sci. 20, 56–59
25. Haynes, S. R., Dollard, C., Winston, F., Beck, S., Trowsdale, J., and David, I. B. (1992) Nucleic Acids Res. 20, 2603
26. Jeannot, F., Wurts, J. M., Le Douarin, B., Chambon, P., and Losson, R. (1997) Trends Biochem. Sci. 22, 151–153
27. Winston, F., and Allis, C. D. (1999) Nat. Struct. Biol. 6, 601–604
28. Bochar, D. A., Savard, J., Wang, W., Laufer, D. W., Moore, P., Cote, J., and Shiekhattar, R. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 1038–1043
29. Sterner, D. E., Grant, P. A., Roberts, S. M., Duggan, L. J., Belseretorskaya, R., Pacella, L. A., Winston, F., Workman, J. L., and Berger, S. L. (1999) Mol. Cell. Biol. 19, 86–98
30. Tomita, A., Towarai, M., Tsuzuki, S., Hayakawa, F., Kosugi, H., Tamai, K., Miyazaki, T., Kinoshita, T., and Sato, H. (2000) Oncogene 19, 444–451
31. Kouzardes, T. (2000) EMBO J. 19, 1176–1179
32. Synichaki, P., Topalidou, I., and Theuret, G. (2000) Nature 404, 414–417
33. Ny [|...](http://www.jbc.org)
The Bromodomain Mediates Transcriptional Intermediary Factor 1α-Nucleosome Interactions
Eumorphia Remboutsika, Ken Yamamoto, Matthias Harbers and Marc Schmutz

J. Biol. Chem. 2002, 277:50318-50325.
doi: 10.1074/jbc.M203759200 originally published online October 15, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M203759200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 67 references, 22 of which can be accessed free at http://www.jbc.org/content/277/52/50318.full.html#ref-list-1