THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 3, Issue of January 15, pp. 1628–1634, 1999

Printed in U.S.A.

High Mobility Group Protein 1 Interacts Specifically with the Core Domain of Human TATA Box-binding Protein and Interferes with Transcription Factor IIB within the Pre-initiation Complex*

(Received for publication, September 10, 1998, and in revised form, October 15, 1998)

Montserrat Sutrias-Grau‡§, Marco E. Bianchi¶, and Jordi Bernués‡‖

From the ‡Departament Biologia Molecular i Cellular, Centre d'Investigació i Desenvolupament-Consejo Superior de Investigaciones Científicas, J. Girona 18-26, 08034 Barcelona, Spain, ¶Dipartimento di Ricerca Biologica e Technologia, San Raffaele Scientific Institute, via Olgettina 58, 20132 Milano, Italy, and ¶Dipartimento di Genetica e di Biologia dei Microorganismi, Universitá di Milano, via Celoria 26, 20133 Milano, Italy

The high mobility group (HMG) box domain has defined a family of proteins, mostly transcription factors, that specifically interacts with DNA on the minor groove and sharply bends it. The founding member of the family, HMG1, does not specifically recognize regular B-DNA but is recruited to DNA by interaction with other transcription factors and TATA box-binding protein (TBP). However, conflicting effects of HMG1 on transcription have been reported. We show that the interaction between HMG1 and TBP is species-specific. This interaction in turn affects the interaction of TBP with transcription factor (TF) IIB and is competed by TFIIA. A primary binding site was mapped to the H2′ α-helix in the highly conserved core domain of human TBP. On HMG1, the primary binding site was only in the HMG box A, and HMG box A was also sufficient to interact with native TFIID. Both HMG boxes efficiently repressed transcription *in vitro* as fusions to the Gal4-DNA binding domain. Additionally, HMG box B showed a weak level of activation at very low amounts. These results suggest a general involvement of HMG1 at the early stages of polymerase II transcription that may result in subtle activation or repression of individual genes.

High mobility group protein 1 (HMG1)[1] is an abundant, highly conserved nuclear protein found in practically all eukaryotes. It is structured in three domains, one C-terminal highly acidic domain and two basic domains, A and B. The structures of the A and B domains have been solved in solution by NMR (1, 2). Both domains adopt a very similar L-shaped structure, formed by two short and one long α-helix, that is known as the HMG box domain. An increasing number of proteins containing one or more HMG box domains have been described; the HMG box domains for which the structure has been solved, such as the ones in LEF-1 or SRY, are very similar to the HMG box domains of HMG1 (3, 4). However, whereas SRY and LEF-1 interact with DNA in the minor groove with a certain sequence specificity, HMG1 (and the related protein HMG2) does not interact specifically with regular B-DNA. Nevertheless, HMG1 shows a clear preference for binding angled structures in the DNA without any sequence specificity, such as cisplatin-modified DNA, bulged DNA, or four-way DNA junctions (5, 6). Structure-specific DNA recognition has also been observed for the HMG box domains of several other proteins such as UBF and SRY (7, 8).

The HMG boxes of HMG1 are also a place for protein-protein interactions. Both HMG1 domains A and B have been reported to interact with the POU domains of Oct2 and HOXD9 (9, 10), and full-length HMG1 has been shown to interact with TBP (11) and recently with p53 (12) and steroid hormone receptors (13).

The fact that many members of the HMG box family are transcription factors (14), along with the interactions of HMG1/2 with several transcription factors, has suggested a role for HMG1/2 in transcription. In this respect, enhancement of progesterone receptor binding to specific DNA sequences (15), reversible repression of basal and activated transcription *in vitro* (11, 16), and co-stimulation of activated transcription both *in vitro* and *in vivo* (9, 10, 17, 18) have been well documented.

Here we describe a remarkably species-specific interaction of HMG1 with human TBP that affects the interaction of TFIIB with hTBP and is competed by TFIIA. The interaction takes place between the highly conserved core domain of hTBP and the HMG1 box A domain; HMG box A also interacts with the high molecular mass TFIID complex in crude nuclear extracts. HMG box A represses transcription *in vitro* on its own, and this activity can be significantly increased by using Gal4-binding domain fusions.

EXPERIMENTAL PROCEDURES

Plasmids—Plasmids pT7-HMG1bA and pT7-HMG1bB, expressing rat HMG1 boxes A and B, respectively, have already been described (19). pET14b-HMG1 was constructed by inserting an *Nde*I-*Bam*HI insert from pT7-HMG1 (20) in pET14b (Novagen). An internal *Nde*I site at position +44 in HMG1 was previously mutated without affecting the amino acidic sequence.

Constructs expressing His_6-tagged human and yeast TBP were provided by A. Hoffmann and R. Roeder (The Rockefeller University, New York) and J.-M. Egly (Institut de Génétique, Biologie Moléculaire et Cellulaire (IGBMC), Illkirch, France), respectively. The human TFIIB expression plasmid was obtained from D. Reinberg (Robert Wood Johnson Medical School, University of Medicine and Dentistry, Piscataway, NJ).

pGST-HMG1box A was constructed by inserting a *Nco*I-*Eco*O109

* This work was supported by Grants CHRX-CT94-0482 and FMRXCT970109 from the European Union and Grants PB96-0812 and UE96-0021 from the Spanish Comisión Interministerial de Ciencia y Tecnología and was carried out within the framework of the "Centre de Referència en Biotecnologia" of the Generalitat de Catalunya. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Doctoral fellow of the Fundación Ramón Areces.

‖ To whom correspondence should be addressed. Tel.: 34-93-400 61 77; Fax: 34-93-204 59 04; E-mail: jbmbmc@cid.csic.es.

[1] The abbreviations used are: HMG1, high mobility group protein 1; cisplatin, *cis*-diamminedichloroplatinum; EMSA, electrophoretic mobility shift assay; FPLC, fast protein liquid chromatography; pol, polymerase; PIC, pre-initiation complex; GST, glutathione *S*-transferase; TBP, TATA box-binding protein; hTBP, human TBP; hcTBP, human core TBP; yTBP, yeast TBP; ycTBP, yeast core TBP; WT, wild type; TF, transcription factor; TAF, TBP-associated factor.

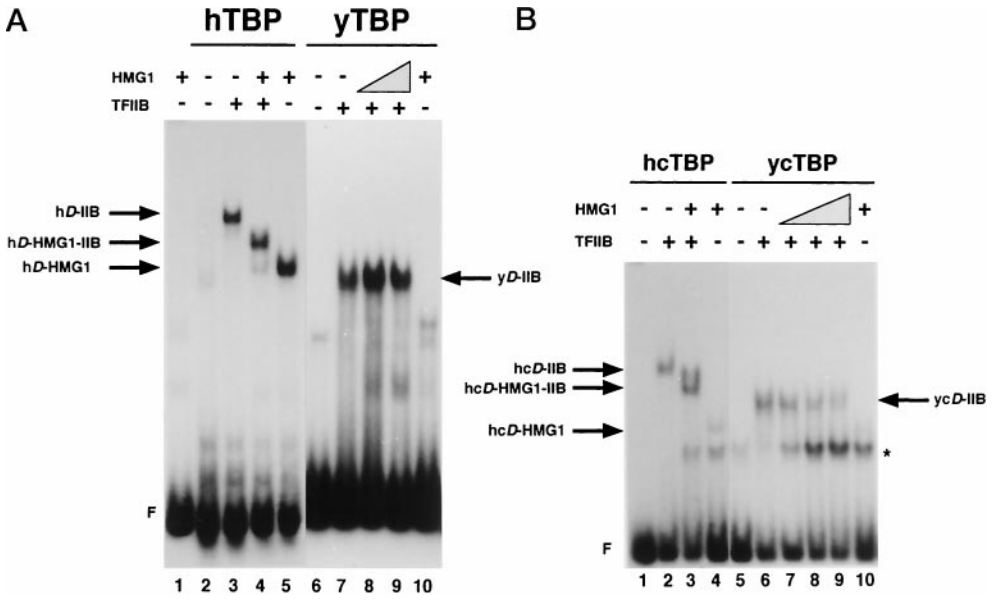

FIG. 1. **HMG1 interacts with hTBP but not with yTBP.** *A*, EMSA of human TBP (10 ng, *lanes 2–5*) and yeast TBP (20 ng, *lanes 6–10*) in the presence of TFIIB (10 ng, *lanes 3* and *7*), HMG1 (30 ng, *lanes 5* and *7*), and both (*lane 4*, TFIIB + 30 ng of HMG1; *lanes 8* and *9*, 10 ng of TFIIB + 30 and 60 ng of HMG1, respectively). *Lane 1* is a control with HMG1 (60 ng) alone. *B*, EMSA of human core TBP (5 ng, *lanes 1–4*) and yeast core TBP (18 ng, *lanes 5–10*) in the presence of TFIIB (10 ng, *lanes 2* and *6*), HMG1 (140 ng, *lanes 4* and *10*), and both (*lane 3*, 10 ng of TFIIB + 35 ng of HMG1; *lanes 7–9*, 10 ng of TFIIB + 35, 70, and 140 ng of HMG1, respectively). *Lanes 1* and *5* are controls for human core and yeast core TBP alone, respectively. The positions of the relevant complexes are indicated; h*D* and hcD and y*D* and yc*D* indicate full-length or core versions of human and yeast TBP, respectively. *F*, free DNA probe; ∗ denotes a nonspecific complex.

(Klenow-filled) fragment from pET14b-HMG1 into pGEX-KG digested with *Hin*dIII (Klenow-filled) and *Nco*I. pGST-HMG1box B was constructed by inserting an *Nde*I (Klenow-filled)-*Hin*dIII fragment obtained from pT7-HMG1bB into a pGEX-KG digested with *Sma*I and *Hin*dIII. pGEX-KG is a derivative of pGEX-2T (Amersham Pharmacia Biotech) with an extended polylinker.

pET14b-Gal4BD-HMG1box A was obtained by ligating the sequence corresponding to domain A, excised from pET14b-HMG1 with *Nde*I and *Ava*I and filled in with Klenow enzyme, to the *Sma*I site of pET14b-Gal4BD; pET14-Gal4BD-HMG1box B was constructed by ligating the sequence corresponding to domain B, excised from pT7-HMG1bB with *Nde*I and *Hin*dIII and filled in with Klenow enzyme, to the *Sma*I site of pET14-Gal4BD.

All constructs were verified by DNA sequencing.

Recombinant Proteins and Extracts—Expression and purification of HMG1 boxes A and B were carried out in *Escherichia coli* BL21(DE3) cells as described (19) except that they were further purified over FPLC-MonoS columns using a 0–1 M KCl gradient in buffer D (20 mM HEPES, pH 7.9, 20% glycerol, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride). HMG1 box A elutes at 0.6 M KCl and box B at 0.2 M KCl. Selected fractions were pooled, dialyzed *versus* 0.1 M KCl-buffer D, aliquoted, and stored at −80 °C. Recombinant His_6-tagged HMG1 was expressed in *E. coli* BL21(DE3) pLysE cells at 30 °C for 2 h and purified over Ni^{2+}-nitrilotriacetate columns exactly as described for human TBP (21). Fractions containing HMG1 were further purified by FPLC over MonoQ columns using a 0.05–1 M KCl gradient in buffer D. HMG1, which elutes at 0.5 M KCl, was dialyzed *versus* 0.1 M KCl-buffer D, aliquoted, and stored at −80 °C.

Expression and purification of yeast and human TBP were done as described (22). Human TBP mutants in pQE vectors were expressed and purified on Ni^{2+}-nitrilotriacetate columns as described above and further purified on FPLC-MonoS columns. For pull-down experiments, the soluble fraction of crude extracts was used without further purification.

Recombinant human TFIIB was expressed in *E. coli* and purified as described (23). TFIIA was purified from HeLa nuclear extracts exactly as described (24).

GST and GST box A and box B were expressed and purified as suggested by the manufacturer (Amersham Pharmacia Biotech).

Pull-down Assays—GST, GST box A, or GST box B were incubated with glutathione-Sepharose beads in 0.1 M NaCl-buffer D containing 0.05% Nonidet P-40. Protein in excess was washed away with the same buffer. For each experiment, 20 μl of beads were incubated for 1 h at 4 °C with extracts in a final volume of 450 μl. After exhaustive washing of the beads with the same buffer, retained proteins were eluted by boiling in SDS-polyacrylamide gel electrophoresis loading buffer. Proteins were separated in SDS-polyacrylamide gels, transferred to nitrocellulose membranes (BA-85, Schleicher & Schuell), probed with specific antibodies, and detected using ECL reagents.

EMSA and Hydroxyl Radical Footprinting—Gel retardation analysis was performed by incubating the proteins and the TATA box WT probe for 30 min at 30 °C (22). Supershifting of the bands included an additional incubation with the antibody for 10 min before loading the gels. Antibodies used were a monoclonal anti-TFIIB (Promega) and 3G3 anti-hTBP monoclonal antibody (a gift from P. Chambon, IGBMC).

Hydroxyl radical footprinting was done as described (25). Briefly, after assembly of complexes under the same conditions as for EMSA but omitting glycerol, samples were subjected to hydroxyl radical digestion conditions, and the reaction was quenched by glycerol addition. Samples were then run on nondenaturing EMSA gels and the corresponding complexes excised from the gels after visualization by autoradiography. DNA was eluted and analyzed in denaturing 20% polyacrylamide gels.

In Vitro Transcription Assays—*In vitro* transcription reactions were done and analyzed as described (21). The 8xGal4-WT DNA template was constructed by multimerization of a pair of synthetic oligonucleotides containing a monomer of the Gal4-binding site sequence (CGGAGTACTGTCCTCCG and CGGAGGACAGTACTCCG) and ligation to the *Eco*RI site, made blunt by repair with Klenow enzyme, of the WT G-less construct previously described (22).

RESULTS

HMG1 Binds Human but Not Yeast TBP—The involvement of HMG1 (and HMG2) in the transcription process has long been a matter of discussion, and apparently opposite results have been reported. Transcription from pol II requires the formation of a pre-initiation complex (PIC). This process is begun by the recognition of the TATA box by TBP. Then, TFIIA and TFIIB join the initial complex, followed by several other factors until the whole set of basal factors plus RNA pol II are ready to start transcription (26).

With the aim of clarifying the role of HMG1 in RNA pol II transcription, we have studied its involvement in several of the very beginning steps of PIC formation.

As shown in Fig. 1*A*, the interaction of HMG1 with human TBP was clearly seen in an EMSA gel, whereas it could not be detected when using yeast TBP (compare *lanes 5* and *10*). Notice that in the gel conditions used, TBP interaction with the

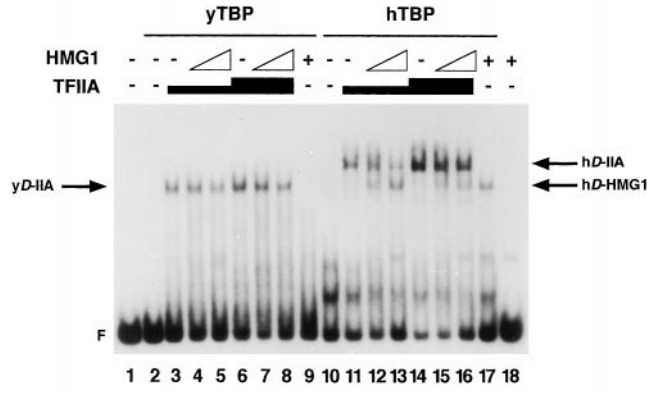

FIG. 2. **Binding of HMG1 and TFIIA to hTBP is mutually exclusive.** EMSA of yeast TBP (20 ng, *lanes 2–9*) in the presence of TFIIA (125 ng, *lanes 3–5*; 180 ng, *lanes 6–8*) and human TBP (10 ng, *lanes 10–17*) in the presence of TFIIA (30 ng, *lanes 11–13*; 60 ng, *lanes 14–16*). Increasing amounts of HMG1 (35 and 100 ng) were added, respectively, to *lanes 4* and *5, 7* and *8, 12* and *13*, and *15* and *16*. *Lanes 9, 17,* and *18* contain 70 ng of HMG1. *Lane 1* contains free DNA probe. Relevant complexes are indicated by *arrows*; h*D* and y*D* indicate human and yeast TBP, respectively. *F,* free DNA probe.

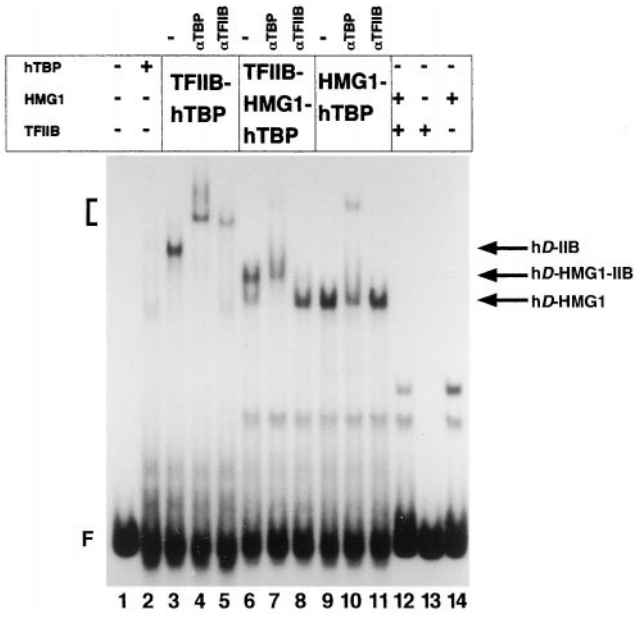

FIG. 3. **Altered arrangement of TFIIB in the hTBP-HMG1-TFIIB complex.** The composition of the TFIIB-hTBP (*lanes 3–5*), TFIIB-HMG1-hTBP (*lanes 6–8*), and HMG1-hTBP complexes (*lanes 9–11*) was assessed in each case by addition of buffer, anti-hTBP, and anti-hTFIIB antibodies, respectively. *Lanes 1* and *2* and *12–14* are controls for free DNA probe, hTBP alone, and HMG1 and TFIIB, either alone or in combination. Complexes are indicated on the *right* (*arrows*). The supershifted complexes are indicated by a *bracket* on the *left*. h*D* indicates human TBP. *F,* free DNA probe.

TATA box on its own was very unstable and could hardly be detected (see Fig. 1, *A* (*lanes 2* and *6*) and *B* (*lanes 1* and *5*)) as originally described (27). The interaction of HMG1 with the hTBP-TATA complex (h*D*) gave rise to a clear complex HMG1-hTBP (h*D*-HMG1) (Fig. 1*A, lane 5*), suggesting that HMG1 stabilized the binding of hTBP to the TATA box. Moreover, this interaction also took place with the TFIIB-hTBP complex (h*D*-IIB), giving rise to the HMG1-hTBP-TFIIB complex (h*D*-HMG1-IIB, *lane 4*). Remarkably, the presence of HMG1 in the HMG1-hTBP-TFIIB complex did not result in a band of lower electrophoretic mobility but rather of intermediate mobility between the hTBP-TFIIB and the HMG1-hTBP complexes (compare *lane 4* with *lanes 3* and *5*). HMG1 did not bind significantly to DNA in the absence of TBP (Fig. 1*A, lane 1*).

Human and yeast TBPs share a highly conserved C-terminal region (the so-called core domain) with 81% sequence identity, whereas the N-terminal domains do not show any significant homology. Fig. 1*B* shows that using human core TBP (hcTBP), HMG1 still interacts with hcTBP and gives rise to a HMG1-hcTBP-TFIIB complex with anomalous electrophoretic mobility as shown above (hc*D*-HMG1-IIB, *lane 3*). The interaction of hcTBP with HMG1 looks weaker in this case (*lane 4*). Yeast core TBP (ycTBP) was also assayed for interaction with HMG1 (*lanes 7–10*) either alone or in the presence of TFIIB. Results show that ycTBP interacted with TFIIB (*lane 6*) but not with HMG1, ruling out any potential interference by the yTBP N terminus. The presence of increasing amounts of HMG1 appeared somehow to interfere with the formation of the ycTBP-TFIIB complex (Fig. 1*B*, *lanes 6–9*). This interference never resulted in the formation of a new complex containing ycTBP and might reflect a very weak interaction that cannot be observed with the full-length yTBP (Fig. 1*A*, *lanes 7–9*). All of these results indicate that the core domain of hTBP contains the primary binding site for HMG1, most likely in a region not conserved in the yeast TBP.

EMSA experiments (Fig. 2) showed no evidence for a hypothetical TFIIA-HMG1-hTBP ternary complex. Instead, bands of the hTBP-TFIIA and hTBP-HMG1 complexes appeared at the same time (h*D*-IIA and h*D*-HMG1, Fig. 2, *lanes 12* and *13* and *15* and *16*), and their intensities were inversely correlated. There was no detectable interaction either with yTBP or yTBP-TFIIA, but there was a faint interference as the amount of HMG1 increased (*lanes 3–5*). This effect became weaker at higher amounts of TFIIA (*lanes 6–8*). Because homologous hTBP-hTFIIA complexes have been reported to be more stable than heterologous yTBP-hTFIIA complexes (28), this effect might reflect a very weak interaction that cannot give rise to a defined band, as suggested above. The same results were obtained when the core versions of yeast and human TBPs were assayed (results not shown). Altogether, the results suggest that HMG1 does not interact with TFIIA and that their binding sites on hTBP may be in close proximity.

The Interaction of HMG1 with hTBP Affects the Arrangement of TFIIB in the PIC—The simplest explanation for the anomalous mobility of the HMG1-hTBP-TFIIB complex may be that the overall arrangement of subunits in the complex was different from the hTBP-TFIIB and hTBP-HMG1 complexes. To test this hypothesis, the hTBP-TFIIB, HMG1-hTBP-TFIIB, and hTBP-HMG1 complexes were assembled and then incubated with monoclonal antibodies against hTBP or TFIIB (Fig. 3). *Lanes 3–5* in Fig. 3 are controls run with the hTBP-TFIIB complex. *Lanes 9–11* show the presence of hTBP in the hTBP-HMG1 complex and the lack of reaction with anti-TFIIB. Note that the amount of the complex supershifted with the anti-hTBP antibody was clearly reduced in the HMG1-hTBP complex (and also in the HMG1-hTBP-TFIIB) when compared with the TFIIB-hTBP complex (Fig. 3, *lanes 10, 7,* and *4,* respectively). Since the antibody used (3G3) recognizes residues 1–17 of hTBP (29), these residues are probably close to HMG1 in the complex. *Lanes 6* and *7* show that the HMG1-hTBP-TFIIB complex could still be supershifted with the anti-hTBP antibody, although at a very moderate level. Upon addition of the anti-TFIIB antibody, the HMG1-hTBP-TFIIB complex was not supershifted but actually disappeared (*lane 8*); at the same time, the band representing the HMG1-hTBP complex became prominent. Unfortunately, the presence of HMG1 in the complexes could not be observed directly because anti-HMG1 antibodies do not supershift HMG1 complexes (results not shown). These results suggest that TFIIB is weakly bound within the complex so that the addition of the anti-TFIIB

antibody (but not the anti-hTBP antibody) is sufficient to displace it.

We obtained more direct evidence on the status of TFIIB in the different complexes by hydroxyl radical footprinting. Fig. 4 shows a clear hTBP footprint encompassing the TATATA sequence in the three complexes. The protection resulting from TFIIB and centered around position −17 could be observed for the TFIIB-hTBP complex but was absent in the HMG1-hTBP-TFIIB complex. This suggests that TFIIB is not stably bound to DNA in the presence of HMG1 and accounts for the reduced stability and the anomalous electrophoretic mobility of the complex shown above. In these experiments, there was no evidence for specific contacts of HMG1 with DNA in any complex. These results indicate that the interaction of HMG1 with hTBP affects the interaction of TFIIB with hTBP.

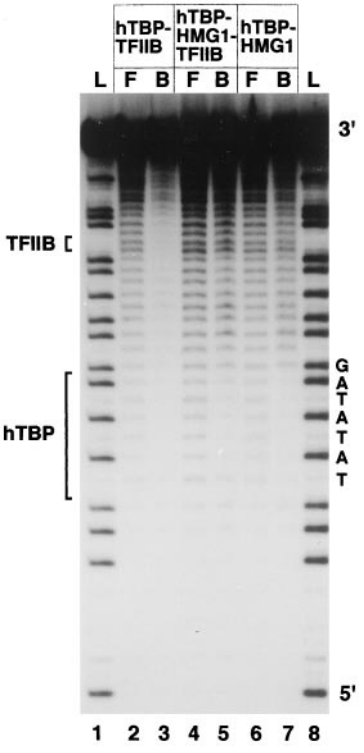

FIG. 4. **Hydroxyl radical footprinting of the TBP-containing complexes.** The three complexes studied, hTBP-TFIIB (*lanes 2* and *3*), hTBP-HMG1-TFIIB (*lanes 4* and *5*), and hTBP-HMG1 (*lanes 6* and *7*) were reacted with hydroxyl radicals as indicated under "Experimental Procedures." Free and bound DNAs (*F* and *B*, respectively) are analyzed on a 20% polyacrylamide sequencing gel. The footprints of hTBP and TFIIB are indicated on the *left*. *L*, G + A ladders. The 5′ to 3′ orientation and the sequence of the TATA box are indicated on the *right*.

HMG1-hTBP Interaction Occurs Primarily between HMG Box A and Helix H2′ of Human TBP—We identified the hTBP segment involved in the interaction with HMG1 by screening the point mutations on the surfaces of hTBP that are not engaged with DNA in the hTBP-TATA box complex. EMSA analysis of mutants in all helical tracts (hTBP-WT, -IIA, -H1, -H1′, and -H2, mostly double point mutations (30)), kindly provided by Bill Tansey and Winship Herr, showed no remarkable effect on HMG1 interaction (not shown). The mutant in helix H2′ (-H2′), although stable in *E. coli*, looked to be incorrectly folded and could not be tested. To circumvent this problem, a second set of single point mutations, kindly provided by A. Berk, was assayed (31). We studied mutations on helix H2′ and concentrated on the nonconservative changes observed between human and yeast TBPs. A pull-down assay using GST fusions to domains A and B of HMG1 was used. Only domain A, encompassing residues 1–89, showed a clear interaction with wild type hTBP (Fig. 5); domain B did not show any significant affinity for hTBP. The interaction was not strongly affected in any hTBP mutant except for the Asn → Glu substitution at position 327. In this case, a 5–10-fold lower affinity was consistently observed. Similar results have been observed in EMSA assays using full-length HMG1. Mutant 327 fully supported basal transcription in *in vitro* assays (results not shown).

All of these results indicate that HMG1 interacts with human TBP primarily by making contact with its HMG box A to the helix H2′ of hTBP, likely involving Asn-327 as an important residue.

HMG1 Interacts with TFIID—TBP has been shown to exist in mammalian cells in the form of several complexes, at least one for each polymerase; there is probably no free TBP in the cell nucleus. For RNA pol II transcription, TBP exists in the form of TFIID, a high molecular mass oligomer (>700 kDa) containing TBP and a set of other polypeptides called TAFs. As shown in Fig. 6, we have found that HMG box A can efficiently pull down TFIID from crude HeLa cell nuclear extracts, as revealed by the presence of hTBP and $TAF_{II}135$ in the retained material. HMG box B did not show any significant affinity for any form of TBP in the same extracts. In similar experiments, we observed no evidence for interaction of HMG box A with

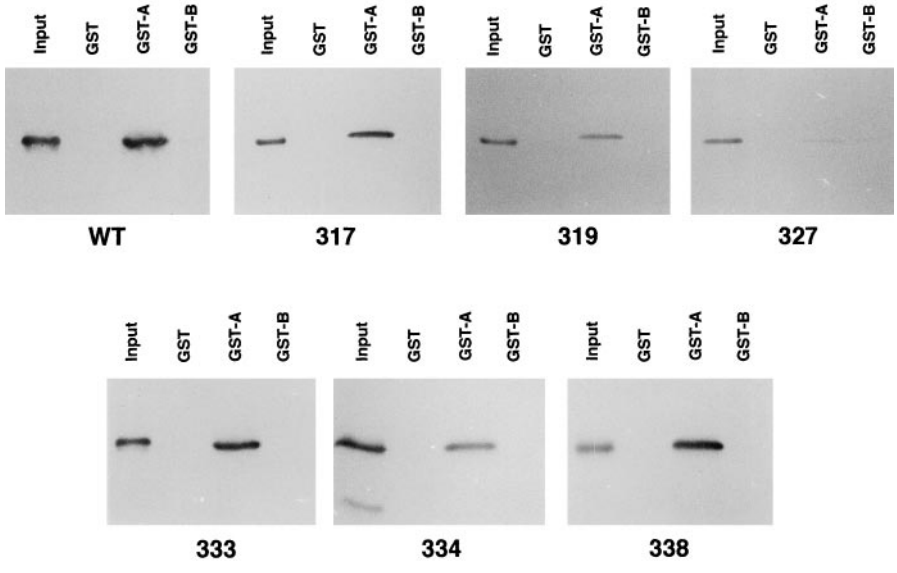

FIG. 5. **HMG1 interacts with hTBP on the H2′ α-helix.** The interaction of HMG1 boxes A and B with several point mutations on the H2′ α-helix of hTBP was assayed by pull-down analysis. The input material and the hTBP retained by GST, GST-HMGbox A, and GST-HMGbox B are shown for each mutant, as revealed by Western blot analysis. *Numbers* refer to the mutated residue. Input lanes contain 10% of the material used per reaction.

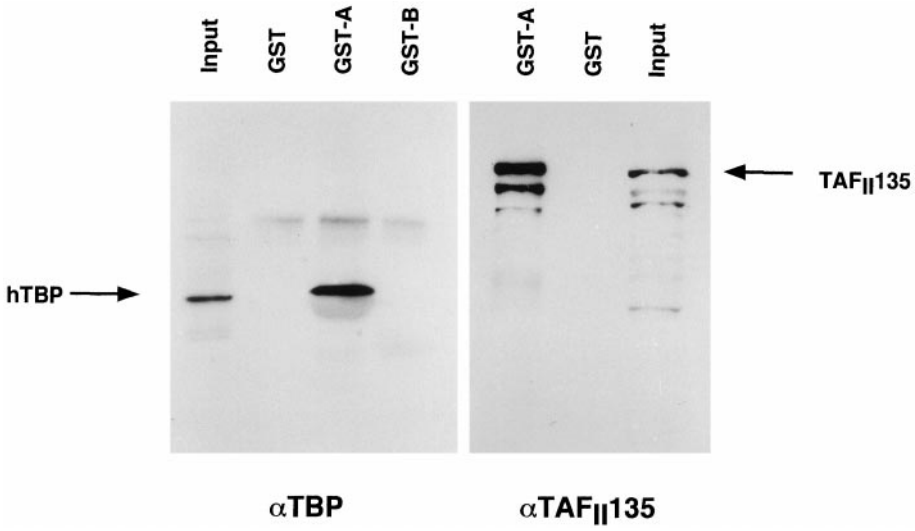

FIG. 6. **HMG box A interacts with TFIID in nuclear extracts.** The interaction of HMG1 boxes A and B with TBP in crude nuclear HeLa cell extracts was analyzed by pull-down assays. The material retained on GST, GST-HMGbox A, and GST-HMGbox B beads was analyzed by Western blot using an anti-hTBP antibody (*left panel*) and an anti-hTAF$_{II}$135 antibody (*right panel*).

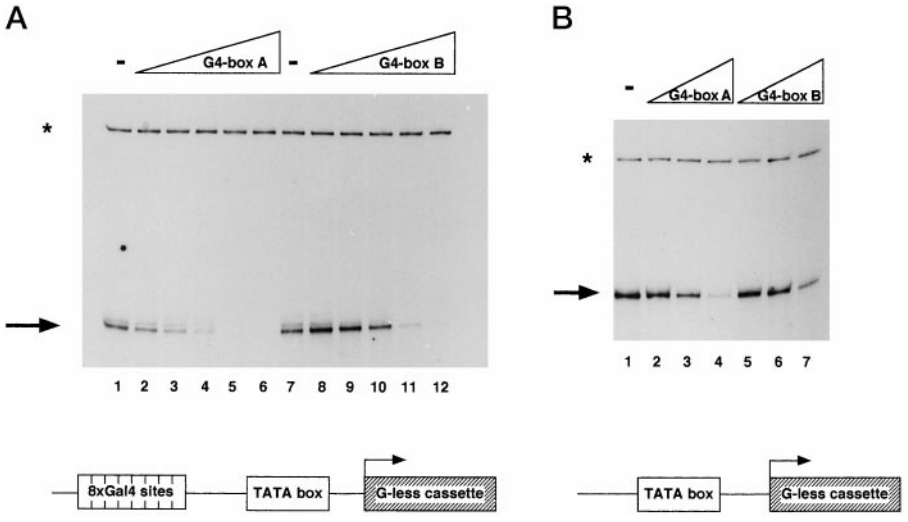

FIG. 7. **Gal4BD-HMGbox A and B fusions show different activities in basal transcription.** *A*, effect of titration of Gal4 boxes A and B on transcription *in vitro* from a template containing 8xGal4 binding sites. *Lanes 1* and *7* are control reactions with no added Gal4 fusions. *Lanes 2–6* and *8–12* contained increasing amounts (30, 60, 120, 300, and 450 ng) of Gal4 box A and Gal4 box B, respectively. *B*, effect of titration of Gal4 boxes A and B on transcription *in vitro* from a template bearing no Gal4 binding sites. *Lane 1* is a control without added Gal4 fusions. *Lanes 2–4* and *5–7* contained increasing amounts (60, 120, and 300 ng) of Gal4 box A and Gal4 box B, respectively. The positions of the full transcripts (*arrows*) and the recovery control (*) are indicated.

TFIIIB, the TBP-containing complex for pol III, suggesting an involvement of HMG1 mostly in RNA pol II transcription (results not shown).

Differences in HMG Boxes A and B on Repression of Basal Transcription When Tethered Close to a Minimal Promoter—The addition of increasing amounts of HMG1 to an *in vitro* transcription reaction has already been shown to result in repression of both basal and activated transcription. However, to obtain these results large amounts of HMG1 or -2 must be added (Refs. 11 and 16; and results not shown). We prepared Gal4-binding domain fusions to domains A and B of HMG1 to increase the efficiency of promoter loading and reduce the amounts required. We used the hybrid proteins in transcription assays *in vitro* with a template with 8xGal4 binding sites (Fig. 7*A*) or a control template with no binding sites (Fig. 7*B*) fused to a minimal promoter. Titration of Gal4 box A (Fig. 7, *A* (*lanes 2–6*) and *B* (*lanes 2–4*)) resulted in repression, which was more efficient on the 8xGal4 template. Gal4 box B titration resulted in an overall repression activity when the template tested had no Gal4 binding sites (Fig. 7*B, lanes 5–7*). However, a two-step behavior was observed when using the 8xGal4 template. At low concentrations, there was no repression; instead, a moderate (around 1.5-fold) although reproducible activation was consistently observed (Fig. 7*A, lanes 8* and *9*). At higher concentrations, a repressing effect was noticed (Fig. 7*A*, *lanes 10–12*). These differences suggest that depending on the way domain B enters the PIC, it may repress or enhance transcription.

At the same protein concentrations, Gal4 box A was more efficient in repressing transcription than Gal4 box B when using the 8xGal4 template. From the remaining transcriptional activities (16.5 and 86.8% for Gal4 box A and Gal4 box B in *lanes 4* and *10* of Fig. 7*A*, respectively), we can estimate that Gal4 box A is around 5-fold more efficient than Gal4 box B in repressing basal transcription. However, this difference practically disappeared when the template carried no Gal4 binding sites (*e.g.* compare *lanes 3* and *6* on Fig. 7*B*). These results suggest a specific repression by domain A targeted to the promoter. Remarkably, and in contrast to domain B, the addition of Gal4 box A never showed any positive effect at any concentration, suggesting again that boxes A and B, although similar, are not equivalent and that they likely play different roles in RNA pol II transcription in the context of the intact HMG1 protein.

DISCUSSION

The involvement of HMG1 (and HMG2) in transcription has been suspected for a long time, and apparently different results have been reported. Depending on the different systems and conditions used, either repression or activation of transcription has been observed *in vitro* (11, 15, 16, 18). The conflicting results may be the effect of the shortcomings of *in vitro* reconstituted systems and thus biologically meaningless, or they

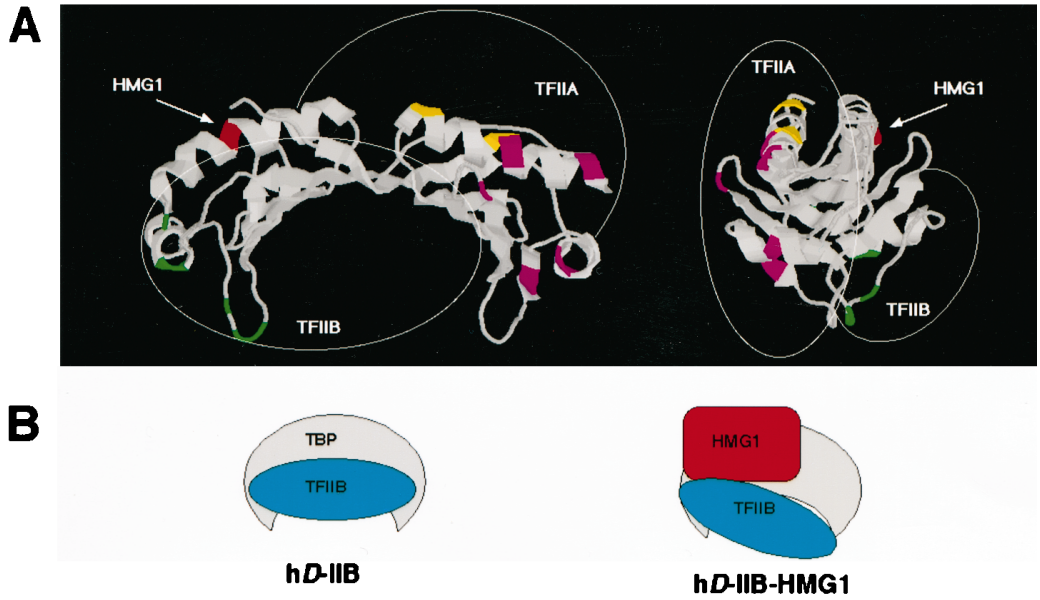

FIG. 8. **Model of the human core TBP interacting with HMG1 and TFIIB.** *A*, front and side views (*left* and *right panels*, respectively) of hcTBP as a schematic drawing of the crystal structure. The position of the residues involved in TFIIB contacts, as reported in the crystal structure, are shown in *green*. The position of the homologous residues involved in contacts with yeast TFIIA in the yIIA-yTBP positions, as described in the crystal structure, are indicated in *magenta*; the positions marked in *yellow* correspond to mutants shown to affect human TFIIA interaction with hTBP by EMSA. The position of HMG1 is shown in *red*, around Asn-327. The approximate areas occupied by TFIIB and TFIIA are indicated by *ovals* and are not intended to be at scale. *B*, schematic drawing of the proposed TFIIB arrangement in the TFIIB-hTBP (*left*) and HMG1-hTBP-TFIIB (*right*) complexes.

may come from subtly different activities of HMG1 on the promoter of different genes.

Here, we have studied in detail the interaction of HMG1 with TBP in PIC formation to gain some clues on the possible mechanisms of action. Ge and Roeder (11) first reported that HMG1 can interact with hTBP. Our results were in agreement with theirs and, surprisingly, showed that this interaction is species-specific. The contact surface of the interacting partners has been mapped; we determined that only box A of HMG1 interacts with hTBP. This is remarkable because to this point domains A and B had been shown to be absolutely equivalent for binding to cruciform and cisplatin-modified DNA (6, 14) and for interacting with HOXD9 and Oct2 (9, 10). The exact surface of HMG box A involved in the interaction with hTBP is not known; however, molecular docking analysis rules out the concave side of the L-shaped domain, which has been shown to interact in the minor groove of DNA (32). HMG1 interacts with human TBP but not detectably with yeast TBP, and the interaction takes place on the core domain. Because of the extensive sequence identity, a nonconserved region within the TBP core domain was a likely candidate to explain the results. In fact, we identified helix H2′ of hTBP as the HMG1 primary binding site; a single point mutation that converts residue 327 from Asn to Glu strongly reduces the interaction. Asn-327 is conserved neither in yeast TBP (Ala) nor in *Drosophila* TBP (Lys); in agreement, we also did not find any detectable interaction of HMG1 with *Drosophila* TBP using pull-down assays (results not shown). Mutation of Asn-327 affects the interaction with HMG1. However, some interaction can still be observed, suggesting that neighboring residues, which are not conserved either, can help in defining the binding specificity. For example, mutation of human Gly-334 (which is Glu in yeast and Lys in *Drosophila*) to Arg also weakly reduces the interaction with HMG box A (Fig. 5, *panel 334*). Note that in *Drosophila* the closest homolog to mammalian HMG1, HMG-D, shows only a very limited sequence homology (14). The same is true for the closest yeast homologs of HMG1, proteins HMO1 and HMO2, which show a weak homology, especially in the HMG box A domain (33). Conversely, both HMG1 and the core domain of TBP are absolutely conserved in mammals and may thus interact in all mammalian species.

The N-terminal region of hTBP probably contributes to the interaction with HMG1, as suggested by the reduced interaction of core TBP compared with full-length TBP and by the limited availability to antibodies of the N-terminal region of hTBP in the hTBP-HMG1 complex compared with the hTBP-TFIIB complex.

HMG1 can clearly interact not only with free TBP but also with TFIID. The simplest interpretation is that the same H2′ helix of TBP is recognized, although possible interactions with TAFs cannot be discounted. In any case, HMG box B does not interact (Fig. 6). Significantly, TBP in the TFIIIB complex is not recognized, implying that any specific biological effect should be restricted to pol II transcription, because HMG1 is excluded from the nucleolus, where pol I transcription takes place (34). In any case, the possibility of additional interactions with other basal transcription factors remains open.

Significant fractions of basal transcription factors and RNA pol II are associated to form the pol II holoenzyme (35–37). Interestingly, the holoenzyme has been reported to be efficiently repressed *in vitro* by the addition of HMG2 (35). We found no evidence for the interaction of the pol II holoenzyme with HMG1, as neither the small subunit of TFIIF (RAP30) or TFIIB was detected in pull-down experiments with crude extracts (results not shown).

The proposed site of interaction of HMG1 on TBP maps very closely in space to the TFIIB binding site on the C-terminal stirrup (38) (see Fig. 8*A*). It is also proximal to the TFIIA binding site, which has been mapped on the opposite side of TBP in the co-crystal structure and on helix H2 by site-directed mutagenesis (31, 39). The TBP-TFIIA interaction surface is still subject to some uncertainty, however, because of different requirements for the binding of human and yeast TFIIAs (40, 41). Despite these caveats, the known structures are compatible with the effects we observe of HMG1 on TFIIB and TFIIA; TFIIA binding to TBP excludes HMG1 binding, whereas HMG1

and TFIIB are not mutually exclusive, but TFIIB is repositioned with respect to the DNA (its footprint disappears) and possibly with respect to TBP (TFIIB becomes more loosely bound and TBP less accessible to specific antibodies). We do not know whether the HMG1-induced state of TFIIB within the PIC corresponds to one of the alternative (open or closed) conformations that have been attributed to TFIIB (42) or represents an additional conformation. In any case, our results suggest that in the hTBP-HMG1-TFIIB complex, TFIIB may be interacting only on the hTBP C-terminal half, likely on the stirrup (residues 284–290 in hTBP) (Fig. 8*B*). In support of this interpretation, no interaction of either HMG box A or box B with TFIIB can be detected in pull-down experiments (results not shown), suggesting that distortion of TFIIB binding to hTBP involves no specific interaction of HMG1 with TFIIB but rather a sterical hindrance.

HMG boxes in general and those of HMG1 in particular are known to interact preferentially with curved DNA (5–8, 14, 32). Therefore, the anomalous electrophoretic mobility of the hTBP-HMG1-TFIIB complex may reflect a change in the bending state of the DNA. We reason that this is unlikely because if HMG1 produces an increase in the bending state of DNA in the complex, this would result in a band of lower, not faster, electrophoretic mobility. A reduction in DNA bending is not to be expected from HMG1 properties and, in addition, would negatively affect TBP interaction with the TATA box, which has not been observed. In any case, the initial binding is because of hTBP, and the effect on DNA, if any, must be a consequence of this event. Further structural analysis is required to ascertain the bending state of the DNA in the complex.

TFIIB is absolutely required for pol II transcription and is also the potential target for several activators (for reviews, see Refs. 26 and 43). The fact that HMG1 affects the normal interaction of TFIIB in the pre-initiation complex may help explain its repressing activity observed *in vitro* (Refs. 11 and 16; this work). On the other hand, the reversal of the TBP-HMG1 interaction brought about by TFIIA might substantially reduce the overall effect of HMG1 on PIC formation and pol II transcription. We have thus tested the effect of the HMG boxes of HMG1 on transcription from a minimal promoter *in vitro*. Even in this highly simplified situation, the results observed are complex. HMG box B has a moderate repressing effect when present in solution at high concentrations and has concentration-dependent activating or repressing effects when it is recruited to the promoter by tethering it to a DNA binding domain. Box A, on the contrary, shows only repressing effects in both cases.

Overall, the HMG1-TBP interaction does not necessarily preclude a successful PIC formation, perhaps as a consequence of TFIIA recruitment. Nonetheless, as some effect is apparent, HMG1 interactions might be biologically relevant for the modulation of PIC formation *in vivo*. HMG1 may help recruit the transcriptional activators with which it interacts (HOX and OCT proteins, nuclear hormone receptors, and p53) (9, 10, 12, 13) to TFIID and/or TFIIB and be ejected by TFIIA afterward, for instance. In fact, the web of interactions within the PIC is so complex that HMG1 most likely will have opposite effects on the transcription of different genes not only *in vitro*, as already shown (18), but *in vivo* as well.

Acknowledgments—We especially thank R. G. Roeder, P. Chambon, J.-M. Egly, D. Reinberg, W. Herr, and A. J. Berk for the generous supply of expression plasmids and specific antibodies. We are very grateful to F. Azorín, J. Portugal, and all members of their groups and to A. Vaquero, B. Piña, J. Casanova, and R. Mantovani for stimulating discussions and criticism. We also thank R. Eritja and the oligonucleotide service for their help and efficiency, Jenny Colom for expert technical assistance, and X. Gomis-Rüth for docking analysis.

REFERENCES

1. Hardman, C. H., Broadhurst, R. W., Raine, A. R. C., Grasser, K. D., Thomas, J. O., and Laue, E. D. (1995) *Biochemistry* **34,** 16596–16607
2. Read, C. M., Cary, P. D., Crane-Robinson, C., Driscoll, P. C., and Norman, D. G. (1993) *Nucleic Acids Res.* **21,** 3427–3436
3. Love, J. J., Li, X., Case, D. A., Giese, K., Grosschedl, R., and Wright, P. E. (1995) *Nature* **376,** 791–795
4. Werner, M. H., Huth, J. R., Gronenborn, A. M., and Clore, G. M. (1995) *Cell* **81,** 705–714
5. Bianchi, M. E., Beltrame, M., and Paonessa, G. (1989) *Science* **243,** 1056–1059
6. Locker, D., Decoville, M., Maurizot, J. C., Bianchi, M. E., and Leng, M. (1995) *J. Mol. Biol.* **246,** 243–247
7. Ferrari, S., Harley, V. R., Pontiggia, A., Goodfellow, P. N., Lovell-Badge, R., and Bianchi, M. E. (1992) *EMBO J.* **11,** 4497–4506
8. Pöhler, J. R. G., Norman, D. G., Bramham, J., Bianchi, M. E., and Lilley, D. M. J. (1998) *EMBO J.* **17,** 817–826
9. Zappavigna, V., Falciola, L., Citterich, M. H., Mavilio, F., and Bianchi, M. E. (1996) *EMBO J.* **15,** 4981–4991
10. Zwilling, S., König, H., and Wirth, T. (1995) *EMBO J.* **14,** 1198–1208
11. Ge, H., and Roeder, R. G. (1994) *J. Biol. Chem.* **269,** 17136–17140
12. Jayaraman, L., Moorthy, N. C., Murthy, K. G. K., Manley, J. L., Bustin, M., and Prives, C. (1998) *Genes Dev.* **12,** 462–472
13. Boonyaratanakornkit, V., Melvin, V., Prendergast, P., Altmann, M., Ronfani, L., Bianchi, M. E., Taraseviciene, L., Nordeen, S. K., Allegretto, E. A., and Edwards, D. P. (1998) *Mol. Cell. Biol.* **18,** 4471–4487
14. Bianchi, M. E. (1994) in *DNA-Protein: Structural Interactions* (Lilley, D. M. J., ed) pp. 177–200, Oxford University Press, Oxford
15. Oñate, S. A., Prendergast, P., Wagner, J. P., Nissen, M., Reeves, R., Pettijohn, D. E., and Edwards, D. P. (1994) *Mol. Cell. Biol.* **14,** 3376–3391
16. Stelzer, G., Goppelt, A., Lottspeich, F., and Meisterernst, M. (1994) *Mol. Cell. Biol.* **14,** 4712–4721
17. Ogawa, Y., Aizawa, S., Shirakawa, H., and Yoshida, M. (1995) *J. Biol. Chem.* **270,** 9272–9280
18. Shykind, B. M., Kim, J., and Sharp, P. A. (1995) *Genes Dev.* **9,** 1354–1365
19. Bianchi, M. E., Falciola, L., Ferrari, S., and Lilley, D. M. J. (1992) *EMBO J.* **11,** 1055–1063
20. Bianchi, M. E. (1991) *Gene* (*Amst.*) **104,** 271–275
21. Bernués, J., Simmen, K. A., Lewis, J. D., Gunderson, S. I., Polycarpou-Schwarz, M., Moncollin, V., Egly, J.-M., and Mattaj, I. W. (1993) *EMBO J.* **12,** 3573–3585
22. Bernués, J., Carrera, P., and Azorín, F. (1996) *Nucleic Acids Res.* **24,** 2950–2958
23. Ha, I., Lane, W. S., and Reinberg, D. (1991) *Nature* **352,** 689–695
24. Meisterernst, M., and Roeder, R. G. (1991) *Cell* **67,** 557–567
25. Lee, S., and Hahn, S. (1995) *Nature* **376,** 609–612
26. Orphanides, G., Lagrange, T., and Reinberg, D. (1996) *Genes Dev.* **10,** 2657–2683
27. Auble, D. T., and Hahn, S. (1993) *Genes Dev.* **7,** 844–856
28. Lee, D. K., Dejong, J., Hashimoto, S., Horikoshi, M., and Roeder, R. G. (1992) *Mol. Cell. Biol.* **12,** 5189–5196
29. Brou, C., Chaudary, S., Davidson, I., Lutz, Y., Wu, J., Egly, J.-M., Tora, L., and Chambon, P. (1993) *EMBO J.* **12,** 489–499
30. Tansey, W. P., Ruppert, S., Tjian, R., and Herr, W. (1994) *Genes Dev.* **8,** 2756–2769
31. Bryant, G. O., Martel, L. S., Burley, S. K., and Berk, A. J. (1996) *Genes Dev.* **10,** 2491–2504
32. Falciola, L., Murchie, A. I. H., Lilley, D. M. J., and Bianchi, M. E. (1993) *Nucleic Acids Res.* **22,** 285–292
33. Lu, J., Kobayashi, R., and Brill, S. J. (1996) *J. Biol. Chem.* **271,** 33678–33685
34. Falciola, L., Spada, F., Calogero, S., Längst, G., Voit, R., Grummt, I., and Bianchi, M. E. (1997) *J. Cell Biol.* **137,** 19–26
35. Chao, D. M., Gadbols, E. L., Murray, P. J., Anderson, S. F., Sonu, M. S., Parvin, J. D., and Young, R. A. (1996) *Nature* **380,** 82–85
36. Maldonado, E., Shiekhattar, R., Sheldon, M., Cho, H., Drapkin, R., Rickert, P., Lees, E., Anderson, C. W., Linn, S., and Reinberg, D. (1996) *Nature* **381,** 86–89
37. Ossipow, V., Tassan, J.-P., Nigg, E. A., and Schibler, U. (1995) *Cell* **83,** 137–146
38. Nikolov, D. B., Chen, H., Halay, E. D., Usheva, A. A., Lee, D. K., Roeder, R. G., and Burley, S. K. (1995) *Nature* **377,** 119–128
39. Tang, H., Sun, X., Reinberg, D., and Ebright, R. H. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 1119–1124
40. Geiger, J. H., Hahn, S., Lee, S., and Sigler, P. B. (1996) *Science* **272,** 830–836
41. Tan, S., Hunziker, Y., Sargent, D. F., and Richmond, T. J. (1996) *Nature* **381,** 127–134
42. Roberts, S. G. E., and Green, M. R. (1994) *Nature* **371,** 717–720
43. Zawel, L., and Reinberg, D. (1995) *Annu. Rev. Biochem.* **64,** 533–561

High Mobility Group Protein 1 Interacts Specifically with the Core Domain of Human TATA Box-binding Protein and Interferes with Transcription Factor IIB within the Pre-initiation Complex

Montserrat Sutrias-Grau, Marco E. Bianchi and Jordi Bernués

J. Biol. Chem. 1999, 274:1628-1634.
doi: 10.1074/jbc.274.3.1628