MOLECULAR AND CELLULAR BIOLOGY, July 1995, p. 3738–3747 Vol. 15, No. 7
0270-7306/95/$04.00+0

Functional Interaction between the POU Domain Protein Tst-1/Oct-6 and the High-Mobility-Group Protein HMG-I/Y

HUBERT LEGER,[1] ELISABETH SOCK,[1] KARIN RENNER,[1] FRIEDRICH GRUMMT,[2] AND MICHAEL WEGNER[1]*

Zentrum für Molekulare Neurobiologie, Universität Hamburg, D-20246 Hamburg,[1] and Institut für Biochemie, Biozentrum, D-97074 Würzburg,[2] Germany

Received 18 January 1995/Returned for modification 14 March 1995/Accepted 28 April 1995

The POU domain protein Tst-1/Oct-6 is a transcriptional activator of human papovavirus JC virus in transient transfections. Because of its endogenous expression in myelinating glia, Tst-1/Oct-6 might also be an important determinant for the glia specificity of JC virus in vivo. Activation of viral early and late genes depends on the ability of Tst-1/Oct-6 to interact with an AT-rich element within the viral regulatory region. Here, we show that this element not only is bound by Tst-1/Oct-6 but, in addition, serves as a binding site for the high-mobility-group protein HMG-I/Y. In the presence of HMG-I/Y, Tst-1/Oct-6 exhibited an increased affinity for this AT-rich element. The specificity of this effect was evident from the fact that no stimulation of Tst-1/Oct-6 binding was observed on a site that did not allow binding of HMG-I/Y. In addition, both proteins interacted with each other in solution. Direct contacts were identified between the POU domain of Tst-1/Oct-6 and a short stretch of 10 amino acids in the central portion of HMG-I/Y. These results point to an accessory role for HMG-I/Y in the activation of JC viral gene expression by the POU domain protein Tst-1/Oct-6. In agreement with such a role, HMG-Y synergistically supported the function of Tst-1/Oct-6 in transient transfections, measured on the early promoter of JC virus or on an artificial promoter consisting of only a TATA box and the common binding element for Tst-1 and HMG-I/Y.

POU domain proteins are important transcriptional regulators. They are involved in conferring developmental or tissue specificity on a number of cellular and viral gene promoters (38, 40, 47). Accordingly, expression of most POU domain proteins is tightly regulated. Tst-1, for instance, is expressed in a highly restricted manner during development and in the adult. Coded for by an intronless gene with features of an expressed retroposon (15, 24), expression of Tst-1 is confined to embryonic stem cells, skin, neuronal subpopulations, and myelinating glia (4, 10, 11, 17, 31, 44). In glial cells, this protein, which is also known as SCIP or Oct-6 (29, 31, 44) and hereafter will be referred to as Tst-1/Oct-6, has been implicated in the regulation of myelin-specific genes and the glia-specific human papovavirus JC virus (16, 30, 48).

JC virus specifically infects glial cells in the central nervous system of immunocompromised individuals, thereby leading to a severe demyelinating disease, which is known as progressive multifocal leukoencephalopathy (27). Part of the viral tropism for glial cells is based on the glia specificity of viral gene expression (22). Tst-1/Oct-6 has been implicated in this glia specificity, because it both binds to the regulatory region of JC virus and activates the viral early and late gene promoters which are contained within this region (48).

Like most POU domain proteins, Tst-1/Oct-6 is an intrinsically weak transcriptional regulator (for a recent review, see reference 47). To compensate for this weakness, Tst-1/Oct-6 has to rely on glia-specific or viral coactivators which interact with either its POU domain or its amino-terminal transactivation region (28, 32, 33, 37). While the glia-specific coactivators have yet to be cloned, the large T antigen of JC virus has been identified as a viral coactivator which stimulates the function of Tst-1/Oct-6 synergistically by interacting directly with its POU domain (37).

Recently, the high-mobility-group protein HMG-I and its splice variant HMG-Y (21) have been implicated in the regulation of a number of cellular gene promoters, including the beta interferon, interleukin-4, interleukin-2 receptor α-chain, lymphotoxin, and E-selectin promoters (3, 9, 18, 26, 45, 50). With the exception of the interleukin-4 gene promoter (3), HMG-I/Y exerts a stimulatory effect on the activities of these promoters. They all contain AT-rich binding sites for HMG-I/Y in close vicinity to binding sites for known transcription factors such as NF-κB, Elf-1, and ATFs, with which HMG-I/Y has been shown to interact directly. In addition, HMG-I/Y often increases the binding affinities of those proteins it physically associates with (6, 26, 45, 50). However, HMG-I/Y can also sterically block the access of proteins to a promoter in the absence of protein-protein interaction (6). Though not a transcriptional regulator by itself, HMG-I/Y is believed to serve as a promoter-specific accessory factor modifying the function of NF-κB, ATFs, and Elf-1 (6, 7, 18, 26, 45, 50).

Here, we show that the promoter-specific interaction of HMG-I/Y with Tst-1/Oct-6 is an important component for the function of Tst-1/Oct-6, providing a novel mechanism for modifying the activity of a POU domain protein by chromatin components.

MATERIALS AND METHODS

Plasmids. cDNA sequences for human and mouse HMG-Y were gifts of R. Eckner and R. Reeves, respectively. A eukaryotic expression vector for human HMG-Y was created by placing the entire cDNA (8) as an *Eco*RI fragment under the control of the cytomegalovirus promoter, thus generating pCMV/HMG-Y. A second eukaryotic expression vector, pCMV/T7HMG-Y, was constructed by using an epitope-tagged version of the coding sequence for mouse HMG-Y (20). The epitope which corresponded to the amino-terminal end of the T7 major capsid protein (T7-Tag; Novagen) and was preceded by a eukaryotic translational start consensus, was fused in frame to the amino terminus of HMG-Y. The other plasmids used in transient transfections include pCMV/

* Corresponding author. Mailing address: ZMNH, Interim I, Pav. 22, Martinistr. 52, D-20246 Hamburg, Germany. Phone: 49 40 4717 4708. Fax: 49 40 4717 4774.

Tst-1, an expression vector for Tst-1/Oct-6, and the luciferase reporter plasmids pJCluc$_{early}$, p36-luc, and siteA-luc (37, 48).

The bacterial expression vector pGEX/HMG-Y contains the coding sequence for mouse HMG-Y fused in frame to glutathione *S*-transferase (GST) as a *Bam*HI-*Eco*RI fragment. Similar fusion proteins were generated for select parts of HMG-Y by using fragments with *Eco*RI and *Hin*dIII sites introduced by PCR at their ends. pGEX/H20-56 expressed amino acids 20 to 56 of HMG-Y as a GST fusion protein. Analogously, pGEX/H32-56 and pGEX/H46-56 expressed amino acids 32 to 56 and 46 to 56, respectively. The corresponding plasmid for the bacterial expression of a GST/Tst-1 fusion protein as well as all plasmids used during in vitro transcription/translation of Tst-1/Oct-6 mutants in reticulocyte lysates have been described previously (37, 48). For in vitro transcription/translation of HMG-Y, the T7-tagged version of mouse HMG-Y (see above) was inserted into pBKS−II+ (Stratagene).

Cell culture, transfections, and luciferase assays. U138 human glioblastoma cells were propagated in RPMI medium supplemented with 10% fetal calf serum (FCS). Five days before transfection, cells were plated at a density of 5×10^5/60-mm plate in Dulbecco's modified Eagle's medium supplemented with 0.5% FCS. Cells were transfected by the calcium phosphate technique (2) with 2 μg of a luciferase reporter plasmid and 2 μg of an expression plasmid for Tst-1, HMG-I/Y, or a combination of both. The total amount of plasmid was kept constant. At 4 h posttransfection, cells were treated for 1 min with 30% (vol/vol) glycerol in phosphate-buffered saline (PBS) and placed in fresh Dulbecco's modified Eagle's medium supplemented with 0.5% FCS. Cells were harvested after 48 h. Extracts were assayed for luciferase activity as described elsewhere (48).

Preparation of nuclear extracts. Nuclear extracts were prepared from Schwann cell cultures or transiently transfected CV1 cells as described elsewhere (41). Cells from two 100-mm plates were washed twice with PBS, scraped from the plates in hypotonic buffer, swollen on ice, and lysed by the addition of 1% Nonidet P-40 and vortexing. Nuclei were pelleted and extracted in 200 μl of ice-cold 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (pH 7.9)–400 mM NaCl–0.1 mM EDTA–0.1 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*,*N*′-tetraacetic acid]–2 mM dithiothreitol–1% Nonidet P-40–2 μg of pepstatin per ml–2 μg of leupeptin per ml–1 μg of aprotinin per ml for 15 min at 4°C under constant rotation.

Electrophoretic mobility shift assay. In general, 0.5 ng of ^{32}P-labeled probe was incubated with protein for 20 min at room temperature in a 20-μl reaction mixture containing 10 mM HEPES (pH 8.0), 5% glycerol, 25 mM NaCl, 5 mM $MgCl_2$, 2 mM dithiothreitol, 0.1 mM EDTA, and 50 ng of poly(dG-dC) as unspecific competitor. Reaction mixtures were loaded onto native 4% polyacrylamide gels and electrophoresed in 0.5× TBE (45 mM Tris, 45 mM boric acid, 1 mM EDTA [pH 8.3]) at 180 V for 1.5 h.

The following oligonucleotides were used as probes: site A (AGGCGG CCTCGGCCTCCTGTATATATAAAAAAAAGGGAAGGGATGGC), site A mutants A2 to A9 (see Fig. 2C), oct$_{GC}$ (GATCTGGCATGCAAAGCGGGCT AG), and muNTS1(48-103) (49). HMG-I/Y purified to near homogeneity from Ehrlich ascites tumor cells (49) and Schwann cell nuclear extracts were used as protein sources in addition to GST/Tst-1 and GST/HMG-Y fusions. Expression of GST fusion proteins from pGEX/Tst-1, pGEX/HMG-Y, pGEX/H20-56, pGEX/H32-56, and pGEX/H46-56 in *Escherichia coli* DH5α and purification procedures were as described elsewhere (37).

In vitro translation. In vitro translation of [^{35}S]methionine-labeled Tst-1/Oct-6, HMG-Y, or GST/HMG-Y protein was carried out with in vitro-transcribed RNA in nuclease-treated rabbit reticulocyte lysate using protocols of the supplier (Promega). For ^{35}S labeling, a T7-Tag sequence (Novagen) had to be added onto the amino terminus of HMG-Y. To obtain deletion proteins of HMG-Y, pBKS/HMG-Y was digested with *Ava*I, *Nar*I, *Sty*I, or *Sfa*NI prior to in vitro transcription. For in vitro transcription of GST/HMG-Y fusion proteins, fragments were generated by a PCR-mediated approach in which a T7 promoter was placed immediately in front of the coding sequence for the respective fusion protein.

Protein-protein interaction. Equal amounts of purified GST or its fusion derivatives (GST/Tst-1 and GST/HMG-Y) were immobilized on glutathione-agarose beads (Sigma). Resins carrying equal amounts of GST, GST/Tst-1, or GST/HMG-Y were incubated for 2 h at 4°C with in vitro-translated ^{35}S-labeled proteins or nuclear extracts in B buffer (100 mM NaCl, 20 mM HEPES [pH 7.8], 2 mM EDTA, 0.2% Nonidet P-40, 5 mM dithiothreitol, 10% glycerol, 1 μg of leupeptin per ml, 1 μg of aprotinin per ml). After extensive washing, resin-bound proteins were separated on sodium dodecyl sulfate (SDS)-polyacrylamide gels (10% polyacrylamide for Tst-1/Oct-6 and 15% polyacrylamide for HMG-Y) and detected by autoradiography (for ^{35}S-labeled proteins) or Western immunoblotting (for nuclear extracts). Western blots were performed with the ECL detection system (Amersham) according to the manufacturer's instructions. Alternatively, in vitro-translated ^{35}S-labeled GST proteins were incubated with purified baculovirus-produced full-length Tst-1 (48a) in the presence of anti-Tst-1 antiserum and protein A-Sepharose beads in B buffer. Coimmunoprecipitated GST proteins were detected after extensive washing as described above for the glutathione-agarose-bound proteins.

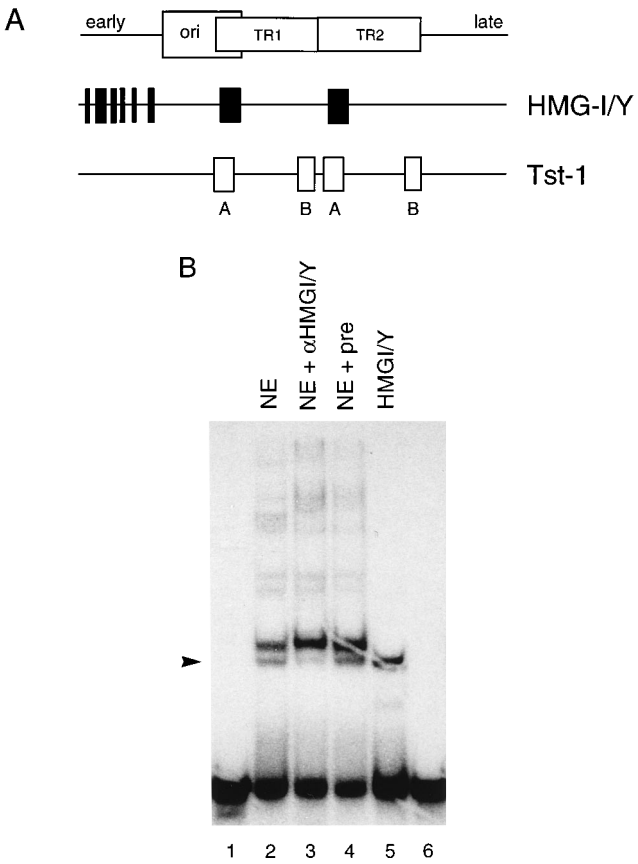
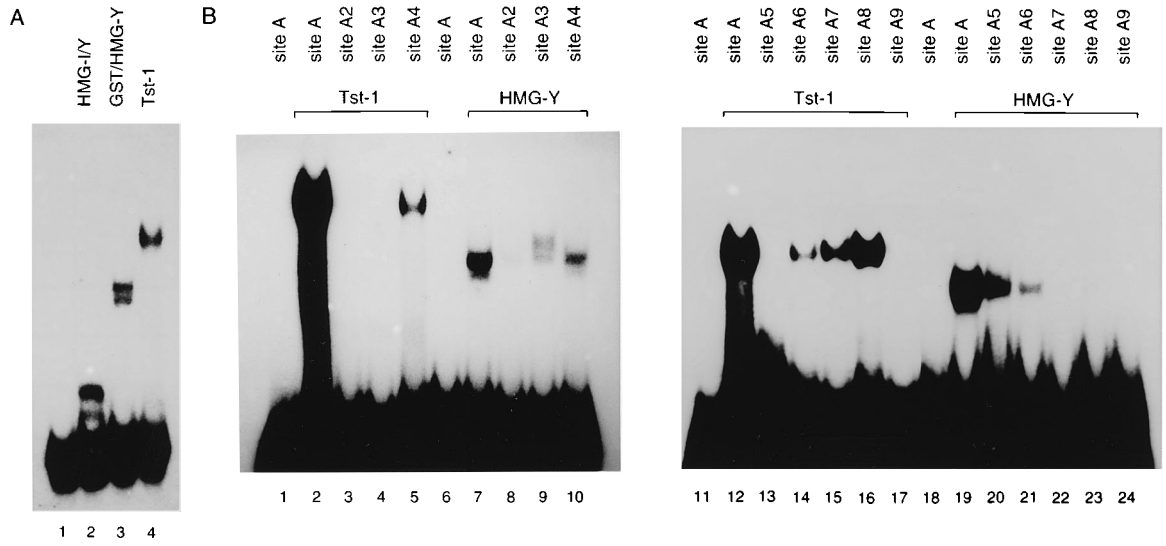


FIG. 1. (A) Schematic representation of the regulatory region of JC virus (Mad-1). The region between map positions 5029 and 279 of the viral genome (12) is flanked by the early (on the left) and late (on the right) viral transcription units. It contains both viral promoters as well as the origin of DNA replication (ori). Solid boxes, potential binding sites for HMG-I/Y (42); open boxes, previously identified binding sites A and B for Tst-1/Oct-6 (48). TR1 and TR2, 98-bp tandem repeats 1 and 2. (B) Electrophoretic mobility shift assays with site A and nuclear extracts. Radiolabeled site A was incubated with purified HMG-I/Y from Ehrlich ascites tumor cells (lane 5) or 2 μg of nuclear extract (NE) prepared from Schwann cells which after expansion in culture had been kept in the absence of forskolin (lanes 2 to 4). For some binding reactions nuclear extracts were preincubated with an antiserum raised against HMG-I/Y (αHMGI/Y) (lane 3) or with preimmune serum (pre) (lane 4). Lanes 1 and 6, free probe. Electrophoresis was done on an 8% polyacrylamide gel. Arrowhead, HMG-I/Y-containing complex.

RESULTS

The high-mobility-group protein HMG-I/Y interacts with a Tst-1/Oct-6 responsive element from the JC virus promoter. We have previously identified two binding sites for Tst-1/Oct-6 within the regulatory region of JC virus, designated site A and site B (Fig. 1A). Each of these sites occurs twice because of the repetition of a 98-bp enhancer (TR1 and TR2 in Fig. 1A). Whereas site A conferred Tst-1/Oct-6-dependent transcriptional activation to a heterologous promoter in transient transfections, site B failed to respond to the presence of Tst-1/Oct-6 (48). Thus, it is likely that site A rather than site B is involved in determining the glia specificity of JC viral gene expression through binding of Tst-1/Oct-6 (48).

To identify glial proteins interacting with Tst-1/Oct-6 in the JC virus promoter region, we initially performed electrophoretic mobility shift assays on nuclear extracts using site A as a probe. In the course of these experiments we reproducibly observed a fast-migrating complex which on high-percentage polyacrylamide gels resolved into two distinct bands (Fig. 1B),



C

site A	AGGCGGCCTCGGCCTCCTGTATATATAAAAAAAGGGAAGGGATGGC
site A2	----------gcg----------
site A3	----------cgc----------
site A4	----------gag----------
site A5	----------gggggg----------
site A6	----------gggg----------
site A7	----------gggg----------
site A8	----------gggg----------
site A9	----------gg----gggg----------

FIG. 2. Electrophoretic mobility shift assays reveal binding of Tst-1/Oct-6 and HMG-I/Y to the same element within the regulatory region of JC virus. (A) Radiolabeled site A was incubated with purified HMG-I/Y from Ehrlich ascites tumor cells (lane 2), recombinant GST/HMG-Y (lane 3), and bacterially expressed Tst-1/Oct-6 (lane 4). Lane 1, probe alone. (B) Binding of bacterially expressed Tst-1/Oct-6 (lanes 2 to 5 and 12 to 17) and recombinant GST/HMG-Y (lanes 7 to 10 and 19 to 24) to site A and its mutants A2 to A9. Lanes 1, 6, 11, and 18, free probe. (C) Sequences of site A and its mutant versions A2 to A9. The binding site for Tst-1/Oct-6, as determined in DNase footprint experiments (48), is overlined.

neither of which contained Tst-1/Oct-6 (data not shown). Fast migration and thermal stability pointed to the presence of the low-molecular-weight HMG-I/Y protein in one of these complexes. Preincubation of extracts with an antiserum directed against HMG-I/Y led to a specific loss of the lower band of the doublet, while the preimmune serum had no such effect. Additionally, only the lower band exhibited the same mobility as HMG-I/Y purified from mouse Ehrlich ascites tumor cells, indicating the presence of HMG-I/Y in this complex.

Like many other binding sites for POU domain proteins, site A is indeed a potential binding site for HMG-I/Y, as judged by its AT richness (42). In fact, binding sites for POU domain proteins and HMG-I/Y are sometimes so similar that in the past human HMG-I/Y has been cloned from a cDNA library as binding to the octamer sequence motif from the immunoglobulin light-chain promoter (8). However, binding sites for POU domain proteins and HMG-I/Y are not completely identical, as can be deduced from Fig. 1A.

Similarly to recombinant Tst-1/Oct-6 and HMG-I/Y from Ehrlich ascites tumor cells, a bacterially expressed GST/HMG-Y fusion protein bound site A in an electrophoretic mobility shift assay (Fig. 2A). As can be deduced from Fig. 2C, the sequence of site A contains in its center a long AT-rich region which is half homopolymeric and half heteropolymeric. To determine the exact binding sites for Tst-1/Oct-6 and HMG-I/Y within this AT-rich region, we performed gel shift assays with mutant versions of site A (Fig. 2C) in which runs of consecutive bases were substituted in or near those parts known from DNase I footprint experiments to be bound by Tst-1/Oct-6 (48).

Only site A8 formed as readily a complex with Tst-1/Oct-6 as did wild-type site A (Fig. 2B). Reduced binding of Tst-1/Oct-6 was observed for a number of other mutants including A4, A6, and A7, while no binding could be detected for mutants A2, A3, A5, and A9. These results are in good agreement with previously published DNase I footprint experiments (48). According to the footprint data, the mutations which exhibit unchanged or reduced Tst-1/Oct-6 binding (A4, A6, A7, and A8) carry substitutions in regions representing the footprint borders. Mutations present in A2, A3, A5, and A9 disrupt the core of the Tst-1/Oct-6 binding site as defined in footprint analyses.

Bacterially expressed GST/HMG-Y showed a distinct binding pattern compared with that of Tst-1/Oct-6. Binding of GST/HMG-Y was severely reduced in all mutants but was more strongly affected in mutants A2, A3, A7, A8, and A9 than in A4, A5, and A6. At high concentrations of GST/HMG-Y, however, residual binding was observed for all mutants (data not shown). Because mutations which severely decrease binding of GST/HMG-Y are spread over the whole AT-rich region including the hetero- and the homopolymeric stretches, it has to be concluded that the whole region is involved in determining the optimal DNA conformation for HMG-I/Y binding. In addition, we observed a reduced mobility of the complex between GST/HMG-Y and A3, suggesting that the conformation of bound HMG-I/Y is altered in this mutant compared with wild-type site A. Thus, while being clearly distinct, binding sites for Tst-1/Oct-6 and HMG-I/Y strongly overlap.

In vitro binding of Tst-1/Oct-6 is stimulated by HMG-I/Y. DNA binding properties of Tst-1/Oct-6 and HMG-I/Y are rather divergent. HMG-I/Y recognizes DNA conformation

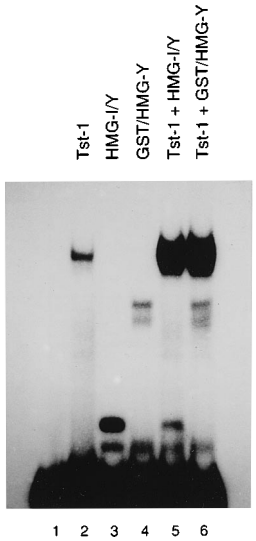


FIG. 3. The presence of HMG-I/Y affects binding of Tst-1/Oct-6 to site A. Site A was incubated with Tst-1/Oct-6 (lane 2), purified HMG-I/Y from Ehrlich ascites tumor cells (lane 3), recombinant GST/HMG-Y (lane 4), Tst-1/Oct-6 and purified HMG-I/Y (lane 5), or Tst-1/Oct-6 and GST/HMG-Y (lane 6). Amounts: Tst-1/Oct-6, 15 ng; HMG-I/Y, 2 ng; GST/HMG-Y, 15 ng. Lane 1, free probe.

through minor groove contacts, whereas Tst-1/Oct-6 interacts with DNA mainly through major groove contacts in a sequence-specific manner (13, 23, 35, 45, 49). To analyze whether the presence of HMG-I/Y exerts any influence on the binding of Tst-1/Oct-6, we performed mobility shift assays with radiolabeled site A in the presence of both proteins. At low concentrations of Tst-1/Oct-6, binding to site A was detectable as a faint complex of characteristic mobility (Fig. 3). Simultaneous addition of a fixed amount of HMG-I/Y resulted in a significant increase of Tst-1/Oct-6 binding to site A. This stimulatory effect was observed not only with HMG-I/Y purified from eukaryotic cells (Fig. 3, lane 5) but also with a bacterially expressed GST/HMG-Y fusion protein (Fig. 3, lane 6). Addition of HMG-I/Y, however, did not alter the mobility of the Tst-1/Oct-6-containing complex. Instead, HMG-I/Y formed a complex of its own characterized by its high mobility. Under no conditions did we observe an additional complex of low mobility in the presence of HMG-I/Y, suggesting that a ternary complex of site A, HMG-I/Y, and Tst-1, if existent, is either transient or unstable under our gel shift conditions.

To investigate this stimulatory effect in more detail, we performed additional studies in which we kept the amount of HMG-I/Y constant while simultaneously increasing the amount of Tst-1/Oct-6 protein. A Tst-1/Oct-6-containing complex was observed with as little as 2 ng of Tst-1/Oct-6 protein in the presence of GST/HMG-Y, whereas in the absence of GST/HMG-Y this complex was first detected with 15 ng of Tst-1/Oct-6 protein, corroborating that HMG-I/Y substantially stimulates Tst-1/Oct-6 binding under our assay conditions (Fig. 4A).

In a reverse experiment we kept the amount of Tst-1/Oct-6 constant while simultaneously increasing the amount of GST/HMG-Y. GST/HMG-Y already exerted a stimulatory effect on Tst-1/Oct-6 binding to site A at concentrations too low to yield a detectable complex between GST/HMG-Y and site A (Fig. 4B, lanes 4 and 5). Tst-1/Oct-6 binding was maximally enhanced at approximately equimolar amounts of both proteins (Fig. 4B, lanes 5 and 6), while very high concentrations of GST/HMG-Y led to a reduction in the strength of the Tst-1/Oct-6-containing complex. Surprisingly, large amounts of GST/HMG-Y also decreased the strength of the complex generated by GST/HMG-Y itself as if under these conditions Tst-1/Oct-6 and GST/HMG-Y would prevent each other from binding to site A. No such effects on Tst-1/Oct-6 binding were observed in the presence of increasing amounts of bovine serum albumin (BSA) (Fig. 4B, lanes 9 to 14).

Stimulation of Tst-1/Oct-6 binding requires HMG-I/Y to bind to DNA. HMG-I/Y not only binds to DNA but is also known to engage in direct physical associations with transcription factors such as NF-κB, ATF-2, and Elf-1 (6, 7, 18, 45). Therefore, HMG-I/Y-dependent stimulation of Tst-1/Oct-6 binding to site A could involve HMG-I/Y binding to DNA as well as interaction of HMG-I/Y with Tst-1/Oct-6. To analyze

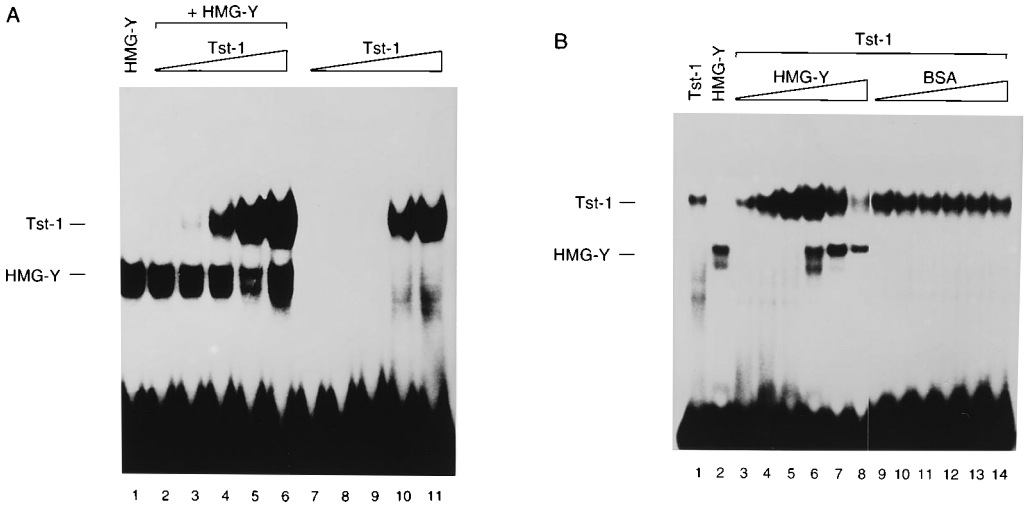


FIG. 4. HMG-I/Y enhances the binding of Tst-1/Oct-6 to site A in electrophoretic mobility shift assays. (A) Site A was incubated with increasing amounts of Tst-1/Oct-6 (lanes 2 and 7, 0.5 ng; lanes 3 and 8, 2 ng; lanes 4 and 9, 5 ng; lanes 5 and 10, 15 ng; lanes 6 and 11, 45 ng) in the presence (lanes 2 to 6) or absence (lanes 7 to 11) of 25 ng of GST/HMG-Y. Lane 1, probe incubated with 25 ng of GST/HMG-Y. (B) Site A was incubated with 15 ng of Tst-1/Oct-6 in the presence of increasing amounts of GST/HMG-Y (lane 3, 2 ng; lane 4, 5 ng; lane 5, 10 ng; lane 6, 25 ng; lane 7, 75 ng; lane 8, 150 ng) or BSA (lane 9, 2 ng; lane 10, 5 ng; lane 11, 10 ng; lane 12, 25 ng; lane 13, 75 ng; lane 14, 150 ng). Lane 1, site A with 15 ng of Tst-1; lane 2, site A with 25 ng of GST/HMG-Y.

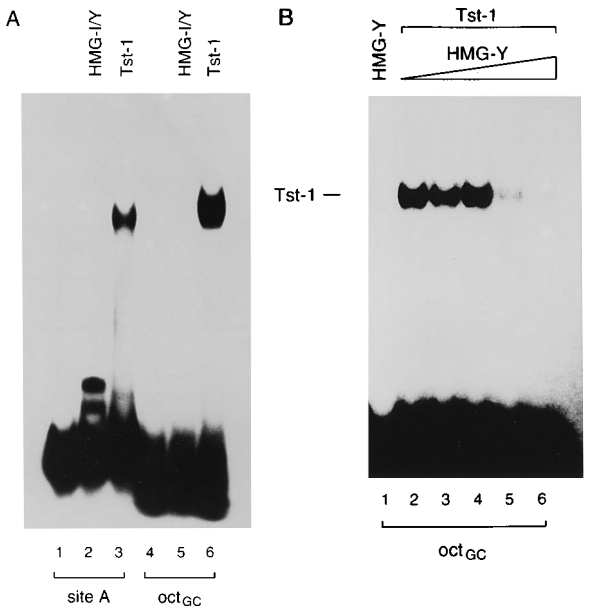


FIG. 5. HMG-I/Y does not enhance the binding of Tst-1/Oct-6 to a specific octamer element. (A) Site A (lanes 1 to 3) and the octamer-containing probe oct$_{GC}$ (lanes 4 to 6) were incubated with eukaryotic HMG-I/Y (lanes 2 and 5) or bacterially expressed Tst-1/Oct-6 (lanes 3 and 6). (B) The octamer-containing probe oct$_{GC}$ was incubated with 15 ng of Tst-1/Oct-6 (lane 2 to 6) in the presence of increasing amounts of GST/HMG-Y (lane 2, 2 ng; lane 3, 10 ng; lane 4, 25 ng; lane 5, 75 ng; lane 6, 150 ng) or with 25 ng of GST/HMG-Y alone (lane 1).

whether HMG-I/Y binding to DNA is a prerequisite for the observed stimulation, we artificially generated a DNA element (oct$_{GC}$) in which the octamer sequence from the simian virus 40 enhancer is flanked by GC-rich sequences. Such a site should still bind Tst-1 but should prohibit binding of HMG-I/Y since no more than three consecutive AT base pairs are present within this site. That this is indeed the case is shown in Fig. 5A, in which binding of HMG-I/Y to site A and oct$_{GC}$ is compared with binding of Tst-1/Oct-6 to the same sites. Purified eukaryotic HMG-I/Y specifically failed to recognize the oct$_{GC}$ sequence while simultaneously binding to site A (Fig. 5A). GST/HMG-Y exhibited a virtually identical behavior (Fig. 5B). Competition experiments revealed that oct$_{GC}$ and site A were equally efficient and specific competitors of Tst-1/Oct-6 binding to site A, with no more than a twofold difference in the effective competitor concentration needed to reduce binding to 50% (data not shown). The existence of conformational differences between Tst-1/Oct-6 bound to oct$_{GC}$ and Tst-1/Oct-6 bound to site A cannot be excluded.

When oct$_{GC}$ was incubated with Tst-1/Oct-6 protein in the presence of increasing amounts of GST/HMG-Y, HMG-I/Y did not enhance Tst-1/Oct-6 binding, but it did so in parallel reactions in which oct$_{GC}$ was replaced by site A (Fig. 5B and data not shown). At low concentrations of GST/HMG-Y, Tst-1/Oct-6 binding to oct$_{GC}$ remained virtually unaffected, before complex formation was strongly repressed at high concentrations of GST/HMG-Y. Therefore, we conclude that HMG-I/Y has to bind to DNA to be able to stimulate Tst-1/Oct-6 binding. We cannot rule out at the moment that a specific conformation adopted by Tst-1/Oct-6 upon binding to site A but not upon binding to oct$_{GC}$ is also important for the observed HMG-I/Y-dependent stimulation of Tst-1/Oct-6 binding to site A.

HMG-I/Y interacts with Tst-1/Oct-6 in the absence of DNA. We analyzed the ability of HMG-I/Y and Tst-1/Oct-6 to interact with each other. Protein-protein interaction was assessed by affinity chromatography in which one of the two proteins was expressed as a GST fusion protein and bound to a glutathione resin while the other protein was passed over the resin and analyzed for its ability to be specifically retained by the GST fusion protein. When passed over a GST/HMG-Y column, ^{35}S-labeled Tst-1/Oct-6 protein bound to the resin, whereas no Tst-1/Oct-6 protein could be recovered from a column containing GST only (Fig. 6A). In the reverse experiment, in vitro-translated ^{35}S-labeled HMG-Y bound specifically to a column carrying GST/Tst-1 but not to a column carrying GST (Fig. 6B).

The presence of contaminating DNA fragments can influence the results from protein-protein interaction studies. It has been argued that inclusion of ethidium bromide in the assay allows a distinction between bona fide protein-protein interactions and interactions requiring the unspecific binding to the contaminating DNA (25). As can be deduced from Fig. 6C, binding of in vitro-translated HMG-Y to GST/Tst-1 is largely unaffected by the presence of up to 200 μg of ethidium bromide per ml, strongly arguing in favor of a bona fide protein-protein interaction between HMG-I/Y and Tst-1/Oct-6.

Interaction between these two proteins was also detectable with nuclear extracts from transfected CV1 cells. Tst-1/Oct-6 was specifically retained from nuclear extracts on a column carrying GST/HMG-Y but not on a column carrying GST only (Fig. 7A). In the reciprocal experiment, murine HMG-Y with a T7-Tag at its amino-terminal end was readily detected in nuclear extracts as binding to a column carrying GST/Tst-1 (Fig. 7B). Again, we did not observe any unspecific interaction

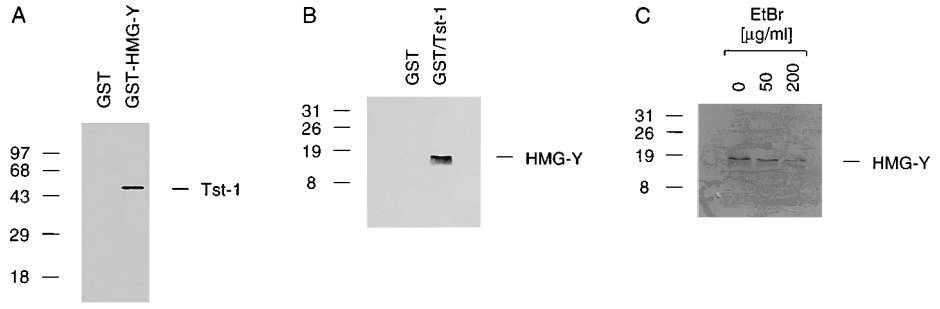


FIG. 6. GST chromatography reveals physical interaction between Tst-1/Oct-6 and HMG-Y. (A) SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of ^{35}S-labeled Tst-1/Oct-6 bound to GST or GST/HMG-Y resins. (B) SDS-PAGE of ^{35}S-labeled HMG-Y bound to GST or GST/Tst-1 resins. (C) ^{35}S-labeled HMG-Y bound to GST/Tst-1 resins in the presence of ethidium bromide (EtBr). EtBr concentrations are given above the lanes. The sizes of molecular mass markers are indicated on the left in kilodaltons.

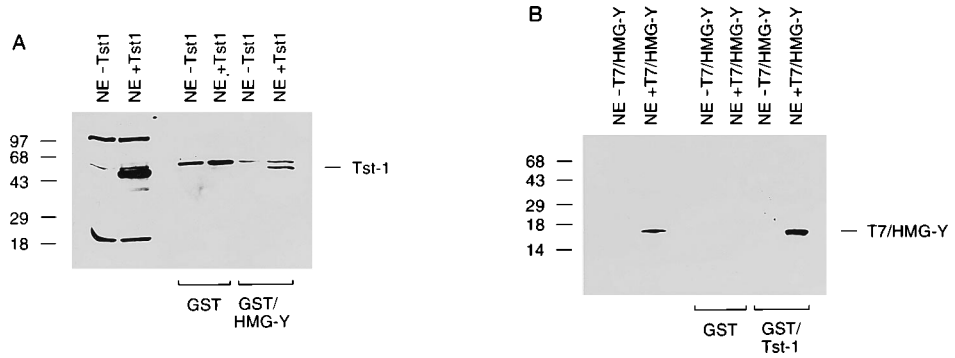


FIG. 7. Interaction between Tst-1/Oct-6 and HMG-Y is detected in nuclear extracts. SDS-polyacrylamide gel electrophoresis of nuclear extracts before and after binding to columns carrying various GST fusion proteins. (A) Nuclear extracts from CV1 cells transfected with pCMV/Tst-1 (NE +Tst1) and nuclear extracts from mock-transfected cells (NE −Tst1) (two left lanes) were passed over columns carrying GST alone or GST/HMG-Y. Resin-bound Tst-1/Oct-6 (four right lanes) was detected in Western blots using a rabbit anti-Tst-1 antiserum and horseradish peroxidase-coupled protein A (both diluted 1:3,000) as primary and secondary antibodies, respectively. (B) Nuclear extracts from CV1 cells transfected with pCMV/T7HMG-Y (NE +T7/HMG-Y) and nuclear extracts from mock-transfected cells (NE −T7/HMG-Y) (two left lanes) were passed over columns carrying GST alone or GST/Tst-1. T7-tagged resin-bound HMG-Y (four right lanes) was detected in Western blots using a mouse monoclonal antibody against the T7-Tag (Novagen) and horseradish peroxidase-coupled anti-mouse immunoglobulin G (both diluted 1:3,000) as primary and secondary antibodies, respectively. The sizes of molecular mass markers are indicated on the left in kilodaltons.

of HMG-Y with the GST part of the fusion protein or the column material.

To further identify the region within Tst-1/Oct-6 which is involved in the interaction with HMG-I/Y, we in vitro translated a series of Tst-1/Oct-6 deletion mutants. After confirming that approximately equal amounts of protein were generated during in vitro translation, we tested each mutant for its ability to be retained on a GST/HMG-Y column (Fig. 8). All amino acids preceding the POU domain (ΔN) could be deleted from the protein without any loss of its ability to interact with HMG-I/Y. Similarly, deletion of all amino acids carboxy terminal to the POU domain (ΔC) had no effect on the interaction. In agreement with this, the POU domain alone without any addition of amino acid sequences to its amino- or carboxy-terminal side (ΔNC) was still fully capable of interacting with HMG-I/Y. Deletion of the POU domain (ΔPOU), on the other hand, abolished interaction between Tst-1/Oct-6 and HMG-I/Y completely. This detrimental effect was observed not only when the whole POU domain was deleted; separate deletion of either the POU-specific domain (ΔPOU_S) or the POU homeodomain (ΔPOU_{HD}) also interfered with the interaction. Therefore, the POU domain is both necessary and sufficient to mediate binding to HMG-I/Y.

A comparable analysis was also performed to map the region within HMG-I/Y involved in the interaction. HMG-I/Y contains three repeats of a basic peptide motif known to be involved in DNA binding (35). In fact, a single isolated peptide motif is able to bind to DNA by itself (13, 35). In addition, HMG-I/Y contains an acidic domain in its very carboxy-terminal part (8, 20). Using various restriction endonucleases, we generated a series of deletion mutants by successively shortening HMG-Y from its carboxy-terminal end (Fig. 9D). All mutants, therefore, lacked the acidic carboxy-terminal domain. The number of intact repeats of the basic peptide motif varied among mutants from only one (Δ46) to all three (Δ86). All mutant proteins were produced by in vitro translation in amounts similar to each other and to the amount of wild-type HMG-Y protein (Fig. 9A). When tested for their ability to be retained on a GST/Tst-1 column, all mutants except the shortest one (Δ46) bound to Tst-1/Oct-6 (Fig. 9B). This indicates that the region which interacts with Tst-1/Oct-6 is contained within the first 56 amino acids of HMG-Y, with the region between amino acids 46 and 56 being important for the interaction. We also tested the mutant proteins for their ability to bind to DNA after establishing that the in vitro-translated full-length HMG-Y bound to DNA with characteristics identical to HMG-I/Y purified from eukaryotic cells (Fig. 9C and data not shown). Mutants Δ86, Δ72, and Δ57 all retained their ability to bind to DNA. However, DNA binding became undetectable in the shortest mutant, Δ46, which contained only

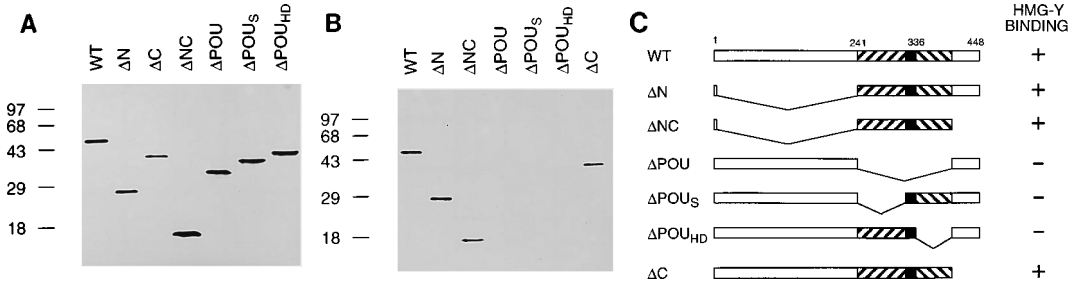


FIG. 8. The POU domain of Tst-1/Oct-6 interacts with HMG-I/Y. (A) SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of ^{35}S-labeled Tst-1/Oct-6 proteins. (B) ^{35}S-labeled Tst-1/Oct-6 wild-type protein and various mutant versions were incubated in similar amounts with resins carrying GST/HMG-Y. Proteins bound to the GST/HMG-Y resin were analyzed by SDS-PAGE. The sizes of molecular mass markers are indicated on the left in kilodaltons. (C) Summary of the Tst-1/Oct-6 mutants and their abilities to interact with HMG-Y. WT, wild-type Tst-1; ΔN, mutant Tst-1/Oct-6 lacking amino acids 4 to 240; ΔNC, POU domain of Tst-1; ΔPOU, mutant Tst-1/Oct-6 lacking amino acids 241 to 395; ΔPOU_S, mutant Tst-1/Oct-6 lacking amino acids 241 to 319; ΔPOU_{HD}, mutant Tst-1/Oct-6 lacking amino acids 336 to 395; ΔC, mutant Tst-1/Oct-6 lacking amino acids 396 to 448.

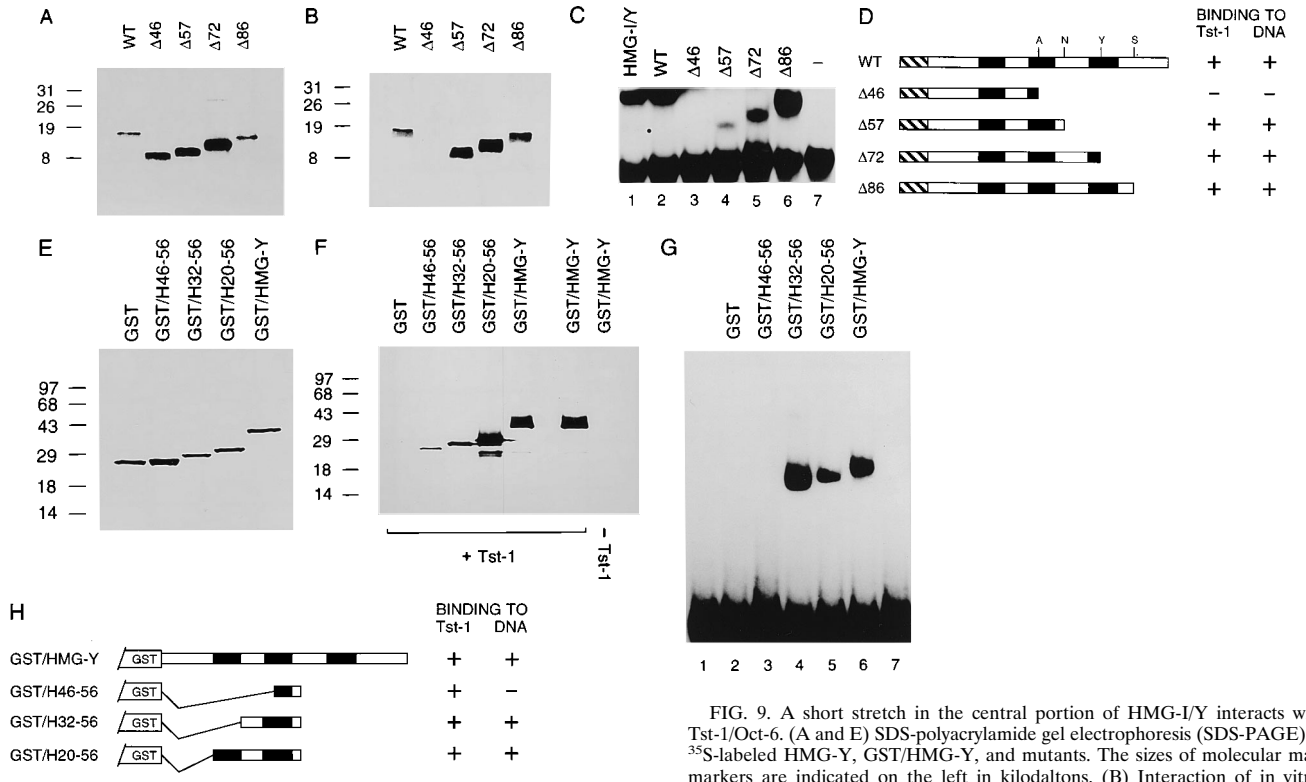


FIG. 9. A short stretch in the central portion of HMG-I/Y interacts with Tst-1/Oct-6. (A and E) SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of ^{35}S-labeled HMG-Y, GST/HMG-Y, and mutants. The sizes of molecular mass markers are indicated on the left in kilodaltons. (B) Interaction of in vitro-translated HMG-Y proteins with Tst-1/Oct-6. Equal amounts of ^{35}S-labeled mutant or wild-type HMG-Y proteins were incubated with a resin carrying GST/Tst-1, before the bound material was analyzed by SDS-PAGE. (C and G) DNA binding of in vitro-translated HMG-Y and GST/HMG-Y proteins in electrophoretic mobility shift assays. An AT-rich region from the nontranscribed spacer of mouse rDNA which previously had been described as a high-affinity binding site for HMG-I/Y (49) was used as a probe. (F) Interaction of in vitro-translated GST/HMG-Y proteins with Tst-1/Oct-6. Equal amounts of ^{35}S-labeled mutant or wild-type GST/HMG-Y proteins were immunoprecipitated with an antiserum against Tst-1/Oct-6 in the presence of recombinant Tst-1/Oct-6 (+ Tst-1). Recombinant Tst-1/Oct-6 was omitted from the last lane (− Tst-1). Coimmunoprecipitated GST proteins were analyzed by SDS-PAGE. WT, in vitro-translated wild-type HMG-Y; Δ46, mutant HMG-Y lacking amino acids 46 to 95; Δ57, mutant HMG-Y lacking amino acids 57 to 95; Δ72, mutant HMG-Y lacking amino acids 72 to 95; Δ86, mutant HMG-Y lacking amino acids 86 to 95; −, unprogrammed reticulocyte lysate; HMG-I/Y, purified HMG-I/Y from Ehrlich ascites tumor cells; GST/HMG-Y, fusion of GST and full-length mouse HMG-Y; GST/H20-56, GST fusion protein containing amino acids 20 to 56 of HMG-Y; GST/H32-56, GST fusion protein containing amino acids 32 to 56 of HMG-Y; GST/H46-56, GST fusion protein containing amino acids 46 to 56 of HMG-Y. (D and H) Schematic representations of mutant HMG-Y and GST/HMG-Y proteins and summaries of their abilities to bind to Tst-1/Oct-6 or DNA. For the generation of HMG-Y mutants the following enzymes were used: A, *Ava*I; N, *Nar*I; Y, *Sty*I; S, *Sfa*NI. Hatched box at the amino terminus, fused T7-Tag (see Materials and Methods). Filled boxes, basic peptide motifs which have been implicated in binding of HMG-I/Y to DNA (35).

the most amino-terminal copy of the three basic peptide motifs present within HMG-I/Y (Fig. 9C). Therefore, the most amino-terminal repeat is not sufficient to obtain detectable levels of DNA binding in the context of the HMG-I/Y protein.

To investigate whether the region between amino acids 46 and 56 of HMG-Y was by itself sufficient to mediate interaction with Tst-1/Oct-6 or whether it was only part of a larger interaction domain, we generated a set of GST/HMG-Y fusion proteins which extended from various amino acid positions in the amino-terminal part of HMG-Y to amino acid 56 (Fig. 9H). All mutant GST/HMG-Y fusion proteins were in vitro translated with efficiencies similar to those of wild-type GST/HMG-Y and GST alone (Fig. 9E). When tested for their ability to bind to DNA in gel shift assays, only the shortest mutant, GST/H46-56, in which amino acids 46 to 56 of HMG-Y were expressed, failed to bind to DNA (Fig. 9G). In agreement with its failure to bind to DNA, GST/H46-56 did not contain a complete copy of the basic peptide motif. Mutants GST/H32-56 and GST/H20-56, which contained amino acids 32 to 56 and 20 to 56, respectively, bound DNA as efficiently as full-length GST/HMG-Y. This indicates that the combination of the first two basic peptide motifs (GST/H20-56) as well as the middle motif alone (GST/H32-56) are sufficient for DNA binding in the context of a GST fusion protein.

Interaction of these GST/HMG-Y mutants with Tst-1/Oct-6 was studied in coimmunoprecipitation assays (Fig. 9F). ^{35}S-labeled GST, GST/HMG-Y, and its mutants were analyzed for their ability to be immunoprecipitated by an antiserum directed against Tst-1/Oct-6. Immunoprecipitation was observed only in the presence of Tst-1/Oct-6 protein (Fig. 9F, compare the last two lanes) and only for GST/HMG-Y but not GST alone. Surprisingly, even the shortest GST/HMG-Y mutant (GST/H46-56), which contained only amino acids 46 to 56, was coimmunoprecipitated, albeit at a lower rate than full-length GST/HMG-Y or the longer mutants. While parts of HMG-Y that are amino terminal to amino acid 46 might thus have a supporting function, the short stretch between amino acids 46 and 56 seems to be the central domain for interaction with Tst-1/Oct-6.

HMG-I/Y and Tst-1/Oct-6 synergistically activate JC virus gene expression in transient transfections. In light of the above-mentioned data, it was important to ask whether any functional interaction between HMG-I/Y and Tst-1/Oct-6 could also be detected in vivo. For this purpose we performed transient transfection experiments on U138 glioblastoma cells. As shown previously, ectopically expressed Tst-1/Oct-6 is able to activate the early promoter of JC virus approximately 10-

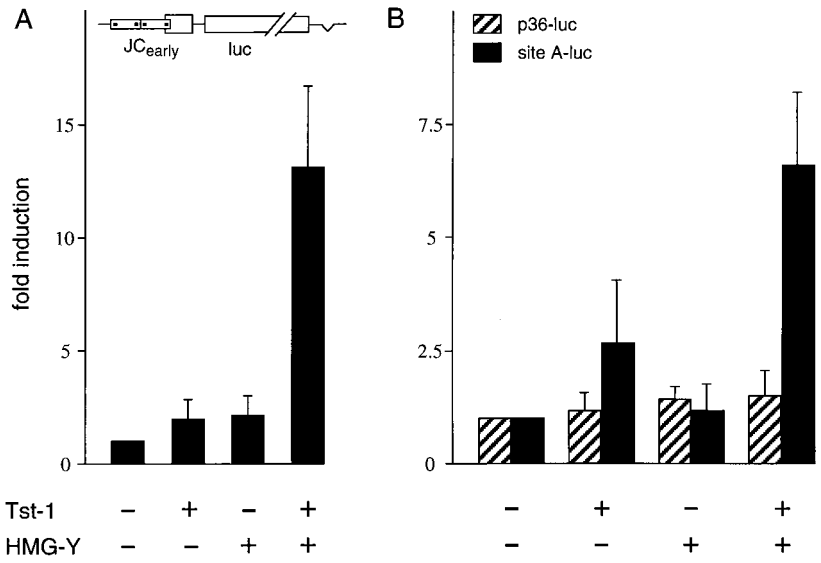


FIG. 10. Early gene expression of JC virus is synergistically activated by Tst-1/Oct-6 and HMG-I/Y. Luciferase reporter plasmids containing the JC virus early promoter (A), a minimal promoter (p36-luc) (B; hatched bars), or a combination of site A and the minimal promoter (siteA-luc) (B; solid bars) were transiently transfected into human U138 cells either alone or in various combinations with expression plasmids for Tst-1/Oct-6 and HMG-Y as indicated. Promoter-dependent expression levels of the reporter were determined by the luciferase activity of cellular extracts in three independent experiments, each performed in duplicate. Results are presented as fold inductions (with standard errors of the means) above the level of luciferase activity obtained in transfections with the reporter plasmid alone, which was assigned an arbitrary value of 1.

fold when these cells are kept and transfected in the presence of 10% FCS (37, 48). However, under these conditions no significant change in the rate of stimulation was observed when expression plasmids for HMG-Y or its antisense RNA were cotransfected (data not shown).

Results were different when transfections were carried out in U138 cells, which had been starved in 0.5% FCS prior to transfection (Fig. 10). Under these low-serum conditions stimulation of the JC virus early promoter by Tst-1/Oct-6 was strongly reduced (Fig. 10A). In fact, Tst-1/Oct-6 induced only a twofold stimulation of promoter activity, very similar to that induced by ectopically expressed HMG-Y. The presence of both Tst-1/Oct-6 and ectopically expressed HMG-Y in transfected cells, however, led to a strong, 13-fold stimulation of the early viral promoter, clearly indicating the existence of transcriptional cooperativity between the two proteins. Such a cooperativity was observed not only with a reporter plasmid in which the luciferase gene was under the control of the JC virus early promoter but also with a reporter in which luciferase gene expression was driven by the combination of site A and an adjacent TATA box (siteA-luc in Fig. 10B). In the absence of site A, no such effect was observed (p36-luc in Fig. 10B). Site A alone was not as efficient in mediating cooperativity between Tst-1/Oct-6 and HMG-Y as the JC virus early promoter, indicating that, in addition to site A, other components of the early promoter or its overall structure might be important for cooperativity between these two proteins.

DISCUSSION

HMG-I/Y was originally identified as a binding protein for highly repetitive AT-rich "satellite" DNA and is one of the major nonhistone components of chromatin in eukaryotic cells (43). High concentrations of HMG-I/Y protein and mRNA are usually detectable only in mammalian cells actively engaged in cell proliferation and mitosis (19–21). In agreement with its high-affinity for AT-rich sequences in vitro, HMG-I/Y protein is localized to G/Q-bands, centromeres, and telomeres of metaphase chromosomes (5). These observations have led to the assumption that HMG-I/Y might actively participate in the dynamic changes of chromatin structure. Other data implicate HMG-I/Y in the control of gene expression and DNA replication (9, 34, 39, 45, 49, 52). Control of cellular transcription by HMG-I/Y is thought to be mediated through general derepression as well as promoter-specific mechanisms (3, 7, 9, 26, 45, 50, 53). HMG-I/Y is uniquely suited for this task because it interacts with both DNA and proteins. Among the proteins known to interact with HMG-I/Y are nucleosome core particles and the transcription factors NF-κB and ATF-2 (6, 7, 36, 50). In both the human beta interferon gene promoter and the E-selectin gene promoter, HMG-I/Y has been shown to bind to AT-rich sequences adjacent to or overlapping with binding sites for NF-κB or ATF-2 and to be involved in cooperative interactions with these transcription factors (7, 26, 45, 50). At the same time HMG-I/Y has been shown to increase the overall transcriptional synergy between different elements within these promoters, an effect which was attributed to the ability of HMG-I/Y to engage in direct protein-protein interactions. ATF-2, for instance, physically associates with HMG-I/Y through its bZip domain (7). As a consequence, HMG-I/Y promotes ATF-2 dimerization and stimulates its binding to the beta interferon promoter (6).

However, HMG-I/Y functions not only as a positive regulator of promoter activity. There is at least one case in which HMG-I/Y has been shown to decrease promoter activity (3). An explanation for this effect was offered by a recent report, showing that there exists a specific isoform of ATF-2 with which HMG-I/Y does not interact (6). While facilitating the binding of other ATF-2 isoforms to the beta interferon gene promoter, HMG-I/Y interferes with binding of this specific isoform probably because of steric hindrance. Thus, it is easily conceivable how HMG-I/Y can modulate promoter activity by preventing some transcription factors from binding to a promoter while stimulating others.

Here, we show that interactions also occur between HMG-I/Y and Tst-1/Oct-6, a member of a class of transcription factors previously not known to interact with HMG-I/Y. In particular, HMG-I/Y was found to stimulate binding of Tst-1/Oct-6 to a DNA element within the regulatory region of JC virus which in the past had been shown to mediate the activation of both viral promoters by Tst-1/Oct-6. Activation of viral gene expression by Tst-1/Oct-6 could be an important determinant in the glia specificity of JC virus gene expression (37, 48), which in turn is one of the main reasons for preferential infection and destruction of oligodendrocytes by JC virus during the demyelinating disease progressive multifocal leukoencephalopathy (27). Our results therefore place HMG-I/Y in the middle of these events.

DNA binding is a critical function of HMG-I/Y without which it cannot stimulate binding of Tst-1/Oct-6 to its site. Despite this fact, we were unable to show simultaneous binding of both Tst-1/Oct-6 and HMG-I/Y to the same site. This is best explained by a transient character or by instability of the ternary complex under our assay conditions. Alternatively, HMG-I/Y and Tst-1/Oct-6 could bind to DNA in a sequential order, with HMG-I/Y binding first and changing DNA conformation in a way that increases affinity for Tst-1/Oct-6. Evidence for such a conformational change is provided by reports of HMG-I/Y-induced DNA bending (45). Binding of Tst-1/Oct-6 would now be followed by a displacement of HMG-I/Y.

HMG-I/Y also interacts with Tst-1/Oct-6 in the absence of DNA. The physical association in solution involves the POU domain of Tst-1/Oct-6. Thus, binding of HMG-I/Y to Tst-1/Oct-6 resembles its interaction with ATF-2 (6, 7) or Elf-1 (18): association in all these cases involves the region of the transcription factor that is involved in DNA binding, namely, the POU domain, the bZip region, and the Ets domain. To extend the comparison between ATF-2 and Tst-1/Oct-6 even further, both the bZip region and the POU domain are known to engage in a number of other protein-protein interactions (1, 7, 14, 37, 47, 51).

In case of ATF-2, interaction with HMG-I/Y also promotes dimerization of ATF-2 (6). POU domain proteins are also able to form dimers (46). Therefore, it is conceivable that the presence of HMG-I/Y facilitates dimerization of Tst-1/Oct-6 with itself or other POU domain proteins. Alternatively, HMG-I/Y could stimulate interaction with other proteins including transcriptional coactivators. In this regard, it is intriguing to speculate that HMG-I/Y affects the synergism between Tst-1/Oct-6 and JC virus T antigen (37).

So far, no attempt has been undertaken to determine the region within HMG-I/Y responsible for physical interaction with transcription factors. Here, we show that a short contiguous stretch of 10 amino acids in the center of the HMG-I/Y protein (amino acids 46 to 56) is sufficient for the interaction with Tst-1/Oct-6. Importantly, this region contained only part of one of the three basic peptide motifs involved in DNA binding (13, 35) and was unable to bind to DNA by itself. Thus, it seems unlikely that these peptide motifs determine the ability of HMG-I/Y to interact with Tst-1/Oct-6. Furthermore, our results indicate that sequences between amino acids 20 and 46 of HMG-Y might have an additional stabilizing effect on the interaction with Tst-1/Oct-6.

The functional significance of the interaction between Tst-1/Oct-6 and HMG-I/Y was underlined by results from transient transfections: under low-serum conditions we observed functional cooperativity between both proteins on the early promoter of JC virus and on an artificial promoter consisting of only site A and a TATA box. Although functional cooperativity between HMG-I/Y and Tst-1/Oct-6 in the context of the JC virus promoter most likely involves multiple interactions with other binding sites and probably other cellular or viral proteins, site A clearly plays a crucial role in this phenomenon. In conclusion, our observations suggest that HMG-I/Y is an important accessory factor for the function of the POU domain protein Tst-1/Oct-6 and therefore might be involved in the activation of JC virus gene expression during the pathogenesis of progressive multifocal leukoencephalopathy by mediating protein-DNA as well as protein-protein interactions in the regulatory region of JC virus.

ACKNOWLEDGMENTS

We thank D. Thanos and T. Maniatis for their gift of anti-HMG-I/Y antiserum and R. Reeves and R. Eckner for providing mouse and human cDNAs for HMG-Y, respectively. D. Kuhl is acknowledged for critical reading of the manuscript.

H.L. was supported part of the time by a fellowship from the DFG-Graduiertenkolleg "Regulation des Zellwachstums." This work was supported by a grant from the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie to M.W.

ADDENDUM

While the manuscript was under review, Zwilling et al. reported the existence of protein-protein interactions between the POU domain protein Oct-2 and the high-mobility-group protein HMG-2 (54). The interaction of Oct-2 with HMG-2 and the interaction of Tst-1/Oct-6 with HMG-I/Y exhibit many common features, including the involvement of the POU domain in the interaction, the HMG-dependent stimulation of POU domain protein binding to DNA, and the absence of a detectable ternary complex between POU domain protein, high-mobility-group protein, and DNA in electrophoretic mobility shift assays. Thus, interaction with nonhistone chromatin proteins might be a common theme in the function of POU domain proteins.

REFERENCES

1. **Bengal, E., L. Ransone, R. Scharfmann, V. J. Dwarki, S. J. Tapscott, H. Weintraub, and I. M. Verma.** 1992. Functional antagonism between c-Jun and MyoD proteins: a direct physical interaction. Cell **68:**507–519.
2. **Chen, C., and H. Okayama.** 1987. High efficiency transformation of mammalian cells by plasmid DNA. Mol. Cell. Biol. **7:**2745–2752.
3. **Chuvpilo, S., C. Schomberg, R. Gerwig, A. Heinfling, R. Reeves, F. Grummt, and E. Serfling.** 1993. Multiple closely-linked NFAT/octamer and HMG I(Y) binding sites are part of the interleukin-4 promoter. Nucleic Acids Res. **21:**5694–5704.
4. **Collarini, E. J., R. Kuhn, C. J. Marshall, E. S. Monuki, G. Lemke, and W. D. Richardson.** 1992. Down-regulation of the POU transcription factor SCIP is an early event in oligodendrocyte differentiation in vitro. Development **116:** 193–200.
5. **Disney, J. E., K. R. Johnson, N. S. Magnuson, S. R. Sylvester, and R. Reeves.** 1989. High-mobility group protein HMG-I localizes to G/Q- and C-bands of human and mouse chromosomes. J. Cell Biol. **109:**1975–1982.
6. **Du, W., and T. Maniatis.** 1994. The high mobility group protein HMG I(Y) can stimulate or inhibit DNA binding of distinct transcription factor ATF-2 isoforms. Proc. Natl. Acad. Sci. USA **91:**11318–11322.
7. **Du, W., D. Thanos, and T. Maniatis.** 1993. Mechanisms of transcriptional synergism between distinct virus-inducible enhancer elements. Cell **74:**887–898.
8. **Eckner, R., and M. L. Birnstiel.** 1989. Cloning of cDNAs coding for human HMG I and HMG Y proteins: both are capable of binding to the octamer sequence motif. Nucleic Acids Res. **17:**5947–5959.
9. **Fashena, S. J., R. Reeves, and N. H. Ruddle.** 1992. A poly(dA-dT) upstream activating sequence binds high-mobility group I protein and contributes to lymphotoxin (tumor necrosis factor-beta) gene regulation. Mol. Cell. Biol. **12:**894–903.
10. **Faus, I., H.-J. Hsu, and E. Fuchs.** 1994. Oct-6: a regulator of keratinocyte gene expression in stratified squamous epithelia. Mol. Cell. Biol. **14:**3263–3275.
11. **Frantz, G. D., A. P. Bohner, R. M. Akers, and S. K. McConnell.** 1994. Regulation of the POU domain gene SCIP during cerebral cortical development. J. Neurosci. **14:**472–485.

12. **Frisque, R. J., G. L. Bream, and M. T. Cannella.** 1984. Human polyomavirus JC virus genome. J. Virol. **51:**458–469.
13. **Geierstanger, B. H., B. F. Volkman, W. Kremer, and D. E. Wemmer.** 1994. Short peptide fragments derived from HMG-I/Y proteins bind specifically to the minor groove of DNA. Biochemistry **33:**5347–5355.
14. **Hai, T., and T. Curran.** 1991. Cross-family dimerization of transcription factors Fos/Jun and ATF/CREB alters DNA binding specificity. Proc. Natl. Acad. Sci. USA **88:**3720–3724.
15. **Hara, Y., A. C. Rovescalli, Y. Kim, and M. Nirenberg.** 1992. Structure and evolution of four POU domain genes expressed in mouse brain. Proc. Natl. Acad. Sci. USA **89:**3280–3284.
16. **He, X., R. Gerrero, D. M. Simmons, R. E. Park, C. R. Lin, L. W. Swanson, and M. G. Rosenfeld.** 1991. Tst-1, a member of the POU domain gene family, binds the promoter of the gene encoding the cell surface adhesion molecule Po. Mol. Cell. Biol. **11:**1739–1744.
17. **He, X., M. N. Treacy, D. M. Simmons, H. A. Ingraham, L. W. Swanson, and M. G. Rosenfeld.** 1989. Expression of a large family of POU domain regulatory genes in mammalian brain development. Nature (London) **340:**35–42.
18. **John, S., R. B. Reeves, J.-X. Lin, R. Child, J. M. Leiden, C. B. Thompson, and W. J. Leonard.** 1995. Regulation of cell-type-specific interleukin-2 receptor α-chain gene expression: potential role of physical interactions between Elf-1, HMG-I(Y), and NF-κB family proteins. Mol. Cell. Biol. **15:** 1786–1796.
19. **Johnson, K. R., J. E. Disney, C. R. Wyatt, and R. Reeves.** 1990. Expression of mRNAs encoding mammalian chromosomal proteins HMG-I and HMG-Y during cellular proliferation. Exp. Cell Res. **187:**69–76.
20. **Johnson, K. R., D. A. Lehn, T. S. Elton, P. J. Barr, and R. Reeves.** 1988. Complete murine cDNA sequence, genomic structure, and tissue expression of the high mobility group protein HMG-I(Y). J. Biol. Chem. **263:**18338–18342.
21. **Johnson, K. R., D. A. Lehn, and R. Reeves.** 1989. Alternative processing of mRNAs encoding mammalian chromosomal high-mobility-group proteins HMG-I and HMG-Y. Mol. Cell. Biol. **9:**2114–2123.
22. **Kenney, S., V. Natarajan, D. Strike, G. Khoury, and N. P. Salzman.** 1984. JC virus enhancer-promoter active in human brain cells. Science **226:**1337–1339.
23. **Klemm, J. D., M. A. Rould, R. Aurora, W. Herr, and C. O. Pabo.** 1994. Crystal structure of the Oct-1 POU domain bound to an octamer site: DNA recognition with tethered DNA-binding modules. Cell **77:**21–32.
24. **Kuhn, R., E. S. Monuki, and G. Lemke.** 1991. The gene encoding the transcription factor SCIP has features of an expressed retroposon. Mol. Cell. Biol. **11:**4642–4650.
25. **Lai, J. S., and W. Herr.** 1992. Ethidium bromide provides a simple tool for establishing genuine DNA-independent protein associations. Proc. Natl. Acad. Sci. USA **89:**6958–6962.
26. **Lewis, H., W. Kaszubska, J. F. DeLamarter, and J. Whelan.** 1994. Cooperativity between two NF-kappa B complexes, mediated by high-mobility-group protein I(Y), is essential for cytokine-induced expression of the E-selectin promoter. Mol. Cell. Biol. **14:**5701–5709.
27. **Major, E. O., K. Amemiya, C. S. Tornatore, S. A. Houff, and J. R. Berger.** 1992. Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clin. Microbiol. Rev. **5:**49–73.
28. **Meijer, D., A. Graus, and G. Grosveld.** 1992. Mapping the transactivation domain of the Oct-6 POU transcription factor. Nucleic Acids Res. **20:**2241–2247.
29. **Meijer, D., A. Graus, R. Kraay, A. Langeveld, M. P. Mulder, and G. Grosveld.** 1990. The octamer binding factor Oct6: cDNA cloning and expression in early embryonic cells. Nucleic Acids Res. **18:**7357–7365.
30. **Monuki, E. S., R. Kuhn, G. Weinmaster, B. Trapp, and G. Lemke.** 1990. Expression and activity of the POU transcription factor SCIP. Science **249:** 1300–1303.
31. **Monuki, E. S., G. Weinmaster, R. Kuhn, and G. Lemke.** 1989. SCIP: a glial POU domain gene regulated by cyclic AMP. Neuron **3:**783–793.
32. **Monuki, E. S., R. Kuhn, and G. Lemke.** 1993. Cell-specific action and mutable structure of a transcription factor effector domain. Proc. Natl. Acad. Sci. USA **90:**9978–9982.
33. **Monuki, E. S., R. Kuhn, and G. Lemke.** 1993. Repression of the myelin P0 gene by the POU transcription factor SCIP. Mech. Dev. **42:**15–32.
34. **Reeves, R., T. S. Elton, M. S. Nissen, D. Lehn, and K. R. Johnson.** 1987. Posttranscriptional gene regulation and specific binding of the nonhistone protein HMG-I by the 3′ untranslated region of bovine interleukin 2 cDNA. Proc. Natl. Acad. Sci. USA **84:**6531–6535.
35. **Reeves, R., and M. S. Nissen.** 1990. The A · T-DNA-binding domain of mammalian high mobility group I chromosomal proteins. A novel peptide motif for recognizing DNA structure. J. Biol. Chem. **265:**8573–8582.
36. **Reeves, R., and M. S. Nissen.** 1993. Interaction of high mobility group-I (Y) nonhistone proteins with nucleosome core particles. J. Biol. Chem. **268:** 21137–21146.
37. **Renner, K., H. Leger, and M. Wegner.** 1994. The POU-domain protein Tst-1 and papovaviral T-antigen function synergistically to stimulate glia-specific gene expression of JC virus. Proc. Natl. Acad. Sci. USA **91:**6433–6437.
38. **Rosenfeld, M. G.** 1991. POU-domain transcription factors, pou-er-ful developmental regulators. Genes Dev. **5:**897–907.
39. **Russnak, R. H., E. P. Candido, and C. R. Astell.** 1988. Interaction of the mouse chromosomal protein HMG-I with the 3′ ends of genes in vitro. J. Biol. Chem. **263:**6392–6399.
40. **Schöler, H. R.** 1991. Octamania: the POU factors in murine development. Trends Genet. **7:**323–329.
41. **Schreiber, E., P. Matthias, M. M. Müller, and W. Schaffner.** 1989. Rapid detection of octamer binding proteins with 'mini-extracts' prepared from a small number of cells. Nucleic Acids Res. **17:**6419.
42. **Solomon, M. J., F. Strauss, and A. Varshavsky.** 1986. A mammalian high mobility group protein recognizes any stretch of six A · T base pairs in duplex DNA. Proc. Natl. Acad. Sci. USA **83:**1276–1280.
43. **Strauss, F., and A. Varshavsky.** 1984. A protein binds to a satellite DNA repeat at three specific sites that would be brought into mutual proximity by DNA folding in the nucleosome. Cell **37:**889–901.
44. **Suzuki, N., H. Rohdewohld, T. Neumann, P. Gruss, and H. R. Schöler.** 1990. Oct-6: a POU transcription factor expressed in embryonal stem cells and in the developing brain. EMBO J. **9:**3723–3731.
45. **Thanos, D., and T. Maniatis.** 1992. The high mobility group protein HMG I(Y) is required for NF-kappa B-dependent virus induction of the human IFN-beta gene. Cell **71:**777–789.
46. **Voss, J. W., L. Wilson, and M. G. Rosenfeld.** 1991. POU-domain proteins Pit-1 and Oct-1 interact to form a heteromeric complex and can cooperate to induce expression of the prolactin promoter. Genes Dev. **5:**1309–1320.
47. **Wegner, M., D. W. Drolet, and M. G. Rosenfeld.** 1993. POU-domain proteins: structure and function of developmental regulators. Curr. Opin. Cell Biol. **5:**488–498.
48. **Wegner, M., D. W. Drolet, and M. G. Rosenfeld.** 1993. Regulation of JC virus by the POU-domain transcription factor Tst-1, implications for progressive multifocal leukoencephalopathy. Proc. Natl. Acad. Sci. USA **90:**4743–4747.
48a. **Wegner, M., and M. G. Rosenfeld.** Unpublished data.
49. **Wegner, M., G. Zastrow, A. Klavinius, S. Schwender, F. Mueller, H. Luksza, J. Hoppe, J. Wienberg, and F. Grummt.** 1989. Cis-acting sequences from mouse rDNA promote plasmid DNA amplification and persistence in mouse cells: implication of HMG-I in their function. Nucleic Acids Res. **17:**9909–9932.
50. **Whitley, M. Z., D. Thanos, M. A. Read, T. Maniatis, and T. Collins.** 1994. A striking similarity in the organization of the E-selectin and beta interferon gene promoters. Mol. Cell. Biol. **14:**6464–6475.
51. **Yang-Yen, H. F., J. C. Chambard, Y. L. Sun, T. Smeal, T. J. Schmidt, J. Drouin, and M. Karin.** 1990. Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction. Cell **62:**1205–1215.
52. **Yang-Yen, H. F., and L. I. Rothblum.** 1988. Purification and characterization of a high-mobility-group-like DNA-binding protein that stimulates rRNA synthesis in vitro. Mol. Cell. Biol. **8:**3406–3414.
53. **Zhao, K., E. Kaes, E. Gonzalez, and U. K. Laemmli.** 1993. SAR-dependent mobilization of histone H1 by HMG-I/Y in vitro: HMG-I/Y is enriched in H1-depleted chromatin. EMBO J. **12:**3237–3247.
54. **Zwilling, S., H. König, and T. Wirth.** 1995. High mobility group protein 2 functionally interacts with the POU domains of octamer transcription factors. EMBO J. **14:**1198–1208.