The Binding Interaction of HMG-1 with the TATA-binding Protein/TATA Complex*

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High mobility protein-1 (HMG-1) has been shown to regulate transcription by RNA polymerase II. In the context that it acts as a transcriptional repressor, it binds to the TATA-binding protein (TBP) to form the HMG-1/TBP/TATA complex, which is proposed to inhibit the assembly of the preinitiation complex. By using electrophoretic mobility shift assays, we show that the acidic C-terminal domain of HMG-1 and the N terminus of human TBP are the domains that are essential for the formation of a stable HMG-1/TBP/TATA complex. HMG-1 binding increases the affinity of TBP for the TATA element by 20-fold, which is reflected in a significant stimulation of the rate of TBP binding, with little effect on the dissociation rate constant. In support of the binding target of HMG-1 being the N terminus of hTBP, the N-terminal peptide of human TBP competes with and inhibits HMG-1/TBP/TATA complex formation. Deletion of segments of the N terminus of human TBP was used to map the region(s) where HMG-1 binds. These findings indicate that interaction of HMG-1 with the Q-tract (amino acids 55–95) in hTBP is primarily responsible for stable complex formation. In addition, HMG-1 and the monoclonal antibody, 1C2, specific to the Q-tract, compete for the same site. Furthermore, calf thymus HMG-1 forms a stable complex with the TBP/TATA complex that contains TBP from either human or Drosophila but not yeast. This is again consistent with the importance of the Q-tract for this stable interaction and shows that the interaction extends over many species but does not include yeast TBP.

The TATA-binding protein is a universal transcription factor that is essential for eukaryotic transcription by all three RNA polymerases (1–4). For RNA polymerase II transcription, the regulation of TBP1 binding to the TATA element is considered a principal determinant in promoter activity and therefore a primary target for regulatory factors. TBP can be considered modular in nature, with its highly conserved C terminus being necessary and sufficient for both binding to the TATA box and basal level transcription (5, 6). In addition all activators and repressors that bind to human TBP (hTBP) are reported to bind to the C terminus (2–4). On the other hand, the interactions of regulatory factors with the 159-residue N terminus in hTBP appear much more limited, and its role in transcriptional regulation is not understood. In the only case that is characterized, it was shown that the N terminus down-regulates hTBP binding to the U6 TATA box, mediates cooperative binding with SNAPc to the U6 promoter, and facilitates an enhanced level of RNA polymerase III transcription of the U6 gene (7, 8). In addition, a monoclonal antibody specific for the Q-tract of the N terminus of hTBP was shown to inhibit selectively in vitro transcription from TATA-containing, but not TATA-less, promoters that were transcribed by RNA pol II or III. This antibody interaction did not affect TBP binding to the TATA box or inhibit the formation of the TFIIA/TFIIB/TBP/TATA complex, which suggests that the N terminus may be available for protein-protein interactions associated with subsequent assembly of the preinitiation complex (9).

HMG-1 is a ubiquitous and highly conserved nuclear protein that has been reported to serve as a transcriptional repressor (10, 11) in some systems, while functioning as a coactivator for RNA polymerase II in other contexts (12–17). Fig. 1A shows that HMG-1 is likewise modular in nature, consisting of three domains. The A- and B-domains, each containing about 80 residues with a high percentage of arginines and lysines, are homologous and structurally comparable and have been shown to bind nonspecifically to DNA (18–21). The C terminus is quite different, being polyanionic, with the last 30 residues being a stretch of exclusively aspartic or glutamic acid residues. This segment reduces binding affinity to DNA and is not required for protein stability (22) but has defined a more definitive functional role.

In this work, we show that the C terminus of HMG-1 and the Q-tract in the N terminus of human TBP are essential for stable HMG-1/TBP/TATA complex formation. HMG-1 increases the affinity of TBP for the TATA element 20-fold, which is reflected in a significant increase in the rate of TBP binding, while having little effect on the lifetime of the complex. This interaction provides a broader spectrum of regulatory controls for TBP binding and promoter activity.

EXPERIMENTAL PROCEDURES

Isolation, Purification, and Characterization of Proteins—Calf thymus HMG-1 and HMG-2 proteins were purified in non-denaturing conditions using salt extraction, selective precipitation with ammonium sulfate, and further purification by high pressure liquid chromatography using a MonoQ column (23, 24).

The expression vector for HMG-1(A-B) didomain (residues 1–176), obtained from M. Bianchi, was transfected into BL21(DE3) cells, and the expressed protein was purified using published protocols (25). The expression vectors, pET-his, pET11d-his, 180hTBP (from

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1 The abbreviations used are: TBP, TATA-binding protein; AdMLP, adenovirus major late promoter; EMSA, electrophoretic mobility shift assay; GST, glutathione S-transferase; HMG-1, high mobility group-1 protein; hTBP, human TATA-binding protein; mAb, monoclonal antibody; Q, glutamine; SNAPc, small nuclear RNA-activating protein complex; pol, polymerase; cTBP, the C terminus of human TBP, residues 160–335; nTBP, the N terminus of human TBP, residues 1–159; dTBP, Drosophila TBP; yTBP, yeast TBP.

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The Acidic C Terminus of HMG-1 Is Essential for Stable HMG-1/TBP/TATA Complex Formation—HMG-1 binds to TBP/TATA to form an EMSA-stable HMG-1/TBP/TATA complex, exhibiting a significantly greater stability than the TBP/TATA complex (24). To investigate the extent to which the acidic C terminus of HMG-1 contributes to the stability of the complex, the binding of HMG-1-(I–215) to TBP was compared with that for the didomain HMG-1(A-B)-(I–176) that lacks the C terminus. The EMSA profile in Fig. 1B compares the binding of HMG-1 (lanes 3–7) and HMG-1(A-B) (lanes 9–14) to the TBP/TATA complex. HMG-1 binding produces a complex with an increased mobility, as reported previously (24). The HMG-1/TBP/TATA complex is evident at an (HMG-1/TBP) molar ratio of 4, whereas it is the sole species at a molar ratio of 40. On the other hand, no complex is detectable with HMG-1(A-B) at a molar ratio as high as 640. A strong new band of increased mobility becomes apparent at these increasingly higher HMG-1(A-B) molar levels (lanes 11–14). Lanes 15–18 confirm that this major band observed in lanes 11–14 is also observed when HMG-1(A-B) is reacted with the DNA probe in the absence of TBP. This indicates that at HMG-1(A-B) molar ratios of 80 or greater, the highly charged HMG-1(A-B) binds directly and preferentially to DNA and not to the TBP. This is consistent with the much higher DNA binding affinity expected for HMG-1(A-B) compared with HMG-1 (28–30). We conclude that the C terminus is essential for the stability of the HMG-1/TBP/TATA complex.

The N Terminus of hTBP Is Required for Stable HMG-1/TBP/TATA Complex Formation—The role of the N terminus of hTBP was examined by comparing the relative binding of HMG-1 to both the full-length hTBP (1–339) and the C-terminal TBP fragment (residues 159–339). Lanes 1–6 in Fig. 2, like the data in Fig. 1, show the strong binding profile for HMG-1 to TBP/TATA, with complete complex formation at a (HMG-1/TBP) molar ratio of 40. However, incubation of HMG-1 with cTBP/TATA complex does not lead to any detectable complex formation, as evident in lanes 9–14. Complexation of HMG-1 with cTBP/TATA could not be detected at (HMG-1/TBP) molar ratios as high as 640. In fact, at ratios of about 200 and higher, HMG-1 inhibits cTBP binding to the TATA-containing probe. Together with this, a band of greater mobility is observed again, which results from HMG-1 binding directly and nonspecifically to the DNA probe. This interpretation is verified by reacting HMG-1 with the DNA probe in the absence of cTBP (lanes 15–17) which produces the same band profile.
HMG-1 Binding Increases the Affinity of hTBP for the TATA Element—The relative stabilities of the TBP/TATA and HMG-1/TBP/TATA complexes were quantitatively compared by titrating the TATA-containing oligonucleotide with TBP, in the absence and presence of saturating levels of HMG-1. The EMSA binding profiles for the two complexes are shown in Fig. 3A. Qualitative examination of the binding at low TBP levels (compare lanes 2 and 11) shows more complex formed in the presence of HMG-1. The band intensity data were used to plot the fraction of each complex formed as a function of TBP concentration (Fig. 3B), from which the corresponding $K_d$ values were determined. The $K_d$ value for the TBP/TATA complex was 1.5 nm, which is comparable to values reported previously (31–34). The corresponding plot for TBP binding in the presence of saturating levels of HMG-1 shows stimulation binding of TBP to the TATA element. Complex formation is observed at significantly lower TBP levels than required for the formation of only TBP to the TATA element. This complex formation reduced the $K_d$ value by about 20-fold, with 50% HMG-1/TBP/TATA complex formation occurring at about 70 pM TBP.

HMG-2 protein is similar to HMG-1 in size and exhibits a high degree of homology, with both proteins being implicated in the regulation of transcription (18–21). However, HMG-2 has eight fewer acidic residues (22 versus 30 in HMG-1) in the C-terminal acidic tract (35, 36). We determined that HMG-2 also stimulates TBP binding, with both proteins exhibiting comparable effects in enhancing TBP binding (data not shown).

Since the $K_d$ value is a reflection of the ratio of $k_{on}/k_{off}$, the impact of HMG-1 on the complex dissociation rate constant was determined. The EMSA profile for dissociation for each complex is shown in Fig. 4A, with the data plotted in Fig. 4B. The dissociation profiles for the complexes differ only slightly, indicating that the presence of HMG-1 has little effect on the half-life of the complex. The values of $k_{off}$ are 160 and 130 min, respectively, for the TBP/TATA and the HMG-1/TBP/TATA complexes. The corresponding values for $k_{on}$ are $7.2 \times 10^{-5}$ and $8.9 \times 10^{-3}$ s$^{-1}$, respectively. The value for the TBP/TATA complex is comparable to previously reported values (33) obtained for TBP dissociation.

This finding would indicate that the effect of HMG-1 on the $K_d$ value should be associated predominantly with an increase in the on-rate for TBP. To obtain an estimate of the relative on-rates and determine if this was generally consistent with the $K_d$ data, the comparative time course of TBP binding was monitored for the two complexes and is shown in Fig. 4C.

Comparison of the relative band intensities at the same time points (e.g. 5 min) shows that, qualitatively, the presence of HMG-1 stimulates the rate of TBP binding. As shown in Fig. 4D, quantitative measurements indicate that HMG-1 clearly stimulates the rate of TBP binding, enhancing the initial rate by about 10-fold. The initial slope for the formation of the HMG-1/TBP/TATA complex represents only an estimate or lower limit value due to the high rate of reaction and difficulty of obtaining consistent data at times less than 1 min. These kinetic data are, however, consistent with the thermodynamic data and indicate that HMG-1 decreases the $K_d$ value by primarily increasing the off-rate kinetics, while having little discernible effect on the dissociation kinetics.

The N-terminal hTBP Polypeptide Inhibits hTBP Binding to HMG-1—It was of interest to provide additional support for the role of the N terminus of TBP as the target for HMG-1. If HMG-1 interacts directly with the N terminus of hTBP and this provides the primary stability for the complex, then the presence of the exogenous N-terminal polypeptide would be expected to inhibit the formation of the HMG-1/TBP/TATA complex. Fig. 5 shows the effect of increasing levels of GST-nTBP (1–159), 34–260 nm (lanes 2–6), when it is preincubated with 80 nm HMG-1 for 30 min on ice, followed by 30 min of incubation with TBP/TATA. Little inhibition is observed at the lower level of nTBP (molar ratio of nTBP/HMG-1 of 0.5; (nTBP/TBP) of 34) (lane 2), whereas progressive inhibition occurs at the higher TBP levels, with complete inhibition of complex formation observed at about the 225 nM level (molar ratio of nTBP/HMG-1 of 3; (nTBP/TBP) of 225) (lane 4–5). The presence of nTBP exhibited no detectable effect on TBP/TATA complex formation (data not shown). These data provide additional support for the N terminus of hTBP as the principal target for HMG-1 binding and for interaction with this region providing the primary stability in complex formation.

Comparative Binding of HMG-1 with N-terminal Deletion
Mutants of hTBP—In order to determine if a particular segment of the N terminus of hTBP might play a predominant role in the HMG-1 interaction, the effect that HMG-1 has on increasing the stability of the TBP/TATA complex was examined using hTBP deletion mutants. The schematic of hTBP and the five N-terminal deletion mutants used are shown in Fig. 6A. The non-conserved 159-residue N terminus can be conveniently divided into three regions as follows: the segment containing the initial residues from 1 to 54 (fragment I); the central region, inclusive of residues 55–95 and containing the Q-tract, which is made up of 34 consecutive glutamine residues (fragment II); and the segment from 96 to 158 (fragment III), which lies between the Q-tract and the conserved C terminus (residues 159–335). The ΔN nomenclature for the mutants is from Mittal and Hernandez (7). The pairs of adjacent lanes in Fig. 6B show the relative stability of the TBP/TATA and the corresponding HMG-1/TBP/TATA complexes. Comparison of lanes 1 and 2 shows that the extent of GST-TBP binding to TATA (lane 1) is significantly increased by complexation with HMG-1. This is essentially the same value obtained when TBP (not in the GST fusion) is used. This indicates that the presence of the GST does not change the HMG-1/TBP binding, which is also what was observed previously with the SNAPc/TBP binding (7, 8). The relative increases for the mutant-TBPs were determined using a PhosphorImager (data not shown) and were compared with this value. The mutants were found to fall into two different groups. HMG-1 increases the stability of the complex formed for both ΔN + I + II (lanes 3 and 4) and ΔN + II (lanes 5 and 6), with the stability being comparable, but slightly less, than that for hTBP itself. This suggests that segment II (the Q-tract) plays the major role in stabilizing the interaction with HMG-1, with segment I providing little additional stability. Fragment ΔN + I (lanes 11 and 12) has a reduced TATA binding affinity, in agreement with previous reports (7, 8) and the addition of HMG-1 has no effect on stability (extended exposure in lanes 11’ and 12’ is shown in the right panel). To ensure that ΔN +
which hTBP (1 nM) and DNA were added to all reactions, and incubation was continued for another 30 min at 30 °C.

I remained capable of binding to the TATA element and was not simply inactivated during the purification procedure. TFIIB was reacted with TBP/TATA to form a stable TFIIB/TBP/TATA complex (data not shown). There is no mobility shift and insignificant intensity change on reaction of HMG-1 with the TBP/TATA complex (lanes 1–3; data not shown). There is no mobility shift on reaction of HMG-1 with the TBP/TATA complex (lanes 4–6). The latter three deletion mutants, ΔN, ΔN + I, and ΔN + III, represent the second group, all of which exhibit no significant interaction with HMG-1. These findings indicate that the Q-tract in segment II is a major target for HMG-1 binding.

Monoclonal Antibody 1C2 Inhibits HMG-1 Binding to hTBP—If the Q-tract is important for the HMG-1 interaction, HMG-1 should compete with an antibody specific to the Q-tract region and reduce or eliminate the formation of a supershifted complex. Antibodies that are targeted to epitopes that are not directly involved in the HMG-1 binding should correspondingly yield a supershifted complex in the presence or absence of HMG-1. Fig. 7A shows the sequence for the first 95 residues in the N terminus of hTBP, highlighting the location of the epitopes for two monoclonal antibodies (mAb). mAb1C2 was originally reported to be specific for residues 53–101, with a schematic description of the TBP deletion mutants of interest. B, comparative binding of HMG-1 with N-terminal deletions of hTBP. All lanes contain 16–22 nM of a GST-TBP fusion protein and AdMLP DNA, in addition to a 40 molar excess of HMG-1 in the even-numbered lanes (+). The N-terminal deletion mutants of hTBP used are indicated above the lanes. Lanes 1 and 2 have full-length TBP, in addition to 640 nM HMG-1 in lane 1. Lanes 3 and 4 have fragment ΔN + I in lane 3. Lanes 5 and 6 contain fragment ΔN + II, in addition to 720 nM HMG-1 in lane 5. Lanes 7 and 8 contain ΔNc-TBP, in addition to 880 nM HMG-1 in lane 7. Lanes 9 and 10 contain fragment ΔN + III, in addition to 880 nM HMG-1 in lane 9. Lanes 11 and 12 contain fragment ΔN + I, in addition to 800 nM HMG-1 in lane 11. Lanes 13 and 14 are control lanes, which have 38 nM GST, in addition to 880 nM HMG-1 in lane 13. Lanes 15 and 16 contain fragment ΔN + II, with and without HMG-1 (lanes 15 and 16), were overexposed and are shown to the right. The asterisk represents fragment ΔN + I/DNA complex.

Fig. 7C shows the titration in which the HMG-1/TBP/TATA complex was preestablished, and increasing levels of antibody were added in an attempt to compete with HMG-1 binding. The
addition of increasing amounts of mAb1C2 to the complex did not displace HMG-1 from the complex. This antibody produced only a marginal band for a supershifted complex at very high levels of antibody, in contrast to that observed in the control lane 5, in which HMG-1 was absent. On the other hand, the comparable experiment in which the preestablished complex was titrated with mAb3G3 showed a consistent band for the supershifted complex in the presence of HMG-1, indicating again that mAb3G3 and HMG-1 bind simultaneously in the complex. Both of these findings are consistent with HMG-1 binding to the Q-tract of the N terminus of TBP.

**HMG-1 Binds Strongly to TATA-binding Proteins That Contain Q-tracts**

Since the data indicate that the Q-tract is the primary target for HMG-1, TBP proteins from other species were investigated to determine if HMG-1 binding correlated with the presence of a Q-tract in the N terminus of TBP and whether this interaction extended more broadly to other species. We examined the HMG-1 interaction with human, *Drosophila*, and yeast TBP, which are shown schematically in Fig. 8A. Both human and *Drosophila* TBP have glutamine tracts, whereas yeast TBP does not.

**FIG.7.** A, binding sites for monoclonal antibodies 3G3 and 1C2 in the N-terminal domain of hTBP. The partial sequence for the N-terminal domain of hTBP is shown, with segments I (residues 1–54) and II (residues 55–95) underlined. The minimal epitopes for mAb3G3 (residues 1–10) and mAb1C2 (residues 53–62 and Q-tract) are shown in bold type and with a dashed line above them. B, HMG-1 competitive binding for preestablished mAb/TBP/TATA complex. Human TBP (1 nM, present in all lanes) was preincubated for 15 min with AdMLP TATA, and then 2 ng of purified mAbs was added followed by an additional 15-min incubation, after which increasing amounts of HMG-1 were added, with incubation continued for another 15 min. HMG-1 is present in lane 1, 160 nM; lanes 4–8 and 12–16, at 13, 20, 40, and 160 nM, respectively. 2 nM of mAb1C2 is added in lanes 3–9, and 2 nM of mAb3G3 is added in lanes 11–17. Lanes 9 and 17 are control lanes with 300 nM of bovine serum albumin in lieu of HMG-1. The positions of TBP/TATA and HMG-1/TBP/TATA are indicated. The asterisk indicates the supershifted band, and NS denotes a nonspecific complex. C, mAb competitive binding for preestablished HMG-1/TBP/TATA complex. Human TBP (1 nM, present in all lanes) and HMG-1 were preincubated for 15 min with AdMLP TATA, after which increasing amounts of mAbs were added and then the incubation was continued for an additional 15 min. HMG-1 is present in lanes 1 and 2.5 nM; lanes 3 and 4; or dTBP (1 nM, lanes 5 and 6; 8.5 nM, lanes 7 and 8), or yTBP (8.7 nM, lanes 9 and 10; 17.5 nM, lanes 11 and 12) is reacted with HMG-1 (80 nM, lane 2; 200 nM, lane 4; 80 nM, lane 6; 680 nM, lane 8; 700 nM, lane 10; 1400 nM, lane 12). All even-numbered lanes contain HMG-1 at a molar excess of 80 with respect to TBP. B, HMG-1 forms a stable EMSA complex with dTBP/TATA. dTBP (1.4 nM) is reacted with AdMLP DNA and HMG-1 (200 nM) in lanes 1–4, with increasing levels of anti-HMG-1 added in lanes 2 and 3. As a negative control, anti-TFIIB was added to dTBP, AdMLP DNA, and HMG-1 in lane 4. TBP, HMG-1, and AdMLP DNA were preincubated for 15 min, and anti-HMG-1 or anti-TFIIB was then incubated for an additional 15 min.

**FIG.8.** A, comparative binding of HMG-1 to hTBP/TATA, dTBP/TATA, and yTBP/TATA. Top panel, schematic representation of the TBP's from human, *Drosophila*, and yeast showing the Q-tracts. Bottom panel, hTBP (1 nM, lanes 1 and 2; 2.5 nM, lanes 3 and 4) or dTBP (1 nM, lanes 5 and 6; 8.5 nM, lanes 7 and 8), or yTBP (8.7 nM, lanes 9 and 10; 17.5 nM, lanes 11 and 12) is reacted with HMG-1 (80 nM, lane 2; 200 nM, lane 4; 80 nM, lane 6; 680 nM, lane 8; 700 nM, lane 10; 1400 nM, lane 12). All even-numbered lanes contain HMG-1 at a molar excess of 80 with respect to TBP. B, HMG-1 forms a stable EMSA complex with dTBP/TATA. dTBP (1.4 nM) is reacted with AdMLP DNA and HMG-1 (200 nM) in lanes 1–4, with increasing levels of anti-HMG-1 added in lanes 2 and 3. As a negative control, anti-TFIIB was added to dTBP, AdMLP DNA, and HMG-1 in lane 4. TBP, HMG-1, and AdMLP DNA were preincubated for 15 min, and anti-HMG-1 or anti-TFIIB was then incubated for an additional 15 min.
HMG-1 Interaction with Human TATA-binding Protein

The rate of TBP binding and the stability of the TBP/TATA interaction within class II eukaryotic promoters are both highly regulated events, of central importance in the decision to commit to and/or initiate transcription. We show that HMG-1 significantly increases the affinity of TBP for the TATA element and stimulates the rate of TBP/TATA binding. In addition, we present a number of lines of evidence that show that HMG-1/TBP/TATA complex formation requires the acidic C terminus of HMG-1 and the Q-tract within the N terminus of TBP. The presence of HMG-1 expands the spectrum of regulatory controls for TBP binding and the promoter-bound complex.

The comparative binding of TBP with HMG-1 and the HMG-1(A-B) peptide, which has the acidic C terminus deleted, demonstrates (Fig. 1) the requirement for the C terminus of HMG-1 for complex formation with TBP/TATA. Only at very high levels of HMG-1(A-B) is there any evidence for a binding interaction, and at these levels, HMG-1(A-B) does not bind to TBP but binds nonspecifically to DNA. This brings out and emphasizes two important functional roles for the C terminus of HMG-1. First, it reduces the binding affinity of HMG-1 for DNA. Second, and more importantly, the C terminus is the critical domain for targeting HMG-1 to bind to TBP and effecting this protein-protein interaction. In contrast to this functional domain, the basic A and B boxes strongly promote nonspecific binding in the minor groove of DNA (38, 39). This is consistent with the findings that the didomain HMG-1(A-B) peptide binds more strongly to DNA. This is due in large part to its high positive charge (+22) relative to HMG-1, which has a more moderate negative charge (+8). These findings, coupled with those from previous studies (10, 12–17, 24), further highlight the distinctly different character of the domains in HMG-1, which may provide the basis for understanding its reported multifunctionalities in context-dependent transcription (40).

Although the functions of the C terminus of hTBP in transcription have been well documented (1–6), defining a role for the N terminus of hTBP has proved to be more difficult. The findings here (Figs. 2 and 3) indicate that the N terminus of hTBP is the primary target for the HMG-1 interaction. The targeting of HMG-1 to the N terminus appears unique when compared with the domain where other regulatory factors bind to TBP. Although TBP interacts with a multitude of repressors and activators, virtually all bind to the C terminus of TBP (2–4). Only in the case of the SNAPc promoter specificity factor does binding occur in the N terminus and in the Q-tract (7).

HMG-1 binds to TBP, both free in solution (10) and when bound to the TATA element. This latter interaction is consistent with its role as a repressor of both basal and Gal4-AH/USA-activated RNA pol II transcription (10). Notwithstanding the present results that show that the HMG-1 binding to the N terminus of hTBP is critical for a stable complex, there is evidence that HMG-1 also has secondary binding interactions with the C terminus of TBP (41). The HMG-1 binding to the N terminus, or both interactions, may inhibit simultaneous binding of HMG-1 and other general factors to TBP. For example, TFIIA can compete off HMG-1 from the HMG-1/TBP/TATA complex (HMG-1 is limiting), but when there are high excesses of HMG-1, an HMG-1/TFIIA/TBP/TATA complex can form (24, 41). In contrast, it has been proposed that TFIIA can dissociate the HMG-1 from HMG-1/TBP/TATA. In this way, increasing the level of TFIIA, but not TFIIA, was shown to restore basal and activated transcription in an in vitro assay (10).

The N terminus of hTBP reduces the binding affinity of TBP for the TATA element (7, 42). Interestingly, this is the same general effect that the C terminus of HMG-1 has on its nonspecific binding to DNA. It has been reported that TFIIA binding to yeast TBP enhances TBP binding to promoter DNA by eliminating the otherwise inhibitory effect of the N terminus in TBP (42). Our data indicate that the binding of HMG-1 to the N terminus of hTBP also increases the affinity of TBP for TATA. Although the result of the binding interaction may be quite complex, the direct HMG-1/TBP interaction may provide a similar effect in reducing an energetically unfavorable interaction between the N terminus and DNA and contributing to the mechanism by which HMG-1 binding stabilizes the complex.

Interestingly, the presence of TFIIA stimulates TBP binding and stabilizes TBP/TATA and facilitates further preinitiation complex assembly that can lead to productive transcriptional initiation. HMG-1 also stimulates the rate of TBP binding and leads to an increased TBP/TATA stability. In contrast, however, HMG-1 leads to the formation of a temporal, transcriptionally inactive promoter complex. This may provide a novel mechanism by which HMG-1 can establish a reversible "poised" but transcriptionally inactive complex that can suppress basal level transcription and, in selected promoters, be in a position to facilitate subsequent activation as proposed previously (43, 44).

EMSA experiments with hTBP deletions and monoclonal antibodies to epitopes in the N terminus were instrumental, and reinforced each other, in defining the Q-tract in hTBP as the decisive segment for HMG-1 binding. Segments I and III have little or no effect on enhancing the stability of the HMG-1/TBP/TATA complex (Fig. 6), whereas segment II greatly enhances complex formation. In further support of this, mAb1C2, which was specific for the Q-tract (9), was unable to bind to TBP in the presence of HMG-1 (Fig. 7). This is consistent with the idea that HMG-1 and mAb1C2 bind to the same or overlapping sites in the Q-tract. In contrast, the binding of mAb3G3 to its epitope in the first 10 residues of the N terminus was

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unaffected by the presence of HMG-1, and its binding super-shifted the complex at all levels of HMG-1.

In one of the first reports that suggested a direct functional role for the N terminus of hTBP in transcription, it was shown that the N terminus mediates cooperative binding with SNAPc to the U6 promoter, resulting in enhanced U6 transcription by RNA polymerase III (7, 8). In this case, segments I and II were implicated as the target for SNAPc interactions. Monoclonal antibody 1C2, which binds hTBP at the same site as does HMG-1, was shown to inhibit in vitro transcription from TATA-containing promoters, from both RNA pol II and III (9). One can speculate from our data that HMG-1 may act as a more general transcriptional repressor, not only repressing transcription carried out by RNA pol II (10), but also repressing RNA pol III transcription for this class of small nuclear RNA genes. For both HMG-1 and mAb1C2, the target is the Q-tract. This suggests that in both these cases, the interaction may obstruct or obviate the function of the Q-tract in important protein-protein or protein-DNA interactions.

The presence of HMG-1 increased the stability of the HMG-1/TBP/TATA complex by about 20-fold, relative to TBP/TATA complex. This is a similar to, but greater than, the increased stability reported for the binding of the general transcription factors TFII B or TFII A to TBP/TATA. In these cases, the affinity of TBP for TATA was increased by up to 10-fold (31–34). There is no universal agreement on these values which suggests that there may be a significant dependence on conditions (32, 34). In the most extensive studies, Pugh et al. (32) have shown that TFII A significantly increases the stability of the TBP/TATA interaction, as reflected by both an increase in the on-rate for TBP binding and a decrease in the off-rate by about a factor of 4 (32).

HMG-1 increased the affinity of the TBP/TATA interaction but without any significant effect on the dissociation kinetics of the complex. The decreased KD value was predominantly a result of HMG-1 stimulating the on-rate constant for TBP. This complexation could provide TBP with a significant kinetic advantage in extending the conditions under which it could gain access to the TATA element. It would permit TBP to bind most promoters under conditions of limiting TBP levels and/or facilitate efficient TBP binding to promoters that lack strong TBP-binding sites.

The interaction of HMG-1 with TBP may have broader physiological importance in light of recent findings that HMG-1 was identified as a component of TFII D (41, 45). HMG-1 was found in association with a crude TFII D fraction from HeLa cells and was shown to serve as a coactivator for herpes simplex virus IP C4 in vitro (45). It was also shown that the GST-A box of HMG-1 was able to pull down TFII D in crude HeLa extract, suggesting that HMG-1 binds to TBP and/or other TFII D components (41). Both these studies suggest that the strong HMG-1 interaction with hTBP described here may not only be retained but may be further reinforced in binding to the multisubunit TFII D complex.

As a result of a lack of HMG-1 interaction with yeast TBP, it has been suggested that HMG-1 binds to TBP in a species-specific manner (41). A comparison made of calf thymus HMG-1 interacting with TBP from human, Drosophila, and yeast found that HMG-1 formed a stable EMSA complex with both human and Drosophila TBP, but not with yeast TBP (Fig. 7). In fact, HMG-1 dissociates yTBP from the TATA element, in a manner similar to that observed with its interaction with human c-TBP (Fig. 2). Although the C terminus of TBP is highly conserved over these species (81% homology) (6, 46–51, 57), the N terminus is generally considered to be highly divergent, with the exception that the N terminus is highly conserved over vertebrates (52–53). A -tract remains in all vertebrates, although its length varies, whereas segments I and II are 78 and 73% identical, respectively, in human (46, 47, 54, 55), mice (52), hamster (7), and two different vipers (53, 56). In addition, the N terminus of Drosophila TBP is longer than that in human TBP (Fig. 8) and is similar in that it contains primarily hydrophilic residues, with very few charged residues (5% of the residues). In addition, it contains two smaller Q-tracts (6 and 8 glutamines, respectively) that are separated by 32 residues (48). However, yeast TBP is quite different, in that the N terminus is significantly shorter, has a large fraction of charged residues (39%), and does not contain a Q-tract (6, 57). The data are consistent with HMG-1 binding to Q-tracts in the N terminus, although the enhanced stability as a result of HMG-1 binding to dTBP is significantly reduced compared with that in hTBP. This is what would be expected as a result of both the reduced sizes and the separation of the two Q-tracts in dTBP. Surprisingly, it was reported that there was no detectable interaction between HMG-1 and dTBP in GST pull-down experiments (41). From the limited findings, calf thymus HMG-1 binding to TBP clearly occurs in different species and may be quite general, extending throughout the vertebrates, to Drosophila, and perhaps to others.

These results lead to a general view for the principal interaction between HMG-1 and hTBP in the HMG-1/TBP/TATA complex. A simple working model (Fig. 9) that is consistent with the current data emphasizes a significant and direct interaction of the acidic residues in HMG-1 with the Q-tract in the N terminus of hTBP. In this interaction, one can hypothesize that the amide hydrogens in the glutamine residues may act as hydrogen bond donors, with the negatively charged carboxylate groups of glutamate/aspartate residues in HMG-1 acting as hydrogen bond acceptors. This “zipper of electrostatic hydrogen bonds” would be expected to provide significant stability to this intermolecular interaction. In addition, the HMG-1 interaction to the N terminus may reduce an energetically unfavorable interaction with DNA. Together with the EMSA data, and the GST-TBP pull-down results that show detectable interactions between the A and B boxes and TBP (data not shown), we propose that although the primary stabilizing interaction involves the C terminus of HMG-1 and the N terminus of hTBP, additional sites in both HMG-1 and hTBP appear to be implicated in the overall complexation (22, 39).

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