

Snf2/Swi2-related ATPase Mot1 drives displacement of TATA-binding protein by gripping DNA

Rebekka O Sprouse¹, Michael Brenowitz²
and David T Auble^{1,*}

¹Department of Biochemistry and Molecular Genetics, University of Virginia Health System, Charlottesville, VA, USA and ²Department of Biochemistry, The Albert Einstein College of Medicine, Bronx, NY, USA

Mot1 is a conserved Snf2/Swi2-related transcriptional regulator that uses ATP hydrolysis to displace TATA-binding protein (TBP) from DNA. Several models of the enzymatic mechanism have been proposed, including Mot1-catalyzed distortion of TBP structure, competition between Mot1 and DNA for the TBP DNA-binding surface, and ATP-driven translocation of Mot1 along DNA. Here, DNase I footprinting studies provide strong support for a 'DNA-based' mechanism of Mot1, which we propose involves ATP-driven DNA translocation. Mot1 forms an asymmetric complex with the TBP core domain (TBPc)–DNA complex, contacting DNA both upstream and within the major groove of the TATA Box. Contact with upstream DNA is required for Mot1-mediated displacement of TBPc from DNA. Using the SsoRad54–DNA complex as a model, DNA-binding residues in Mot1 were identified that are critical for Mot1–TBPc–DNA complex formation and catalytic activity, thus placing Mot1 mechanistically within the helicase superfamily. We also report a novel ATP-independent TBPc displacement activity for Mot1 and describe conformational heterogeneity in the Mot1 ATPase, which is likely a general feature of other enzymes in this class.

The EMBO Journal (2006) 25, 1492–1504. doi:10.1038/sj.emboj.7601050; Published online 16 March 2006

Subject Categories: chromatin & transcription

Keywords: ATPase; Mot1; Snf2; Swi2; TBP

Introduction

Mot1 is an essential transcriptional regulator in *Saccharomyces cerevisiae* that directly interacts with the TATA-binding protein (TBP) in solution and when both proteins are bound to promoter DNA (Davis *et al.*, 1992; Auble *et al.*, 1994; Poon *et al.*, 1994; Adamkewicz *et al.*, 2001; Darst *et al.*, 2001; Pereira *et al.*, 2003). A remarkable property of Mot1 is its ability to utilize the energy derived from the hydrolysis of ATP to displace TBP bound to a TATA Box sequence on DNA. This activity rationalizes Mot1's

function as a transcriptional repressor (Auble *et al.*, 1994). The requirement for Mot1 to activate transcription of some genes (Collart, 1996; Madison and Winston, 1996; Prelich, 1997; Andrau *et al.*, 2002; Dasgupta *et al.*, 2002) can also be explained at least in part by the ability of Mot1 to eject inactive TBP-containing complexes from activated promoters (Geisberg *et al.*, 2002; Dasgupta *et al.*, 2005).

Mot1 is a member of the Snf2/Swi2 ATPase family (Eisen *et al.*, 1995). Enzymes in this class participate in all of the fundamental processes of DNA metabolism, utilizing ATP hydrolysis to alter contacts between proteins and DNA (Vignali *et al.*, 2000). Many enzymes in this family reposition nucleosomes, structurally alter them, or remove them entirely from DNA (Becker and Horz, 2002; Narlikar *et al.*, 2002). The changes that occur in nucleosomal DNA follow multiple cycles of ATP hydrolysis driving changes in DNA twist or bulge propagation over the surface of the nucleosome (Flaus and Owen-Hughes, 2003). These observations have given rise to the idea that these ATPases function as molecular motors. While the utilization of ATP hydrolysis to drive changes in DNA topology is well established, relatively little is known about how the chemical energy from ATP hydrolysis is used to generate mechanical force driving disruption of protein–DNA interactions (Smith and Peterson, 2005).

Mot1-catalyzed dissociation of the TBP–DNA complex affords a simplified system for defining how this chemical–mechanical coupling occurs. Mot1 neither translocates processively along DNA nor functions by propagating changes in DNA structure (Auble and Hahn, 1993; Auble and Steggerda, 1999; Adamkewicz *et al.*, 2001; Darst *et al.*, 2001). Mot1 stabilizes TBP binding to nonconsensus TATA sequences in the absence of ATP (Gumbs *et al.*, 2003; Klejman *et al.*, 2005). Thus, rather than manipulating DNA structure, Mot1 may use ATP hydrolysis to distort TBP structure (Adamkewicz *et al.*, 2001; Darst *et al.*, 2003; Gumbs *et al.*, 2003). Such a mechanism would represent a novel use of such an ATPase. Yeast Mot1 and its human homolog, BTAF1, compete with DNA for binding to TBP (Pereira *et al.*, 2001; Darst *et al.*, 2003). This competition has given rise to an alternative, but not mutually exclusive, suggestion that the interaction between Mot1 and the TBP DNA-binding surface contributes to the Mot1/BTAF1 regulatory mechanism (Pereira *et al.*, 2001; Darst *et al.*, 2003). A third idea posits that Mot1, as a member of the helicase superfamily (Eisen *et al.*, 1995), uses a mechanism of ATP-driven DNA translocation to disrupt the interaction between TBP and DNA (Darst *et al.*, 2001). In support of the DNA translocation model, the recent crystal structures of the Snf2/Swi2-related ATPase Rad54 from zebrafish (Thoma *et al.*, 2005) and *Sulfolobus* (Durr *et al.*, 2005) revealed structural similarity between the ATPase and helicase catalytic cores (Durr *et al.*, 2005). However, in the SsoRad54–DNA complex, the ATPase domains were in an unexpected arrangement in which residues required for ATP binding and hydrolysis were misaligned and the ATP binding cleft was unformed (Durr

*Corresponding author. Department of Biochemistry and Molecular Genetics, University of Virginia Health System, 1300 Jefferson Park Avenue, Room 6213, Charlottesville, VA 22908-0733, USA.
Tel.: +1 434 243 2629; Fax: +1 434 924 5069;
E-mail: dta4n@virginia.edu

Received: 6 October 2005; accepted: 20 February 2006; published online: 16 March 2006

et al, 2005). Durr *et al* (2005) proposed a model reconciling this structure with helicase activity in which domain rotation induced by ATP binding and hydrolysis results in translocation of the enzyme along DNA.

In this study, we test a model for Mot1 action based on the SsoRad54–DNA structure and conclude that Mot1 is a non-processive DNA-translocating enzyme. These results also provide support for the biochemical significance of ATPase domain rotation observed in the SsoRad54–DNA crystal structure. Moreover, a novel ATP-independent displacement activity of Mot1 was observed, which appears to arise at least in part from conformational variability in the ATPase and is likely a general feature of enzymes in this class.

Results

These studies employed purified, bacterially expressed, yeast TBP and highly purified Mot1, which was obtained from a yeast overexpression strain (Figure 1A; Darst *et al*, 2001). Using these proteins, TBP–DNA and Mot1–TBP–DNA ternary complexes were detectable by electrophoretic mobility shift assays (Figure 1B and C). The addition of unlabeled competitor DNA demonstrated that preformed TBP–DNA complexes dissociated over the time course, whereas the detectable ternary complexes were markedly more stable. In contrast, addition of ATP resulted in Mot1-catalyzed displacement of both proteins from DNA in less than 2 min. While these assays confirm the general features of Mot1’s activity, quantitative studies are problematic using this method because of the time required to load the gels and the instability of TBP–DNA complexes during electrophoresis (Hoopes *et al*, 1992). We therefore turned to DNase I footprinting, a techni-

que that circumvents these limitations and identifies the regions of protein contact on the DNA in solution (Galas and Schmitz, 1978).

Structural organization of the Mot1–TBPc–DNA complex

DNase I footprint titration experiments were conducted in which increasing concentrations of TBP core domain (TBPc) were titrated against radiolabeled DNA bearing the high-affinity TATA Box sequence TATAAAAG (Figure 2A and B). TBPc provides full function in yeast cells (Reddy and Hahn, 1991) and is displaced by Mot1 from DNA indistinguishably from full-length TBP (Auble and Hahn, 1993; Darst *et al*, 2001). The tighter DNA binding affinity of TBPc compared to full-length TBP improved the reproducibility and robustness of DNase I protection and thereby facilitated this quantitative analysis. The measured K_d of 3.8 ± 0.6 nM measured for the TBPc–DNA interaction and the crispness of the observed protection pattern demonstrate high affinity and specificity of this interaction under these experimental conditions.

Titration of Mot1 to the TBPc–DNA complex resulted in an extension of the DNase I footprint upstream from the TATA Box (Figure 2C). Quantitation of the bands uniquely protected from DNase I by Mot1 yields an apparent K_d of 1.5 ± 0.5 nM for its association with the TBPc–DNA complex (Figure 2D). This reaction includes both *intrinsic* (i.e., Mot1–DNA interactions) and *cooperative* (i.e., Mot1–TBPc interactions) contributions since Mot1 does not detectably bind to DNA in the absence of TBPc (Ackers *et al*, 1983; Darst *et al*, 2001; and data not shown). To probe the Mot1–TBPc–DNA ternary complex in detail, autoradiograms such as that

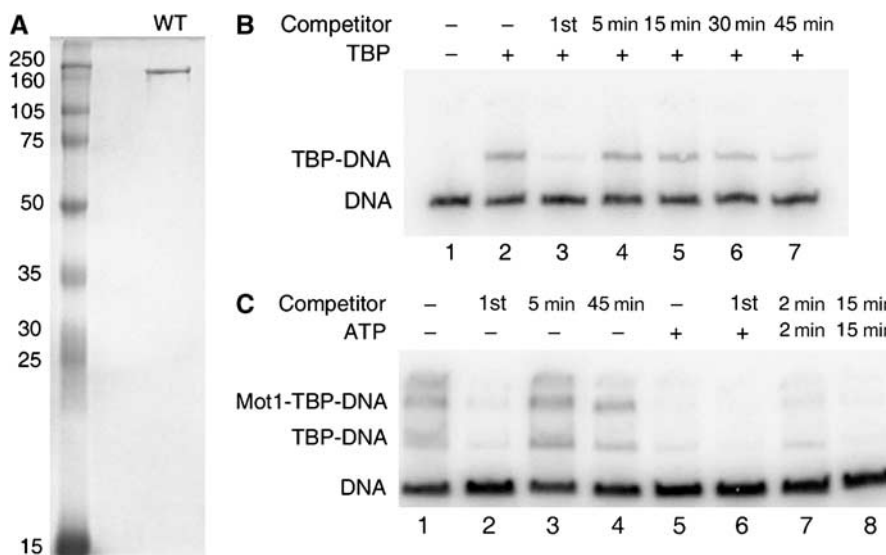


Figure 1 (A) A representative 12% protein gel showing the purified wild-type Mot1 protein used in these studies. The gel was stained with Coomassie blue and molecular weight standards are shown on the left. (B) Electrophoretic mobility shift assay using radiolabeled DNA (<1 nM) combined with 10 nM TBP where indicated. Unlabeled competitor TATA DNA was added prior to the addition of TBP (lane 3; 1st), or was incubated with preformed TBP–DNA complexes for the indicated times prior to loading on the gel (lanes 4–7). (–) indicates that the component was not added. The positions of the free DNA and TBP–DNA complex are shown on the left. Quantitation of the TBP–DNA complex revealed a dissociation half-time of ~17 min (not shown). (C) An electrophoretic mobility shift experiment in which TBP (10 nM) was preincubated with radiolabeled DNA (<1 nM) as in (A), followed by addition of Mot1 (10 nM) to all reactions for 2 min. In lanes 3, 4, 7, and 8, unlabeled competitor TATA DNA was then added with or without 25 μ M ATP for the indicated times prior to loading on the gel. Competitor DNA was added prior to TBP in the reactions in lanes 2 and 6. The positions of the free DNA, TBP–DNA, and Mot1–TBP–DNA complexes are indicated.

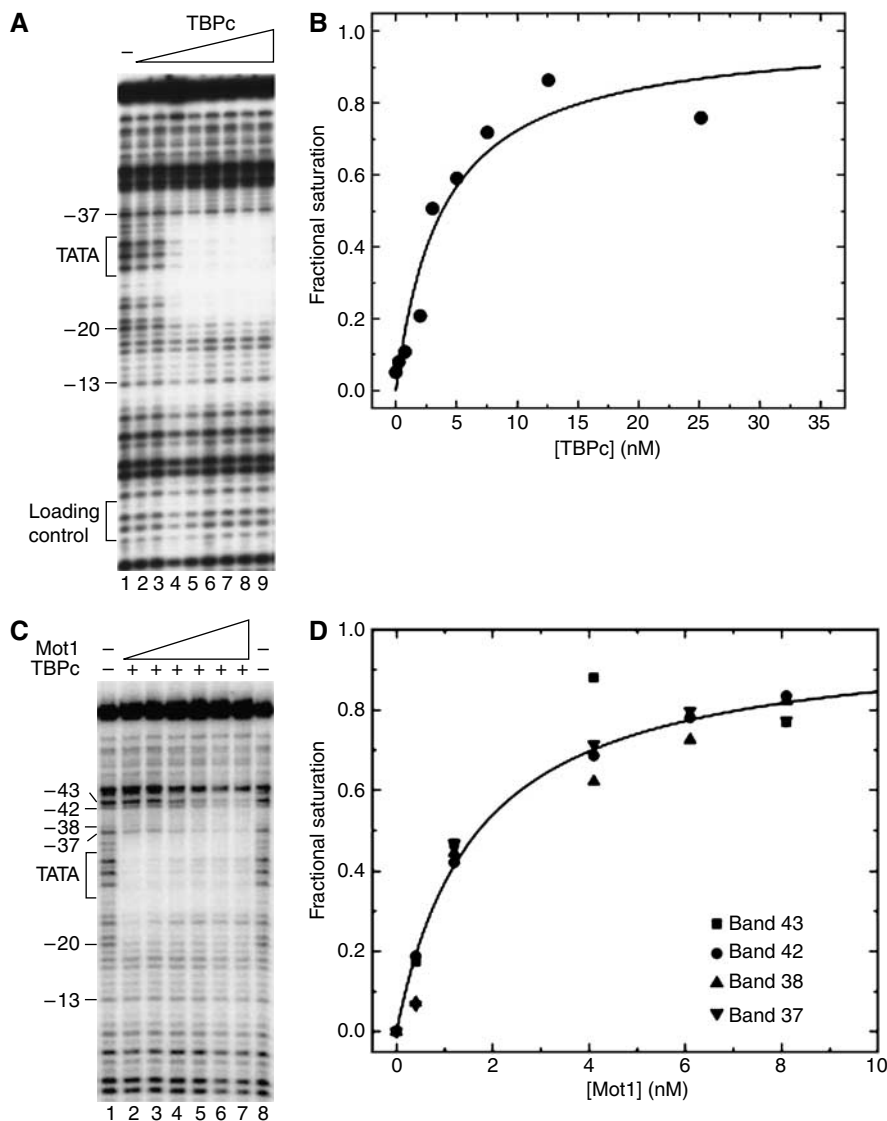


Figure 2 (A) An autoradiogram showing a DNase I footprint experiment in which TBPC was titrated to DNA. Lane 1 shows free DNA. Reactions in lanes 2–9 contained 0.25, 0.75, 2.0, 3.0, 5.0, 7.5, 12.6, or 25.1 nM TBPC, respectively. (B) Analysis of the experiment in (A), yielding an apparent K_d of 3.8 ± 0.6 nM for the TBPC–DNA interaction. Quantitation was performed by block analysis (Brenowitz *et al*, 1986) of the TATA band intensities normalized for differences in lane loading using the bands in the indicated region of the gel. (C) An autoradiogram showing a DNase I footprint titration experiment in which Mot1 was titrated to the TBPC–TATA complex. Lanes 1 and 8 show the free DNA. Lanes 2–7 included TBPC at 8.8 nM and Mot1 at 0.0, 0.4, 1.2, 4.1, 6.1, or 8.1 nM Mot1 for lanes 2–7, respectively. The positions of those upstream bands whose DNase I reactivity reflects only Mot1 binding are shown to the left of the autoradiogram (see Figure 3). A decrease in TATA Box protection upon addition of Mot1 was also observed; this is quantitated in Figure 3. (D) An isotherm calculated from (C) for the binding of Mot1 to the TBPC–DNA complex. The changes in density of the bands corresponding to nucleotides 37, 38, 42, and 43 quantitated individually were globally analyzed against the Langmuir polynomial to yield a best-fit value of $K_d = 1.5 \pm 0.5$ nM as depicted in the simulated curve.

shown in Figure 2C were quantitated with single-nucleotide resolution (Takamoto *et al*, 2004; Das *et al*, 2005).

The extent of DNase I protection for the DNA alone, TBPC–DNA, and TBPC–Mot1–DNA complexes is shown in Figure 3. The TBPC footprint extends from nucleotides 36 to 19, bracketing the TATA Box. The addition of Mot1 yielded unique upstream protections at nucleotides 37, 38, 42 and 43. Owing to band compression at more upstream positions, the upstream edge of the Mot1 footprint was not determined in this analysis, but the overall pattern of upstream protection closely resembles that reported previously (Darst *et al*, 2001). Neither protein affected the DNase I reactivity of nucleotides

downstream of 19. These observations are consistent with both proteins directly contacting the DNA; this conclusion is supported by crosslinking studies that are discussed below (Figure 8). We also noted that the extent of DNase I protection within the TATA Box decreased as Mot1 binding increased (Figure 2C and data not shown). This decrease in TATA Box protection was observed for every nucleotide protected by TBPC (Figure 3). The Mot1-mediated increase in DNase I reactivity within the TATA Box could result from a conformational change in which TBPC is partially lifted from the TATA Box, or alternatively, some TBPC may be displaced by Mot1 in the absence of ATP hydrolysis.

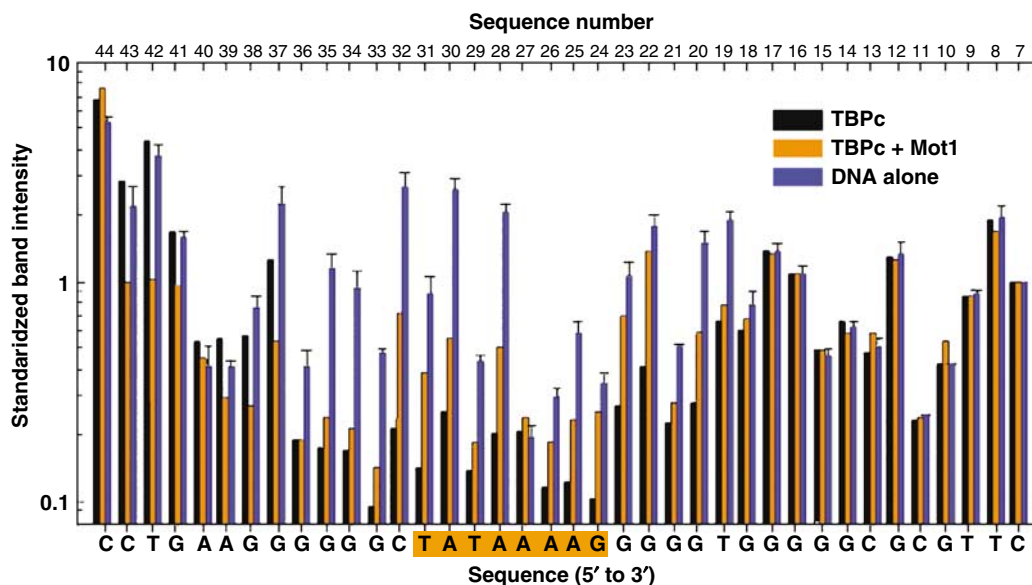


Figure 3 A histogram showing quantitation of the relative intensity of the electrophoretic bands within and surrounding the TATA Box for DNA alone (blue), TBPC alone (black), and TBPC + Mot1 (orange) quantitated with single-nucleotide resolution. The data were derived from averaging lanes 1 and 8, or 2 and 7 of Figure 2C, respectively. The error bars on the free DNA data are the standard deviation of the average of the two lanes. The alterations in DNase I digestion pattern induced by TBP and Mot1 quantified here were observed in multiple independent experiments (not shown).

Unexpected heterogeneity in the Mot1–TBPC–DNA complex

To distinguish between these models, kinetics experiments were performed in which displaced TBPC was trapped with unlabeled competitor DNA, allowing dissociation to proceed to completion. In these experiments, TBPC was added to the DNA and allowed to equilibrate for 20 min, and then Mot1 was added at the indicated concentrations and allowed to equilibrate for 2 min before the addition of competitor DNA. In the absence of Mot1, the typically slow rate of TBPC dissociation was observed, following an exponential decay with a half-time of 17.9 ± 1.8 min. This reaction was minimally affected by ATP ($t = 12.2 \pm 1.2$ min; Figure 4A).

When the experiment was conducted with Mot1 present and ATP added together with the competitor DNA, dissociation was fast with a half-time of 0.91 ± 0.09 min in a reaction that is also described as an exponential decay (Figure 4B; see Supplementary Figures S1 and S2 for representative autoradiograms and ATP hydrolysis rate under these conditions). Surprisingly, in the absence of ATP, dissociation of the Mot1–TBPC–DNA complex was biphasic (Figure 4B). A fast phase characterized by a half-life of $1.8 (+1.0, -0.7)$ min comprises about a third of the reaction amplitude. This fast phase was followed by a slow one characterized by a half-life of $92.2 (+23.6, -16.4)$ min. The fast phase represents a 10-fold destabilization of the TBPC–DNA complex, whereas the slow phase represents 5-fold stabilization. Since neither rate corresponds to that observed for TBPC alone, these dissociation events are unique to the ternary complex. The uniqueness of this dissociation behavior is supported by kinetic analysis of the mot1–505 mutant protein that harbors mutations in conserved residues of the ATP-binding Walker B motif (D1408A, E1409A, H1411A; Eisen *et al*, 1995) destroying ATPase activity. Dissociation of mot1–505–TBPC–DNA complexes in the presence of ATP was also biphasic; the dissociation progress curve for this reaction was very similar to that of

wild-type ternary complexes in the absence of ATP (compare Figure 5 with Figure 4B). Clearly, the biphasic dissociation behavior is not owing to residual Mot1 catalytic activity resulting, for instance, from ATP contamination. The biphasic ternary complex dissociation data explain why nuclease sensitivity increases in the TATA Box in proportion to increasing Mot1: Mot1 can dissociate a proportion of the TBPC–DNA complexes in the absence of ATP. These results also argue against the possibility that the increased TATA Box reactivity (Figure 2C) results from a conformational change in TBP induced by Mot1.

Perturbation of the linker connecting ATPase subdomains affects ternary complex heterogeneity

The biphasic kinetic behavior suggests that in the absence of ATP, wild-type Mot1 assembles into at least two types of ternary complexes with different stabilities. While this heterogeneity could arise from conformational differences in any of the ternary complex components, recent structural studies of the homologous Snf2/Swi2 family member SsoRad54 (Durr *et al*, 2005) point to Mot1. The ATPase subdomains of SsoRad54 are rotated in the crystal structure $\sim 180^\circ$ away from the positions required for establishment of the ATP binding cleft (Durr *et al*, 2005; Figure 6A and B, ‘open’ and ‘closed’ forms). This suggests that Snf2/Swi2 ATPase domains can undergo functionally significant rotational changes. The sequences of SsoRad54 and Mot1 align over the entire length of their ATPase domains with an expectation value of 5×10^{-71} . This high degree of homology strongly supports the hypothesis that these proteins have the same overall fold and that the Mot1 ATPase domain can likewise undergo a functionally significant rotational change in conformation.

This hypothesis was tested by analysis of the activity of mot1–24 (Madison and Winston, 1996), a conditional mutant protein harboring a single amino-acid change within the hinge connecting the subdomains proposed to undergo rotation (Figures 6A and B). Although mot1–24 footprints

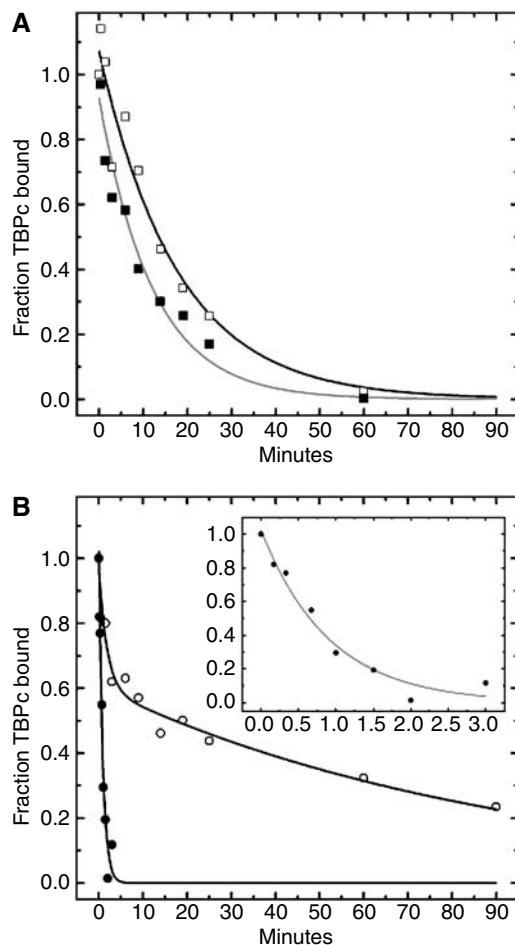


Figure 4 (A) The rate of TBPC dissociation was monitored by DNase I footprinting in the absence (open symbols) and presence (filled symbols) of 25 μ M ATP. The half-time values determined for the best fit to a single exponential decay are 17.9 ± 1.8 and 12.2 ± 1.2 min, respectively. (B) The rate of dissociation of the TBPC-Mot1-DNA complex in the absence (open symbols) and presence (solid symbols) of 25 μ M ATP. The ATP-mediated dissociation reaction is well described by a single exponential decay characterized by a half-time of 0.91 ± 0.09 min. In contrast, the progress curve measured in the absence of ATP is biphasic and characterized by half-times of 1.8 ($-0.5, +0.7$) and 92.2 ($-12.0, +17.0$) min and amplitudes of 0.37 ± 0.6 and 0.63 ± 0.5 , respectively. The fit of the data to a double compared with single exponential decay was significantly better as judged by the distribution of residuals (not shown) and χ^2 values (2.18×10^{-3} versus 1.26×10^{-2} , respectively).

with TBPC-DNA indistinguishably from wild-type Mot1 in the absence of ATP (Figure 6C), the dissociation kinetics of the mot1-24-TBPC-DNA ternary complex differ significantly from wild-type ternary complex kinetics (Figure 6D). Notably, the amplitude of the fast kinetic phase is barely distinguishable and the slow phase is ~ 2.5 -fold less stable than ternary complexes formed with wild-type Mot1. The absence of appreciable heterogeneity in the stability of mot1-24 ternary complexes suggests that the wild-type heterogeneity in ternary complex stability is owing to structural heterogeneity in Mot1 conformation. Because mot1-24 had no detectable ATPase activity (Figure 6E), we suggest that the linker mutation impairs conversion of the catalytically inactive 'open' conformation to the catalytically active 'closed' conformation, and that both conformational forms can interact with TBPC-DNA.

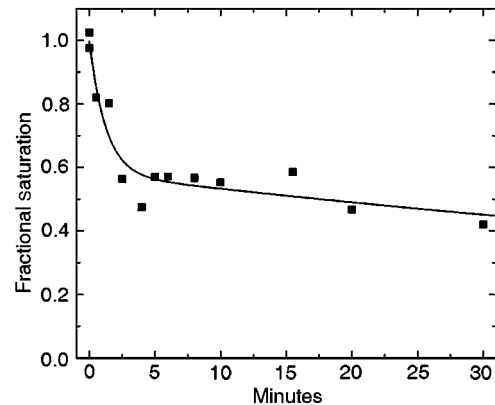


Figure 5 The rate of dissociation of TBPC-mot1-505-DNA complexes in the presence of 25 μ M ATP. The progress curve is biphasic and characterized by half-times of 1.25 ($-0.37, +0.48$) and 120.1 ($-47.9, +227.8$) min and amplitudes of 0.42 ± 0.06 and 0.58 ± 0.05 , respectively.

Catalysis of TBPC-DNA dissociation by Mot1 requires upstream DNA

If an interaction of Mot1 with upstream DNA is functionally important for Mot1-mediated catalysis of TBPC displacement, then Mot1 should not remove protein bound to a DNA template lacking the upstream footprinted sequence. Two templates with truncated upstream DNA were tested (Figure 7A; templates 5-3 and 6-1); the TBPC footprint and binding affinity were indistinguishable when compared to the full-length RW2 probe (not shown). However, significant differences were observed when Mot1 was added to pre-formed TBPC-DNA complexes. The control template displayed the expected ATP-dependent Mot1-mediated displacement of TBPC from the DNA (Figure 7B; RW2). Mot1 removed essentially all of the bound TBPC following 1.0 min incubation with ATP, consistent with Figure 4. In contrast, Mot1 displaced relatively little of the TBPC bound to the short DNA templates after 5.0 min ATP incubation.

Kinetic experiments (Figure 7D) demonstrated that the half-time of ATP-dependent Mot1-mediated TBPC displacement from the 5-3 template was 9.5 ($-1.1, +1.3$) min, a value comparable to that measured in the absence of Mot1. This result confirms that the DNA upstream of the TATA Box is critical for ATP-dependent Mot1-mediated catalysis of TBPC-DNA dissociation under these experimental conditions. As expected, the requirement for upstream DNA for ATP-dependent Mot1-mediated displacement was also observed for the full-length TBP (Figure 7C).

The ATP-independent displacement of TBPC from the 5-3 template by Mot1 was characterized by the biphasic progress curve observed for the long DNA (Figure 7E). Approximately 30% of the dissociation reaction occurred with a half-time of 3.8 ($-1.5, +2.4$) min while the remainder occurred with a half-time of 50.7 ($-10.1, +12.4$) min. Thus, the ATP-independent dissociation of TBPC-DNA by Mot1 does not have an upstream DNA requirement.

Site-specific photocrosslinking of Mot1 to DNA

To test directly the hypothesis that Mot1 intimately contacts DNA, photocrosslinking was performed to determine if Mot1 could be covalently bound to DNA probes with 5-iodouracil (5-IdU; Malkov and Camerini-Otero, 1995) clusters positioned

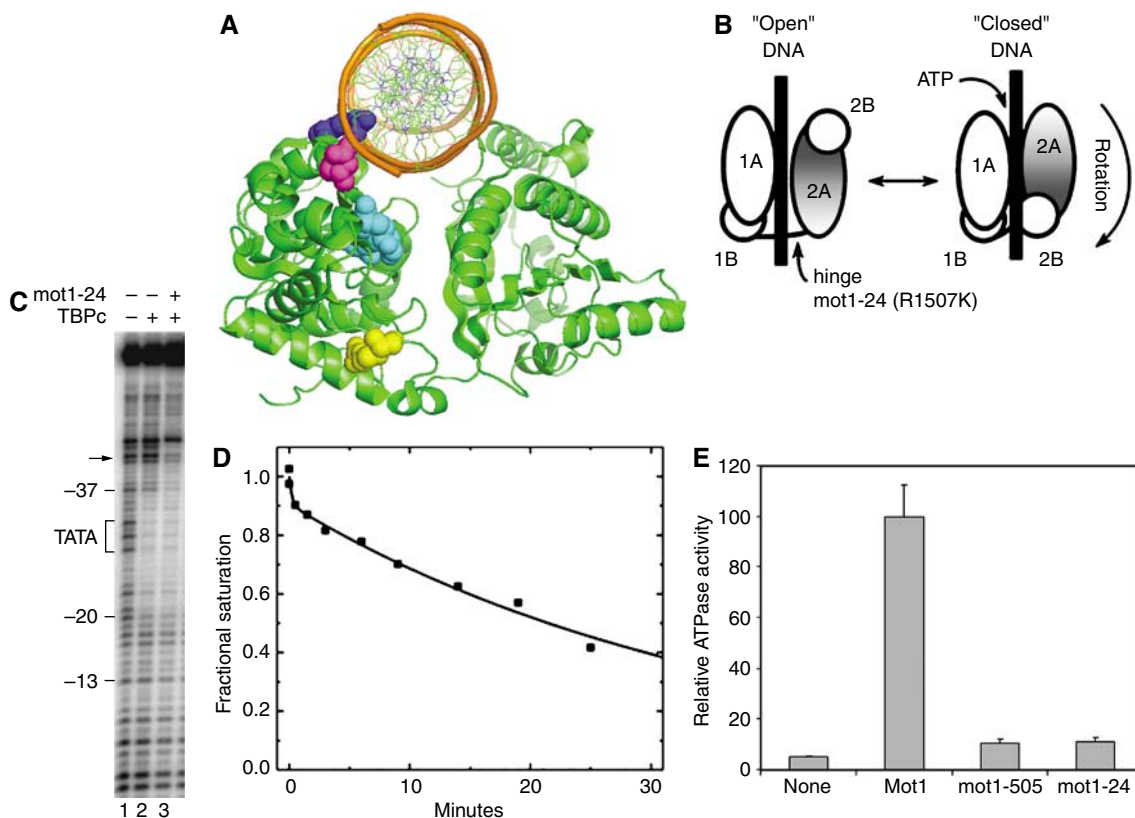


Figure 6 (A) Ribbon representation of Sso-Rad54 bound to DNA (Durr *et al*, 2005). View along the ATP binding cleft shows domain 1 (left) and domain 2 (right) connected by a flexible hinge (bottom). Homologous residues corresponding to mutations analyzed in this study are shown as colored spheres: mot1-24 (hinge region, yellow); mot1-505 (Walker B motif, cyan); mot1-508 and mot1-509 (DNA contacting residues in domain 1A; blue and magenta, respectively). (B) Model for Mot1 ATPase conformational change. Interaction of the ATPase with DNA (thick bar) is mediated primarily by residues in domain 1A. In the open conformation, domain 2A is oriented with ATP-binding residues pointing outward. An $\sim 180^\circ$ rotation of domain 2A establishes the ATP binding cleft between domains 1A and 2A, and swings the Snf2/Swi2-specific 1B and 2B domains in position to push on DNA during the power stroke. A hinge connects the two pairs of domains and is the location of the R1507K mutation. Model is based on the SsoRad54-DNA structure (Durr *et al*, 2005) and the PcrA-DNA-AMPNP structure (Velankar *et al*, 1999). (C) DNase I footprinting experiment with reactions that contained 8.8 nM TBPC and/or 9 nM mot1-24 as indicated. Arrow indicates upstream protection induced by mot1-24. mot1-24 alone had no effect on the DNase I digestion pattern (not shown). (D) The rate of dissociation of the TBPC-mot1-24-DNA complex in the presence of 25 μ M ATP. The progress curve is biphasic and characterized by half-times of 0.23 (-0.17 , $+0.42$) and 36.2 (-2.5 , $+3.1$) min and amplitudes of 0.09 (-0.02 , $+0.03$) and 0.91 (-0.03 , $+0.01$), respectively. (E) Relative TBP-stimulated ATPase activity of wild-type Mot1, mot1-505, and mot1-24. 'None', no Mot1 added. Average activity \pm standard deviation derived from two or more assays is shown. TBP (100 nM) was present in all reactions.

on either strand, within or upstream of the TATA Box (Figure 8A). Irradiation of ternary complexes formed with native unsubstituted DNA at the 5-IdU-selective wavelength of 312 nm did not yield crosslinked Mot1, but crosslinking to Mot1 was detected with irradiation with 254 nm UV light, presumably via thymine residues in the probe (Figure 8B, lanes 1-3). Since 5-IdU crosslinks across the major groove, TBPC crosslinking was not detected, as expected (Figure 8B-D, and data not shown).

As shown in Figure 8B-D, photocrosslinking between Mot1 and 5-IdU-substituted probes #1 and #3 was detected, whereas no crosslinking was detected to probes #2 and #5 (not shown). Among these probes, photocrosslinking of Mot1 to probes #1 and #3 was most robust, #4 and #7 less substantial, and #6 only slightly above background. The crosslinking of Mot1 to probes with 5-IdU substituted in only certain positions suggests that Mot1 occupies a specific position(s) in the ternary complex. Crosslinking to probes #1 and #3 was specific for 312 nm light, and was dependent on the presence of TBPC. While crosslinking was not significantly affected by the presence of ATP (Figure 8C), the

absence of an ATP-dependent effect was expected because of the long irradiation time required for detection of crosslinked products and the fact that TBPC can rebind DNA following its displacement by Mot1 in the absence of a competitor (Auble and Hahn, 1993; Darst *et al*, 2003; Gumbs *et al*, 2003). Importantly, crosslinking of Mot1 to probes #1 and #4 shows that the protein contacts the major groove of the upstream DNA, consistent with the results shown in Figures 2 and 7. Crosslinking between Mot1 and probes #3 and #7 shows that Mot1 contacts the major groove of the TATA Box, suggesting that the TATA Box is sandwiched between the two proteins.

Inhibition of DNA binding by preincubation of Mot1 and TBPC

The unexpected interaction between Mot1 and TATA Box DNA is consistent with results suggesting an interaction between Mot1 and the TBP DNA-binding surface (Darst *et al*, 2001; Pereira *et al*, 2001; Klejman *et al*, 2005). Under conditions where TBP and TBPC bind rapidly and tightly to TATA Box bearing DNA (Figure 9, reaction scheme 1),

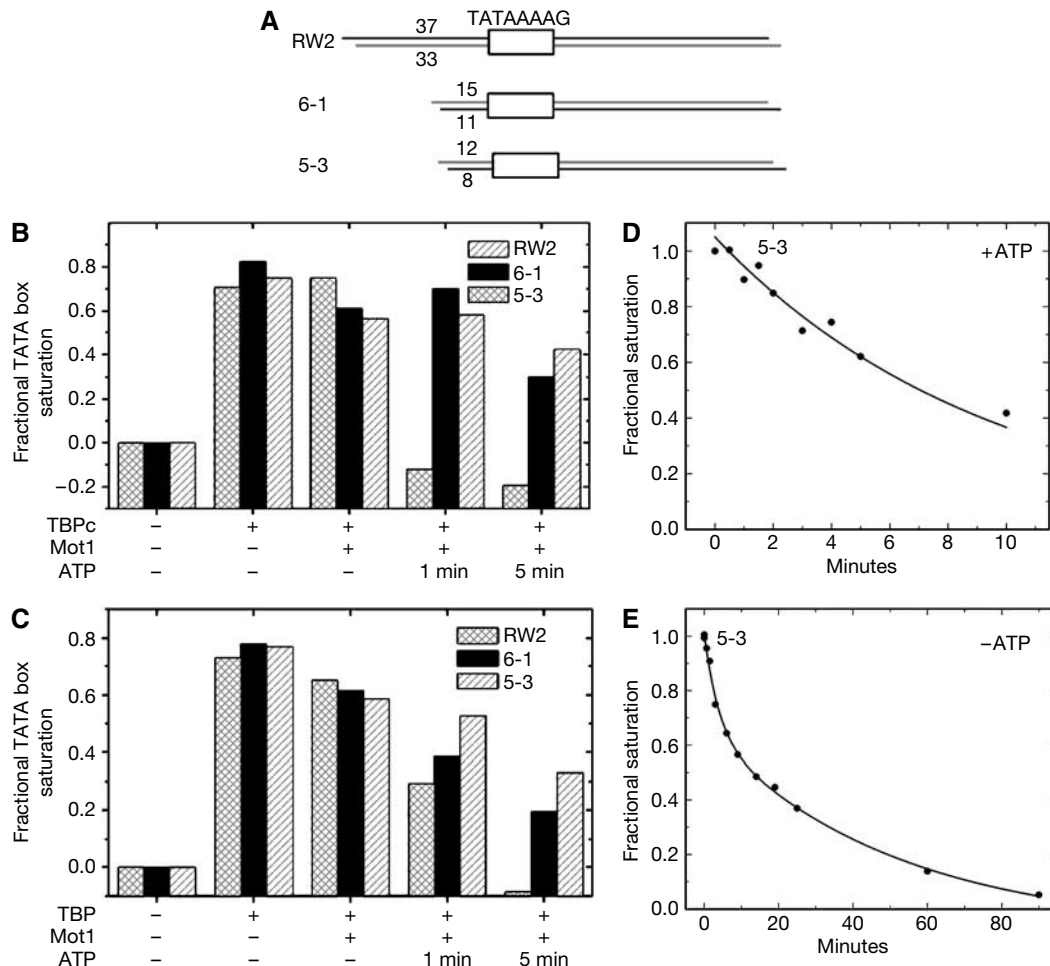


Figure 7 Upstream DNA is critical for Mot1's ATP-dependent dissociation activity. (A) A schematic representation of three DNA molecules possessing identical TATA and downstream DNA sequences, but different lengths of DNA upstream of the TATA Box. The length of each upstream strand is indicated in nucleotides. (B, C) The TATA Box occupancy as assayed by DNase I footprinting of TBPC and TBP, respectively, on the three DNA templates. The concentrations of TBPC, TBP, Mot1, and ATP used were 8 nM, 12 nM, 8 nM, and 25 μ M, respectively. ATP was added for 1 or 5 min as indicated. Representative autoradiograms for this experiment are shown in Supplementary Figure S3. (D) Rate of dissociation of the Mot1-TBPC-DNA ternary complex in the presence of ATP. The dissociation reaction is well described by a single exponential with a half-time of 9.5 (–1.1, +1.3) min. (E) Rate of dissociation of the Mot1-TBPC-DNA ternary complex in the absence of ATP. The curve is biphasic and is characterized by half-times of 3.8 (–1.5, +2.4) and 50.7 (–10.1, +12.4) min.

detectable TATA Box occupancy was not observed when Mot1 was preincubated with these proteins (Figure 9, reaction scheme 2). Thus, Mot1 appears to wrap around the TBPC DNA-binding surface whether it is occupied or unoccupied by TATA DNA.

Conserved DNA-binding residues in Mot1 are important for catalysis of TBPC-DNA dissociation

Conserved SsoRad54 residue R547 appears critical for interaction with DNA (Durr *et al*, 2005; see Figure 6A). To determine if Mot1 interacts with DNA similarly to SsoRad54 and to determine whether this residue is important for Mot1's activity, the homologous residue, R1391, was converted to alanine and the resulting mutant, mot1-508, was assayed for function. As shown in Figure 10A and B, mot1-508 was defective for ternary complex formation as judged by both DNase I footprinting and gel shift assays. While mot1-508 had some ability to catalyze displacement of TBP from DNA in the presence of ATP (Figure 10B, lanes 14 and 15), its displacement of TBPC from DNA was 6.8-fold

slower than the reaction catalyzed by wild-type Mot1 (compare Figure 10C with Figure 4B). Interestingly, mot1-508 supports viability (Figure 10D), suggesting that a high catalytic efficiency is not essential *in vivo*. mot1-508 also possessed TBP-stimulated ATPase activity that was equivalent to wild-type Mot1 (Figure 10E), indicating that this mutant is defective in coupling ATP hydrolysis to displacement.

SsoRad54 N569 (Figure 6A) is a conserved residue implicated in DNA-binding and DNA-stimulated ATPase activity (Durr *et al*, 2005). To further test the SsoRad54-DNA complex as a model for Mot1 function, the homologous residue in Mot1, N1415, was converted to isoleucine and the resulting mutant, mot1-509, assayed for function. Consistent with a predicted defect in DNA binding, mot1-509 was impaired in ternary complex formation as well as ATP-dependent TBPC-DNA dissociation (Figures 11A and B). Like mot1-508, mot1-509 supported yeast cell viability, but overexpression of mot1-509 exerted an approximate 10-fold dominant-negative inhibitory effect on cell growth, consistent with the

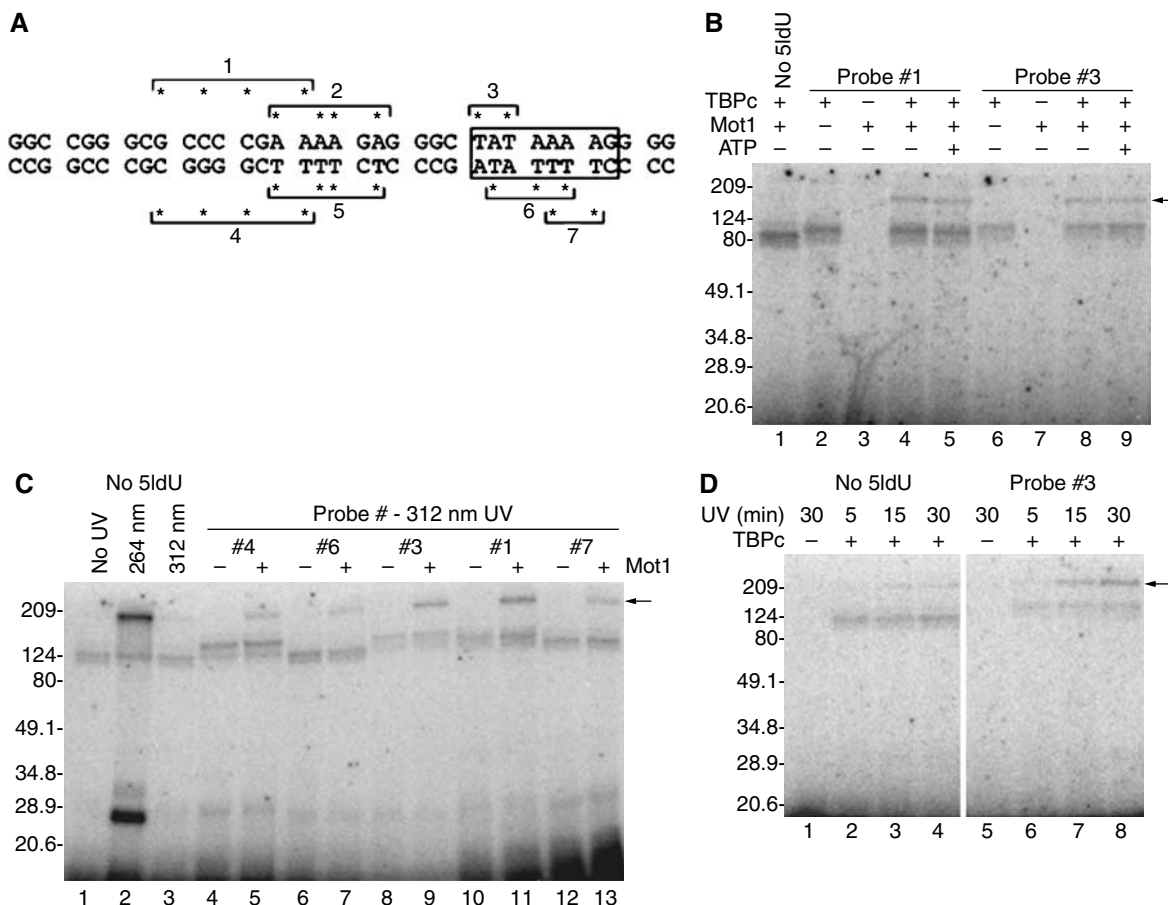


Figure 8 Site-specific photocrosslinking of Mot1 to DNA. (A) DNA probes used. The native, unsubstituted sequence is shown. Asterisks indicate positions of 5-IdU substitution. (B) Radiolabeled DNAs (~0.5 nM) were incubated with 8 nM TBPc ± 8 nM Mot1 and irradiated as indicated. Mot1 was added to all three reactions with unsubstituted DNA ('no 5-IdU') and as indicated for the others. Reactions were irradiated and products resolved on a 10% protein gel; crosslinked products were visualized by PhosphorImager analysis. There was no detectable crosslinking of Mot1 to probe nos. 2 and 5 (not shown). (C) Reactions were performed as in (B) with 312 nm UV light and the components as indicated. (D) Reactions were performed as in (B) with 312 nm UV irradiation for the times indicated. All reactions contained Mot1. In panels (B–D), arrow indicates the Mot1–DNA crosslinked product.

impaired catalytic activity observed *in vitro* (Figure 11C; Auble *et al*, 1997). In addition, mot1-509 displayed reduced TBP-stimulated ATPase activity as predicted from its location within the 'Switch' region adjacent to the ATP binding site in SsoRad54 (Durr *et al*, 2005). Taken together, these results support a model in which Mot1 engages DNA similarly to SsoRad54 and functions as an ATP-driven DNA-translocating enzyme.

Discussion

Gene transcription is regulated by complex cooperative and bidentate protein–protein and protein–DNA interactions that are critical to biological activity. A consequence of cooperative protein–DNA complexes is that their lifetimes are dramatically increased relative to the individual binding interactions (Matthews, 1992; Ptashne, 2004). An unusual aspect of the biochemistry of TBP is its slow association and dissociation kinetics (Hoopes *et al*, 1992), characteristics that may either enhance or impede its function and regulation. Since time as well as space is important in controlling gene expression, it is clear why Mot1-catalyzed TBP recycling is essential for transcriptional control *in vivo*.

Mot1–TBPc–DNA complex heterogeneity: implications for Snf2/Swi2 ATPase conformation and function

The complexity present in the dissociation of Mot1–TBPc–DNA ternary complexes was surprising (Figure 4B). The biphasic dissociation curve reflects one population of molecules whose lifetime is reduced compared to the TBPc–DNA complex alone and a second population whose lifetime is extended by Mot1. The extended lifetime of the ternary complex is consistent with cooperative interaction between Mot1 and TBPc upon their simultaneous binding to DNA; the slowed dissociation results from the energy contributed by binding cooperativity (Matthews, 1992).

The intriguing observation from this analysis is that Mot1 can displace a proportion of DNA-bound TBPc by a novel ATP-independent mechanism. In principle, many aspects of ternary complex conformational change could explain the heterogeneity in dissociation behavior, but the behavior of the mot1-24 linker mutant supports the hypothesis that ternary complex heterogeneity is due (at least in part) to differences in the conformation that Mot1 adopts in solution. The mutation in mot1-24 is a conservative amino-acid change in the linker connecting the ATPase subdomains that have been proposed to rotate with respect to one another

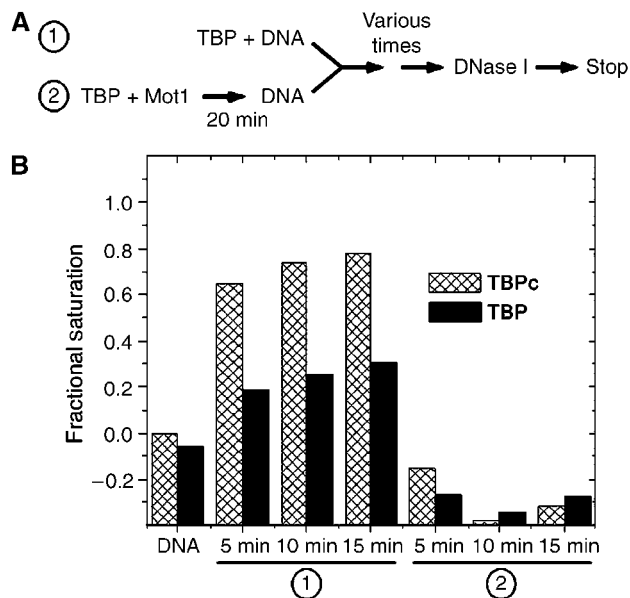


Figure 9 The effect on DNA binding of the order of Mot1 addition to TBP or TBPC was assayed by DNase I footprinting by combining the components in the order indicated in (A). The differences in the apparent saturation of TBP and TBPC seen in (B) reflect their different binding affinities (data not shown). The reactions contained 8.8 nM TBPC without (1) or with (2) 19.2 nM Mot1 or 15 nM TBP without (1) or with (2) 23 nM Mot1. Radiolabeled TATA-containing DNA was then added to each reaction for 5, 10, or 15 min prior to sampling the occupancy of the TATA Box with DNase I. Quantitation of TATA binding is shown in the bar graph. See Supplementary Figure S4 for a representative autoradiogram.

to allow ATP binding. We suggest that the linker defect impairs the interconversion of these conformational states; a bias favoring the ATPase-inactive 'open' conformation (Durr *et al*, 2005; Figure 6B) explains why *mot1-24* has no detectable ATPase activity. ATPase domain swiveling has been observed in other ATPases (e.g., actin) and as these enzymes are built upon a similar scaffold, domain rotation may well be a general property of ATPase function (Egelman, 2003).

Most of the ternary complexes formed with *mot1-24* are more stable than the TBPC-DNA complex, even though ATP is present in the reaction along with *mot1-24*. On the other hand, in the presence of ATP, *mot1-24*-TBPC-DNA complexes are substantially less stable than ternary complexes formed with wild-type Mot1 in the absence of ATP. These results suggest that the *mot1-24* mutant is locked in a conformation of intermediate activity. This conclusion is consistent with the observation that *mot1-24* cells are viable (although growth impaired), likely due to the ability of the mutant to destabilize a small proportion of DNA-bound TBP. In addition, *mot1-24* may have residual ATPase activity below our detection limits. Given the ability of the linker mutant to dramatically affect the proportion and stability of ternary complexes, we propose that the ATP-independent mechanism is a property of a particular conformational state of the Mot1 ATPase, and that this behavior is a general feature of Snf2/Swi2 ATPases. It is not clear whether the ATP-dependent and fast phase of the ATP-independent dissociation reactions represent the same or distinct mechanisms. Since ATP-dependent displacement requires upstream

DNA but the fast phase of the ATP-independent reaction does not, there must be mechanistic differences between these two reactions. Future work will be directed at understanding if the ATP-independent reaction efficiently displaces certain types of TBPC-DNA complexes, and whether the ATP-dependent and -independent reactions of Mot1 have different functions dependent upon the DNA sequence, orientation, or affinity of TBP, or perhaps the association of TBP with other regulatory factors.

This study was facilitated by the use of TBPC, which has greater stability *in vitro* than full-length TBP. Could the nonessential N-terminus of TBP affect recognition or catalysis by Mot1? As judged by gel mobility shift analysis, Mot1 appears to function equivalently using either TBPC or TBP (Auble *et al*, 1997; Darst *et al*, 2003). Additionally, the requirement for upstream DNA, and biochemical defects of *mot1-508* and *mot1-509* were observed for both TBPC and TBP. Interestingly, the Mot1-specific DNase I protection clearly evident in the Mot1-TBPC-DNA ternary complex is not as robust in the complex with TBP (data not shown). This observation is consistent with the lesser stability of the ternary complex with TBP compared with TBPC (Auble and Steggerda, 1999) and the lesser affinity of TBP-TATA versus TBPC-TATA complexes (M Brenowitz and co-workers, unpublished data). In the absence of ATP, the Mot1 complexes with TBPC-DNA are likely more stable than Mot1-TBP-DNA complexes due to the higher intrinsic affinity of TBPC for DNA. Despite these differences, the underlying mechanisms used by Mot1 to remove TBPC and TBP from DNA are indistinguishable in every respect so far examined.

Mot1's catalytic mechanism falls within the paradigm for ATP-driven DNA translocation

The Snf2/Swi2-related ATPases utilize the energy derived from ATP hydrolysis to disrupt protein-DNA complexes and thereby facilitate the establishment of new complexes (reviewed in Vignali *et al*, 2000). In contrast to the DNA topology-based mechanisms employed by many chromatin-remodeling enzymes, Mot1 does not apparently use ATP hydrolysis to induce DNA bending, twisting, or strand separation through the TATA Box (Adamkewicz *et al*, 2000; Darst *et al*, 2001). Rather than exploiting interactions with both TBP and DNA, it has been suggested that Mot1 might utilize ATP hydrolysis to distort TBP structure and thereby reduce the affinity and specificity of TBP for DNA (Adamkewicz *et al*, 2000; Gumbs *et al*, 2003). Despite the lack of direct experimental support for the 'TBP distortion' model of Mot1 action, it is possible that the Mot1 'power stroke' induces a transient change in TBP structure that facilitates its dissociation from DNA. While the intrinsic fluorescence of the C-terminal core of TBP shows that its structure is rigid (Khrapunov *et al*, 2002; Rashidzadeh *et al*, 2003; Khrapunov and Brenowitz, 2004), it is conceivable that Mot1 perturbs either the TBP stirrups or the beta strands connecting the subdomains.

Mot1 and TBP were shown to bind to short duplex DNAs with nonconsensus TATA sequences (Gumbs *et al*, 2003). Similar results were obtained using BTAF1 and a different nonconsensus TATA probe (Klejman *et al*, 2005). These results were interpreted to reflect a relaxation of TBP binding 'specificity' by Mot1 or BTAF1, perhaps by an alteration of either TBP or DNA structure (Gumbs *et al*, 2003;

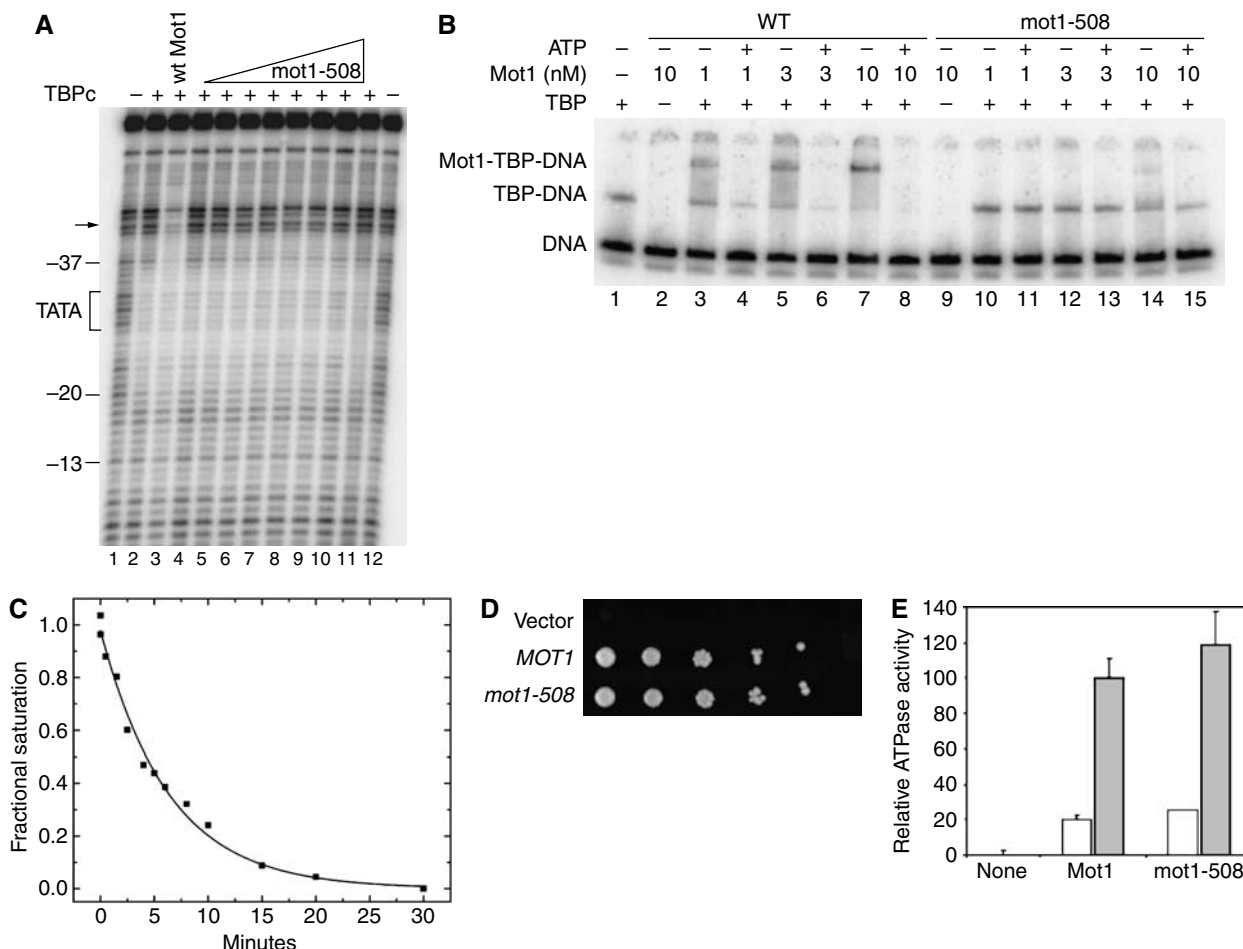


Figure 10 *mot1-508* forms unstable ternary complexes and is defective for TBPC-DNA dissociation. (A) DNase I footprinting experiment in which wild-type Mot1 (9 nM, lane 3) or varying concentrations of *mot1-508* were added to preformed TBPC-DNA complexes for 2 min prior to DNase I digestion. Reactions in lanes 4–10 contained 0.5, 1.2, 2, 4, 8, 12, or 16 nM *mot1-508*, respectively. TBPC concentration was 8.8 nM. Arrow indicates upstream protection due to wild-type Mot1 but not *mot1-508*. (B) Electrophoretic mobility shift assay using radiolabeled DNA, 10 nM TBP, 25 μ M ATP, and wild-type Mot1 or *mot1-508* at the indicated concentrations. TBP and DNA were incubated together for 20 min followed by the addition of Mot1 \pm ATP as indicated for 25 min prior to loading on the gel. Positions of the free DNA, TBP-DNA, and Mot1-TBP-DNA complexes are indicated. (C) The rate of dissociation of TBPC-*mot1-508*-DNA complexes in the presence of 25 μ M ATP. The progress curve is adequately fit by a single exponential decay with a half-time of 6.17 min ($-0.5, +0.55$). (D) *mot1-508* supports yeast cell viability. A *MOT1* shuffling strain (Darst *et al*, 2003) was transformed with plasmid-borne wild-type *MOT1*, *mot1-508*, or vector alone. Strains were spotted onto synthetic media containing 5-fluoroorotic acid in 10-fold serial dilutions. (E) *mot1-508* possesses wild-type ATPase activity. The bar graph shows relative ATPase activity in the absence (white bars) or presence (gray bars) of 100 nM TBP. ‘None’ refers to the ATPase activity of TBP without added Mot1.

Klejman *et al*, 2005). This change in specificity is most likely a manifestation of the cooperativity inherent in the assembly of Mot1-TBP-DNA complexes in the absence of ATP (Figure 2D). Greater stabilization of TBP binding to DNA by Mot1 is expected to compensate for TBP’s weaker intrinsic affinity for the TATA Box (Ackers *et al*, 1983). As shown by photocrosslinking (Figure 8), the weak binding of TBP to certain short DNAs is likely stabilized by Mot1 because Mot1 interacts with both TBP and duplex DNA opposite the TBP binding site.

Footprinting assays reflect the competition between the protein(s) being studied and an exogenous probe, in this case the enzyme DNase I. While the reactivity of DNase I is sensitive to the local structure of the DNA, it predominantly reports the solvent accessibility of the DNA backbone. Since DNase I is an enzyme, its footprints are typically larger than the protein-DNA interface being probed; contacts between the DNA and bound protein(s) reside within the nuclease

protection. Three lines of evidence support the inference that the Mot1-specific upstream extension of the TBP-DNA DNase I protection reflects direct contact of the protein with the DNA and that Mot1 utilizes this ‘grip’ on DNA to induce TBPC-DNA dissociation. First, upstream DNA is required for ternary complex formation and Mot1-mediated dissociation of TBPC-DNA (Figure 7). This observation is consistent with results showing that DNA upstream of the TATA Box stabilizes Mot1-TBP-DNA complex formation (Gumbs *et al*, 2003). Second, Mot1 specifically crosslinks to a DNA probe with 5-IdU substitutions in the upstream DNA (Figure 8). Third, mutation of conserved residues in Mot1 that contact DNA in the SsoRad54-DNA crystal structure decrease the affinity of Mot1 for TBPC-DNA and reduce the ATP-dependent displacement activity of Mot1 compared to wild-type Mot1 (Figures 10 and 11). Taken together, these results argue strongly against the previous conclusion that Mot1’s catalytic mechanism derives from the apparently reduced DNA-binding

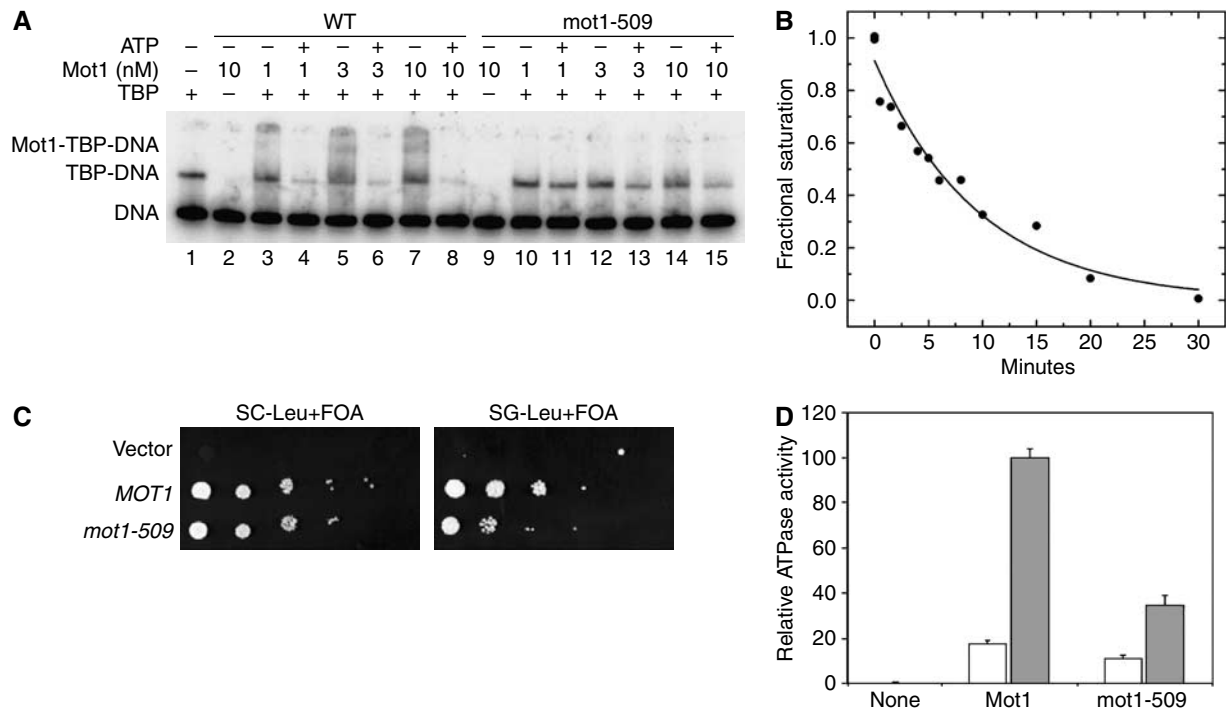


Figure 11 mot1-509 forms unstable ternary complexes and exhibits reduced TBP-stimulated ATPase activity. (A) Electrophoretic mobility shift assay performed as in Figure 10B, but with mot1-509 at the indicated concentrations. (B) The rate of dissociation of TBPC-mot1-509-DNA complexes in the presence of 25 μ M ATP. The curve is a single exponential characterized by a half-time of 9.6 ± 0.95 min. (C) A MOT1 shuffling strain was transformed with plasmid-borne copies of wild-type MOT1, mot1-509, or vector alone. In the left panel, the indicated MOT1 alleles were under the control of the MOT1 promoter. In the right-hand panel, the indicated alleles were under the control of the GAL1 promoter. Strains were spotted in 10-fold serial dilutions on synthetic media containing either glucose (SC; left panel) or galactose (SG; right panel). (D) The bar graph shows relative ATPase activity of mot1-509 compared to wild-type Mot1 in the absence (white bars) or presence (gray bars) of 100 nM TBP.

selectivity of the Mot1-TBP complex and that upstream DNA is unimportant for Mot1's ATP-driven displacement reaction (Gumbs *et al*, 2003).

SsoRad54 possesses an ATP-hydrolyzing core that is structurally similar to the catalytic core of DExx box helicases (Durr *et al*, 2005), with Snf2/Swi2-specific subdomains fused to the conserved catalytic core (Figure 6B). Comparison of the SsoRad54-DNA structure to that of the PcrA helicase-DNA-AMPNP complex (Velankar *et al*, 1999) led to the proposal that ATP hydrolysis by Snf2/Swi2 ATPases drives translocation along DNA (Durr *et al*, 2005). Our study provides biochemical support for this model and suggests that Mot1 uses this mechanism to displace TBPC from DNA. Since Mot1 does not detectably bind to DNA on its own (Adamkewicz *et al*, 2001; Darst *et al*, 2001; Gumbs *et al*, 2003) and the cooperative interaction between Mot1 and TBP stabilizes the ternary complex, step-wise translocation of Mot1 into the TBP-DNA interface would dissociate the components from DNA without requiring the processivity of chromatin remodeling (Fan *et al*, 2003; Kassabov *et al*, 2003; Schwanbeck *et al*, 2004). This model fits well with previous work demonstrating that Mot1 does not detectably translocate along duplex DNA over distances of ~ 50 -100 bp, whereas its ability to displace two adjacent TBPs is consistent with short-range, nonprocessive tracking (Auble and Steggerda, 1999).

mot1-508 and mot1-509 have quantitative defects in ternary complex formation that are predicted by the homology with SsoRad54; nonetheless, these proteins retain some catalytic activity, explaining why these alleles support yeast

cell viability. Formation of the SsoRad54-DNA complex is mediated by a network of interactions (Durr *et al*, 2005), no single one of which may be absolutely essential. Moreover, the proposed catalytic mechanism of Durr *et al* invokes an ATP-driven 'push' along the DNA minor groove, suggesting that the actual catalytic step does not involve formation of new, specific interactions between the ATPase and DNA. Thus, we suggest that there are no residues in Mot1 that only make specific contacts with DNA during the ATP-driven power stroke.

The mechanism of Mot1 action proposed here fits well with the general mechanism of DNA-translocating ATPases, of which Mot1 is a member. Interestingly, the size of the upstream Mot1 footprint is roughly the size expected if ATPase domain 1A engages upstream DNA in a manner similar to that used by SsoRad54 to contact DNA (Durr *et al*, 2005). We therefore favor the idea that the Mot1 ATPase engages upstream DNA, whereas the TATA Box interaction is mediated by another surface of the protein. Interaction of Mot1 with the TATA Box may help orient and stabilize its binding to TBPC-DNA. Alternatively, contact between Mot1 and the TATA Box may serve a novel function in propelling TBPC-DNA displacement.

Materials and methods

Recombinant proteins

Full-length yeast TBP (TBP) and TBPC were obtained from *Escherichia coli* overexpression strains using previously described

procedures (Darst *et al*, 2001; Darst *et al*, 2003). Protein concentrations were determined by Bradford protein assays using BSA standards. Plasmid-borne alleles of *mot1-24*, *mot1-505*, *mot1-508*, and *mot1-509* for phenotypic analysis and protein overexpression were constructed by standard PCR and subcloning procedures and verified by DNA sequencing. Mot1 was obtained by affinity chromatography using extracts obtained from a yeast overexpression strain (Auble *et al*, 1997; Darst *et al*, 2001). Mot1 concentrations were determined by quantitating the amount of the Mot1 polypeptide by densitometry of Coomassie blue-stained protein gels using BSA to calibrate the signal. The proteins used were >90% pure as judged by Coomassie staining of protein gels.

DNase I footprinting

Unless otherwise noted, reactions were performed using a singly end-labeled 110 bp DNA restriction fragment containing the AdMLP TATA Box sequence TATAAAAG. The fragment was obtained by excision from plasmid pRW2 using *EcoRI* and *XbaI*, followed by fill-in of the *EcoRI* end using the Klenow fragment of DNA polymerase I and [α - 32 P]dATP and purification on a 6% nondenaturing polyacrylamide gel. Each 20 μ l reaction contained ~0.5 nM radiolabeled DNA in 4 mM Tris-HCl (pH 8.0), 60 mM KCl, 5 mM MgCl₂, 4% glycerol, 0.1% Brij 58, 1 mM DTT, 0.005 mg/ml poly[dG-dC], 100 μ g/ml BSA, and 0.005% bromophenol blue. TBPC, TBP, and/or Mot1 were added as indicated in the legends to the figures. ATP was added where indicated to a final concentration of 25 μ M. Reactions were incubated at 21.5 \pm 1.0°C. The electrophoretic mobility shift assays were performed as described previously (Darst *et al*, 2001).

DNase I digestion was performed for 10 s by adding 1 μ l of an appropriate dilution of DNase I (Worthington) in 10 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 10 mM CaCl₂, and 20% glycerol. Kinetic analysis required the rapid addition of several reagents in prescribed orders and times. Multiple solutions were added reproducibly to the same tube with time intervals of 10 s using preloaded pipettes. Reactions were terminated by the addition of 20 μ l 5 M ammonium acetate and 120 μ l 100% ethanol. Following ethanol precipitation, DNA pellets were washed with 80% ethanol, dried, and resuspended in formamide sample buffer, heated at 100°C for 5 min, and resolved on 8% denaturing polyacrylamide gels. The radiolabeled bands were visualized by autoradiography and quantitated using a Molecular Dynamics PhosphorImager. The sequence corresponding to the DNase I bands was determined from parallel chemical sequencing reactions.

Quantitation of the band densities was accomplished using either block analysis (Brenowitz *et al*, 1986) or single band peak fitting (Takamoto *et al*, 2004; Das *et al*, 2005; Figure 2). Isotherms were derived from equilibrium titrations and analyzed as described (Brenowitz *et al*, 1986; Dhavan *et al*, 2002). Kinetic progress curves were derived from time-dependent experiments and analyzed as described (Mollah and Brenowitz, 2000; Dhavan *et al*, 2002). The block of bands at the center of the TATA Box were quantitated for the kinetics experiments (e.g., Figure 2A, bands marked 'TATA').

The fractional saturation ranges between the free DNA (zero) and the ternary complex (1.0). Nonlinear least-squares analysis of the data was conducted using OriginPro v7.5 SR4 (OriginLab Corporation). The best-fit values and 65% confidence intervals are reported.

ATPase assays

ATP hydrolysis was measured under the same conditions as were used for footprinting, except the reactions contained radiolabeled ATP rather than radiolabeled DNA. Each 20 μ l reaction contained 0.2 mCi [γ - 32 P]ATP (3000 Ci/mmol); total ATP concentration was 25 μ M. Following incubation at 21.5 \pm 1.0°C for various times, reactions were terminated by addition of EDTA to 0.5 mM and ATP to 0.6 mM. Reactions were spotted onto Bakerflex PEI plates and developed in 0.6 M KH₂PO₄ (pH 3.4). The extent of ATP hydrolysis was quantitated by imaging with a PhosphorImager. Rates of hydrolysis were calculated under conditions in which the extents of ATP hydrolysis were linearly related to reaction time and proportional to the concentration of Mot1. Data analysis was performed using Kaleidagraph software.

Photocrosslinking

DNA probes were assembled by annealing synthetic deoxyoligonucleotides containing 5-IdU substitutions as indicated in Figure 7A. The duplexes were end-labeled on the 5-IdU-substituted strand by fill-in using [α - 32 P]dCTP and the Klenow fragment of DNA polymerase I, then purified by preparative electrophoresis on 8% nondenaturing polyacrylamide gels. Reactions were assembled in the same buffer as for footprinting but without bromophenol blue, and contained ~30 000 c.p.m. radiolabeled DNA, 9 nM TBPC, 13 nM Mot1, and 25 μ M ATP as indicated. Reactions were irradiated with a 264 nm lamp (model UVGL58) or in a Stratalinker fitted with 312 nm UV bulbs. Irradiation was conducted at 21.5 \pm 1.0°C for 60 min, unless otherwise noted. Following irradiation, reactions were terminated by additions of SDS sample buffer and reaction products were resolved on 10% protein gels and visualized using a phosphorimager.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online.

Acknowledgements

This work was supported by NIH Grants GM55763 to DTA and GM39929 to MB. We are grateful to Arindam Dasgupta for purification of Mot1, to Fred Winston for the *mot1-24* plasmid, to Joel Hockensmith for photocrosslinking advice, to Karl-Peter Hopfner and Ed Egelman for discussions and to Josh Smith, Staton Wade, Pieter deHaseth, Russell Darst, Ian Willis, and Greg Prelich for comments on this manuscript.

References

- Ackers GK, Shea MA, Smith FR (1983) Free energy coupling within macromolecules. The chemical work of ligand binding at individual sites in co-operative systems. *J Mol Biol* **170**: 223–242
- Adamkewicz JI, Hansen KE, Prud'homme WA, Davis JL, Thorner J (2001) High-affinity interaction of yeast transcriptional regulator, Mot1, with TATA Box-binding protein (TBP). *J Biol Chem* **276**: 11883–11984
- Adamkewicz JI, Mueller CGF, Hansen KE, Prud'homme WA, Thorner J (2000) Purification and enzymic properties of Mot1 ATPase, a regulator of basal transcription in the yeast *Saccharomyces cerevisiae*. *J Biol Chem* **275**: 21158–21168
- Andrau J-C, Van Oevelen CJC, Van Teeffelen HAAM, Weil PA, Holstege FCP, Timmers HTM (2002) Mot1p is essential for TBP recruitment to selected promoters during *in vivo* gene activation. *EMBO J* **21**: 5173–5183
- Auble DT, Hahn S (1993) An ATP-dependent inhibitor of TBP binding to DNA. *Genes Dev* **7**: 844–856
- Auble DT, Steggerda SM (1999) Testing for DNA tracking by MOT1, a SNF2/SWI2 protein family member. *Mol Cell Biol* **19**: 412–423
- Auble DT, Hansen KE, Mueller CGF, Lane WS, Thorner J, Hahn S (1994) Mot1, a global repressor of rna polymerase II transcription, inhibits TBP binding to DNA by an ATP-dependent mechanism. *Genes Dev* **8**: 1920–1934
- Auble DT, Wang D, Post KW, Hahn S (1997) Molecular analysis of the SNF2/SWI2 protein family member MOT1, an ATP-driven enzyme that dissociates TATA-binding protein from DNA. *Mol Cell Biol* **17**: 4842–4851
- Becker PB, Horz W (2002) ATP-dependent nucleosome remodeling. *Ann Rev Biochem* **71**: 247–273
- Brenowitz M, Senear DF, Shea MA, Ackers GK (1986) Quantitative DNase footprint titration: a method for studying protein-DNA interactions. *Methods Enzymol* **130**: 132–181
- Collart MA (1996) The NOT, SPT3, and MOT1 genes functionally interact to regulate transcription at core promoters. *Mol Cell Biol* **16**: 6668–6676
- Darst RP, Dasgupta A, Zhu C, Hsu J-Y, Vroom A, Muldrow TA, Auble DT (2003) Mot1 regulates the DNA binding activity of free TATA-binding protein in an ATP-dependent manner. *J Biol Chem* **278**: 13216–13226

- Darst RP, Wang D, Auble DT (2001) Mot1-catalyzed TBP-DNA disruption: uncoupling DNA conformational change and role of upstream DNA. *EMBO J* **20**: 2028–2040
- Das R, Laederach A, Perlman SM, Herschlag D, Altman RB (2005) SAFA: semi-automated footprinting analysis software for high-throughput quantification of nucleic acid footprinting experiments. *RNA* **11**: 344–354
- Dasgupta A, Darst RP, Martin KJ, Afshari CA, Auble DT (2002) Mot1 activates and represses transcription by direct, ATPase-dependent mechanisms. *Proc Natl Acad Sci USA* **99**: 2666–2671
- Dasgupta A, Juedes SA, Sprouse RO, Auble DT (2005) Mot1-mediated control of transcription complex assembly and activity. *EMBO J* **24**: 1717–1729
- Davis JL, Kunisawa R, Thorner J (1992) A presumptive helicase (MOT1 gene product) affects gene expression and is required for viability in the yeast *Saccharomyces cerevisiae*. *Mol Cell Biol* **12**: 1879–1892
- Dhavan GM, Crothers DM, Chance MR, Brenowitz M (2002) Concerted binding and bending of DNA by *Escherichia coli* integration host factor. *J Mol Biol* **315**: 1027–1037
- Durr H, Korner C, Muller M, Hickmann V, Hopfner K-P (2005) X-ray structures of the *Sulfolobus solfataricus* SWI2/SNF2 ATPase core and its complex with DNA. *Cell* **121**: 363–373
- Egelman EH (2003) A tale of two polymers: new insights into helical filaments. *Nat Rev Mol Cell Biol* **4**: 621–630
- Eisen JA, Sweder KS, Hanawalt PC (1995) Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. *Nucleic Acids Res* **23**: 2715–2723
- Fan H-Y, He X, Kingston RE, Narlikar GJ (2003) Distinct strategies to make nucleosomal DNA accessible. *Mol Cell* **11**: 1311–1322
- Flaus A, Owen-Hughes T (2003) Mechanisms for nucleosome mobilization. *Biopolymers* **68**: 563–578
- Galas DJ, Schmitz A (1978) DNase footprinting: a simple method for the detection of protein-DNA binding specificity. *Nucleic Acid Res* **5**: 3157–3170
- Geisberg JV, Moqtaderi Z, Kuras L, Struhl K (2002) Mot1 associates with transcriptionally active promoters and inhibits association of NC2 in *Saccharomyces cerevisiae*. *Mol Cell Biol* **22**: 8122–8134
- Gumbs OH, Campbell AM, Weil PA (2003) High-affinity DNA binding by a Mot1p-TBP complex: implications for TAF-independent transcription. *EMBO J* **22**: 3131–3141
- Hoopes BC, LeBlanc JF, Hawley DK (1992) Kinetic analysis of yeast TFIID-TATA Box complex formation suggests a multi-step pathway. *J Biol Chem* **267**: 11539–11547
- Kassabov SR, Zhang B, Persinger J, Bartholomew B (2003) SWI/SNF unwraps, slides, and rewraps the nucleosome. *Mol Cell* **11**: 391–403
- Khrapunov S, Brenowitz M (2004) Comparison of the effect of water release on the interaction of the *Saccharomyces cerevisiae* TATA binding protein (TBP) with 'TATA Box' sequences composed of adenosine or inosine. *Biophys J* **86**: 371–383
- Khrapunov S, Pastor N, Brenowitz M (2002) Solution structural studies of the *Saccharomyces cerevisiae* TATA binding protein (TBP). *Biochemistry* **41**: 9559–9571
- Klejman MP, Zhao X, van Schaik FMA, Herr W, Timmers HTM (2005) Mutational analysis of BTAF1-TBP interaction: BTAF1 can rescue DNA-binding defective TBP mutants. *Nucleic Acid Res* **33**: 5426–5436
- Madison JM, Winston F (1996) Evidence that Spt3 functionally interacts with Mot1, TFIIA, and TBP to confer promoter-specific transcriptional control in *Saccharomyces cerevisiae*. *Mol Cell Biol* **17**: 287–295
- Malkov VA, Camerini-Otero RD (1995) Photocross-links between single-stranded DNA and *Escherichia coli* RecA protein map to loops L1 (amino acid residues 157–164) and L2 (amino acid residues 195–209). *J Biol Chem* **270**: 30230–30233
- Matthews KS (1992) DNA looping. *Microbiol Rev* **56**: 123–136
- Mollah AKMM, Brenowitz M (2000) Quantitative DNase I kinetics footprinting. In *Protein-DNA Interactions—A Practical Approach*, Travers A, Buckle M (eds), pp 281–290. Oxford: IRL Press at Oxford University Press
- Narlikar GJ, Fan H-Y, Kingston RE (2002) Cooperation between complexes that regulate chromatin structure and transcription. *Cell* **108**: 475–487
- Pereira LA, Klejman MP, Timmers HTM (2003) Roles for BTAF1 and Mot1p in dynamics of TATA-binding protein and regulation of RNA polymerase II transcription. *Gene* **315**: 1–13
- Pereira LA, van der Knaap JA, van den Boom V, van den Heuvel FA, Timmers HT (2001) TAF(II)170 interacts with the concave surface of TATA-binding protein to inhibit its DNA binding activity. *Mol Cell Biol* **21**: 7523–7534
- Poon D, Campbell AM, Bai Y, Weil PA (1994) Yeast Taf170 is encoded by MOT1 and exists in a TBP-TAF complex distinct from TFIID. *J Biol Chem* **269**: 23135–23140
- Prelich G (1997) *Saccharomyces cerevisiae* BUR6 encodes a DRAP1/NC2 α homolog that has both positive and negative roles in transcription *in vivo*. *Mol Cell Biol* **17**: 2057–2065
- Ptashne M (2004) *Genetic Switch: Phage Lambda Revisited*. New York: Cold Spring Harbor Laboratory Press
- Rashidzadeh H, Khrapunov S, Chance MR, Brenowitz M (2003) Solution structure and interdomain interactions of the *Saccharomyces cerevisiae* 'TATA binding protein' (TBP) probed by radiolytic protein footprinting. *Biochemistry* **42**: 3655–3665
- Reddy P, Hahn S (1991) Dominant negative mutations in yeast TFIID define a bipartite DNA-binding region. *Cell* **65**: 349–357
- Schwabeck R, Xiao H, Wu C (2004) Spatial contacts and nucleosome step movements induced by the NURF chromatin remodeling complex. *J Biol Chem* **279**: 3993–39941
- Smith CL, Peterson CL (2005) A conserved Swi2/Snf2 ATPase motif couples ATP hydrolysis to chromatin remodeling. *Mol Cell Biol* **25**: 5880–5892
- Takamoto K, Chance MR, Brenowitz M (2004) Semi-automated, single-band peak-fitting analysis of hydroxyl radical nucleic acid footprint autoradiograms for the quantitative analysis of transitions. *Nucleic Acid Res* **32**: E119
- Thoma NH, Czyzewski BK, Alexeev AA, Mazin AV, Kowalczykowski SC, Pavletich NP (2005) Structure of the SWI2/SNF2 chromatin-remodeling domain of Rad54. *Nat Struct Mol Biol* **12**: 350–356
- Velankar SS, Soultanas P, Dillingham MS, Subramanya HS, Wigley DB (1999) Crystal structures of complexes of PcrA DNA helicase with a DNA substrate indicate an inchworm mechanism. *Cell* **97**: 75–84
- Vignali M, Hassan AH, Neely KE, Workman JL (2000) ATP-dependent chromatin-remodeling complexes. *Mol Cell Biol* **20**: 1899–1910