Saccharomyces cerevisiae HMO1 interacts with TFIID and participates in start site selection by RNA polymerase II

Koji Kasahara, Sewon Ki, Kayo Aoyama, Hiroyuki Takahashi and Tetsuro Kokubo*

Division of Molecular and Cellular Biology, International Graduate School of Arts and Sciences, Yokohama City University, Yokohama, 230-0045, Japan

Received October 11, 2007; Revised November 11, 2007; Accepted November 12, 2007

ABSTRACT

Saccharomyces cerevisiae HMO1, a high mobility group B (HMGB) protein, associates with the rRNA locus and with the promoters of many ribosomal protein genes (RPGs). Here, the Sos recruitment system was used to show that HMO1 interacts with TBP and the N-terminal domain (TAND) of TAF1, which are integral components of TFIID. Biochemical studies revealed that HMO1 copurifies with TFIID and directly interacts with TBP but not with TAND. Deletion of HMO1 (Δhmo1) causes a severe cold-sensitive growth defect and decreases transcription of some TAND-dependent genes. Δhmo1 also affects TFIID occupancy at some RPG promoters in a promoter-specific manner. Interestingly, over-expression of HMO1 delays colony formation of taf1 mutants lacking TAND (taf1ΔTAND), but not of the wild-type strain, indicating a functional link between HMO1 and TAND. Furthermore, Δhmo1 exhibits synthetic growth defects in some spt15 (TBP) and toa1 (TFIIB) mutants while it rescues growth defects of some sua7 (TFIIB) mutants. Importantly, Δhmo1 causes an upstream shift in transcriptional start sites of RPS5, RPS16A, RPL23B, RPL27B and RPL32, but not of RPS31, RPL10, TEF2 and ADH1, indicating that HMO1 may participate in start site selection of a subset of class II genes presumably via its interaction with TFIID.

INTRODUCTION

Transcriptional initiation of protein-coding (class II) genes by RNA polymerase II (Pol II) involves a set of general transcription factors (GTFs: TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH), as well as other cofactors including mediator, histone-modifying enzymes and ATP-dependent chromatin remodeling complexes (1–3). Upon transcriptional activation, gene-specific activators recruit the active forms of these components to the promoter region surrounding the transcriptional initiation site of target genes (4,5). Despite considerable investigation, details of the molecular mechanisms underlying transcriptional activation remain unclear. TFIID, which is composed of the TATA box-binding protein (TBP) and 14 TBP-associated factors (TAFs), not only recognizes a set of core promoter elements, but also serves as one of the most important targets for activators (6). The TAF1 N-terminal domain, TAND, binds to the concave and convex surfaces of TBP, thereby inhibiting TBP binding to the TATA element (7). The loading of TBP onto the promoter is a key regulatory step for activation (8–10) and, thus, we proposed that activators reverse the TAND–TBP interaction, enabling formation of a productive TFIIA–TBP–TATA complex (11).

High mobility group B (HMGB) proteins constitute a subgroup of non-histone HMG proteins in eukaryotic chromatin. They contain one or more HMG box domains (12,13) that are distinctive DNA-binding motifs, in which the global fold is well conserved and comprises three helices arranged in an L-shape (14,15). HMGB proteins are involved in diverse biological processes, including transcription, recombination and DNA repair, by facilitating assembly of nucleoprotein complexes that are required for these processes (16–18). Notably, some yeast and human HMGB proteins are transcriptional coactivators that stabilize the TBP/TFIID–TFIIA–promoter complex (19,20).

Saccharomyces cerevisiae expresses seven HMGB proteins, HMO1, HMO2 (also called NHP10), NHP6A, NHP6B, ABF2, ROX1 and IXR1. Among these proteins, only HMO1 is involved in both RNA polymerase I (Pol I)- and Pol II-mediated transcription (21–23). HMO1 binds to promoters of most ribosomal protein genes (RPGs, 97 of 138) as well as promoters of 386 non-RP target loci over the entire genome (22,23). Our recent work also revealed that 138 RPGs can be classified into 13 distinct groups based on HMO1-abundance at the
promoter and the HMO1-dependence of FHL1 and/or RAP1 binding to the promoter (23). Both FHL1 and/or RAP1 are key regulators of RPG transcription (24–30). Interestingly, FHL1 binds to most of the HMO1-enriched and transcriptionally HMO1-dependent RPG promoters in an HMO1-dependent manner, whereas it binds to HMO1-limited RPG promoters in an HMO1-independent manner (23).

This study shows that HMO1 interacts physically and genetically with TAND and TBP/TFIID. Genetic analyses also indicate a close relationship between the functions of HMO1 and TAND, TBP, TFIIB and TFIIA. Surprisingly, Δhmo1 rescues growth defects of some sua7 (TFIIB) mutants and causes an upstream shift in transcriptional start sites of some genes, e.g. RPS5, RPS16A, RPL23B, RPL27B and RPL32, but not of others, e.g. RPS31, RPL10, TEF2 and ADHI. Similarly, tfg1/tfg2 (TFIIF) (31–33) and sua7 mutations rescue the growth defect of some tfg1/tfg2 (TFIIF) (31–33) and sua7 mutations rescue the growth defect of some tfg1/tfg2 (TFIIF) (31–33) mutants, and also shift the start site of ADHI upstream. Thus, it is likely that HMO1 participates in the start site selection of a subset of class II genes by a mechanism that differs from that of TFIIF and Pol II.

MATERIALS AND METHODS

Yeast strains

Standard techniques were used for growth and transformation of yeast (38). The yeast strains used in this study are listed in Supplementary Table 1. The Δspt15 strain (YTK271) has been described previously (39). YTK271, YTK8264, YTK8270, YTK8166, YTK8269 and YTK8273 were used as parental strains for exchange of plasmids encoding wild-type TBP, TFIIB or TFIIA with plasmids encoding mutant proteins. YKK74 was generated as described previously (23).

Targeted disruption of HMO1 was performed by PCR-based gene deletion (40) using the primers TK4022 and TK4023. The oligonucleotides used in this study are listed in Supplementary Table 2. HMO1 disrupted strains YTK8468, YTK8166, YTK8269 and YTK8273 were generated from Y13.2, YTK271, YTK8264 and YTK8270, respectively, using HIS3 as the selectable marker. Using kanMX as the selectable marker, targeted disruptions of SUA7 (primers TK7770-TK6225) and TOA1 (primers TK7768-TK5565) were performed in the H2451 strain (41) to generate the YTK8264 and YTK8270 strains, respectively.

A number of strains were generated using a plasmid shuffle technique, whereby, for example, pYN1/TAF1 (URA3 marker) was replaced with a TRP1-marked plasmid, by growing transformants on 5-fluoroorotic acid (5-FOA)-containing plates. Using the plasmids pM2715/TAF1, pM2722/taf1Δ2-86 or pM2727/taf1Δ1-86, the YTK8472, YTK8473 and YTK8474 strains, respectively, were generated from YTK8468 and the YTK8469, YTK8470 and YTK8471 strains, respectively, were generated from Y13.2, YKK237, YKK238, YKK241 and YKK247 were generated from Y22.1 (41) using the pM1169/TAF1, pM977/taf1Δ41-73, pM1002/taf1Δ8-42 or pM1001/taf1Δ10-73 plasmids, respectively.

Construction of plasmids for the Sos recruitment system

A 0.65 kb fragment containing TAND (1–208 aa) was amplified by PCR using the TK2697 and TK2698 primers, and then ligated into a blunt-ended BamHI site in pSos (Stratagene), generating the bait plasmid pM2718. A 0.89 kb fragment containing the GAL1 promoter, a myristylation signal and the CYC1 terminator of pMyr (Stratagene) was amplified by PCR using the TK3528 and TK3529 primers, and ligated into the pPV1U site of pRS426 (42), forming the pM2721 vector. As prey, yeast cDNA was prepared using a cDNA synthesis kit (Stratagene), digested with EcoRI-XhoI and then ligated into a similarly digested pM2721.

DNA fragments (0.73 kb) encoding TBP were amplified by PCR using the TK4348-TK936 primers, then digested and ligated into the BamHI-Sall sites of pSos, forming pM2875 or amplified using the TK21-TK936 primers, digested and ligated into the EcoRI-Sall sites of pMyr, forming pM2719. A 0.74 kb fragment encoding HMO1 was amplified by PCR using the TK3807 and TK3808 primers, digested, and ligated into the EcoRI-XhoI site of pM2721, forming pM2754.

Construction of plasmids for the GST pulldown assay

The plasmid encoding His-TBP (pM1578) has been described previously (39). A 0.73 kb fragment encoding TBP was amplified by PCR using the TK1 and TK2 primers, digested, and ligated into the BamHI-EcoRI sites of pGEX-2T (GE Healthcare Biosciences), generating pM4673 (encoding GST-TBP). Similarly, the expression plasmids pM2773 (GST-TAND; 6–208 aa) and pM2823 (GST-HMO1, 1–246 aa) were constructed by ligating a 0.6 kb BamHI (internal)-NotI fragment from pM2718 and a 0.74 kb EcoRI-XhoI fragment from pM2754, respectively, into pGEX-6P-1 (GE Healthcare Biosciences). The expression plasmid pM5214 (His-HMO1) was constructed by ligating a 0.74 kb EcoRI-XhoI fragment from pM2754 into pCold 1 (TaKaRa).

Construction of plasmids for genetic studies

TAF1 plasmids. pM1169 (TAF1/pRS314), pM1001 (taf1Δ10-73/pRS314), pM1002 (taf1Δ8-42/pRS314), pM977 (taf1Δ41-73/pRS314), pM1689 (taf1Δ2-86/pRS314) and pM1657(taf1Δ2-186/pRS314) have been described previously (43,44). The NeoI-NruI fragment of pM1169 was replaced with the NeoI-EcoRV fragment of pBS1479, generating pM2711. A 0.82 kb fragment was amplified by PCR from pM1169 using the TK120 and TK3516 primers, digested and ligated into the NeoI site of pM2711, generating pM2715 (TAF1-TAP/pRS314). The 1.6 kb BlnI-PstI fragments of pM1689 and pM1657 were replaced with the BlnI-PstI fragment of pM2715, generating pM2722 and pM2727, respectively.

HMO1 plasmids. pM2897 was constructed by ligating a 0.74 kb EcoRI-XhoI fragment from pM2754 into similarly digested p414-TEF (45). SacI-NaeI fragments
from pM2897 (1.8 kb), pM2899 (2.05 kb), pM2933 (1.5 kb) and p414-GPD (1.3 kb) were ligated into similarly digested pRS315 (46), generating pM2949 (TEF1 promoter-driven HMO1/pRS315), pM2950 (TDH3 promoter-driven HMO1/pRS315), pM2956 (TDH3 promoter-driven VTC1/pRS315) and pM2959 (TDH3 promoter only/pRS315), respectively. pM2899 was constructed by ligating a 0.74 kb EcoRI-XhoI fragment from pM2754 into similarly digested p414-GPD (45). A 0.45 kb fragment encoding VTC1 was amplified by PCR using the TK2340 and TK2341 primers, digested and ligated into the BamHI-PstI sites of p414-GPD, generating pM2933.

**TBP, TFIIB and TFIIA plasmids.** Plasmids expressing wild-type TBP or its mutants in yeast cells have been described previously (39). In this study, the following plasmids were generated to express TFIIB, TFIIA and their mutants. A 3.0 kb fragment containing SUA7 was amplified from genomic DNA by PCR using the TK5948 and TK5949 primers, digested and ligated into the XbaI-XhoI sites of pRS316 and pRS315, generating pM4326 and pM5283, respectively. pM5283 was subjected to site-specific mutagenesis to generate sua7 alleles. The TK7848, TK7849, TK7850, TK7851, TK7852 and TK7853 primers were used to generate the pM5287 (E62K), pM5288 (R78C), pM5289 (K190E), pM5290 (K201E), pM5291 (S53P) and pM5292 (G247V) plasmids, respectively.

A 1.9 kb fragment containing TOA1 was amplified from genomic DNA by PCR using the TK1518 and TK1519 primers, digested and ligated into the BamHI site of pRS316 and pRS315, generating pM5281 and pM5276, respectively. Subsequently, pM5276 was subjected to site-specific mutagenesis to generate toal alleles. The TK7845, TK7846, TK7847, TK7856 and TK7857 primers were used to generate the pM5293 (K255A, R257A, K259A), pM5294 (S220A, S225A, S232A), pM5295 (W285A), pM5296 (Δ217–227) and pM5297 (Δ55–215)/GGSGG linker) plasmids, respectively.

**A screen for TAND-interacting proteins using the Sos recruitment system.**

The Sos recruitment system (The CytoTrap™ Two-Hybrid System, Stratagene) (47) was used to identify factor(s) that could interact with TAND or TAF1. pM2718 (pSos-TAND) was used as bait to screen a yeast cDNA library constructed in pM2721. Saccharomyces cerevisiae cdc25H (aa 1–246) were raised in rabbits directed against HMO1 (aa 1–227) and pM2957 (S220A, S225A, S232A), pM2958 (W285A), pM2959 (Δ217–227) and pM2960 (Δ55–215)/GGSGG linker) plasmids, respectively.

**Protein expression and the GST pulldown assays.**

His-TBP was expressed in Escherichia coli BL21(DE3) (Novagen), as described previously (48). His-HMO1 was expressed in E. coli XL1-Blue (Stratagene). Cells were induced with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) and incubated for 24 h at 15°C in 50 ml of LB broth. GST, GST-tagged TAND (6–208 aa), GST-tagged HMO1 and GST-tagged TBP were expressed in E. coli XL1-Blue. Cells were induced with 0.4 mM IPTG and incubated for 3 h at 37°C (GST, GST-TAND, GST-HMO1) or at 30°C (GST-TBP) in 50 ml of LB broth. Cultures were harvested and pellets resuspended in 500 μl of 0.06 M KCl/Buffer PB [20 mM HEPES–KOH pH 7.9, 0.5 mM EDTA, 6 mM MgCl2, 0.1% Nonidet P-40, 20% (v/v) glycerol, 7 mM 2-mercaptoethanol and 1 mM phenylmethylsulfonyl fluoride (PMSF)]. Following sonication, lysates were clarified by centrifugation and the supernatants subjected to GST pulldown assays without further purification.

To study the interactions between TAND (6–208 aa), TBP and HMO1, a bacterial lysate containing His-HMO1 or His-TBP was mixed with one containing GST-TAND, GST-TBP, GST-HMO1 or GST, in 500 μl of 0.1 M KCl/Buffer PB, and then incubated at 4°C for 120 min. Following addition of 10 μl glutathione-Sepharose™ 4B (GE Healthcare Biosciences), the incubation was continued for a further 120 min, at which time the beads were washed three times with 1 ml 0.1 M KCl/Buffer PB. Beads were boiled in SDS sample buffer to elute bound protein, and the eluates were separated using 10% SDS-PAGE, followed by immunoblotting with polyclonal anti-HMO1 and anti-TBP antibodies.

**TAP purification.**

TAP-tagged HMO1 was purified as described previously (49) with minor modifications. A detailed protocol is available upon request.

**Immunoblot analyses.**

Immunoblotting and preparation of polyclonal antibodies directed against TAF1, TAF11 and TBP have been described previously (7,50,51). Polyclonal antibodies directed against HMO1 (aa 1–246) were raised in rabbits using gel-purified His-HMO1 expressed in E. coli.

**Chromatin immunoprecipitation (ChIP) analyses.**

ChIP analysis was conducted according to the Hahn laboratory protocol (http://www.fhrc.org/science/labs/hahn/methods/mol_bio_meth/hahnlab_ChIP_method.html), with minor modifications. A detailed protocol is available upon request.

PCR amplification conditions were: 94°C for 1.5 min; 27 cycles of 94°C for 15 s, 55°C for 30 s and 72°C for 30 s and a final extension at 72°C for 7 min. PCR products were separated using 5% non-denaturing PAGE and stained with SYBR Green I (Invitrogen). Each band was
quantified using an LAS-1000 Plus image analyzer (Fuji Film), and the ratio of IP/input was calculated. The PCR primer pairs used to amplify the following genes were: RPS5, TK8935/TK9382; RPS31, TK4692/TK4625; RPL10, TK5031/TK5032; RPL3, TK5035/TK5036; RPL23B, TK8993/TK8994; RPL27B, TK8997/TK8998; RPL32, TK8449/TK8450 and the transcribed region of POL1 (27) (asterisk), TK3506/TK3507.

Northern blot analyses

Northern blot analyses of several endogenous genes were performed as described previously (50). For detection of Northern blot analyses of several endogenous genes were performed as described previously (50). For detection of Northern blot analyses of several endogenous genes were performed as described previously (50).

Other primer pairs used were: RPL32, TK4446/TK4447; GAL1, TK253/TK254.

RPL23B, RPL27B and 25S rRNA were also detected by northern blot analysis. The probes were generated by 5'-end labeling gene-specific oligonucleotides with [32P]ATP using the T4 polynucleotide kinase as described previously (23).

 Primer extension analyses

Transcription start sites were mapped by primer extension analysis as described previously (52). Extension reactions were conducted using M-MLV reverse transcriptase (TaKaRa) and [γ-32P]ATP-labeled oligonucleotide primers. All primers were used both as a primer to generate the DNA sequencing ladder and for primer extension reactions. The primers used were: TK3212 (RPS5), TK3214 (ADH1), TK5414 (RPL10), TK7981 (TEF2), TK9588 (RPL32), TK9430 (RPS31), TK9589 (RPL27B), TK9590 (RPL23B) and TK9591 (RPS16A). The cDNA products were analyzed on a 6.5% polyacrylamide DNA sequencing gel. Gels were exposed to imaging plates (BAS2500, Fuji Film) for quantitation and scanning of electrophoretic images.

RESULTS

HMO1 interacts with TFIIID

In this study, the Sos recruitment system (SRS) (47) was used to screen for novel proteins that bind to TAND. The Sos system was chosen because it does not depend on transcription, whereas the standard yeast two-hybrid system does (11,53). Temperature-sensitive S. cerevisiae cdc25H carrying pSos-TAND (1–208 aa) was transformed with the modified pMyr library. After excluding revertants and transformants carrying CDC25 or cdc25 suppressor genes, 75 independent clones expressing Myr-tagged fusion proteins were identified for further analysis. These 75 clones included 15 clones carrying the entire HMO1 coding region and expressing the HMO1 protein. Because the cDNA library was generated using an oligo (dT) primer and all 15 clones were full length, the N-terminal segment of HMO1 may be critical for interactions with TAND.

The interaction between HMO1 and TAND appears to be specific, because co-transformation with pMyr-HMO1 and pSos-TAND rescues the growth defect of S. cerevisiae cdc25H at 37°C, while co-transformation with pMyr-HMO1 and pSos (Figure 1A) or pSos-TAF1 (354–817 aa; data not shown) does not. Saccharomyces cerevisiae cdc25H is also viable at 37°C in the presence of pMyr-HMO1 and pSos-TBP (Figure 1A), suggesting that HMO1 interacts with TBP. In this assay, the strength of the interaction between HMO1 and TAND/TBP is indistinguishable from that of the well-characterized interaction between TAND and TBP (Figure 1A).

Direct binding between HMO1 and TBP was demonstrated using recombinant proteins in GST pulldown assays (Figure 1B, lanes 4 and 8). Using this method, there was no evidence of direct interaction between HMO1 and TAND (Figure 1B, lane 3), suggesting that HMO1 interacts indirectly with TAND. This is consistent with previous studies showing that HMO1 interacts with TAF11 (54) and TAF13 (55,56). We also examined whether HMO1 co-purifies with TFIIID. A small amount of TAF1 (~0.01%), TAF11 (~0.01%) and TBP (~0.001%) were recovered with HMO1-TAP purified from cell extracts (Figure 1C). These results suggest that HMO1 regulates transcription of class II genes via an interaction with TFIIID.

Genetic interaction between HMO1 and TAND

Because biochemical studies indicate that HMO1 does not interact directly with TAND (Figure 1), we examined whether there is a genetic interaction between these two factors. On rich medium, we compared the growth properties of wild-type, single (Δhmo1, Δtaf1ΔTAND) and double (Δhmo1 Δtaf1ΔTAND) deletion mutants (Figure 2A). Previous studies demonstrated that Δhmo1 strains exhibit slower growth rates than wild-type strains over a wide range of temperatures (21,57). In contrast, our Δhmo1 strain demonstrated a cold-sensitive growth phenotype (Figure 2A), presumably due to its specific genetic background. A slight (but reproducible) decrease in growth rate was observed when Δhmo1 was combined with taf1ΔTAND [i.e. TAND1 + 2 (Δ2–86) or TAND1 + 2 + 3 (Δ2–186)] (Figure 2A).

When an HMO1 expression plasmid was transfected into wild-type and taf1ΔTAND strains, the number of transformants appeared to be significantly lower in taf1ΔTAND (10–73 aa) than in the wild-type strain (Figure 2B), due to delayed rates of colony formation in the former strain. This strain-specific toxicity was also HMO1-specific, because it was not observed with empty vector ( ), or with a plasmid overexpressing an unrelated protein (VTC1). HMO1 was also overexpressed in wild-type and taf1ΔTAND mutant strains [TAND1 (8–42 aa), TAND2 (41–73 aa) or both (10–73 aa)] from the TEF1 and TDH3 promoters. The results confirmed that HMO1 overexpression is toxic to cells that are deficient in TAND (Figure 2C). One interpretation of these data is that HMO1 and TAND1 perform antagonistic roles in
regulating TBP. These genetic data support the hypothesis that HMO1 is involved in transcription of class II genes via its interaction with TBP/TAND1/TFIID.

The effect of Δhmo1 on TFIID-dependent transcription

To test the hypothesis that HMO1 plays a role in TFIID-dependent transcription, the expression of several TAND-dependent genes was examined in wild-type, and in single (Δhmo1, taf1ΔTAND) or double (Δhmo1 taf1ΔTAND) deletion mutants cultured at 25°C, 30°C or 35°C (Figure 3A). As described previously (21), the steady-state levels of 25S rRNA were lower in the Δhmo1 strain at 25°C and 30°C than in the wild-type strain (compare lanes 1 and 4). Expression of PHO4, PHO12, HIS4 and RPS5 were also HMO1-dependent at 25°C, 30°C and 35°C (compare lanes 1 and 4). In contrast, expression of ACT1 was relatively HMO1-independent, decreasing significantly only in the Δhmo1 taf1ΔTAND strains at 25°C (compare lanes 2, 3 and lanes 5, 6). Furthermore, expression of PHO4 was restored in Δhmo1 taf1ΔTAND strains at 35°C (lanes 5 and 6, percentages are indicated in the histogram). The results

Figure 2. Genetic interaction between HMO1 and TAND. (A) Effect of Δhmo1 and taf1ΔTAND on growth. Strains carrying a combination of HMO1 or Δhmo1 and TAF1, taf1ΔTAND (2–86 aa) or taf1ΔTAND (2–186 aa), as indicated at the left, were spotted onto YPD plates at three dilutions and grown at 25°C, 30°C or 35°C for 3 days. (B) The effect of HMO1 overexpression on growth. Strains carrying TAF1 or taf1ΔTAND (10–73 aa) were transformed with pM2950 or pM2956 to overexpress HMO1 or VTC1, respectively, or with empty plasmid (pM2959, asterisk, negative control). A region of each plate containing transformants cultured at 30°C for 2.5 days is shown. (C) TAND1 or TAND2 are required to reduce the toxicity of HMO1 overexpression. As shown in B, strains carrying TAF1, taf1ΔTAND (8–42 aa), taf1ΔTAND (41–73 aa) or taf1ΔTAND (10–73 aa) were transformed with fixed amounts (100 ng) of empty plasmid (−) or plasmid overexpressing HMO1 (pM2949 and pM2950) from the TEF1 or TDH3 promoters, respectively. Following incubation at 30°C for 2.5 days, the number of transformants were counted.

Figure 1. HMO1 interacts with components of TFIID. (A) HMO1 interacts with TAND and TBP in the Sos recruitment system. *Saccharomyces cerevisiae* cdc25H was transformed with the indicated combinations of plasmids and grown on appropriately supplemented 2% galactose and 1% raffinose minimal medium at 25°C or 37°C (right panel) for 5 days. Note the revertant colonies (false positives) that did not grow uniformly in inoculated areas. (B) HMO1 binds TBP, but not TAND. GST pulldown assays were performed by incubating HMO1 (200 pmol in lanes 2–4) or TBP (40 pmol in lanes 6–8) with GST-TAND (20 pmol in lanes 3 and 7), GST-TBP (20 pmol in lane 4), GST-HMO1 (25 pmol in lane 8) and GST (60 pmol in lanes 2 and 6). Aliquots of 4% of the total input of HMO1 and TBP are shown in lanes 1 and 5, respectively. Proteins were separated by 10% SDS–PAGE and visualized by immunoblotting using antibodies specific for the proteins indicated. (C) HMO1 co-purifies with TFIID. Cell lysates prepared from strains expressing TAP-tagged or un-tagged HMO1 were purified as described previously (49) with minor modifications. Purified fractions containing TAP-tagged (lane 3) or un-tagged (lane 2) HMO1 were separated by 7% (TAF1), 10% (TAF11) or 12% (HMO1, TBP) SDS–PAGE and visualized by immunoblotting using antibodies against the proteins indicated at the left. TAP-tagged lysates (0.01% of input) are shown in lane 1.
are consistent with the hypothesis that HMO1 and TAND play antagonistic roles in PHO84 transcription. A similar, but weaker effect was observed for PHO12.

The role of HMO1 in transcription of the inducible GAL1 gene was also investigated. Here, expression was measured as a function of time after shift from raffinose to galactose-containing medium in wild-type, and in single (Δhmo1, taflΔTAND) or double (Δhmo1 taflΔTAND) deletion mutant strains (Figure 3B). Deficiency in HMO1 (Δhmo1) caused a decrease (or delay) in expression of GAL1. Deficiency in TAND had a similar but more severe effect, and the effects of these two mutations were additive. These results indicate that HMO1 and TAND function together in GAL1 transcription. Significantly, mutations in HMO1 and TAND also had additive effects on transcription of HIS4 and RPS5 (Figure 3A). Collectively, these data indicate that HMO1 and TAND may regulate transcription of distinct subsets of TFIIID-dependent genes in antagonistic or cooperative manners.

The effects of Δhmo1 and/or taflΔTAND on TFIIID occupancy at several RPG promoters

Recent studies have shown that HMO1 binds to many RPG promoters (22,23). We have proposed that 138 RPGs can be classified into 13 distinct groups based on HMO1-abundance at their promoters and the HMO1-dependence of FHL1 and/or RAP1 binding to the promoters (23). For instance, HMO1 is abundant at promoters of RPS5, RPL23B, RPL27B and RPL32, but not RPS31, RPL3 and RPL10 (23). FHL1 binds to the former group in an HMO1-dependent manner, whereas it binds to the latter group in an HMO1-independent manner (23). Intriguingly, however, HMO1-dependency of transcription of these RPGs does not correlate with HMO1 binding or HMO1-dependent recruitment of FHL1. (Figure 4B) (23). Thus, we examined whether TFIIID occupancy at these promoters is correlated with the HMO1-dependence of transcription. For this purpose, chromatin immunoprecipitation (ChiP) analyses were conducted in wild-type, and in single (Δhmo1, taflΔTAND) or double (Δhmo1 taflΔTAND) deletion mutants expressing TAP-tagged TAF1 or TAF1ΔTAND that were cultured at 25°C in rich medium (Figure 4A).

The results demonstrate that TFIIID occupancy is greater at RPS5, RPS31 and RPL10 than at RPL3, RPL23B, RPL27B and RPL32 (Figure 4B), suggesting that TFIIID-abundance is not correlated with HMO1-abundance or HMO1-dependent recruitment of FHL1. Intriguingly, taflΔTAND weakened transcription of these RPGs, whereas it enhanced TFIIID occupancy at the promoters, irrespective of whether HMO1 was intact or not (compare open and dark gray bars in Figure 4B). These data are in good agreement with previous results suggesting that TAND is inhibitory for TFIIID binding but necessary for efficient transcription (11,51,58).

Δhmo1 decreased transcription and TFIIID occupancy at these promoters in TAF1 strains (open bars in Figure 4B). However, in taflΔTAND strains, Δhmo1 decreased transcription of RPS31, RPL3 and RPL10 but increased TFIIID occupancy at these promoters (dark gray
We investigated the genetic relationship between TBP, TFIIB, and TFIIA.

Genetic interaction between HMO1 and TAND/TFIID

We investigated the genetic relationship between HMO1 and SPT15 (TBP; Figure 5A), SUA7 (TFIIB; Figure 5B) and TOA1 (TFIIA; Figure 5C). To construct strains deficient in SPT15 and/or HMO1, plasmids were shuffled in Δspt15 and Δhmo1 Δspt15 backgrounds. In the newly constructed strains, a URA3-marker plasmid expressing SPT15 was replaced with a TRP1-marker plasmid expressing wild-type or mutant spt15 (39). The viability of these strains was tested at 30°C, 34°C and 37°C in rich medium (Figure 5A). The presence of the Δhmo1 defect altered the growth of some but not all spt15 mutant strains. The affected strains were spt15-E236P, K239L, F237D, K138T/Y139A, N159L and P65S, demonstrating an allele-specific genetic interactions between HMO1 and TAND/TFIID. The affected strains were spt15-E236P, K239L, F237D, K138T/Y139A, N159L and P65S, demonstrating an allele-specific genetic interactions between HMO1 and TAND/TFIID.
and TBP. Because five of six (all except K239L) of the affected spt15 mutant alleles are deficient in Pol II-dependent but not Pol I-dependent transcription (59–63), these results indicate that HMO1 may be more intimately involved in the TBP-mediated core promoter function of class II genes than of the 35S rRNA gene. Consistent with this hypothesis, HMO1 binds specifically to the promoter regions of class II genes whereas it binds more broadly to the entire 35S rRNA gene (22,23). The allele-specific interactions between HMO1 and SPT15 suggest a highly specific interaction between these two genes/proteins.

TFIIB mutants were also tested for genetic interactions with Δhmo1 (Figure 5B). Intriguingly, Δhmo1 rescued the growth defects of sua7-K190E (64), K201E (64) and R78C (65) at 30/35°C, 30/37°C and 37°C, respectively. A similar, but weaker interaction was observed for sua7-E62K (65) at 37°C. These results indicate that HMO1 and TFIIB have antagonistic roles, as do HMO1 and TAND (Figures 2B, C and 3A) and
that HMO1 may compete with TFIIB and TAND for binding to TBP.

Similar analyses were performed for several TFIIA mutants (Figure 5C). The results indicate a genetic interaction between Δhmo1 and toal-Δ(55–215) (66) (35/37°C), toal-K255A/R257A/K259A (67) (35/37°C) and toal-W285A (68) (37°C). In summary, these results indicate that HMO1 may regulate transcription of class II genes by affecting the formation and/or stability of the TFIIA–TFIIB–TBP/TFIID–promoter complex.

Δhmo1 restores transcription of class II genes in several sua7 mutants

The results described above showed that Δhmo1 reduces the growth of some spt15 and toal mutants (Figure 5A and C), while it restores the growth of some sua7 mutants (Figure 5B). Thus, we asked whether the effect of Δhmo1 on transcription of class II genes is consistent with these growth phenotypes. Indeed, Δhmo1 impairs transcription of some genes in the spt15 and toal mutants whereas it restores transcription in the sua7 mutants.

Northern blot analysis was conducted for the strains described above (Figure 5). The results show that Δhmo1 significantly reduced the expression of HIS4 at 25°C and 37°C in the spt15-E236P and -K239L mutants (Supplementary Figure 1A). A similar but weaker effect of Δhmo1 was observed in the spt15-F237D and -N159L mutants (Supplementary Figure 1A). In contrast, the spt15-K138T/Y139A mutation almost completely abolished HIS4 expression at both temperatures even in the HMO1 strain (Supplementary Figure 1A). Such a detrimental effect of Δhmo1 in the spt15 mutants seems to be specific for a subset of genes since it was observed for HIS4 but not for TEF2 (Supplementary Figure 1A), even though HMO1 binds to both promoters at similar levels (23). These results indicate that HMO1 plays different roles in transcription at different promoters.

Consistent with the growth phenotypes, Δhmo1 restored the expression of RPS5 at 25°C and 37°C in the sua7-K190E and -K201E mutants (Supplementary Figure 1B). A similar but much weaker effect of Δhmo1 was observed in the sua7-E62K and -R78C mutants (Supplementary Figure 1B). Furthermore, Δhmo1 restored the expression of HIS4 in the sua7-K190E (37°C), -K201E (37°C), -E62K (25/37°C) and -R78C (25/37°C) mutants (Supplementary Figure 1B). Δhmo1 also restored the expression of TEF2 in the sua7-K190E and -K201E mutants (25/37°C) (Supplementary Figure 1B). Previous studies have shown that the sua7-K190E and -K201E mutations are defective in forming a stable TFIIB–TBP–DNA complex while sua7-E62K and -R78C mutations shift the transcriptional start site downstream (64,65). Therefore, these results indicate that HMO1 could restore a range of transcriptional defects caused by different types of sua7 mutations.

Finally, northern blot analyses showed that Δhmo1 significantly reduced expression of HIS4 (25/37°C) and RPS5/TEF2 (25/37°C) in the toal-Δ(55–215) and toal-W285A mutants, respectively (Supplementary Figure 1B).

Thus, we conclude that the effects of Δhmo1 on growth and on transcription of a subset of class II genes are well correlated in several spt15, sua7 and toal mutants.

HMO1 participates in start site selection of a subset of class II genes

The results described above indicate that HMO1 and TFIIB function antagonistically in growth and transcription. Since some TFIIB mutants like sua7-E62K and -R78C cause a downstream shift in transcriptional start site (64,65), we examined whether Δhmo1 caused an upstream shift, i.e. a shift in the opposite direction. Transcriptional start sites of RPS5, RPL32, RPS31 and RPL10 were examined by primer extension analysis in some of the strains described in Figure 5B (Figure 6A–D). The electrophoretic images were scanned and quantified to compare the positions of transcriptional start sites (Figure 6E–H). The sua7-K190E mutant shifted transcriptional start site of these four genes slightly downstream (compare lanes 1, 3 and 5 in Figure 6A–D), whereas Δhmo1 alone shifted the transcriptional start site of RPS5 and RPL32, but not of RPS31 and RPL10, upstream (compare lanes 1 and 2 in Figure 6A–D). These results indicate that HMO1 participates in start site selection of a subset of genes. Indeed, a similar upstream shift in transcriptional start site was observed for RPS16A, RPL23B and RPL27B, but not for TEF2 and ADH1 (data not shown, Supplementary Figure 2). Notably, Δhmo1 also shifted the transcriptional start sites of the same set of genes, i.e. RPS5, RPS16A, RPL23B, RPL27B and RPL32, upstream in the sua7-K190E mutant, and less strongly than in the SUA7 strain (Figure 6A and B, data not shown). In contrast, the upstream shift in transcriptional start sites of these genes induced by Δhmo1 was quite weak or almost undetectable in the sua7-K190E strain (Figure 6A and B, data not shown). Similar results were obtained when the experiments were conducted at different temperatures (i.e. 30°C, 35°C and 37°C) (Supplementary Figure 2, data not shown).

These observations could explain, at least in part, how Δhmo1 restored the growth of the sua7-R78C mutant at 37°C (Figure 5B). Furthermore, given that Δhmo1 cannot cause a strong upstream shift in the sua7-K190E mutant, stable TFIIB–TBP–DNA complex formation must be important for transcriptional initiation from upstream regions. HMO1 is enriched at the promoters of RPS5, RPS16A, RPL23B, RPL27B and RPL32 but limited at those of RPS31, RPL10, TEF2 and ADH1 (23). Therefore, HMO1 may play more direct and important roles in start site selection at HMO1-enriched promoters than at HMO1-limited promoters.

DISCUSSION

In this study, we identified HMO1 as a TAND-interacting protein using the Sos recruitment system (Figure 1A) and showed that HMO1 binds directly to TBP and TFIID (Figure 1B and C). Our evidence suggests that HMO1 may function with TAND either antagonistically or...
Figure 6. The effect of Δhmo1 on start site selection of class II genes in sua7 mutants. (A) Transcriptional start sites of RPS5 in Δhmo1 and/or sua7 mutants. Total RNA (20 μg) from strains containing the alleles indicated at the top was isolated 2 h after a temperature shift to 37°C from 30°C before being subjected to primer extension analysis. The position of a major transcriptional start site (C at −37 numbered relative to the A (+1) of the start codon ATG) is indicated by the asterisk. (B) Transcriptional start sites of RPL32 in Δhmo1 and/or sua7 mutants. Primer extension analysis was done as described in A. A major transcriptional start site at −365 is indicated by the asterisk. (C) Transcriptional start sites of RPS31 in Δhmo1 and/or sua7 mutants. Primer extension analysis was done as described in A. A major transcriptional start site at −57 is indicated by the asterisk. (D) Transcriptional start sites of RPL10 in Δhmo1 and/or sua7 mutants. Primer extension analysis was done as described in A. A major transcriptional start site at −21 is indicated by the asterisk. (E) Each lane of the electropherogram shown in A was scanned and quantified by densitometry (Multi Gauge ver.3.0, Fuji Film) (SUA7, left panel; sua7-R78C, center panel; sua7-K190E, right panel). The solid and broken lines represent the results obtained from HMO1 and Δhmo1 strains, respectively. Asterisks indicate the peaks that correspond to the major transcriptional start sites described in A. The upstream regions were expanded and are shown in the lower panels to make the differences between HMO1 and Δhmo1 strains more evident. (F) Each lane of the electropherogram shown in B was scanned and presented as described in E. (G) Each lane of the electropherogram shown in C was scanned and presented as described in E. (H) Each lane of the electropherogram shown in D was scanned and presented as described in E.
cooperatively in supporting cell growth (Figure 2) and transcription of a subset of class II genes (Figure 3). Furthermore, allele-specific interactions of αhmol with spt15, sua7 and toa1 indicate that HMO1 may be involved in TFIIA–TFIIB–TBP/TFIID–DNA complex formation (Figure 5, Supplementary Figure 1).

NHP6A/B, another well-characterized HMGB protein in yeast, may also enhance assembly of the
TFIIA–TBP–DNA complex (20,69). Does NHP6A/B play a role similar to HMO1 in transcription? Substitutions of TBP residues F237/K239, K138/Y139 or P65 exhibited synthetic growth defects in \( /C1^{hmo1} \) cells (Figure 5A). Similarly, these substitutions were lethal in \( /C1^{nhp6a/b} \) cells (70). However, \( /C1^{hmo1} \) exhibited no apparent synthetic phenotype with \( /C1^{nhp6a/b} \) (57). These results suggest that HMO1 and NHP6A/B are not functionally redundant, but that they may instead have complementary functions, for example, in regulating different subsets of class II genes via similar mechanisms. In fact, each plays a crucial role in Pol I (21) and Pol III (71) transcription, respectively.

\( /C1^{hmo1} \) caused growth defects in some, but not all, of the TBP mutants that are lethal with \( taf1^{TAND} \). For instance, the TBP mutations N159D, V161A and S118L have growth defects in combination with \( taf1^{TAND} \) (39), but not with \( /C1^{hmo1} \) (Figure 5A). These observations indicate that HMO1 and TAND have both shared and unique functional roles, which is consistent with the observation that the synthetic defect between \( taf1^{TAND} \) and \( /C1^{hmo1} \) is relatively weak (Figure 2A).

One of the most intriguing findings of this study is that \( /C1^{hmo1} \) causes an upstream shift in transcriptional start sites of a subset of genes (Figure 6, data not shown). This phenotype is uncommon and has previously been found in mutations of four polypeptides within the preinitiation complex (PIC), i.e. the TFG1 and TFG2 subunits of TFIIIF (31–33) and the RPB2 and RPB9 subunits of Pol II (34–37). Conversely, mutations in the amino-terminal B-finger region (e.g. residues E62, F63, R64, F66, R78 and V79) of TFIIIB (64,65,72) and in the RPB1 subunit (e.g. residues K332, R344 and N445 in the active site) of Pol II (73,74) cause a downstream shift in transcriptional start site. Mutations of TFG1, TFG2, RPB2 and RPB9 could rescue growth defects and suppress the downstream start site shift caused by mutations of \( SUA7 \) (TFIIB) and RPB1. Similarly, our current study showed that \( /C1^{hmo1} \) could rescue the growth and transcriptional defects of some TFIIB mutants (Figures 5 and 6, and Supplementary Figure 1). Thus, it will be important in future studies to determine whether \( /C1^{hmo1} \) and mutations of TFIIF/Pol II cause upstream start site shifts by similar or different mechanisms.

The dimerization domain (i.e. the interface between TFG1 and TFG2) of TFIIF and the B-finger of TFIIB lie within the active site of Pol II in the PIC (32,74,75). In contrast, the position of the G369 residue of RPB2, whose mutation causes an upstream start site shift and suppresses the growth and transcriptional defects of the TFIIB mutant, was located near RPB9 but distal to the active site of Pol II (76). However, mutations of RPB2 and RPB9 may impair interactions between TFIIF and Pol II (33). Furthermore, a mutation in the switch 2 region of Pol II (i.e. \( rpb1-R344A \)) that causes a downstream start site shift destabilizes a short RNA–DNA hybrid in the active site and thereby increases the frequency of abortive initiation (74). Based on these and other observations, an intriguing model was recently proposed for the roles...
played by the B-finger of TFII B, TFII F and the switch 2 region of Pol II (74). In this model, the nascent short RNA–DNA hybrid is initially stabilized by the B-finger of TFII B, and continued synthesis of RNA induces a conformational change in Pol II, presumably mediated by TFII F. Subsequently, the switch 2 region is repositioned in the active site to displace the B-finger and then stabilize the 3′-end of the RNA–DNA hybrid. Thus, mutations of the B-finger and switch 2 region that fail to stabilize the RNA–DNA hybrid in the active site would increase the frequency of abortive initiations and result in re-initiation from sites farther downstream. Mutations of TFII F and Pol II that disrupt the appropriate TFII F–Pol II interactions may generate an initiation complex that more readily undergoes the conformational change to start transcription from sites farther upstream than normal.

One possible explanation for the phenotypes of Δhmo1 is that the absence of HMO1 disrupts the appropriate TFII F–Pol II interactions indirectly, as proposed for RPB2 and RPB9 mutants. However, HMO1 may be involved in TFII A–TFII B–TBP;TFII D–DNA complex formation, as described above. In addition, the ADH1 transcriptional start site was shifted in TFII B, TFII F, and Pol II mutants, but not in the Δhmo1 mutant (Supplementary Figure 2). Thus, the alternative hypothesis that the absence of HMO1 may facilitate TFII A–TFII B–TBP;TFII D–DNA complex formation at sites farther upstream than normal should be considered. In this regard, it is notable that TBP (SPT15) and several components of SAGA, e.g. SPT3, SPT7, SPT8 and SPT20, were originally isolated as SPT genes that suppress Spt- phenotypes, indicating that mutations in TBP or SAGA weakened transcription of the transposon Ty or δ element, but enhanced transcription of adjacent genes (77,78). Although the precise mechanisms by which these mutations could induce a promoter shift from the transposon to an adjacent gene remain unknown, it is likely that TBP binding may be altered to prefer the latter promoter. Hence, it is also possible that Δhmo1 may alter the site of TBP binding upstream by similar mechanisms.

Recently, NHP6A/B was shown to serve as a Pol III initiation fidelity factor, since Δnhp6a/b cells lose initiation fidelity (i.e. generate many ectopic initiation sites) at some, but not all, tRNA genes (79). Biochemical analyses suggest that NHP6A/B may directly promote accurate binding of TFII B to the correct position, or indirectly via the function of TFII C. Although HMO1 does not show similar activity in the Pol III system (79), it may play an analogous role in the Pol II system, e.g. by promoting correct binding of TBP/TFII D at a subset of class II gene promoters.

The functions of HMO1 and NHP6A/B in start site selection appear to be promoter-specific (Figure 6, Supplementary Figure 2, data not shown) (79). Notably, the effects of TFII B and Pol II mutations are also promoter-specific; they shifted transcriptional start sites of ADH1 and CYC1 but not of HIS3 (72,73). Mutation analyses using ADH1–HIS3 hybrid promoters revealed that the feature that confers sensitivity to TFII B mutations is encoded in the sequence surrounding the start site and not by the spacing between the TATA element and the start site (72). Similar but more intricate promoter-specific effects were observed for a E51 mutation (corresponding to E62 in yeast) within the B-finger of human TFII B; this mutation shifted the transcriptional start site of the AdE4 promoter downstream and that of HIV-LTR upstream, even though it did not affect the transcriptional start sites of AdML and hIGFII promoters (80). Consistent with yeast studies, hybrid promoter analysis showed that these promoter-specific effects were determined by the sequence surrounding the start site (i.e. initiator) (80). Intriguingly, the same mutation shifted transcriptional start site of HIV-LTR downstream (i.e. in the opposite direction) when a single base-pair substitution was introduced at a specific position in the initiator region of this promoter (80). These observations indicate a common mechanism of TFII B-mediated start site selection that is conserved from yeast to human, although the identity of the human counterpart of HMO1 still remains unclear (21–23).

Our previous study showed that HMO1-abundance and HMO1-dependence of transcription and FHL1-recruitment were determined by the promoter sequence itself, at least in the case of RPS5 and RPL10 (23). Thus, the promoter sequence may also determine the sensitivity of start site selection to Δhmo1. Further studies are required to elucidate the full role played by HMO1 in transcription, and, particularly, in start site selection in RPGs and other HMO1-dependent class II genes.

ACKNOWLEDGEMENTS

We would like to thank Dr H. Iwasaki, Dr T. Kobayashi and members of our laboratory for advice and comments on this work. We also thank Dr A. G. Hinnenbusch, Dr Y. Nakatani and Dr J. Heitman for yeast strains, and M. Funk, Y. Ohyama, A. Kobayashi, M. Yuhki, Y. Tsukihashi, S. Takahata, S. Tomita and A. Okada-Murabayashi for plasmids. The TAP plasmid was obtained from CellZome (Heidelberg). This study was supported by a grant from the 2007 Strategic Research Project (No.W19021) of Yokohama City University, and grants from the Japan Society for the Promotion of Science, the Ministry of Education, Culture, Sports, Science and Technology of Japan, CREST of the Japan Science and Technology Corporation, and the Mitsubishi Foundation. Funding to pay the Open Access publication charges for this article was provided by the Japan Society for the Promotion of Science.

Conflict of interest statement. None declared.

REFERENCES

1. Mellor, J. (2005) The dynamics of chromatin remodeling at promoters. Mol. Cell, 19, 147–157.
2. Hahn, S. (2004) Structure and mechanism of the RNA polymerase II transcription machinery. Nat. Struct. Mol. Biol., 11, 394–403.
3. Taatjes, D.J., Marr, M.T. and Tjian, R. (2004) Regulatory diversity among metazoan co-activator complexes. Nat. Rev. Mol. Cell Biol., 5, 403–410.
4. Ptaschke,M. (2005) Regulation of transcription: from lambda to eukaryotes. Trends Biochem. Sci., 30, 275–279.
5. Govind,C.K., Yoon,S., Qi,H., Govind,S. and Hinnebusch,A.G. (2005) Simultaneous recruitment of coactivators by Gen4p stimulates multiple steps of transcription in vivo. Mol. Cell Biol., 25, 5626–5638.
6. Matangkasombut,O., Buratowski,R.M., Swilling,N.W. and Buratowski,S. (2005) Bromodomain factor 1 corresponds to a missing piece of yeast TFIIH. Genes Dev., 14, 951–962.
7. Kotani,T., Miyake,T., Tsukihashi,Y., Hinnebusch,A.G., Nakatani,Y., Kawaichi,M. and Kokubo,T. (1998) Identification of highly conserved amino-terminal segments of dTAFII230 and yTAFI1145 that are functionally interchangeable for inhibiting TBP-DNA interactions in vitro and in promoting yeast cell growth in vivo. J. Biol. Chem., 273, 32254–32264.
8. Kuras,L. and Struhl,K. (1999) Binding of TBP to promoters in vivo is stimulated by activators and requires Pol II holoenzymes. Nature, 399, 609–613.
9. Li,X.Y., Virbasius,A., Zhu,X. and Green,M.R. (1999) Enhancement of TBP binding by activators and general transcription factors. Nature, 399, 605–609.
10. Kim,J. and Iyer,V.R. (2004) Global role of TATA box-binding protein recruitment to promoters in mediating gene expression profiles. Mol. Cell Biol., 24, 8104–8112.
11. Kotani,T., Banno,K., Ikura,M., Hinnebusch,A.G., Nakatani,Y., Kawaichi,M. and Kokubo,T. (2000) A role of transcriptional activators as antirepressors for the autoinhibitory activity of TATA box binding protein transcription factor IID. Proc. Natl Acad. Sci. USA, 97, 7178–7183.
12. Bustin,M. (2001) Revised nomenclature for high mobility group (HMG) chromosomal proteins. Trends Biochem. Sci., 26, 152–153.
13. Thomas,J.O. and Travers,A.A. (2001) HMG1 and 2, and related ‘architectural’ DNA-binding proteins. Trends Biochem. Sci., 26, 167–174.
14. Weir,H.M., Kraulis,P.J., Hill,C.S., Raine,A.R., Laue,E.D. and Reeves,R. and Adair,J.E. (2005) Role of high mobility group proteins NHP6A/B potentiate promoter-specific transcriptional activation in vivo and assembly of preinitiation complexes in vitro. Trends Biochem. Sci., 26, 442–450.
15. Hull,M.W., McKune,K. and Woychik,N.A. (1995) RNA polymerase II subunit RPB9 is required for accurate start site selection. Genes Dev., 9, 481–490.
16. Sun,Z.W. and Hampsey,M. (1996) Functional interaction between TFIIH and the Rpb9 (SSU73) subunit of RNA polymerase II in Saccharomyces cerevisiae. Nucleic Acids Res., 24, 2560–2566.
17. Ziegler,L.M., Khapersky,D.A., Ammerman,M.L. and Ponticelli,A.S. (2003) Yeast RNA polymerase II lacking the Rbp9 subunit is impaired for interaction with transcription factor II F. J. Biol. Chem., 278, 48950–48956.
18. Amberg,D.C., Burke,D.J. and Strathern,J.N. (2005) Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
19. Kobayashi,A., Miyake,T., Ohyama,Y., Kawaichi,M. and Kokubo,T. (2001) Mutations in the TATA-binding protein, affecting transcriptional activation, show synthetic lethality with the TAF145 gene lacking the TAF N-terminal domain in Saccharomyces cerevisiae. J. Biol. Chem., 276, 395–405.
20. Longtime,M.S., McKenzie,A. III, Demarini,D.J., Shah,N.G., Wach,A., Brachat,A., Philippsen,P. and Pringle,J.R. (1998) Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast, 14, 953–961.
21. Kokubo,T., Swanson,M.J., Nishikawa,J.I., Hinnebusch,A.G. and Nakatani,Y. (1998) The yeast TAF145 inhibitory domain and TFIIA competitively bind to TATA-binding protein. Mol. Cell Biol., 18, 1003–1012.
22. Christianson,T.W., Sikorski,R.S., Dante,M., Shero,J.H. and Hieter,P. (1992) Multifunctional yeast high-copy-number shuttle vectors. Gene, 110, 119–127.
23. Takahata,S., Ryu,H., Ohtsuki,K., Kasahara,K., Kawaichi,M. and Kokubo,T. (2003) Identification of a novel TATA element-binding protein binding region at the N terminus of the Saccharomyces cerevisiae TFIIA protein. J. Biol. Chem., 278, 45888–45902.
24. Kasahara,K., Kawaichi,M. and Kokubo,T. (2004) In vivo synthesis of Taflp lacking the TAF N-terminal domain using alternative transcription or translation initiation sites. Genes Cells, 9, 709–721.
25. Mumberg,D., Muller,R. and Funk,M. (1995) Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. Gene, 156, 119–122.
26. Sikorski,R.S. and Hieter,P. (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics, 122, 19–27.
51. Takahata, S., Kasahara, K., Kawaichi, M. and Kokubo, T. (2004) A comprehensive analysis of protein-protein interaction map of the budding yeast: a comprehensive protein interactome. Mol. Cell Biol., 24, 2866–2882.

52. Carey, M. and Smale, S.T. (2000) A proteasome component, in vivo and in vitro. Mol. Cell Biol., 20, 623–627.

53. Chang, C., Gonzalez, F., Rothermel, B., Sun, L., Johnston, S.A. and Chang, J. (1995) Nhp6 is a transcriptional activation-defective TBP mutations on transcription initiation in vivo. Mol. Cell Biol., 15, 1234–1243.

54. Uetz, P., Giot, L., Cagney, G., Mansfield, T.A., Judson, R.S., Knight, J.R., Lockshon, D., Narayan, V., Srinivasan, M., Pochart, P., et al. (2000) A comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae: a targeted proteomics approach. Mol. Cell Biol., 20, 1979–1986.

55. Care, M. and Smale, S.T. (2000) Transcriptional Regulation in Eukaryotes-Concepts, Strategies, and Techniques. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

56. Aronheim, A., Zandi, E., Hennemann, H., Elledge, S.J. and Karin, M. (1997) Mutational analysis of the D1/E1 core helices and the conserved N-terminal region of yeast transcription factor IIIA (TFIIB): identification of an N-terminal mutant that stabilizes TATA-binding protein-TFIIB-DNA complexes. Mol. Cell Biol., 17, 6784–6793.

57. Pinto, L., Wu, W.H., Na, J.G. and Hampsey, M. (1994) Characterization of sua7 mutations defines a domain of TFIIH involved in transcription start site selection in yeast. J. Biol. Chem., 269, 30569–30573.

58. Kim, T.K., Hashimoto, S., Kelleher, R.J. III, Flanagan, P.M., Roeder, R.G. and Nakatani, Y. (1993) Drosophila 230-kDa TFIID subunit, a functional homolog of the human cell cycle gene product, negatively regulates DNA binding of the TATA box-binding subunit of TFIID. Genes Dev., 7, 1033–1046.

59. Kang, J.J., Auble, D.T., Ranish, J.A. and Hahn, S. (1995) Analysis of the yeast transcription factor TFIIA: distinct functional regions and a polymerase II-specific role in basal and activated transcription. Mol. Cell Biol., 15, 1234–1243.

60. Ranish, J.A., Yudkovsky, N. and Hahn, S. (1999) Intermediates in formation and activity of the RNA polymerase II preinitiation complex: holoenzyme recruitment and a postrecruitment role for the TATA box and TFIIB. Genes Dev., 13, 49–63.

61. Belousov, V., Chakravarti, K., Kovalchuk, A. and Ponticelli, A.S. (2004) A functional role for the switch 2 region of yeast RNA polymerase II in transcription start site utilization and abortive initiation. J. Biol. Chem., 279, 4427–4440.

62. Bushnell, D.A., Westover, K.D., Davis, R.E. and Kornberg, R.D. (2004) Functional basis of transcription: an RNA polymerase II-TFIIH co-corecetyl 4.5 Angstroms. Science, 303, 983–988.

63. Cramer, P., Bushnell, D.A. and Kornberg, R.D. (2001) Structural basis of transcription: RNA polymerase II at 2.8 angstrom resolution. Science, 291, 1863–1876.

64. Bangur, C.S., Pardee, T.S. and Ponticelli, A.S. (1997) Mutational analysis of the D1/E1 core helices and the conserved N-terminal region of yeast transcription factor IIIA (TFIIB): identification of an N-terminal mutant that stabilizes TATA-binding protein-TFIIB-DNA complexes. Mol. Cell Biol., 17, 6784–6793.

65. Pinto, L., Wu, W.H., Na, J.G. and Hampsey, M. (1994) Characterization of sua7 mutations defines a domain of TFIIH involved in transcription start site selection in yeast. J. Biol. Chem., 269, 30569–30573.

66. Solow, S.P., Lezina, L. and Lieberman, P.M. (1999) Phosphorylation of TFIIA stimulates TATA binding protein-TATA interaction and contributes to maximal transcription and viability in yeast. Mol. Cell Biol., 19, 2846–2852.

67. Biswas, D., Imbalzano, A.N., Eriksson, P., Yu, Y. and Stillman, D.J. (2004) Role for Nhp6, Gcn5, and the Swi/Snf complex in stimulating formation of the TFIIA-DNA complex. Mol. Cell Biol., 24, 8312–8321.

68. Eriksson, P., Biswas, D., Yu, Y., Stewart, J.M. and Stillman, D.J. (2004) TATA-binding protein mutants that are lethal in the absence of the Nhp6 high-mobility-group protein. Mol. Cell Biol., 24, 6419–6429.

69. Majovski, R.C., Khapersky, D.A., Ghazy, M.A. and Ponticelli, A.S. (2005) A functional role for the switch 2 region of yeast RNA polymerase II in transcription start site utilization and abortive initiation. J. Biol. Chem., 280, 34917–34923.

70. Kassavetis, G.A. and Steiner, D.F. (2006) Nhp6 is a transcriptional activation-defective TBP mutations can be restored by creating a canonical TATA element within the promoter region of the TUB2 gene. Mol. Cell Biol., 20, 2385–2399.

71. Gather, S., Kasahara, K., Kawaichi, M. and Kokubo, T. (2004) A comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae. Nature, 403, 623–627.

72. Faitar, S.L., Brodie, S.A. and Ponticelli, A.S. (2001) Promoter-specific shifts in transcription initiation conferred by yeast TFIIH mutations are detected by the sequence in the immediate vicinity of the start sites. Mol. Cell Biol., 21, 4427–4440.

73. Winston, F. and Sudarsanam, P. (1998) The SAGA of Spt proteins suppressors of Saccharomyces cerevisiae encode replacements of conserved residues within the largest subunit of RNA polymerase II and affect transcription start site selection similarly to sua7 (TFIIB) mutations. Mol. Cell Biol., 14, 226–237.

74. Majovski, R.C., Khapersky, D.A., Ghazy, M.A. and Ponticelli, A.S. (2005) A functional role for the switch 2 region of yeast RNA polymerase II in transcription start site utilization and abortive initiation. J. Biol. Chem., 280, 34917–34923.

75. Bushnell, D.A., Westover, K.D., Davis, R.E. and Kornberg, R.D. (2004) Structural basis of transcription: an RNA polymerase II-TFIIH co-corecetyl 4.5 Angstroms. Science, 303, 983–988.

76. Cramer, P., Bushnell, D.A. and Kornberg, R.D. (2001) Structural basis of transcription: RNA polymerase II at 2.8 angstrom resolution. Science, 291, 1863–1876.

77. Winston, F. and Sudarsanam, P. (1998) The SAGA of Spt proteins and transcriptional analysis in yeast: past, present, and future. Cold Spring Harb. Symp. Quant. Biol., 63, 553–561.

78. Yamaguchi, Y., Narita, T., Inukai, N., Wada, T. and Handa, H. (2001) Nhp6, an HMG1 protein, functions in SNR6 transcription by RNA polymerase III in S. cerevisiae. Mol. Cell, 7, 309–318.

79. Majovski, R.C., Khapersky, D.A., Ghazy, M.A. and Ponticelli, A.S. (2005) A functional role for the switch 2 region of yeast RNA polymerase II in transcription start site utilization and abortive initiation. J. Biol. Chem., 280, 34917–34923.

80. Kassavetis, G.A. and Steiner, D.F. (2006) Nhp6 is a transcriptional activation-defective TBP mutations on transcription initiation in vivo. Mol. Cell Biol., 15, 5461–5469.