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The Glutamine-Rich Activation Domains of Human Sp1 Do Not Stimulate Transcription in *Saccharomyces cerevisiae*

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Eukaryotic transcriptional activators have been classified on the basis of the characteristics of their activation domains. Acidic activation domains, such as those in the yeast GAL4 or GCN4 proteins and the herpes simplex virus activator VP16, stimulate RNA polymerase II transcription when introduced into a variety of eukaryotic cells. This species interchangeability demonstrates that the mechanism by which acidic activation domains function is highly conserved in the eukaryotic kingdom. To determine whether such a conservation of function exists for a different class of activation domain, we have tested whether the glutamine-rich activation domains of the human transcriptional activator Sp1 function in the yeast *Saccharomyces cerevisiae*. We report here that the glutamine-rich domains of Sp1 do not stimulate transcription in *S. cerevisiae*, even when accompanied by human TATA-box binding protein (TBP) or human-yeast TATA-box binding protein hybrids. Thus, in contrast to the case for acidic activation domains, the mechanism by which glutamine-rich domains stimulate transcription is not conserved between *S. cerevisiae* and humans.

Eukaryotic RNA polymerase II is assisted by three classes of auxiliary factors to achieve accurate and regulated transcription of protein-coding genes. The first class comprises at least seven distinct factors, designated the general transcription factors (GTFs), that are required for the ordered assembly of an active preinitiation complex (reviewed in reference 32). This complex, also known as the basal transcription machinery, is by itself capable of accurate TATA-dependent transcription in vitro.

Regulation of the basal machinery is achieved by the combined actions of two additional classes of factors. The first of these includes the promoter-specific regulatory factors that in many cases have been shown to function as transcriptional activators. Typically these activators are comprised of separable domains involved in transcriptional activation and DNA binding (2, 13, 18). The binding of an activator to specific DNA sequences in a promoter serves to bring an activation domain into the vicinity of an assembling transcription complex. Enhanced transcription is then achieved by the activator acting in conjunction with the third and most recently discovered class of factors termed coactivators, which are required for activated but not for basal transcription (reviewed in references 11, 25, 26). Coactivator activity has been found to be contained within the set of polypeptides known as TAFs that are tightly associated with the TATA box-binding protein (TBP). The combined actions of an activator and TAFs on the basal machinery result in an increase in the formation of preinitiation complexes or the rate of transcription initiation from such complexes.

Activators have been generally classified on the basis of the features of their activation domains. Acidic activators, such as the *Saccharomyces cerevisiae* proteins GCN4 and GAL4 and the herpes simplex virus activator VP16, are characterized by the relatively high content of aspartate and glutamate residues in their activation domains. In addition to acidic activation domains, glutamine-rich, proline-rich, and isoleucine-rich activation domains have also been identified; these are contained in the mammalian activators Sp1 and CTF and the *Drosophila* activator NTF-1, respectively (1, 21).

Although the precise mechanisms by which the various classes of activation domains stimulate transcription are not known, it is clear that the mechanism for acidic activation domains is conserved between yeast and higher eukaryotic cells. This is evidenced by the fact that acidic activation domains derived from activators native to a particular eukaryotic species have been shown to function when introduced into different eukaryotic cells. For example, the activation domain of the yeast activator GAL4 has been shown to activate transcription in *Drosophila* (10), tobacco (20), hamster (17), and human (30) cells. Conversely, acidic activation domains derived from some mammalian activators, including the herpes simplex virion protein VP16 and the Jun oncoprotein, efficiently stimulate transcription in *S. cerevisiae* (3, 29).

This species interchangeability for acidic activation domains prompted us to address whether another class of activation domain, the glutamine-rich domains of human Sp1, would also display function across species. In this paper we demonstrate that the activation domains of Sp1 do not stimulate transcription in *S. cerevisiae* even when accompanied by human TBP or human-yeast TBP hybrids. The results suggest that, unlike the case for acidic activation domains, the mechanism by which glutamine-rich domains stimulate transcription is not conserved between *S. cerevisiae* and humans. This lack of conservation reflects the absence or functional divergence of at least one factor in *S. cerevisiae* required for Sp1 function.

MATERIALS AND METHODS

Plasmids. The yeast expression vector pYX315 was constructed by digesting the yeast-*Escherichia coli* shuttle vector pRS315 (*CEN6 LEU2*) (27) with *Xho*I and *Bam*HI, filling in the recessed ends with Klenow, and inserting an approximately 700-bp blunt-ended fragment that contains the yeast *DED1* promoter and downstream polylinker sequences (derived from YCp88 [13]) fused to translational termination codons and the *GCN4* transcription termination sequences. pYX315-Sp1 was constructed by isolating the 3-kb *Xba*I-*Hin*dIII fragment of

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TABLE 1. Yeast strains used in this work

Strain	Genotype or description	Source or reference
KY320	*MAT***a** *ura3-52 ade2-101 trp1-Δ1 lys2-801 leu2*::*PET56 his3-Δ200*	5
KY1365	*MAT***a** *ura3-52 trp1-Δ1 leu2*::*PET56 gcn4-Δ1 his3-Δ86,189*	23
FP102	*MAT***a** *ura3-52 ade2-101 trp1-Δ1 lys2-801 leu2*::*PET56 his3-Δ30*	This work
FP103	FP102 + pHX-Sp1 (*LEU2*)	This work
FP104	*MAT***a** *ura3-52 ade2-101 trp1-Δ1 lys2-801 leu2*::*PET56 his3-Δ102,GC*	This work
FP105	FP104 + pHX-Sp1 (*LEU2*)	This work
FP110	KY1365 + pYX315 (*LEU2*)	This work
FP112	KY1365 + pYX315-Sp1/GCN4 (*LEU2*)	This work
FP113	FP102 + pYX315 (*LEU2*)	This work
FP114	FP102 + pYX315-Sp1 (*LEU2*)	This work
FP115	FP102 + pYX315-GCN4/Sp1 (*LEU2*)	This work
FP116	FP104 + pYX315 (*LEU2*)	This work
FP117	FP104 + pYX315-Sp1 (*LEU2*)	This work
FP118	FP104 + pYX315-GCN4/Sp1 (*LEU2*)	This work
FP119	FP102 + pYX315-Sp1 (*LEU2*) + p2DN-1 h/h (*TRP1*)	This work
FP120	FP102 + pYX315-Sp1 (*LEU2*) + p2DN-1 h/y (*TRP1*)	This work
FP121	FP102 + pYX315-Sp1 (*LEU2*) + p2DN-1 y/h (*TRP1*)	This work
FP122	FP104 + pYX315-Sp1 (*LEU2*) + p2DN-1 h/h (*TRP1*)	This work
FP123	FP104 + pYX315-Sp1 (*LEU2*) + p2DN-1 h/y (*TRP1*)	This work
FP124	FP104 + pYX315-Sp1 (*LEU2*) + p2DN-1 y/h (*TRP1*)	This work
BYΔ2-12	*MAT***a** *ura3-52 ade2-101 trp1-Δ1 lys2-801 leu2*::*PET56 spt15-Δ2* + p2DN-1 h/h/y (*TRP1*)	7
FP126b	*MAT***a** *ura3-52 ade2-101 trp1-Δ1 lys2-801 leu2*::*PET56 spt15-Δ2 his3-Δ30* + p2DN-1 h/h R231K (*TRP1*)	This work
FP127b	*MAT***a** *ura3-52 ade2-101 trp1-Δ1 lys2-801 leu2*::*PET56 spt15-Δ2 his3-Δ102,GC* + p2DN-1 h/h R231K (*TRP1*)	This work
FP154	FP126b + pYX315 (*LEU2*)	This work
FP155	FP126b + pYX315-Sp1 (*LEU2*)	This work
FP156	FP126b + pYX315-GCN4/Sp1 (*LEU2*)	This work
FP157	FP127b + pYX315 (*LEU2*)	This work
FP158	FP127b + pYX315-Sp1 (*LEU2*)	This work
FP159	FP127b + pYX315-GCN4/Sp1 (*LEU2*)	This work

pSp1-778C (containing the entire Sp1 cDNA) (16), filling in the recessed ends with Klenow, and inserting into the unique *Bam*HI site (made blunt) downstream of the *DED1* promoter in pYX315. pYX315-GCN4/Sp1, containing an in-frame fusion of the GCN4 activation domain to the DNA-binding domain of Sp1, was constructed by replacing the 1.9-kb *Bam*HI fragment of pYX315-Sp1 (removing amino acids 1 to 610 that contain the entire Sp1 activation region [16]) with a 701-bp partial *Bam*HI fragment from YCp88-Sc4400 (encoding GCN4 amino acids 1 to 224 [23]). pYX315-Sp1/GCN4, containing an in-frame fusion of the entire Sp1 activation region to the DNA-binding domain of GCN4, was constructed by replacing the 1.1-kb *Bam*HI-*Eco*RI fragment of pYX315-Sp1 (removing amino acids 611 to 778) with a 450-bp *Bam*HI-*Eco*RI fragment from YCp88-Sc4400 (encoding GCN4 amino acids 225 to 281). pHX-Sp1, containing Sp1 under the control of the yeast ADH1 promoter, was constructed by isolating the 3-kb *Xba*I-*Hin*dIII fragment of pSp1-778C, filling in the recessed ends with Klenow, and inserting in between the *Hin*dIII sites (made blunt, deleting the GAL4 sequences) of the vector pGAD424 (2μ *Leu2*; Clontech Laboratories, Inc.). The human and human-yeast TBP constructs used in this study have been described previously; p2DN-1 h/h, p2DN-1 h/y, and p2DN-1 y/h, all with *TRP1* as a selectable marker, were designated constructs number 2, 4, and 3, respectively, in reference 7. p2DN-1 h/h R231K is a spontaneous derivative of p2DN-1 h/h and contains a single arginine-to-lysine substitution at position 231 (8). For the construction of yeast strains containing a minimal *his3* promoter with or without Sp1 binding sites (GC boxes), two integration plasmids were utilized. The first, YIp55-Sc4600 (containing the *his3-Δ30* allele [22]), contains a minimal *his3* promoter, deleted for promoter sequences between −123 and −55 (relative to the start of *his3* transcription) with a unique *Eco*RI linker at the junction (10 bp upstream of the TATA element). This plasmid also contains a unique *Bam*HI site at position −447. To introduce Sp1 binding sites into the *his3* promoter, we amplified by PCR an approximately 100-bp fragment, containing six GC boxes, from plasmid Sp1-32-Inr (28). The sequences amplified normally contain an AP-1 site adjacent to the GC boxes, a site known to be recognized by transcription factors in *S. cerevisiae*. Therefore, we replaced this AP-1 site during the PCR amplification with a *Bam*HI site. The PCR product was digested with *Bam*HI and *Eco*RI and inserted between the unique *Bam*HI and *Eco*RI sites in YIp55-Sc4600. The resulting plasmid, YIp55-GC*his3*, contains the six GC boxes positioned between 30 to 85 bp upstream of the TATA element.

Strains and phenotypic analysis. The *his3* reporter genes of YIp55-Sc4600 and YIp55-GC*his3* were introduced into strain KY320 (MAT**a** *ura3-52 ade2-101 trp1-Δ1 lys2-801 leu2*::*PET56 his3-Δ200*) by a two-step gene replacement, generating strains FP102 and FP104, respectively. FP102 and FP104 were transformed to Leu^+ with plasmid pHX-Sp1, generating strains FP103 and FP105, or with plasmids pYX315, pYX315-Sp1, and pYX315-GCN4/Sp1, generating strains FP113 through FP118 (Table 1). Strain KY1365 (relevant genotype, *ura3-52 gcn4-Δ1 his3-Δ86,189* [23]) was transformed to Leu^+ with plasmids pYX315 and pYX315-Sp1/GCN4 to generate strains FP110 and FP112, respectively. To construct strains expressing both Sp1 and either human TBP or a human-yeast TBP hybrid, strains FP114 (FP102 + pYX315-Sp1) and FP117 (FP104 + pYX315-Sp1) were grown in liquid medium lacking leucine (0.68% yeast nitrogen base without amino acids, 2% glucose, 25 μg of adenine per ml, 100 μg of lysine per ml, 80 μg of tryptophan per ml, 25 μg of uracil per ml [SD + Ade, Lys, Trp, Ura]) and transformed with the appropriate human TBP plasmid (*TRP1*), and transformants were isolated and purified on medium lacking leucine and tryptophan (SD + Ade, Lys, Ura, 2% agar). To test for Sp1 function in strains deleted for the endogenous yeast TBP (*SPT15*) and containing only the human TBP mutant R231K, we first introduced the *his3* reporter genes of YIp55-Sc4600 and YIp55-GC*his3* into strain BYΔ2-12 (*MAT***a** *ura3-52 ade2-101 trp1-Δ1 lys2-801 leu2*::*PET56 spt15-Δ2* + p2DN-1 h/h/y [*TRP1*] [7]) by a two-step gene replacement. Using a plasmid-shuffle approach, p2DN-1 h/h R231K was substituted for p2DN-1 h/h/y, generating strains FP126b and FP127b. FP126b and FP127b were transformed to Leu^+ with plasmids pYX315, pYX315-Sp1, and pYX315-GCN4/Sp1, generating strains FP154 through FP159 (Table 1).

To assay for activated *his3* transcription, strains were purified on medium lacking leucine (FP113 through FP118, FP154 through FP159) or lacking leucine and tryptophan (FP119 through FP124). Isolated colonies were then streaked on aminotriazole (AT) plates (SD + Ade, Lys, Ura, 2% agar, 5 mM AT), and the plates were incubated at 30°C for 3 days. To test for inhibition of transcription, strains were purified on minimal medium lacking leucine and then were tested for their relative growth rates on rich medium (1% yeast extract, 2% peptone, 2% dextrose, 2% agar [YPD]) or on minimal medium lacking histidine (SD + Ade, Leu, Lys, Ura, Trp, 2% agar). The results shown in Fig. 1 to 5 were identical for four colonies tested for each strain.

Preparation of whole-cell extracts and Western blotting (immunoblotting). Strains were grown to mid-log phase in glucose minimal medium lacking leucine (SD + Ade, His, Lys, Trp, Ura). Cells were harvested and whole-cell extracts were prepared as described previously (7). Total protein from each extract (75 μg) was fractionated on a sodium dodecyl sulfate–8% polyacrylamide gel and electroblotted onto Immobilon-P PVDF membrane (Millipore). Affinity-purified anti-Sp1 polyclonal antibody (Santa Cruz Biotechnology) was used at a final

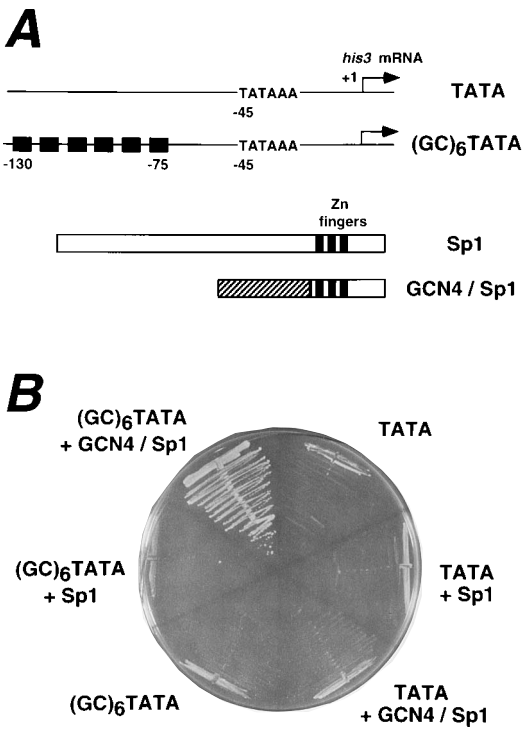

FIG. 1. Sp1 fails to stimulate transcription of the *his3* gene in yeast cells. (A) Diagrammed are the structures for the minimal *his3* promoter (TATA), the *his3* promoter containing six Sp1 binding sites upstream of the TATA element ($[GC]_6$TATA), and the relative structures of Sp1 and a hybrid GCN4/Sp1 activator, containing the acidic activation domain of GCN4 (hatched area) fused to the DNA-binding domain of Sp1 (see Materials and Methods for constructions). (B) Strains containing the indicated *his3* gene and harboring either expression vector pYX315 (no insert), pYX315-Sp1, or pYX315-GCN4/Sp1 were tested for their ability to grow on media containing 5 mM AT, a competitive inhibitor of the *his3* gene product (starting from upper right and moving clockwise, FP113 to FP118). Shown are the growth phenotypes after 3 days at 30°C.

concentration of 1 μg/ml, followed by a 1:15,000 dilution of goat anti-rabbit immunoglobulin G-specific antibody conjugated to horseradish peroxidase (Jackson Laboratories). The membrane was developed with Amersham ECL detection kits and exposed to Amersham Hyperfilm-MP.

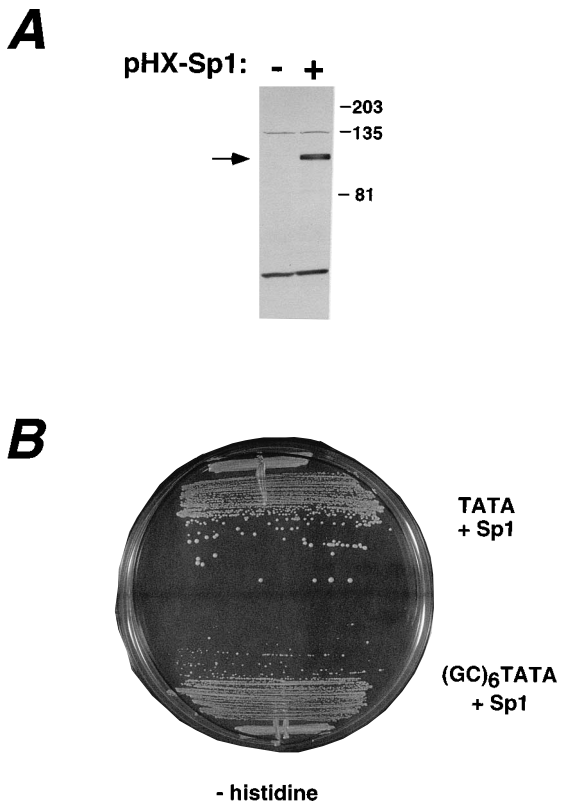

FIG. 2. Expression of Sp1 in *S. cerevisiae*. (A) Western blot analysis. Strain FP104 was transformed with control vector pRS425 (lane 1, −) or with pHX-Sp1 (lane 2, +), which contains the entire Sp1 cDNA under the control of the yeast ADH1 promoter. Whole-cell extracts (75 μg) were prepared and fractionated on a sodium dodecyl sulfate–8% polyacrylamide gel, electroblotted onto polyvinylidene difluoride membrane, and incubated with affinity-purified anti-Sp1 polyclonal antibody (Santa Cruz Biotechnology) followed by secondary goat anti-rabbit immunoglobulin G-specific antibody conjugated to horseradish peroxidase (Jackson Laboratories). The membrane was developed with Amersham ECL detection kits and exposed to Amersham Hyperfilm-MP. The arrow indicates the mobility of Sp1; the mobilities of molecular size marker proteins (in kilodaltons) are indicated at the right. (B) Overexpression of Sp1 inhibits *his3* transcription in a GC box-dependent manner. Strains containing the indicated *his3* gene and harboring pHX-Sp1 (top, FP103; bottom, FP105) were tested for their ability to grow on media lacking histidine. The plate was incubated at 30°C for 4 days.

RESULTS

Human Sp1 does not stimulate transcription in *S. cerevisiae*. To determine whether the glutamine-rich activation domains of human Sp1 function in yeast cells, we constructed yeast strains that contained either a minimal *his3* promoter (TATA only, FP102) or contained a *his3* promoter with six Sp1 binding sites (GC boxes) upstream of the TATA element (FP104) (Fig. 1A). *his3* was chosen as a reporter gene for Sp1 function since transcriptional activation of *his3* can be easily monitored by a sensitive and well-established plate assay utilizing AT, a competitive inhibitor of the *his3* gene product. The test strains were initially transformed with a yeast expression vector expressing the full-length mammalian Sp1 cDNA (*LEU2* as a selectable marker) or with the expression vector alone as a control. Leu^+ transformants were selected on medium lacking leucine, purified once on the same medium, and then were tested for their ability to grow on medium containing 5 mM AT. The results demonstrated that Sp1 did not impart an increase in AT resistance in either test strain (Fig. 1B). The use of AT at 5 mM with these strains would have allowed for as low as a twofold increase in *his3* expression to be readily detected (unpublished results). We also examined the properties of a hybrid GCN4/Sp1 protein, designated pYX315-GCN4/Sp1, that contains the GCN4 acidic activation domain (amino terminal) fused in frame to the DNA-binding domain of Sp1 (carboxy terminal) (Fig. 1A). When introduced into strains FP102 and FP104, this construct stimulated *his3* transcription, but only in the strain containing GC boxes in the *his3* promoter (Fig. 1B). This result demonstrated that the DNA-binding domain of Sp1 does bind to its cognate sequence in *S. cerevisiae*.

Further evidence for Sp1 binding to the GC boxes was obtained by analyzing the overexpression of Sp1. Plasmid pHX-Sp1, containing Sp1 under the control of the yeast ADH1 promoter, was transformed into strains FP102 and FP104. Western blot analysis demonstrated that the Sp1 synthesized was of the expected molecular size for full-length protein (approximately 95 kDa; Fig. 2A). When tested for activation of *his3* transcription, the overexpression of Sp1 did not result in an enhanced ability to grow in the presence of 5 mM AT for either strain (data not shown). More significantly, when tested for the ability to grow on medium lacking histidine, the strain containing only the TATA element in the *his3* promoter grew well when harboring pHX-Sp1 (Fig. 2B) whereas overexpres-

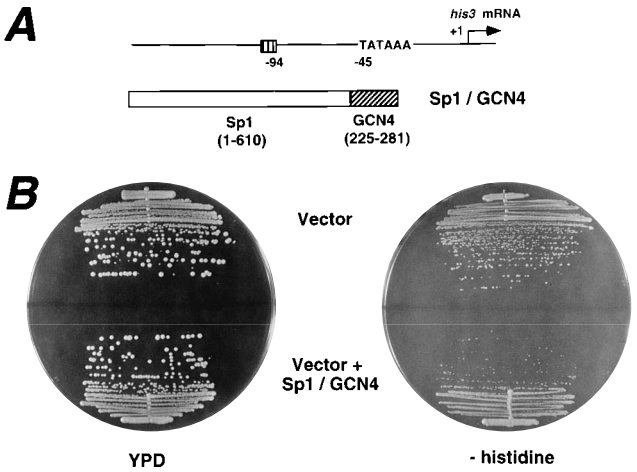

FIG. 3. Inhibition of *his3* transcription by an Sp1 activation region/GCN4 DNA-binding domain fusion protein. (A) Diagrammed are the structures of the *his3* promoter containing a single GCN4 binding site and of a hybrid Sp1/GCN4 activator containing the entire Sp1 activation region fused to the DNA-binding domain of GCN4. (B) Strains containing the above-diagrammed *his3* gene, a deletion of the endogenous *gcn4* gene, and harboring either vector pYX315 (strain FP110) or pYX315-Sp1/GCN4 (strain FP112) were tested for their ability to grow on rich medium (YPD) or on medium lacking histidine. The YPD plate was incubated at 30°C for 3 days; the −histidine plate was incubated for 5 days.

sion of Sp1 in the strain containing the GC boxes in the *his3* promoter resulted in a significant reduction in the growth rate on plates lacking histidine (Fig. 2B). These results demonstrated that Sp1 binds to the *his3* promoter in vivo and partially inhibits *his3* transcription. A similar result was obtained in the analysis of a second hybrid activator, designated pYX315-Sp1/GCN4. This construct contains the entire Sp1 activation region (amino terminal, containing both glutamine-rich regions) fused to the DNA-binding domain of GCN4 (carboxy terminal) (Fig. 3A). When introduced into a strain deleted for *gcn4* and containing a *his3* promoter with a single GCN4 binding site, this Sp1/GCN4 hybrid did not allow for growth on 5 mM AT (data not shown). Rather, the strain harboring pYX315-Sp1/GCN4 grew more slowly on medium lacking histidine than the corresponding test strain transformed with the expression vector alone as a control (Fig. 3B). The observed inhibition with this construct is identical to that seen with similarly expressed GCN4 mutants defective for transcriptional activation (13). We conclude from these combined results that the glutamine-rich activation regions of Sp1 do not activate transcription in *S. cerevisiae*.

Test for Sp1 activation in *S. cerevisiae* containing endogenous yeast TBP along with human or yeast-human TBP hybrids. Biochemical experiments have shown that yeast and human TBP differ in their ability to support an Sp1 response in a human reconstituted system (24). This led us to test whether the introduction of human TBP or human-yeast TBP hybrids into *S. cerevisiae* along with Sp1 would result in an Sp1-dependent stimulation of *his3* transcription. Strains FP102 and FP104 were first transformed with the Sp1-expressing plasmid pYX315-Sp1 and subsequently with a human or human-yeast TBP plasmid (see Materials and Methods). The plasmids used express either the full-length human TBP (h/h), a hybrid TBP containing the human amino-terminal region fused to the yeast conserved carboxy-terminal region (h/y), or a hybrid containing the amino-terminal yeast region fused to the human conserved region (y/h) (Fig. 4A) (7). Strains containing both the Sp1 plasmid and a human TBP plasmid were purified once,

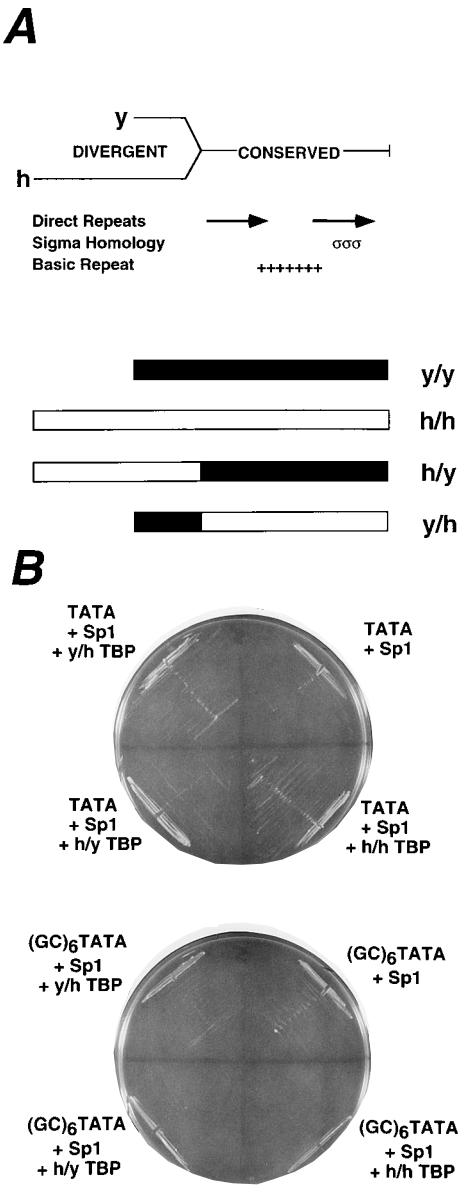

FIG. 4. Test for Sp1 activation of *his3* in strains doubly transformed with Sp1 and either human TBP or a human-yeast TBP hybrid. (A) The structures of the TBP molecules are presented schematically with sequences derived from yeast (solid) and human (open) TBP indicated. (B) Strains containing a *his3* gene with a minimal promoter (TATA, top plate) or with a promoter containing six Sp1 binding sites upstream of the TATA element ($[GC]_6$TATA, bottom plate) were doubly transformed with pYX315-Sp1 along with the indicated TBP plasmid (top plate, strains FP114, FP119 to 121; bottom plate, strains FP117, FP122 to 124). Shown are the growth phenotypes on media containing 5 mM AT after 3 days at 30°C.

maintaining selection for the presence of both plasmids, and then were tested for their ability to grow in the presence of 5 mM AT. As shown in Fig. 4B, in no case did the strains acquire an enhanced ability to grow in the presence of AT. These results indicate that the presence of human or human-yeast TBP is not sufficient to allow Sp1 activation function in *S. cerevisiae*.

Test for Sp1 activation in *S. cerevisiae* containing a human TBP mutant as the sole source of TBP. The above results demonstrated that the introduction of human TBP or human-

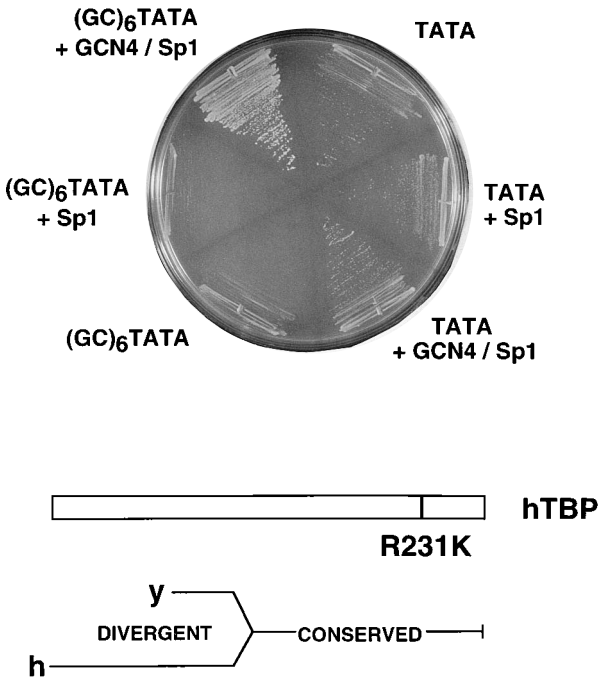

FIG. 5. Test for Sp1 activation of *his3* in strains containing human TBP R231K as the sole source of TBP. The strains contained a deletion of the yeast TBP gene (*SPT15*) and harbored a plasmid encoding the human TBP R231K mutant. The strains also contained a *his3* gene with either a minimal promoter (TATA) or a promoter with six Sp1 binding sites upstream of the TATA element and harbored either expression vector pYX315 (no insert), pYX315-Sp1, or pYX315-GCN4/Sp1 (containing the acidic activation domain of GCN4 fused to the DNA-binding domain of Sp1). Shown are the growth phenotypes on media containing 5 mM AT after 3 days at 30°C (starting from upper right and moving clockwise, FP154 to FP159).

yeast TBP hybrids along with Sp1 did not result in Sp1 activation of *his3*. In earlier work, we have shown that these TBP derivatives are expressed to the same level as endogenous yeast TBP (7). To circumvent the possibility that the endogenous yeast TBP was competing with the introduced human TBP at the *his3* promoter and masking an Sp1 response, we performed a final series of experiments in yeast strains deleted for the endogenous yeast TBP. Since human TBP cannot support cell growth of such a strain (7), our analysis was performed with a human TBP mutant that contains a single arginine-to-lysine substitution at position 231. This mutant is capable of supporting yeast cell viability as the sole source of TBP, presumably because of an enhanced ability to mediate transcription of Pol I, Pol III, and TATA-less Pol II promoters (8). Yeast strains that harbored this derivative as the sole source of TBP and contained either a minimal *his3* promoter or a *his3* promoter with six GC boxes upstream of the TATA element were constructed. These strains were transformed with constructs expressing Sp1 or the GCN4 activation domain/Sp1 DNA-binding domain hybrid and analyzed on AT as previously described. As shown in Fig. 5, activated *his3* transcription was evidenced only with the GCN4/Sp1 hybrid activator in the strain containing GC boxes.

DISCUSSION

We have shown that the human transcriptional activator Sp1, which contains two glutamine-rich activation domains, does not stimulate transcription of a *his3* reporter gene in *S. cerevisiae*. In contrast, a hybrid GCN4/Sp1 activator, containing the acidic activation domain of GCN4 fused to the DNA-binding domain of Sp1, did activate transcription of such a reporter in a GC box-dependent fashion. Overexpression of Sp1 did not reveal any activation activity but rather was found to inhibit *his3* transcription in a GC box-dependent fashion. Similarly, a reciprocal Sp1/GCN4 hybrid, containing the entire activation region of Sp1 fused to the DNA-binding domain of GCN4, was found to inhibit transcription of a *his3* gene containing a single GCN4 binding site in the promoter. The introduction of human TBP or various human-yeast TBP hybrids along with Sp1 into *S. cerevisiae* in no case resulted in any detectable Sp1 activation of *his3*. We conclude that the glutamine-rich activation domains of Sp1 do not stimulate transcription of the yeast RNA polymerase II transcription machinery. Our results are consistent with those recently reported examining the activity of a GAL4/Sp1 fusion protein in *S. cerevisiae* (19).

The failure of the Sp1 glutamine-rich regions to stimulate yeast Pol II transcription could be the result of several possibilities. First, Sp1 is known to be posttranslationally modified by both O-linked glycosylation and phosphorylation in HeLa cells (14, 15). Therefore, Sp1 might not function in *S. cerevisiae* because of the lack of either of these modifications. This is unlikely to be the explanation for the lack of detectable Sp1 activity, since *E. coli*-synthesized Sp1 still possesses significant activity in vitro (16). A second possibility is that Sp1 does not function in *S. cerevisiae* because of the divergence between yeast and human TBPs. In this regard, it has been recently demonstrated that the glutamine-rich regions of Sp1 can bind directly and specifically to the conserved domain of human TBP but much less strongly to yeast TBP (9). However, our results reported here utilizing human-yeast TBP hybrids, and more significantly, the human TBP R231K mutant, argue strongly that the divergence between human and yeast TBP is not the underlying cause of the inability of Sp1 to activate in *S. cerevisiae*. A more likely explanation is that Sp1 requires additional factors for function that are absent in *S. cerevisiae* or whose function is not conserved between humans and *S. cerevisiae*.

It has been reported that acidic, proline-rich, and glutamine-rich activation domains appear to function during multiple steps of preinitiation complex assembly in a human reconstituted system in vitro (6). These steps include an initial and direct recruitment of the general transcription factor TFIIB, and a second (or more) step after TFIIB association that requires the presence of TAFs. Since acidic activators display species interchangeability for function, it suggests that the mechanisms and components involved in these steps stimulated by acidic activation domains are conserved between *S. cerevisiae* and humans. In contrast, the lack of Sp1 function in *S. cerevisiae* suggests that one or more of these steps are not mechanistically conserved for the glutamine-rich activation domains. Thus, Sp1 might not function in *S. cerevisiae* because (i) Sp1 is unable to increase the recruitment of yeast TFIIB, (ii) Sp1 is unable to functionally interact with one of the other yeast GTFs recruited after TFIIB; or (iii) *S. cerevisiae* lacks a functional equivalent of at least one of the TAFs required for Sp1 activity. In this regard, it has been shown that the Sp1 glutamine-rich activation domains can specifically interact with the *Drosophila* TAF110 (12), that the combination of two of the *Drosophila* TAFs (TAF250 and TAF110) along with TBP can support partial Sp1 activation (31), and that the combination of three of the *Drosophila* TAFs (TAF250, TAF150, and TAF110) along with TBP can support robust Sp1 activation (4). The continued characterization of the factors involved in activation should provide useful information to-

wards understanding the basis for evolutionary conservation or divergence in the actions of various activators.

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REFERENCES

1. **Attardi, L. D., and R. Tjian.** 1993. *Drosophila* tissue-specific transcription factor NTF-1 contains a novel isoleucine-rich activation motif. Genes Dev. **7:**1341–1353.
2. **Brent, R., and M. Ptashne.** 1985. A eukaryotic transcriptional activator bearing the DNA specificity of a prokaryotic repressor. Cell **43:**729–736.
3. **Chasman, D. I., J. Leatherwood, M. Carey, M. Ptashne, and R. D. Kornberg.** 1989. Activation of yeast polymerase II transcription by herpesvirus VP16 and GAL4 derivatives in vitro. Mol. Cell. Biol. **9:**4746–4749.
4. **Chen, J.-L., L. D. Attardi, C. P. Verrijzer, K. Yokomori, and R. Tjian.** 1994. Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. Cell **79:**93–105.
5. **Chen, W., and K. Struhl.** 1988. Saturation mutagenesis of a yeast his3 "TATA element": genetic evidence for a specific TATA-binding protein. Proc. Natl. Acad. Sci. USA **85:**2691–2695.
6. **Choy, B., and M. R. Green.** 1993. Eukaryotic activators function during multiple steps of preinitiation complex assembly. Nature (London) **366:**531–536.
7. **Cormack, B. P., M. Strubin, A. S. Ponticelli, and K. Struhl.** 1991. Functional differences between yeast and human TFIID are localized to the highly conserved region. Cell **65:**341–348.
8. **Cormack, B. P., M. Strubin, L. A. Stargell, and K. Struhl.** 1994. Conserved and non-conserved functions of yeast and human TATA-binding proteins. Genes Dev. **8:**1335–1343.
9. **Emili, A., J. Greenblatt, and C. J. Ingles.** 1994. Species-specific interaction of the glutamine-rich activation domains of Sp1 with the TATA box-binding protein. Mol. Cell. Biol. **14:**1582–1593.
10. **Fischer, J. A., E. Giniger, T. Maniatis, and M. Ptashne.** 1988. GAL4 activates transcription in *Drosophila*. Nature (London) **332:**853–856.
11. **Hernandez, N.** 1993. TBP, a universal eukaryotic transcription factor? Genes Dev. **7:**1291–1308.
12. **Hoey, T., R. O. Weinzierl, G. Gill, J. L. Chen, B. D. Dynlacht, and R. Tjian.** 1993. Molecular cloning and functional analysis of *Drosophila* TAF110 reveal properties expected of coactivators. Cell **72:**247–260.
13. **Hope, I. A., and K. Struhl.** 1986. Functional dissection of a eukaryotic transcriptional activator protein, GCN4 of yeast. Cell **46:**885–894.
14. **Jackson, S. P., J. J. MacDonald, S. Lees-Miller, and R. Tjian.** 1990. GC box binding induces phosphorylation of Sp1 by a DNA-dependent protein kinase. Cell **63:**155–165.
15. **Jackson, S. P., and R. Tjian.** 1988. O-glycosylation of eukaryotic transcription factors: implications for mechanisms of transcriptional regulation. Cell **55:**125–133.
16. **Kadonaga, J. T., A. J. Courey, J. Ladika, and R. Tjian.** 1988. Distinct regions of Sp1 modulate DNA binding and transcriptional activation. Science **242:**1566–1570.
17. **Kakidani, H., and M. Ptashne.** 1988. GAL4 activates gene expression in mammalian cells. Cell **52:**161–167.
18. **Keegan, L., G. Gill, and M. Ptashne.** 1986. Separation of DNA binding from the transcription-activating function of a eukaryotic regulatory protein. Science **231:**699–704.
19. **Kunzler, M., G. H. Braus, O. Georgiev, K. Seipel, and W. Schaffner.** 1994. Functional differences between mammalian transcription activation domains at the yeast GAL1 promoter. EMBO **13:**641–645.
20. **Ma, J., E. Przibilla, J. Hu, L. Bogorad, and M. Ptashne.** 1988. Yeast activators stimulate plant gene expression. Nature (London) **334:**631–633.
21. **Mitchell, P., and R. Tjian.** 1989. Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. Science **245:**371–378.
22. **Ponticelli, A. S., and K. Struhl.** 1990. Analysis of *Saccharomyces cerevisiae his3* transcription in vitro: biochemical support for multiple mechanisms of transcription. Mol. Cell. Biol. **10:**2832–2839.
23. **Pu, W. T., and K. Struhl.** 1991. Highly conserved residues in the bZIP domain of yeast GCN4 are not essential for DNA binding. Mol. Cell. Biol. **11:**4918–4926.
24. **Pugh, B. F., and R. Tjian.** 1990. Mechanism of transcriptional activation by Sp1: evidence for coactivators. Cell **61:**1187–1197.
25. **Pugh, B. F., and R. Tjian.** 1992. Diverse transcriptional functions of the multisubunit eukaryotic TFIID complex. J. Biol. Chem. **267:**679–682.
26. **Sharp, P. A.** 1992. TATA-binding protein is a classless factor. Cell **68:**819–821.
27. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
28. **Smale, S. T., and D. Baltimore.** 1989. The "initiator" as a transcription control element. Cell **57:**103–113.
29. **Struhl, K.** 1988. The JUN oncoprotein, a vertebrate transcription factor, activates transcription in yeast. Nature (London) **332:**649–650.
30. **Webster, N., J. R. Jin, S. Green, M. Hollis, and M. Chambon.** 1988. The yeast UAS_G is a transcriptional enhancer in human HeLa cells in the presence of the GAL4 *trans*-activator. Cell **52:**169–178.
31. **Weinzierl, R. O., B. D. Dynlacht, and R. Tjian.** 1993. Largest subunit of *Drosophila* transcription factor IID directs assembly of a complex containing TBP and a coactivator. Nature (London) **362:**511–517.
32. **Zawel, L., and D. Reinberg.** 1993. Initiation of transcription by RNA polymerase II: a multi-step process. Prog. Nucleic Acids Res. Mol. Biol. **44:**67–108.