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Yeast GAL11 protein is a distinctive type transcription factor that enhances basal transcription *in vitro*

(core promoter/general regulatory factor 1/GAL4 derivatives/*Saccharomyces cerevisiae*)

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ABSTRACT **The yeast auxiliary transcription factor GAL11, a candidate for the coactivator, was partially purified from yeast cells, and its function was characterized in a cell-free transcription system. The partially purified GAL11 protein stimulated basal transcription from the *CYC1* core promoter by a factor of 4–5 at the step of preinitiation complex formation. GAL11 protein also enhanced transcription activated by general regulatory factor 1, GAL4-AH, or GAL4-VP16 to the same extent as the basal transcription. Therefore, the apparent potentiation of the activators by GAL11 was attributable to the stimulation of basal transcription. The wild-type GAL11 protein (but not a mutant-type protein) produced in bacteria stimulated transcription as effectively as GAL11 from yeast. These results suggest that GAL11 functions as a positive cofactor of basal and activator-induced transcription in a cell-free transcription system.**

Eukaryotic class II promoters consist of two functionally distinct elements—core promoters and enhancers [termed upstream activation sequences (UASs) in yeast]. Core promoters contribute to basal and faithful transcription by interacting with the general transcription factors that include RNA polymerase II and a set of proteins required for setting the accurate initiation site (1, 2). Enhancers and UASs provide DNA sequences recognized by specific DNA-binding proteins that regulate transcriptional efficiency of the genes (3). However, it remains to be elucidated how the DNA-binding proteins transmit their regulatory signals to the general transcription factors. It is conceivable that the DNA-binding proteins exert their effects either through intermediary factors termed coactivators (sometimes called mediators or adaptors) or directly on the general transcription factors (4). Candidates for coactivators, which are required for the activator-induced transcription but not for the basal transcription, have been identified in yeast (5–9) and mammalian cells (10–13).

We had previously reported that the regulatory protein GAL11 of the yeast *Saccharomyces cerevisiae* (14) is an auxiliary activator for the galactose-inducible genes (15) and later suggested it to be a candidate for the coactivator (16); GAL11 protein transmits the activation signals of two distinct activators, namely GAL4, an activator of the galactose-inducible genes (17), and general regulatory factor 1 (GRF1), a transcription factor required for activation of various genes (18, 19) and for repression of the silent mating-type cassettes (20). Successive studies (16) suggested the possibility that GAL11 stabilized the interaction between GAL4 or GRF1 and the general transcription factors only when UASs were far from the TATA box, since *GAL11* did not exert its function on GAL4 or GRF1 bound close to the TATA element. In parallel, Himmelfarb *et al.* (21) isolated a mutant that potentiates a weak activator GAL4-AH. Surprisingly, the mutation is a missense mutation within *GAL11* and was therefore designated *GAL11P* (P stands for potentiator). They suggest a direct interaction between GAL11 and GAL4 molecules and suggest that the complex of the two proteins is a strong activator. They further find that *GAL11* is required for normal functioning of PPR1, an activator for the *URA3* gene. Puzzlingly, *GAL11* is also involved in the transcriptional repression of genes, since a loss-of-function mutation of *GAL11*, called *spt13*, restores the transcription of genes inactivated by insertion of the yeast transposon Ty (22).

To get insight into the complex pleiotrophic functions of *GAL11 in vivo*, we analyzed the role of GAL11 biochemically. We have partially purified GAL11 protein and studied how it works in a cell-free transcription system. We have found that GAL11 enhances the basal transcription as well as the transcription activated by GAL4-AH, GAL4-VP16, or GRF1. These results suggest that, since GAL11 regulates the basal transcription, it is not a *bona fide* coactivator, but rather it belongs to a distinctive class of basal transcription factors.

MATERIALS AND METHODS

Construction of Plasmids. Template DNAs for *in vitro* transcription were constructed as follows. To create pSK113, pGAL4CG$^-$ (23) was digested with *Xho* I and *Sma* I, and the liberated *CYC1* promoter–G-free cassette fusion was cloned into the *Xho* I and *Xba* I (blunt-ended by the Klenow fragment) sites of pGEM-7Zf(+) (Stratagene). The 5′ end of the *CYC1* promoter of pSK113 is nucleotide position −138 with respect to the translation initiation site at +1 (24). pSK114 was constructed by replacing the *Xho* I–*Ava* II region (from −138 to −68) of pSK113 with the *Xho* I–*Ava* II fragment (from −248 to −68) of the authentic *CYC1* promoter (24). Two tandem copies of GRF1-binding sequence found in telomeres (UAS_{TEL}, see ref. 18) or a single copy of GAL4-binding sequence (UAS_G) of pGAL4CG$^-$ were inserted between the *Bam*HI and *Eco*RI sites of pSK113 and pSK114, such that the distance between the UAS and the TATA box (position −123; see ref. 24) becomes 29 and 132 base pairs (bp), respectively. The UAS-less template pSK115 was constructed by deleting the region between the *Bam*HI and *Eco*RI sites of pSK113. pJJ460 (25) has essentially the same construction to pSK115, except that pJJ460 carries a shorter G-free sequence. GAL11-overexpression plasmid pSK7 was constructed by subcloning the 4.4-kilobase (kb) *Hpa* I fragment of pYM1201 (15) into pTV3 (26). We have recently found errors at three positions in the 5′ region of the *GAL11* nucleotide sequence, correction of which led to an elongation of the coding region beyond the previous 5′ end by 117 codons (15, 27). The major start site of GAL11 mRNA was

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Abbreviations: UAS, upstream activation sequence; GRF1, general regulatory factor 1; GST, glutathione *S*-transferase.

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located at −193 with respect to the first ATG at +1 (data not shown). A TATA box-like sequence (TATATTA) was found 60-bp upstream of the major transcription start site. Plasmid pGST-G11, a fusion gene of a glutathione *S*-transferase (GST) gene and *GAL11*, was constructed by inserting the 3.5-kb *Dra* I–*Sca* I fragment (from −9 to +3496) of pYM1201 encompassing the entire open reading frame (ORF) of *GAL11* (15, 27) into the *Sma* I site of pGEX-3X (Pharmacia). pGST-G11m was constructed by removal of the 192-bp *Eco*RV–*Nru* I fragment (from −2595 to −2786) from pGST-G11. Reporter plasmid pSK235 was an integrating-type plasmid derived from pLG670Z (28), a YEp24 plasmid bearing a *CYC1-lacZ* fusion. pSK236 and pSK237 contained UAS_{TEL} at the *Xho* I site (at −248) and at the *Sph* I site (at −138; converted to a *Xho* I site) of pSK235, respectively.

Purification of GAL11 Protein. The recovery of GAL11 protein was determined by immunoblot probed with anti-GAL11 antibody raised against a GAL11 peptide produced in *Escherichia coli*. Cells of strain 20B-12 (*MATa pep4-3 trp1*) harboring pSK7 were grown in 8 liters of tryptophan-omission medium (15) to late logarithmic phase, harvested, and washed once with cold distilled water. (The following manipulations were performed at 4°C, and all buffers contained 1 mM dithiothreitol and protease inhibitor mixture; ref. 29). Cells were suspended with an equal volume of buffer A [0.2 M Tris chloride/1 M ammonium sulfate/2 mM EDTA/20% (vol/vol) glycerol, pH 8.5] and were disrupted with glass beads as described (18). Glass beads were washed with an equal volume of buffer A, and the combined cell lysate was clarified by centrifugation at 10,000 × *g* for 10 min and then at 100,000 × *g* for 1 hr. Clear supernatant was added to a half volume of saturated ammonium sulfate solution, stirred for 40 min, and centrifuged at 50,000 × *g* for 45 min. The precipitate (240 mg of protein) was dissolved with buffer B (50 mM Tris chloride, pH 8.0/1 mM EDTA/0.1% Nonidet P-40/10% glycerol) to give a protein concentration of 4 mg/ml, and the solution was directly loaded on a 90-ml DE-52 (Whatman) column equilibrated with buffer B containing 0.08 M NaCl. The column was washed with the same buffer and developed with a linear gradient from 0.08 M to 0.25 M NaCl in buffer B. The NaCl concentration of the pooled fractions (0.15–0.2 M NaCl containing 26 mg of protein) was adjusted to 0.35 M by addition of 1 M NaCl in buffer B, and the sample was fractionated on a 6-ml heparin agarose (Bethesda Research Laboratories) column with 0.35–0.8 M NaCl gradient in buffer B. Fractions in the range from 0.4 M to 0.5 M NaCl were pooled (3.9 mg of protein) and applied to an 80-ml Sepharose CL-4B (Pharmacia) column equilibrated with buffer D (10 mM Hepes·NaOH, pH 7.6/1 mM EDTA/0.1% Nonidet P-40/10% glycerol) containing 0.1 M potassium acetate. GAL11-containing fractions were collected (0.6 mg of protein), and the potassium phosphate concentration of the pooled fraction was adjusted to 0.02 M. The sample was loaded on a 1-ml hydroxylapatite (Bio-Rad) column equilibrated with buffer E (10 mM Hepes·NaOH, pH 7.6/0.1 M potassium acetate/0.03% Nonidet P-40/10% glycerol) containing 0.02 M potassium phosphate. GAL11 was eluted at 0.05 M potassium phosphate in a 0.02–0.15 M potassium phosphate gradient in buffer E. The GAL11-containing fraction was applied on a 1-ml Mono S (Pharmacia) column equilibrated with 0.1 M potassium acetate in buffer D. The column was developed with a 0.1–0.5 M potassium acetate gradient, and 0.5-ml fractions were collected. GAL11 protein was eluted at 0.35 M potassium acetate in buffer D and stored at −80°C.

GST-GAL11 fusion protein was expressed in *E. coli* strain JM109 carrying pGST-G11 or pGST-G11m as described (30) except that the expression was induced for 3 hr by the addition of 0.5 mM isopropyl thiogalactoside. Cells from 250 ml of culture were suspended with 5 ml of buffer A and disrupted by sonication. Cleared lysate was diluted 1:4 with cold distilled water, and the concentration of Triton X-100 and pH were adjusted to 0.2% and 7.0, respectively. Fusion protein was purified on a 1.5-ml glutathione agarose (Sigma) column (30) followed by Mono S column chromatography as above. GST-GAL11 and GST-GAL11m were eluted at 0.35 and 0.25 M potassium acetate from a Mono S column, respectively.

Purification of GAL4 Derivatives. GAL4-AH and GAL4-VP16 were expressed in *E. coli* strain JM109 and purified as described (31).

***In Vitro* Transcription.** Yeast nuclear extract (29) was prepared from strain HS301 (*MATa ura3-52 leu2 trp1 prb1-1122 pep4-3 prc1-407 gal2 gal11::LEU2*), which was constructed by disrupting the *GAL11* gene of strain BJ2168. Transcription assay was carried out for 1 hr at 20°C in a 20-μl reaction mixture containing 40 ng of template DNA and 70 μg of protein from yeast nuclear extract in 50 mM Hepes·KOH, pH 7.3/80 mM potassium acetate/5 mM magnesium acetate/5 mM $MgSO_4$/5 mM EGTA/0.5 mM EDTA/2 mM dithiothreitol/0.05% Nonidet P-40/10% glycerol/0.2 unit of Inhibit-ACE (5′–3′)/4 mM phospho*enol*pyruvate/0.4 mM ATP/0.4 mM CTP/0.1 mM 3′-*O*-methyl-GTP/10 μM UTP/5 μCi (185 kBq) of [α-^{32}P]UTP (400 Ci/mmol). Transcripts were isolated and analyzed by 6% polyacrylamide/7 M urea gel as described (23). In our assay system, basal transcription reaction produced 0.12 fmol of transcripts from 18.7 fmol of template, for 0.006 transcript per template. All of the experiments described here were performed at least three times.

RESULTS

***In Vivo* Effect of GAL11 Null Mutation on the Expression of GRF1-Binding Site-Bearing Reporter Genes.** We have previously shown that the *gal11* mutations result in reduced expression of some of the genes whose transcription is activated by GRF1 and that the expression of a reporter gene *PYK1-xylE* bearing a GRF1-binding site is reduced by a factor of 7–8 in *gal11* (16). However, since this reporter contained the authentic 5′ upstream sequence of *PYK1*, it was conceivable that some factor(s) other than GRF1 participated in the function of *GAL11*. To test whether *GAL11* directly regulates the activation function of GRF1, we employed a simple reporter gene, *CYC1-lacZ*, since its expression seemed to be controlled only by the UAS introduced upstream of its core promoter (28). Two tandem repeats of GRF1-binding sequence (UAS_{TEL}) were inserted at 132- or 29-bp upstream of the TATA box of *CYC1*, and these reporters were integrated into the *ura3* gene of wild-type or *gal11* null yeasts. The expression of the UAS_{TEL}-*CYC1-lacZ* reporter genes was activated about 60- to 90-fold in the wild-type yeast probably because of binding of GRF1 to the UAS_{TEL}. In the *gal11* mutant, the β-galactosidase activity of each reporter was reduced by a factor of 1.6 or 2.2 in comparison with that in the wild-type yeast (Table 1). If the reporters were introduced into the yeasts as a multicopy plasmid, the expression was reduced similarly in *gal11* (data not shown). Since, the effect of *GAL11* on the expression of the UAS_{TEL}-*CYC1-lacZ* reporter genes was smaller than the previous observation with *PYK1* as a reporter gene (16), we suggested that the effect of *GAL11* was amplified by some factor(s) other than GRF1 in the case of the native *PYK1* gene or by difference of the promoter context between *PYK1* and *CYC1*. Note that the expression of *CYC1-lacZ* without a UAS was also reduced in the *gal11* mutant (Table 1). In contrast to this integrated reporter, the *gal11* mutation increased the expression of *CYC1-lacZ* on a multicopy plasmid by a factor of 1.5 (data not shown). Similar results were documented by Chen *et al.* (32). This difference might be due to the structural environment of the reporter gene—i.e., chromosomal or

Table 1. Effect of *GAL11* on the expression of *CYC1-lacZ* reporters

Distance between UAS_{TEL} and TATA box	β-galactosidase activity		Ratio *GAL11*
	GAL11	*gal11*	*gal11*
no UAS	1.0	0.4	2.5
Proximal, 29 bp	88	54	1.6
Distal, 132 bp	66	30	2.2

The yeast strains HSY5-3C (*MATα ade his1 leu2 ura3 trp1*) and its isogenic *gal11* null mutant HSY5-3B (*gal11::LEU2*) were used as hosts for analysis of β-galactosidase activity. The reporter plasmids, pSK235, pSK236, and pSK237, were digested with *SmaI* and integrated into a *ura3* locus of HSY5-3B and HSY5-3C. Three independent transformants were grown to an optical density at 600 nm (OD_{600}) of 1.0 in glucose-containing synthetic medium lacking uracil (15). Assay of β-galactosidase activity was performed as described (28), and units of activity were obtained from the following formula: $OD_{420} \times 1000/(OD_{600} \times$ time in minutes).

episomal state. Since the chromosomal reporter gene should reflect the natural gene regulation more faithfully than the episomal reporter, we suggest that *GAL11* stimulated the core promoter activity of *CYC1*.

Transcriptional Stimulation by GAL11 Protein. To elucidate the function of GAL11 on transcription regulation biochemically, we first purified GAL11 protein from yeast cells. Fig. 1 shows profiles of SDS/polyacrylamide gel electrophoresis of total proteins as well as immunoblot analysis of fractions from the final-step Mono S column chromatography. The 130-kDa band, which was concentrated in fractions 21–24, is the GAL11 protein as judged by the reactivity with anti-GAL11 antibody as well as by its molecular size deduced from the amino acid sequence (15, 27). Thus, we obtained 3 μg of the GAL11 protein in a partially purified fraction starting from 240 mg of soluble protein with an approximate recovery of 10%.

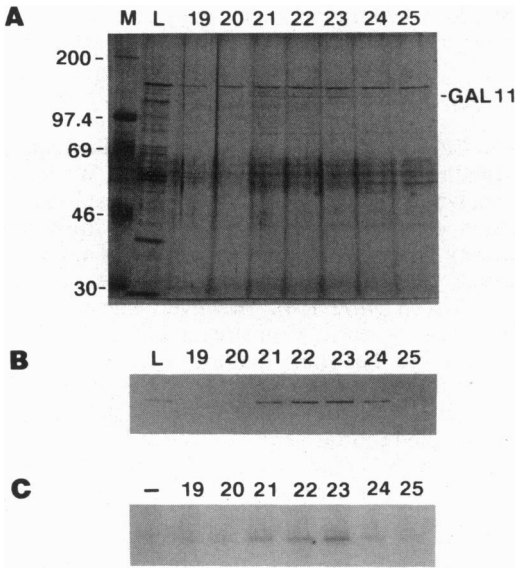

FIG. 1. Partially purified GAL11 protein stimulates basal transcription. Proteins from the hydroxylapatite (lane L) and the Mono S column fractions (numbered lanes) were separated on a SDS/8% polyacrylamide gel and visualized by silver staining (*A*) or analyzed by immunoblotting probed with anti-GAL11 antibody (*B*). The amounts of the samples applied are 16 μl for silver staining and 8 μl for immunoblotting. Lane M contains protein molecular size markers (Amersham), whose molecular sizes in kDa are indicated to the left. Position of the GAL11 protein is indicated to the right. (*C*) Transcription reactions were carried out in the presence (indicated by numbers) or absence (–) of Mono S fractions (5 μl) by using the UAS-less *CYC1* promoter as a template. Transcripts were separated on polyacrylamide/urea gel and visualized by autoradiography. The concentration of template DNA in the reaction is 2 ng/μl, which is much lower than that used in other transcription systems (10–20 ng/μl; refs. 23 and 25). To equalize the approximate molar ratio of the template DNA to GAL11 protein, we reduced the amount of the template DNA to 40 ng, which is equimolar to 6 ng of GAL11 protein contained in 5 μl of fractions 22 and 23. The transcriptional stimulation by GAL11 protein was not significantly affected by salt concentration from 70 to 120 mM potassium acetate or by the amount of the nuclear extract from 60 to 120 μg of protein per 20-μl reaction mixture (data not shown).

To examine the effect of GAL11 protein on transcription, we employed the *in vitro* transcription system developed from yeast nuclei (23, 29). Nuclear extract was prepared from a *gal11*-null strain, and the *CYC1*/G-free sequence fusion was used as a template (23). First we analyzed the effect of GAL11 protein on the basal transcription from the *CYC1* core promoter. Transcription was stimulated by the addition of fractions 21–24 of the Mono S column chromatography, and the stimulatory activity exactly coincided with the peak fractions of GAL11 protein (Fig. 1*C*). This result indicated that GAL11 protein stimulated the basal transcription *in vitro*. Note that the efficiency of stimulation was influenced by a molar ratio between the template DNA and GAL11 protein and that GAL11 protein stimulated transcription about 4-fold under a reaction condition containing equimolar amounts of the template DNA and GAL11 protein (Fig. 1*C*, fractions 22 and 23).

GAL11 Protein Stimulates Activator-Induced Transcription. We then studied the effect of GAL11 on the transcription activated by GRF1 or GAL4 derivatives, since normal functioning of these activators required *GAL11 in vivo* (16). We used template DNAs containing UAS_{TEL} at 29 or 132 bp upstream of the TATA box of *CYC1*. The templates with UAS_{TEL} placed at the proximal or distal positions were transcribed more efficiently than the UAS-less template by a factor of 4 or 2, respectively (Fig. 2*A*). This activation was concluded to be mediated by endogenous GRF1 in the nuclear extracts (data not shown). Densitometric analysis of the autoradiograms revealed that GAL11 stimulated the basal as well as activated transcription by a factor of 3–4 over the control level. As a consequence, addition of GAL11 activated the transcription of the proximal and distal UAS_{TEL} templates by factors 15 and 7, respectively, over the basal

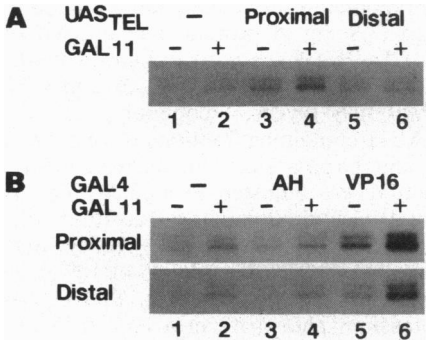

FIG. 2. GAL11 protein stimulates transcription activated by GRF1 or GAL4 derivatives. (*A*) Templates containing no UAS (lanes 1 and 2) or the UAS_{TEL} at the proximal (lanes 3 and 4) or distal (lanes 5 and 6) positions relative to the TATA box of *CYC1* promoter were transcribed *in vitro* in the presence (lanes 2, 4, and 6) or absence (lanes 1, 3, and 5) of GAL11 (5 μl of Mono S fraction 23). Transcripts were analyzed as Fig. 1*C*. (*B*) Template DNAs bearing a single UAS_G at the proximal (*Upper*) or distal (*Lower*) positions were transcribed in the absence (lanes 1 and 2) or presence of 1 pmol of GAL4-AH (lanes 3 and 4) or 0.2 pmol of GAL4-VP16 (lanes 5 and 6). Reactions were run in the presence (lanes 2, 4, and 6) or absence (lanes 1, 3, and 5) of GAL11 (5 μl of Mono S fraction 23).

transcription of the UAS-less templates in the absence of GAL11.

We also investigated the stimulatory effect of GAL11 on the GAL4 derivative-induced transcription, using templates carrying a UAS_G. GAL4-VP16 enhanced the transcription of the proximal and distal UAS_G template about 5- and 3-fold, respectively (Fig. 2*B*). The transcription activated by GAL4-VP16 was further enhanced an additional 4-fold by addition of GAL11. This is approximately the same fold stimulatory effect of GAL11 on the basal transcription. Although GAL4-AH activated transcription less than GAL4-VP16, GAL11 stimulated transcription to a similar extent. We suggest, therefore, that the apparent potentiation of activators by GAL11 was the consequence of stimulation of the basal transcription. Note that the activated transcription was always greater in templates carrying UAS at the proximal position compared with those carrying UAS at the distal position, irrespective of presence or absence of GAL11.

Wild-Type GAL11 Protein but Not Mutant-Type Protein Stimulates Transcription. GAL11 protein purified from yeast was contaminated with other proteins, and it might be suspected that the observed transcription stimulatory effect was due to the proteins other than GAL11. To exclude this possibility, we purified recombinant GST-GAL11 fusion proteins from *E. coli* (Fig. 3*A*). As a control, we also purified a mutant-type GAL11 protein, which deleted only 64 amino acids and could not complement *gal11* null mutations for all the known mutant phenotypes *in vivo* (data not shown). As shown in Fig. 3*B*, the recombinant protein containing full-length GAL11 peptide was as active as the authentic GAL11 protein purified from yeast with respect to the basal transcription as well as to the transcription activated by GAL4-VP16 (lanes 4 and 8). By contrast, the mutant fusion protein exhibited neither activity (lanes 3 and 7). These results strongly suggested that GAL11 itself is responsible for the *in vitro* stimulatory activity.

Probable Involvement of GAL11 Protein in the Transcription Initiation Step. To investigate which step of the transcription reaction was stimulated by GAL11, we conducted a template commitment assay whose protocol is shown in Fig. 4. We used two templates composed of the *CYC1* promoter fused to G-free sequences of different lengths, which produced 370-nucleotide (long) or 280-nucleotide (short) transcripts. To allow the preinitiation complex to be formed, two templates were preincubated separately for 60 min with yeast nuclear extract. One was incubated in the presence of GAL11, while the other was incubated in its absence. The two reaction mixtures were then mixed, and elongation was initiated at 0 or 30 min after mixing. When GAL11 protein was added along with the elongation reaction mixture, no stimulation was observed (data not shown). When the long template was preincubated with nuclear extract in the presence of GAL11, while the short template was preincubated with extract alone, long transcripts were predominantly produced after the two reaction mixtures were combined (Fig. 4, lane 2). After a 30-min challenge with the short template, long transcripts were also predominantly produced over the short transcripts (lane 5). Similarly preincubation of the short template with nuclear extract in the presence of GAL11 yielded short transcripts predominantly over the long transcripts either at 0 or 30 min after mixing (lanes 3 and 6). These results indicated that GAL11 enhanced either the formation or the stability of preinitiation complexes. We may further speculate that GAL11 was irreversibly integrated in the preinitiation complex, since if GAL11 could have been readily dissociated from the preinitiation complex, it should have enhanced the transcription from the challenged template.

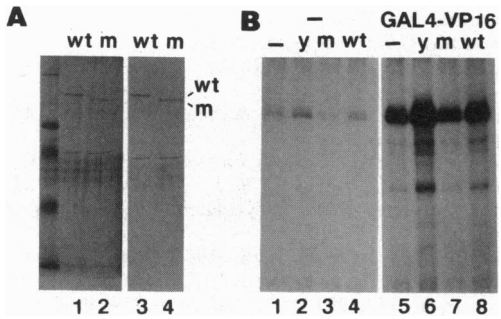

FIG. 3. Recombinant GAL11 protein stimulates transcription. (*A*) The wild-type (wt) (lanes 1 and 3) or mutant-type (m) (lanes 2 and 4) GST-GAL11 fusion proteins produced in *E. coli* (10 ng of protein) were separated on a SDS/8% polyacrylamide gel and visualized by silver staining (lanes 1 and 2) or analyzed by immunoblot probed with anti-GAL11 antibody (lanes 3 and 4). The positions of the fusion proteins are indicated to the right. (*B*) Template DNA containing a single UAS_G at the proximal position was transcribed in the absence (lanes 1–4) or presence of 0.2 pmol GAL4-VP16 (lanes 5–8). The GAL11 protein from yeast (y) (lanes 2 and 6; 5 μl of Mono S fraction 23), mutant-type fusion protein (lanes 3 and 7), or wild-type (lanes 4 and 8) fusion protein (6 ng of protein) was added to the reactions.

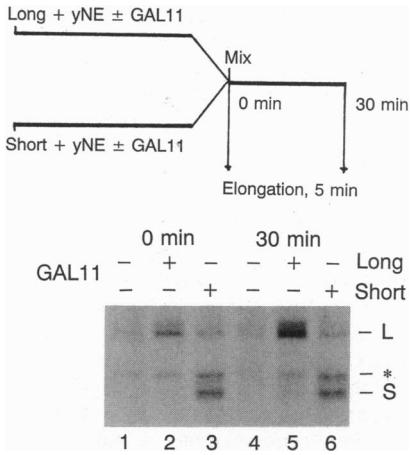

FIG. 4. GAL11 protein functions during the preinitiation step. (*Upper*) Strategy of the template commitment assay. Two templates containing a *CYC1* promoter fused to a long (pSK115) or short (pJJ460) G-free cassette were separately preincubated with yeast nuclear extract in the presence or absence of GAL11 protein for 60 min. Two preincubation mixtures were then combined, and aliquots were removed at the indicated time. Elongation was initiated by addition of four ribonucleotides and allowed to proceed for 5 min. (*Lower*) Result of the template commitment assay. GAL11 protein (5 μl of Mono S fraction 23) was added to the preincubation mixture containing long (lanes 2 and 5) or short (lanes 3 and 6) templates. Elongation was started at 0 (lanes 1–3) or 30 min (lanes 4–6) after the mixing. Positions of transcripts are indicated by L (long) and S (short). The asterisk indicates read-through transcripts from the short template (23).

DISCUSSION

By using a partially purified GAL11 protein from yeast, we have shown that GAL11 was able to enhance the basal transcription as well as the activator-induced transcription in a cell-free transcription system. Recombinant GAL11 protein fused to GST also stimulated transcription as yeast GAL11 did. However, a mutant GST-GAL11 fusion, deleted of a region required for normal functioning of *GAL11 in vivo*, failed to enhance the transcription *in vitro*, indicating that GAL11 protein was really responsible for the *in vitro* activity. Furthermore, the template commitment assay indicated that GAL11 functioned at the step of preinitiation complex for-

mation and suggested that it might be involved in the preinitiation complex. We have suggested that the observed enhancement of the activator-induced transcription by GAL11 was the direct consequence of enhancement of the basal transcription. Consistently, the expression of UAS_{TEL}-*CYC1-lacZ* reporter genes was reduced a similar extent to that of a UAS-less *CYC1-lacZ* reporter in the *gal11* null yeast.

A question arises then; what determines the spectrum of *GAL11* function? In the case of the GAL4-dependent genes, *GAL1*, *GAL7*, or *GAL10* but not *GAL80* are under the control of *GAL11 in vivo* (14, 15). Previously, we considered that the GAL11 dependency was related to the distance between the UAS and the TATA box and showed that the normal functioning of GAL11 was required only when the UAS-TATA distance was long in UAS_G-*PHO5-lacZ* or *PYK1-xylE* reporter genes (16). Such a dependency was not observed in UAS_{TEL}-*CYC1-lacZ* reporter genes in the present work. Also in a cell-free transcription system, GAL11 protein was equally effective whether the UAS-TATA distance in template DNA was long (132-bp) or short (29-bp). Therefore, the dependency of GAL11 functioning on the UAS-TATA distance has to be reexamined systematically. The GAL11 dependency is presumably explained by the promoter context of the genes, since GAL11 protein stimulated the basal transcription from the core promoter of *CYC1 in vivo* as well as *in vitro*. Recently, the requirement for some of the basal transcription factors has been found to vary with the core promoter: transcription factor IIE is an essential component for the basal transcription of the adenovirus major late gene but is dispensable for that of the immunoglobulin heavy chain gene (33). In the case of GAL11, preliminary experiments indicated that it stimulated transcription from the core promoter of *GAL7* (*GAL11*-dependent gene) but not from *GAL80* (*GAL11*-independent gene) *in vitro* (H.S., and T.F., unpublished data). We suggest therefore that GAL11 protein functions as a basal transcription factor of some genes and that the GAL11 dependency is determined by the promoter context of the genes. This is consistent with the observations that the function of *GAL11* is pleiotropic (15, 16, 21, 22). Since GAL11 protein did not bind a specific DNA sequence as judged by footprint analysis (H.S., unpublished results), it may regulate the basal transcription by protein–protein interactions with the basal or general transcription factors. However, we have not been able to exclude the possibility that the GAL11 dependency of a gene was determined by species of activators as postulated (16, 21, 34). Other combinations of activators and promoters should be studied to clarify the connection between GAL11 and activators.

On the basis of present experiments, we suggest GAL11 to be a distinctive class of transcription factor that enhances the basal transcription. Factors exhibiting such an activity have been identified in mammalian cells and termed "positive cofactor" (2, 35). The positive cofactor stimulates basal transcription by itself. In combination with the negative cofactor that inhibits the basal transcription, it increases a net activation of the activator-induced transcription (35). The character of GAL11 revealed by *in vivo* and *in vitro* studies suggests to us that GAL11 is a yeast counterpart of the positive cofactor, and it enhances transcription of some genes in combination with an unidentified yeast negative cofactor.

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