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Biochemistry

The yeast GAL11 protein binds to the transcription factor IIE through GAL11 regions essential for its *in vivo* function

(*Saccharomyces cerevisiae*/RNA polymerase II holoenzyme)

HIROSHI SAKURAI*†, YOUNG-JOON KIM‡§, TOMOKO OHISHI*, ROGER D. KORNBERG‡, AND TOSHIO FUKASAWA¶‖

*Laboratory of Molecular Genetics, Keio University School of Medicine, 35 Shinanomachi Shinjuku-ku, Tokyo 160, Japan; ‡Department of Cell Biology, Stanford University School of Medicine, Stanford, CA 94305; and ¶Kazusa DNA Research Institute, 1532–3 Yanauchino, Kisarazu, Chiba 292, Japan

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ABSTRACT **The *GAL11* gene encodes an auxiliary transcription factor required for full expression of many genes in yeast. The *GAL11*-encoded protein (Gal11p) has recently been shown to copurify with the holoenzyme of RNA polymerase II. Here we report that Gal11p stimulates basal transcription in a reconstituted transcription system composed of recombinant or highly purified transcription factors, TFIIB, TFIIE, TFIIF, TFIIH, and TATA box-binding protein and core RNA polymerase II. We further demonstrate that each of the two domains of Gal11p essential for *in vivo* function respectively participates in the binding to the small and large subunits of TFIIE. The largest subunit of RNA polymerase II was coprecipitated by anti-hemagglutinin epitope antibody from crude extract of *GAL11* wild type yeast expressing hemagglutinin-tagged small subunit of TFIIE. Such a coprecipitation of the RNA polymerase subunit was seen but in a greatly reduced amount, if extract was prepared from *gal11* null yeast. In light of these findings, we suggest that Gal11p stimulates promoter activity by enhancing an association of TFIIE with the preinitiation complex in the cell.**

The *GAL11* gene of the yeast *Saccharomyces cerevisiae* is required for full expression of many but not all genes. Loss-of-function mutations of *GAL11* result in pleiotropic defects but not in lethality on rich medium (1–4). Biochemical analyses indicated that the protein encoded by *GAL11* (Gal11p) stimulates basal transcription in cell-free systems developed from yeast whole cell or nuclear extracts and suggested a direct interaction between Gal11p and a basal transcription factor(s) (5, 6). As a support of this idea, it has recently been found that Gal11p copurifies with "holoenzyme" of RNA polymerase II (PolII), which is composed of core PolII, some of the basal transcription factors, nine SRB (suppressor of RNA polymerase B) proteins (7, 8), global transcription factors Sin4p (9) and Rgr1p (10), and over a dozen of as yet unknown proteins (11–14). The PolII holoenzyme is capable of catalyzing basal as well as activator-dependent transcription from various genes *in vitro* in the presence of the remaining basal factors, and therefore it is assumed to be a preassembled form of the preinitiation complex *in vivo* (7, 15). Components of the yeast holoenzyme, however, vary depending on the purification strategy to some extent (7, 11, 12). Nevertheless, TATA box-binding protein (TBP) and TFIIE are always missing, while Gal11p is universally present in these enzyme preparations (11–13). By contrast, mammalian PolII holoenzyme, which has recently been reported to exist in rat liver cells (16), contains all components required for promoter-specific transcription initiation.

In the present study, we show that Gal11p stimulates basal transcription in a highly purified reconstituted transcription system. We also demonstrate that Gal11p makes contacts with TFIIE through Gal11p regions that are essential for its *in vivo* function. These results lead us to hypothesize that Gal11p contributes to the association between the PolII holoenzyme and TFIIE *in vivo* and thereby regulates transcriptional efficiency of certain classes of genes.

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MATERIALS AND METHODS

Yeast Strains. The yeast strains used in this study were as follows: HS301 (a *leu2 trp1 ura3-52 pep4-3 prb1-1122 prc1-407 gal2 gal11::LEU2*), which was a *gal11* null strain derived from BJ2168. The construction of HS301 was described previously (5). HS304 and HS305 were *tfa2* null derivatives (*tfa2::LEU2*) of BJ2168 harboring pSK461 (YCp/*ADH1*-HA-*TFA2*) and pSK462 (YCp/*ADH1-TFA2*), respectively. HS16 (α *ade2-1 his3-11 leu2-1, 112 trp1-1 ura3-1 can1-100 gal11::LEU2*) was a *gal11* null strain derived from NOY396 (17). HS25 and HS26 were *tfa2* null derivatives (*tfa2::ADE2*) derived from NOY396 and HS16, respectively; both strains harbored pSK461.

Plasmid Constructions. Plasmids expressing influenza virus hemagglutinin (HA) epitope-tagged and untagged *TFA2* were constructed as follows: The *Nco*I–*Bam*HI fragment of pET-*TFA2* (18) was blunt-ended and subcloned downstream of the *ADH1* promoter (*Pvu*II site) of pVT102U (19) to yield pSK460. The HA-tagged variant of TFA2 (pSK459) was created by subcloning the blunt-ended *TFA2* fragment between the *Xho*I (filled-in) and *Pvu*II sites of pVT102UHA, which contains an HA epitope, MYPYDVPAYASL, between the *Bam*HI and *Xho*I sites of pVT102U. The *Sph*I fragments of pSK459 and pSK460 containing the *ADH1-TFA2* construct were cloned into the *Sph*I site of YCp50 (see ref. 20) to yield pSK461 and pSK462, respectively.

Plasmids expressing deletions of Gal11p were constructed from pSK7 (5), which is a *TRP1*-marked YEp plasmid, pTV3 (see ref. 20), harboring *GAL11*. Plasmids bearing fusions of glutathione *S*-transferase (GST) and various regions of *GAL11* were constructed from pGST-G11 (5) and pGEX2T or pGEX3X (Pharmacia). Details of plasmid construction are available upon request.

***In Vitro* Transcription Assay.** *In vitro* transcription reactions reconstituted with TBP (60 ng), TFIIB (30 ng), TFIIE (40 ng), TFIIF (40 ng), TFIIH (30 ng), and PolII (150 ng) were performed as described (11), except that the concentration of template DNA was changed as indicated in the figure legends. The above components were purified from *Escherichia coli* as recombinant proteins except TFIIF, TFIIH, and PolII, which were purified from yeast cells. Gal11p was purified from yeast as described (5).

Abbreviations: PolII, RNA polymerase II; TBP, TATA box-binding protein; HA, hemagglutinin; GST, glutathione *S*-transferase.

†Present address: School of Health Sciences, Faculty of Medicine, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa 920, Japan.
§Present address: Samsung Biomedical Research Institute, Center for Basic Research, Kangnam-ku, Ilwon-dong 50, Seoul 135–230, Korea.
‖To whom reprint requests should be addressed.

Immunopurification of Gal11p-Interacting Protein. The *gal11* null strain HS301 harboring pTV3 (YEp vector) or pSK7 (pTV3 harboring *GAL11*) was grown to mid-logarithmic phase, and nuclear extracts were prepared as described (5). Six milligrams of protein was loaded on 600 μl of an anti-Gal11p antibody-conjugated CH-Sepharose (Pharmacia) column equilibrated with buffer E-0.1 [20 mM Hepes·KOH, pH 7.6/20% glycerol/1 mM EDTA/1 mM dithiothreitol and protease inhibitors (5); molarity of potassium acetate is indicated after the hyphen]. After extensive washing with the same buffer, proteins retained on the column were eluted with buffer E-1.2 and dialyzed against buffer E-0.1.

Immunoprecipitation Experiments. Nuclear extract was diluted to 5 mg of protein per ml with buffer E-0.1. One milligram of protein was incubated with antibody immobilized resin as indicated in the figure legends for 3 hr on a rotating wheel. After washing the resin with buffer E-0.1 containing 0.1% Nonidet P-40, bound proteins were eluted with buffer E-1.0 and subsequently with 0.2 M glycine·HCl (pH 2.3). The proteins were subjected to SDS/PAGE, immunoblotted, and visualized by use of an enhanced chemiluminescence (ECL) detection system (Amersham).

Protein Affinity Chromatography. Full-length and various deleted forms of Gal11p were produced in *E. coli* as a fusion with GST and purified on glutathione agarose (Sigma) as described (21). Each of the fusion proteins was immobilized on the resin. The protein-bound resin was equilibrated with buffer E-0.1 and mixed with the recombinant TFIIE (400 ng of protein), TBP (200 ng of protein), or TFIIB (200 ng of protein) for 1 hr on ice. After washing the resin with buffer E-0.1, the bound proteins were eluted with buffer E-0.5, subjected to SDS/PAGE, and visualized by silver staining. [^{35}S]Methionine-labeled Tfa1p and Tfa2p were produced by using TNT T7 *in vitro* transcription/translation system (Promega) from templates pET-*TFA1* and pET-*TFA2*, respectively. The labeled protein was incubated with the resin as indicated. The bound proteins were eluted with SDS sample buffer and fractionated by SDS/PAGE and detected by autoradiography.

RESULTS

Gal11p Stimulates Basal Transcription in a Reconstituted System. Our previous observation suggested that purified Gal11p stimulates basal transcription in cell-free transcription systems developed from yeast whole cell or nuclear extracts through direct interaction between Gal11p and a basal transcription factor(s) (5, 6). However, a possibility remained to be excluded that the observed stimulation of transcription by Gal11p was due merely to anti-inhibitor activity in the crude system. Indeed, Li *et al.* (14) have found that concomitant loss of Gal11p, Sin4p, and unknown protein p50 from a holo PolII preparation due to a deletion of *SIN4* in the cell affects neither the basal nor the activator-dependent transcription in the reconstituted system. This result has been taken by Li *et al.* (14) as evidence that Gal11p opposed a possible inhibitor in the crude nuclear extract in the previous experiments of Sakurai *et al.* (5). To rule out such a possibility, we studied the function of purified Gal11p in a transcription system reconstituted with recombinant or highly purified basal factors and core PolII (11, 22). As seen in Fig. 1*A* (lanes 1–4), addition of increasing amounts of Gal11p to the reaction mixture resulted in stimulation of transcription from a template containing the yeast *CYC1* core promoter fused to G-free cassette. Gal11p also stimulated transcription from the core promoter of *GAL1*, *PYK1*, adenovirus major late, or *Schizosaccharomyces pombe ADH1* genes (Fig. 1*B*). In the reconstituted transcription system, activator proteins fail to enhance transcription, presumably because of the lack of mediators (23). In fact, GAL4-VP16 did not activate transcription from the template bearing the cognate binding site (Fig. 1*A*, lane 5; compare transcriptional efficiency of UAS_{GAL4}-*CYC1* with that of UAS_{GCN4}-*CYC1*). Gal11p stimulated transcription equally from these two templates (compare Fig. 1*A*, lanes 5 and 6), and showed no significant enhancement of transcription of UAS_{GAL4}-*CYC1* compared with that of UAS_{GCN4}-*CYC1* (Fig. 1*A*, lane 6). These results unequivocally indicate that Gal11p is responsible for enhancement of the basal transcription but does not function as a mediator or as an anti-inhibitor. The exact reason for discrepancy between this and the result of Li *et al.* (14) remains unknown.

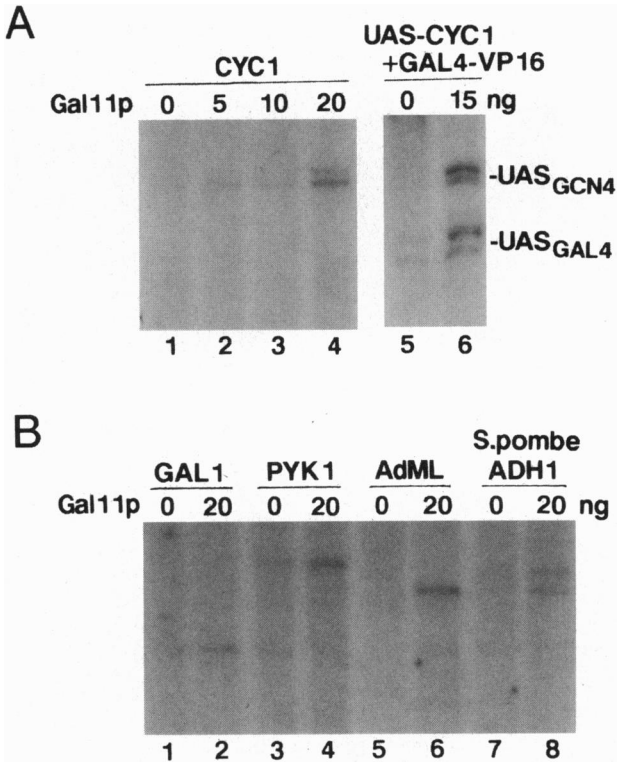

FIG. 1. Gal11p enhances basal transcription in a reconstituted transcription system. (*A*) Transcription reaction was carried out by using template DNA (2 μg/ml) containing the *S. cerevisiae CYC1* promoter fused to G-free cassette (pSK115, ref. 5). Increasing amounts of Gal11p were added in lanes 1 to 4. In lanes 5 and 6, the reaction mixtures contained GAL4-VP16 (30 ng) and two templates (each 2 μg/ml); one bears a Gal4p-binding sequence upstream the *CYC1* promoter (pJJ470), while the other bears two copies of a Gcn4p-binding sequence (pS(GCN4)^{2}CG−) (11). Gal11p was added to the reaction mixture of lane 6. (*B*) Template DNA (2 μg/ml) contained the promoter of *GAL1* (lanes 1 and 2), *PYK1* (lanes 3 and 4), adenovirus major late (lanes 5 and 6), and *S. pombe ADH1* (lanes 7 and 8), each of which was fused to G-free cassette (22). Transcription was conducted in the absence (odd-numbered lanes) or presence (even-numbered lanes) of Gal11p.

Gal11p Contacts TFIIE. To investigate which basal transcription factor might interact with Gal11p, nuclear extract was prepared from a yeast strain overproducing this protein. The extract was loaded on an anti-Gal11p antibody-immobilized column, and proteins retained on the column were eluted with a high salt buffer. To test whether the eluted sample contained any of the basal factors, a complementation analysis was performed with a series of reconstituted transcription mixtures, each of which lacked one of the basal factors or PolII. As shown in Fig. 2*A*, the eluted sample successfully reconstituted transcriptional activity only in the reaction mixture lacking TFIIE (lane 6). The weak complementation observed in TFIIB- or TFIIF-lacking mixtures was not reproducible (see lanes 14 and 18; data not shown). When Gal11p-free extract was loaded on the column, proteins retained on the column

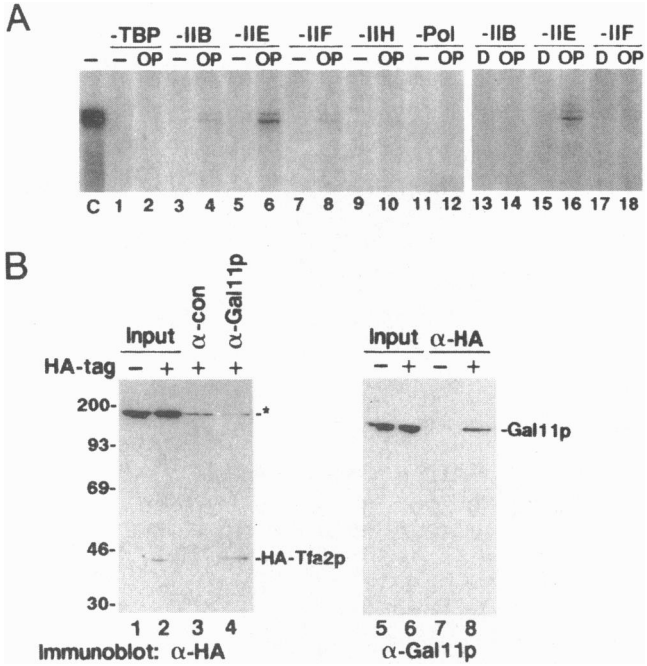

FIG. 2. Gal11p interacts with TFIIE. (*A*) Complementation of basal transcription factor activity by Gal11p-interacting proteins. Nuclear extracts were prepared from a *GAL11*-overexpressing strain (HS301[YEp-*GAL11*]; OP) or a *gal11* null strain (HS301[vector]; D). The high salt eluate of the anti-Gal11p antibody column was added to the transcription mixture lacking each of the basal factors as indicated at the top. In control reactions (−), plain buffer was added. Transcription reaction was performed using pSK115 (5) as a template (10 μg/ml). The reaction in the lane labeled C contained all the basal factors. (*B*) Immunoprecipitation analysis. Nuclear extracts were prepared from a strain expressing *TFA2* (HS305[YEp-*GAL11*]; lanes 1, 5, and 7) or HA-*TFA2* (HS304[YEp-*GAL11*]; lanes 2, 3, 4, 6, and 8). Control antibody (α-con, lane 3), anti-Gal11p antibody (α-Gal11p, lane 4), and anti-HA antibody (α-HA, lanes 7 and 8) were preincubated with Protein A-Sepharose (Pharmacia). Extract was mixed with the resin, and immunoprecipitates were prepared. Input and precipitated proteins were subjected to SDS/PAGE, and immunoblotting was performed with anti-HA antibody (lanes 1–4) or anti-Gal11p antibody (lanes 5–8). Positions of HA-Tfa2p and Gal11p are indicated to the right. The asterisk indicates an artifactual band of immunoblotting. Molecular masses are given in kDa to the left.

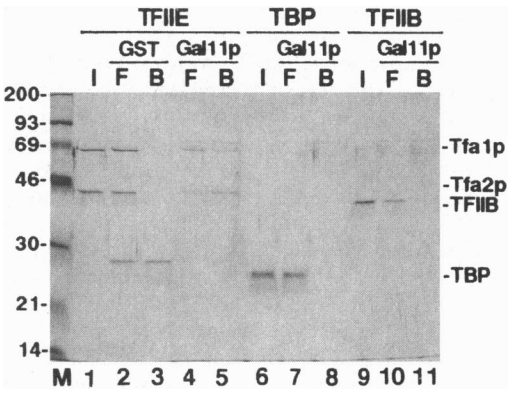

FIG. 3. Direct interaction between Gal11p and TFIIE. GST–Gal11p fusion protein was immobilized on glutathione agarose resin and incubated with TFIIE (lanes 4 and 5), TBP (lanes 7 and 8), or TFIIB (lanes 10 and 11). As a control, GST-immobilized resin was incubated with TFIIE (lanes 2 and 3). Input (I, lanes 1, 6, and 9), unbound (F, lanes 2, 4, 7, and 10), or bound proteins (B, lanes 3, 5, 8, and 11) were subjected to SDS/PAGE and visualized by silver staining. Positions of Tfa1p, Tfa2p, TBP, and TFIIB are indicated to the right. Molecular masses of standard markers (lane M) are given in kDa to the left.

failed to restore the transcriptional activity in the TFIIE-lacking system (compare lanes 15 and 16). These results suggest that TFIIE is retained on the column through an association with Gal11p.

This hypothesis was subjected to a more direct test by an immunoprecipitation assay as follows (Fig. 2*B*). In these experiments, we used a nuclear extract prepared from a strain expressing HA-tagged *TFA2* (*TFA2* encodes the small subunit of TFIIE; ref. 18). In agreement with the complementation analysis above, anti-Gal11p antibody, but not the control antibody, immunoprecipitated HA-Tfa2p from the extract (compare lanes 3 and 4). The reciprocal was also true in that anti-HA antibody immunoprecipitated Gal11p only in the presence of HA-tagged Tfa2p (lane 8). These results demonstrate the direct association between Gal11p and TFIIE *in vivo*.

Direct interaction between Gal11p and TFIIE was also studied *in vitro* by performing protein affinity chromatography experiments (Fig. 3). Gal11p was produced as a fusion protein with GST in *E. coli* (5), and the GST–Gal11p fusion was immobilized on glutathione agarose and incubated with purified TFIIE (18). After extensive washing, bound proteins were eluted with a high salt buffer, subjected to SDS/PAGE, and visualized by silver staining. Both subunits of TFIIE (Tfa1p and Tfa2p; ref. 18) were retained on the GST–Gal11p immobilized resin (lane 5) but not on the control GST resin (lane 3). TBP and TFIIB, however, failed to bind Gal11p under these conditions (lanes 6–11).

Regions Required for Binding to TFIIE. Yeast TFIIE is a heterotetramer consisting of the 66-kDa subunit Tfa1p and the 43-kDa subunit Tfa2p (18, 24). We performed experiments to test which subunit(s) associates with Gal11p. Each subunit was produced by *in vitro* transcription/translation reactions in the presence of [^{35}S]methionine, and the labeled peptides were used for protein affinity chromatography. As shown in Fig. 4*B*, both subunits of TFIIE were retained on the GST–Gal11p immobilized resin (lane F) but not on the control GST resin (lane G). Next we analyzed the region of Gal11p that mediates the binding to each subunit of TFIIE. Fusion proteins of GST and various regions of Gal11p were prepared, each of which was immobilized on glutathione agarose, and protein affinity chromatography was carried out as above. Fig. 4*B* shows that Tfa1p and Tfa2p bind the regions from 116 to 255 (domain B, lane 3) and from 866 to 929 (domain A, lane 8) of Gal11p, respectively.

Previously, we showed that domain A is essential for the function of Gal11p to enhance transcription *in vivo* as well as *in vitro* (5). To determine whether or not domain B was also involved in the *GAL11* function *in vivo*, we carried out a deletion analysis (Fig. 4*A*). Each *GAL11* derivative containing various deletions was expressed in a *gal11* null strain harboring a reporter gene *GAL7-lacZ*. Since the full expression of *GAL7-lacZ* depends on the normal function of *GAL11* (1, 6), the β-galactosidase activity was 11-fold higher in the cell harboring the wild-type *GAL11* gene than in the *gal11* null strain. Partial deletions of domain B (del.48/186 and del.187/618) showed a marginal effect on the expression of *GAL7-lacZ*. By contrast, total deletions of domain B (del.48/326 and del.48/618) resulted in a significant decrease of the expression of reporter gene (22% and 11% of the wild-type level, respectively). Similarly, domain A from 866 to 929 was also required for full expression of the reporter gene. The observed loss of *GAL11* function in these deletions was not due to a failure to produce stable proteins in the cell, because all the deletions yielded Gal11p with the expected molecular sizes judged by an immunoblot experiment (data not shown). These results clearly show that the regions required for binding of TFIIE coincide with the regions essential for the function of *GAL11* *in vivo*.

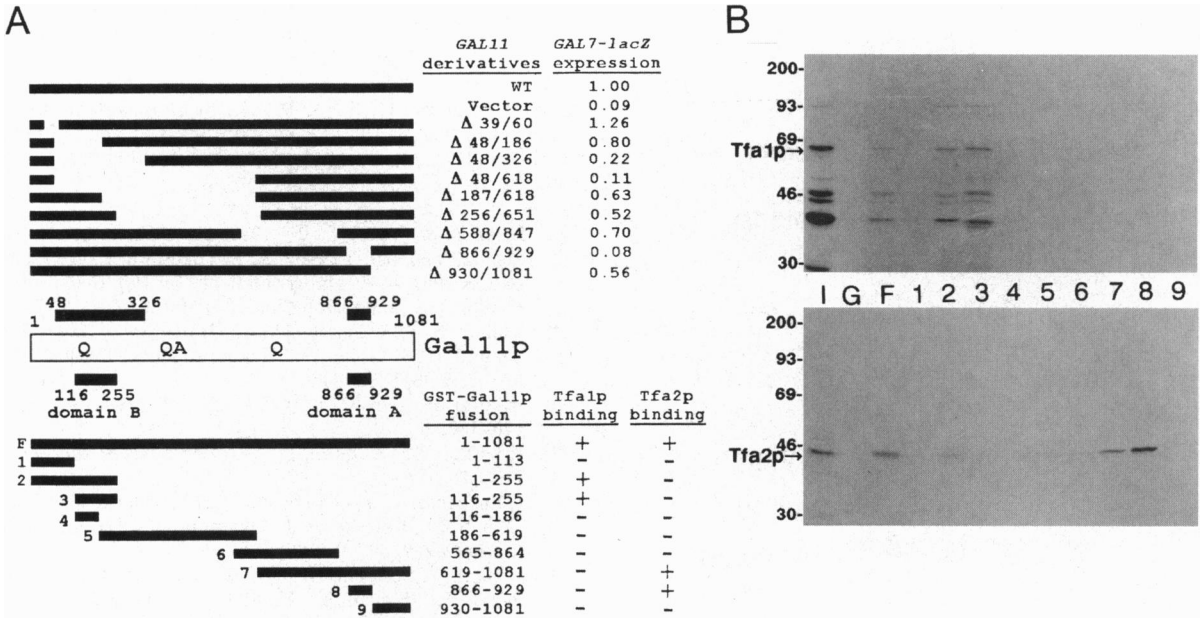

FIG. 4. Gal11p interacts with TFIIE at the regions of Gal11p essential for its *in vivo* function. (*A*, *Upper*) The solid bars represent the full-length *GAL11* (WT) or its derivatives with various deletions. The figures under "*GAL11* derivative" indicate the numbers of N-terminal and C-terminal amino acid residues of the deletion. The figures under "*GAL7-lacZ* expression" indicate the relative activity of β-galactosidase taking the wild-type value (88 units) as a unity (17). The vacant vector is shown as "vector." (*A*, *Middle*) Open bar represents the Gal11 protein, and "Q" and "QA" indicate glutamine stretch and glutamine-alanine repetitive sequence, respectively (2). The solid bars on the open bar indicate the regions necessary for the *in vivo* function of Gal11p as revealed in the deletion analysis above. The thick bars under the open bar indicate the regions responsible for binding with TFIIE as revealed in protein affinity chromatography below. The figures at the ends of each bar indicate the numbers of amino acid residues. (*A*, *Lower*) Solid bars represent Gal11 parts of GST–Gal11p fusions. The numbers of amino acid residues at the N and C termini of the Gal11 peptide are indicated to the right. The results of protein affinity chromatography are summarized under "Tfa1p binding" and "Tfa2p binding; (+) and (−) indicate successful and unsuccessful bindings to each subunit of TFIIE. (*B*) Regions of Gal11p involved in binding of each subunit of TFIIE *in vitro*. Each of [^{35}S]methionine-labeled Tfa1p (*Upper*) or Tfa2p (*Lower*) was incubated with immobilized GST (lane G), GST–Gal11p (lane F), and GST–Gal11p fusion derivatives shown in *A* (lanes 1–9). Input (lane I) and bound proteins were subjected to SDS/PAGE and visualized by autoradiography. The figures at the center of the panel represent the GST–Gal11p fusion numbers that correspond to those in A lower. Arrows indicate the positions of Tfa1p and Tfa2p. Molecular masses are given in kDa to the left.

In contrast to the above conclusion, Barberis *et al.* (13) argued that N-terminal region including domain B (Fig. 4*A*) was dispensable for the Gal11p function. However, their data (in figure 7*C* in their paper) actually show a small but significant difference in activity between the full-length Gal11p and the construct with an N-terminal deletion [construct (261–351)+(799–1081)]. Perhaps, the high background value for a *gal11* null yeast strain carrying the control vector may have made the difference less apparent, leading to those authors to ignore the difference. Also, the data given in figure 7*A* of their paper (13) show that LexA fused to the N-terminus of Gal11p (141–503) stimulated the expression of the cognate reporter gene 21-fold over the background, suggesting the importance of the N-terminal domain in the Gal11p function. Thus, we believe that their data are not inconsistent with our conclusion.

Gal11p Is Involved in Association Between TFIIE and PolII Holoenzyme. Despite the fact that Gal11p copurifies with PolII holoenzyme (11, 13), PolII did not appear to be efficiently retained on the anti-Gal11p antibody column (Fig. 2*A*). We therefore searched for the presence of PolII (subunits) in immunoprecipitates prepared from yeast nuclear extract with anti-Gal11p antibody. Fig. 5*A* shows that immunoprecipitation of Gal11p by anti-Gal11p antibody results in coprecipitation of Rpb1p, the largest subunit of PolII (compare lanes 3 and 4). This result is in agreement with the fact that Gal11p is integrated in the PolII holoenzyme. If Gal11p contained in the holoenzyme binds TFIIE, it could be involved in association between TFIIE and the holoenzyme. This hypothesis is further supported by the following observations (Fig. 5*B*). When HA-Tfa2p was immunoprecipitated from an extract of *GAL11* wild-type yeast, precipitates contained Rpb1p and Gal11p (lane 4). By contrast, a much smaller amount of Rpb1p was detected in precipitates from an extract of *gal11* null yeast (lane 3).

DISCUSSION

We have shown that Gal11p stimulates basal transcription in the reconstituted system and that it interacts with the basal transcription factor TFIIE *in vivo* as well as *in vitro*. Each of the two domains of Gal11p, A and B, which are essential for the function of Gal11p *in vivo*, participates in the binding of the small and large subunits of TFIIE, respectively. In this context, it should be noted that the amino acid sequences of two essential domains of Gal11p are well-conserved in *Kluveromyces lactis* Gal11p (25). We have also shown that anti-Gal11p antibody coprecipitates PolII. The mammalian TFIIE is known to tightly bind PolII (26, 27). By contrast, the interaction between the yeast TFIIE and PolII, which is so weak that it could not be detected by the conventional methods (24), has recently been demonstrated by the surface plasmon resonance in a range of 0.1 micromolar (D. A. Bushnell, personal communication). In accordance with these findings, yeast PolII holoenzyme always lacks TFIIE irrespective of the preparation procedure (11, 12), whereas mammalian holo-PolII contains TFIIE (16). On the other hand, domain A maps within the region required for association of Gal11p with the holoenzyme (13). Therefore, domain A may have dual roles, one to interact with TFIIE, the other to interact with a component(s) of the holoenzyme. From these considerations, we assume that Gal11p assists in recruiting TFIIE to holo PolII and stabilizes the binding of TFIIE to an initiation complex in the cell.

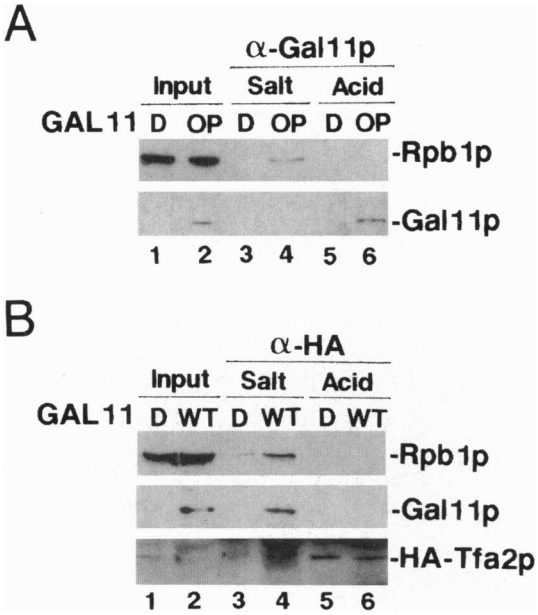

FIG. 5. Gal11p mediates interaction between PolII holoenzyme and TFIIE. (*A*) Extracts prepared from a *gal11* null strain (HS301[vector]; D, lanes 1, 3, and 5) and a *GAL11*-overexpressing strain (HS301[YEp-*GAL11*]; OP, lanes 2, 4, and 6) were incubated with anti-Gal11p-conjugated CH-Sepharose. Input (lanes 1 and 2) and bound proteins eluted with buffer E-1.0 (Salt, lanes 3 and 4) and 0.2 M glycine-HCl (pH 2.3) (Acid, lanes 5 and 6) were subjected to immunoblot analysis with antibody against C-terminal domain of Rpb1p (*Upper*). The filter was reprobed with anti-Gal11p antibody (*Lower*). (*B*) The extracts prepared from a *gal11* null strain (HS26; D, lanes 1, 3, and 5) and a wild-type strain (HS25; WT, lanes 2, 4, and 6) were incubated with anti-HA-coupled Protein A-Sepharose as in *A*. The filter was also probed with anti-HA (*Lower*).

Although *GAL11* is required for full expression of many genes, expression of some genes is unaffected by *gal11* null mutations (2, 6). This gene-specific functioning of *GAL11* has recently been found to be determined by the structure of the core promoter: Only canonical TATA-containing genes but not atypical TATA-containing or TATA-less genes are under the control of *GAL11*. In fact, all the genes tested in Fig. 1 of the present paper bear the typical TATA box. In addition, sequences encompassing the transcription initiation site affects the *GAL11*- dependency of a gene (H.S., T.O., and T.F., unpublished data). Knowing that there are specific interactions between Gal11p and TFIIE, the molecular mechanism by which *GAL11* functions in a gene-specific fashion may be clarified based on the role of TFIIE in transcription initiation. In higher eukaryotes, TFIIE is known to recruit TFIIH to the preinitiation complex and thereby regulate the activities of helicase, ATPase, and protein kinase of TFIIH at the step of promoter clearance (28–31). In a cell-free transcription system, TFIIE is not always required for transcription initiation; the requirement perhaps depends on the structure of start site region (31–33). We imagine that the gene-specific functioning of Gal11p, at least in some cases, may be correlated to the requirement of TFIIE for the initiation.

A major question that remains to be answered is how Gal11p regulates basal transcription. Since Gal11p makes contacts with both subunits of TFIIE, it is tempting to speculate that Gal11p can induce a conformational change in TFIIE that ultimately influences the ability of TFIIE to regulate TFIIH activity and/or stabilizes the interaction between the basal factors in the preinitiation complex. Whatever the mechanism, the present work has demonstrated a case in which promoter activity may be regulated by auxiliary transcription factors like Gal11p through a direct interaction with one of the basal factors without involvement of any DNA-bound activator.

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