Yeast Gal11 and Transcription Factor IIE Function through a Common Pathway in Transcriptional Regulation*

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The global transcription regulator Gal11, a component of RNA polymerase II holoenzyme, is required for full expression of many genes in yeast. We previously reported that Gal11 binds the small (Tfa2) and large (Tfa1) subunits of the general transcription factor (TF) IIE through Gal11 functional domains A and B, respectively. Here we demonstrate that the C-terminal basic region in Tfa2 is responsible for binding to domain A, whereas both the N-terminal hydrophobic and internal glutamic acid-rich regions in Tfa1 are responsible for binding to domain B. Yeast cells bearing a C-terminal deletion encompassing the Gal11-interacting region in each of the two TFIIE subunits, being viable, exhibited no obvious phenotype. In contrast, combination of the two deletions (TFIIE-ΔC) showed phenotypes similar to those of gal11 null mutations. The levels of mRNA from TATA-containing genes, but not from TATA-less genes, were lowered in TFIIE-ΔC to an extent comparable to that in the gal11 null mutant. Combination of TFIIE-ΔC with a gal11 null mutation did not result in an enhanced effect, suggesting that both TFIIE and Gal11 act in a common regulatory pathway. In a reconstituted cell-free system, Gal11 protein stimulated basal transcription in the presence of wild-type TFIIE. Such a stimulation was not seen in the presence of TFIIE-ΔC.

Recent biochemical studies have strongly suggested that Saccharomyces cerevisiae RNA polymerase II consists of a complex with the subset of the general transcription factors, various general transcription regulators (such as Srb proteins, Gal11, Sin4, Rgr1, and Rox3), and as yet unknown proteins. Although two forms of the holoenzyme that differ slightly in components have been reported, the TATA-binding protein (TBP) and transcription factor (TF) IIE are consistently missing, whereas Srb proteins and Gal11 are always present in this form of RNA polymerase II. Both types of the holoenzyme stimulate basal as well as activated transcription in vitro (1–8). Bulk mRNA synthesis is shut down in temperature-sensitive srb mutant cells upon transfer to the restrictive temperature, suggesting that the holoenzyme is involved in transcription of most RNA polymerase II-transcribed genes in the cell (9). When either Gal11 or Srb proteins are tethered to a gene by fusing these proteins to the DNA-binding domain of LexA, the chimeric molecules become potent activators of the target gene. This observation implies that recruitment of the holoenzyme to a promoter is an important pathway of transcriptional activation (10–13). The Swi-Snf complex, which is found in one type of the holoenzyme (14), but not in the other (15), has been proposed to function as a chromatin-remodeling factor and therefore to be responsible for the apparent chromatin remodeling activity of the holoenzyme (16). Both the significance of the two forms of the holoenzyme and the role of the known holoenzyme-associated global regulators (such as Srb proteins and Gal11) in regulating transcription remain to be elucidated.

The GAL11 gene is not essential for growth of yeast, yet GAL11 loss-of-function mutations cause a variety of phenotypes, such as slow utilization of galactose, sucrose, and non-fermentable carbon sources (17–19); sporulation defect (18); inefficient production of α-pheromone (20); suppression of yeast transposon Ty-insertion mutations (20); stabilization of minichromosomes (21); and suppression of defective silencing mutations (22). Gal11 protein is a component of both forms of the holoenzyme (1, 11) and is also a subunit of another type of RNA polymerase II-containing complex, which contains transcription regulators Cdc73 and Pafl, but not Srb proteins (23).

We have recently shown (24) that purified Gal11 stimulates basal transcription in a cell-free system of Sayre et al. (25) that consists of recombinant or highly purified general transcription factors (TBP, TFIIB, TFIIE, TFIIF, and TFIH) and RNA polymerase II, and that Gal11 makes contact with TFIIE in vivo as well as in vitro. Each of two domains of Gal11 (domains A and B), which are essential for its in vivo function, participates in the binding to the small (Tfa2) and large (Tfa1) subunits (26) of TFIIE, respectively (24).

Although several genetic studies suggested that Gal11 was also involved in transcriptional repression in vivo (20, 22), no biochemical evidence has so far been available to support direct involvement of Gal11 in the repression.

In this study, we have determined which regions of both subunits of TFIIE are involved in the binding to Gal11 by constructing deletion mutants of Tfa1 and Tfa2 that fail to interact with Gal11. Based on genetic as well as biochemical analyses using the mutant forms of TFIIE generated from these genes, we suggest that interaction with TFIIE is essential for the function of Gal11 and that the two factors function in a common regulatory pathway of transcription.

EXPERIMENTAL PROCEDURES

Yeast Strains, Media, and Genetic Methods—The yeast strains used are listed in Table I. Rich glucose (YPD), enriched synthetic, synthetic complete, and 5-fluoroorotic acid-containing media were prepared as described (24, 27, 28). Galactose utilization of cells was assayed using EBoGal medium (18).

Plasmid shuffling experiments were carried out as described (28). Synthesis of the α-factor was tested by the halo assay (29). MATa cells

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1 The abbreviations used are: TBP, TATA-binding protein; TF, transcription factor; GST, glutathione S-transferase; HA, hemagglutinin; PAGE, polyacrylamide gel electrophoresis.
Gal11-TFIIE Interaction

### Table I Yeast strains and plasmids used

| Strain      | Genotype                   |
|-------------|----------------------------|
| HS25        | MATa ade2-1 his3-11 leu2-4,112 trg1-1 ura3-1 can1-100 tfa2::ADE2 (pSK461)* |
| HS26        | gal11::LEU2 derivative of HS25 |
| HS27        | HS26 harboring pSK491       |
| HS28        | MATa ade2-1 his3-11 leu2-4,112 trg1-1 ura3-1 can1-100 tfa1::ADE2 tfa2::LEU2 (pSK457) |
| HS29        | MATa ade2-1 his3-11 leu2-4,112 trg1-1 ura3-1 can1-100 tfa1::ADE2 tfa2::LEU2 (pSK492, pSK711) |
| HS30        | gal11::URA3 derivative of HS29 |
| HS31        | gal11::URA3 derivative of HS40 |

### Plasmid Description

| Plasmid    | Description                                      |
|------------|--------------------------------------------------|
| pSK457     | URA3-marked high copy number plasmid bearing TFA1 and TFA2 |
| pSK461     | URA3-marked centromeric plasmid bearing ADH1-HAHis-TFA2 |
| pSK490     | HIS3-marked centromeric plasmid bearing deletion 48-326 of GAL11 |
| pSK492     | TRP1-marked centromeric plasmid bearing ADH1-HAHis-TFA1 |
| pSK703     | TRP1-marked centromeric plasmid bearing ADH1-HAHis-tfa1-N304 |
| pSK711     | HIS3-marked centromeric plasmid bearing ADH1-HAHis-TFA2 |
| pSK713     | HIS3-marked centromeric plasmid bearing ADH1-HAHis-tfa2-N302 |

* Plasmids indicated in parentheses are carried.

(10⁵ cells) were spotted onto a lawn (3 × 10⁵ cells) of the α-factor-sensitive strain RC629 (MATa sst1-2) (29).

Plasmids—A plasmid expressing the domain A polypeptide of Gal11 (pSK720) was constructed by subcloning the HpaII-NruI fragment of GAL11 (amino acids 716–929) into pQE32 (QIAGEN Inc.). The full-length Gal11 expression construct (pSK721) was created by subcloning the BamHI-SspI fragment (amino acids 1–255 of Gal11) of pGST-G11 (30) into pQE32 (QIAGEN Inc.). The full-length Gal11 expression construct (pSK722) was created by subcloning the BamHI-BstEII fragment of pGST-G11 into pQE32. Plasmid pSK491, which is a HIS3-marked centromeric plasmid bearing domain B-deleted GAL11, was constructed by subcloning a fragment of GAL11 with deletion between amino acids 48 and 326 (24) into pRS313 (31).

Plasmids bearing fusions of glutathione S-transferase (GST) and various regions of TFA1 were constructed from pSK492 or its 3′-end deletion derivatives (32) and pQE-2X or pQE-3X (Pharmacia Biotech Inc.). The derivatives of GST/Tff2 fusions were constructed from pSK461 (24); pSK708, pSK709, or pSK710 (see below); and pQE-2X or pQE-3X.

Plasmid pSK457 bearing the TFA1 and TFA2 genes was constructed by subcloning the Smal-XbaI (blunt-ended) fragment of TFA1 (26) and the SalI-XhoI fragment of TFA2 (26) into the PvuII and SalI/NheI sites of Yepl24 (35), respectively (Table I). Other TFA1 and TFA2 derivatives listed in Table I contain hemagglutinin (HA) epitope and polyhistidine (HAHIS) tags at the N termini and are expressed under the control of the ADH1 promoter (24, 26, 32). Plasmid pSK492 was a derivative of the TRP1-marked centromeric plasmid pRS314 (31) bearing ADH1-HAHis-TFA1 (32). Plasmids pSK702 and pSK703 were the same as pSK492 except that they contained tfa1-N417 and tfa1-N304 in place of wild-type TFA1, respectively (32). Plasmid pSK490, a TRP1-marked centromeric plasmid bearing ADH1-HAHis-TFA2, was constructed by subcloning of the blunt-ended SphI fragment of pSK461 (24) into the PvuII sites of pRS314 (31). Plasmids bearing 3′-end deletion derivatives of TFA2 were constructed from pSK490 by digestion with exonuclease III from the 3′-end of the Tfa2 coding region. The derivatives containing the N-terminal 318, 302, and 288 amino acids of Tfa2 were designated as pSK708, pSK709, and pSK710, respectively. Plasmids pSK711, pSK712, pSK713, and pSK714 were the same as pSK490, pSK490, pSK709, and pSK710, respectively, except that they contained HIS3 in place of TRP1.²

Protein Affinity Chromatography—Full-length or variously deleted forms of Tfa1 or Tfa2 were produced in Escherichia coli JM109 cells as fusions with GST and purified on glutathione-agarose (Sigma) as described (24). Each of the fusion proteins was immobilized on the resin, and the resin was equilibrated with buffer A (20 mM Hepes-KOH, pH 7.6, 1 mM EDTA, 1 mM dithiothreitol, 20% glycerol, and 1 mM phenylmethylsulfonyl fluoride) containing 0.1 mM potassium acetate (buffer A-0.1). Extracts containing the domain A or B polypeptide of Gal11 were prepared from JM109 cells harboring pSK720 or pSK721, respectively. The extracts were mixed with the fusion protein-immobilized resin for 1 h on ice. After washing the resin with buffer A-0.1, bound proteins were extracted and subjected to SDS-polyacrylamide gel electrophoresis (PAGE) followed by immunoblot analysis (24, 30).

Immunoprecipitation Experiment—Whole cell extracts were prepared by disruption of cells in glass beads in a buffer containing 0.1 mM Hepes-KOH, pH 7.6, 0.4 mM potassium acetate, 10 mM EDTA, 1 mM dithiothreitol, 30% glycerol, and 1 mM phenylmethylsulfonyl fluoride. The extracts were diluted to 2 mg of protein/ml with buffer A, and 3 mg of protein were incubated with anti-HA antibody-immobilized protein A-Sepharose (Pharmacia Biotech Inc.) in buffer A-0.1 for 5 h on a rotating wheel. After washing the resin with buffer A-0.1 containing 0.1% Nonidet P-40, bound proteins were eluted with 0.5 M glycine HCl, pH 2.7. The proteins were subjected to SDS-PAGE and analyzed by immunoblotting using the ECL system (Amersham Life Science, Inc.).

Primer Extension Analysis—Total RNA was isolated as described (27, 32) and quantified by the absorbance at 260 nm. The integrity of the RNA sample was confirmed by methylene blue staining after agarose gel electrophoresis. Primer extension analysis was carried out with specific primers for the ACT1, CYH2, GAL4, GAL80, and MF1 genes as described (27, 32). The nucleotide sequences of primers for CTS1 and HIS4 are TGAGTTGACCCCATAAACAGC and TCTTCTTAC-TTATCCATGAGGGC, respectively. The relative amount of accurate transcripts was determined by densitometric analysis of the autoradiograms (Shimadzu Model CS-9000).

Protein Purification—Full-length Gal11 protein was expressed in JM109 cells harboring pSK722 by addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 25°C. The following manipulations were performed below 4°C, and all buffers contained 1 mM phenylmethylsulfonyl fluoride. The cells (from a 2-liter culture) were suspended in 20 ml of Tris-buffered saline (20 mM Tris-Cl, pH 7.6, and 150 mM NaCl) and lysed by sonication. After addition of 2 ml of 10% Triton X-100, the lysate was cleared by centrifugation and incubated with 1 ml of Ni²⁺-nitrilotriacetic acid-agarose (QIAGEN Inc.) for 5.5 h. The slurry was washed in a column with wash buffer (20 mM imidazole, 0.1 M Hapes-KOH, pH 7.6, 0.1 M potassium acetate, and 0.1% Nonidet P-40), and then bound proteins were eluted with elution buffer (200 mM imidazole, 0.1 M Hapes-KOH, pH 7.6, 0.5 M potassium acetate, 10% glycerol, and 0.1% Nonidet P-40). The pooled fraction was diluted 3-fold with buffer B (10 mM Hepes-KOH, pH 7.6, 1 mM EDTA, 10% glycerol, 1 mM dithiothreitol, and 0.1% Nonidet P-40) and loaded onto a 0.5-ml Q-Sepharose column (Pharmacia Biotech Inc.) equilibrated with buffer B containing 0.1 M potassium acetate (buffer B-0.1). After washing with buffer B-0.5, Gal11 protein was eluted with buffer B-0.5. The pooled fraction was diluted 3-fold with buffer B and loaded on a 0.5-ml Q-Sepharose column (Pharmacia Biotech Inc.) equilibrated with buffer B-0.15. The flow-through fraction was loaded onto a 20-ml Sepharose CL-4B column (Pharmacia Biotech Inc.) equilibrated with buffer B-0.3, and Gal11-containing fractions were pooled. The yield was ~0.1 mg/liter of starting culture.

The recombinant proteins Tfa1, Tfa2, Tfa1-N304, and Tfa2-N302 were produced in BL21(DE3) cells and purified by Ni²⁺-nitrilotriacetic acid-agarose chromatography as described (26). The pooled fractions of Tfa1, Tfa2, and Tfa2-N302 were dialyzed against buffer A-0.1. The Ni²⁺-nitrilotriacetic acid-agarose fraction of Tfa1-N304 was loaded onto a Q-Sepharose column equilibrated with buffer A-0.1. After washing with buffer A-0.25, Tfa1-N304 was eluted with buffer A-0.4.

² Details of plasmid construction are available upon request.
In Vitro Transcription Assay—The in vitro transcription reaction was reconstituted with the following components in a 20-µl reaction mixture as described (24) at 180 mM potassium acetate. The transcription proteins were recombinant yeast TBP (60 ng), recombinant TFII B (30 ng), TFIIH (0.5 µ; Mono Q fraction, gift from Drs. Jesper Svedrup and Roger Kornberg) (24), RNA polymerase II holoenzyme (1 µg; Mono Q fraction) (1) prepared from a gal11 null strain (HS301) (30), recombinant TFII E or TFII E-Δ (20 ng), and recombinant Gal11 (20 ng). The proteins TBP, TFII B, and RNA polymerase II holoenzyme were a gift from Drs. Young-Joon Kim and Roger Kornberg. The template DNA (40 ng) contained the core promoter region of GAL7 (pSK164), and the transcripts were analyzed by primer extension as described (35). The relative amount of transcripts was measured by a Fuji BAS-1000 imaging analyzer.

RESULTS

Interaction between Domain B of Gal11 and Tfa1—We have previously shown that two domains of Gal11 from amino acids 866 to 929 (domain A) and from amino acids 116 to 255 (domain B) are required for the normal function of Gal11 in vivo (24). Domains A and B are involved in the interaction with the Tfa2 (43-kDa subunit) and Tfa1 (66-kDa subunit) proteins (26) of the yeast TFII E subunits, respectively (24). To dissect Tfa1 and Tfa2 for regions that mediate the binding to Gal11, we constructed various deletion derivatives of the TFII E subunits, which were then subjected to protein affinity chromatography with Gal11.

First, Tfa1 was prepared as a fusion with GST in E. coli and immobilized on glutathione-agarose. A bacterial extract containing the domain B polypeptide of Gal11 (amino acids 1–255) (Fig. 1A) was incubated with resin immobilizing GST/Tfa1 fusion protein. After extensive washing with buffer containing 0.1 M potassium acetate, bound proteins were extracted and subjected to SDS-PAGE and then analyzed by immunoblotting with anti-Gal11 antibody. As shown in Fig. 1B, the domain B polypeptide was retained on the GST/Tfa1 resin (lane 3). The domain B polypeptide was not detected in the bound fraction prepared from the control GST resin (lane 2). Both N-terminal deletion derivatives, GST1/200C and GST1/322C, showed a modest binding to the domain B polypeptide, whereas no trace amount of the domain B polypeptide was detected in the bound fraction from the GST1/409C-immobilized resin (lanes 4–6). A C-terminal deletion derivative, GST1/N417, captured the domain B polypeptide as efficiently as full-length Tfa1 (lane 7). Further deletions extending to the N terminus (GST1/N304, GST1/N189, GST1/N122, and GST1/N55) resulted in a decrease in the binding activity (lanes 8–11). The fusion protein bearing the region from residues 322 to 406 (GST1/322–406) captured the domain B polypeptide as efficiently as GST1/200C and GST1/322C (lane 12). These results indicate that both the N-terminal (residues 1–55) and internal (residues 322–406) amino acids of Tfa1 are involved in the binding to domain B of Gal11. The former is characterized by the abundance of hydrophobic residues (isoleucine, leucine, and valine), and the latter by the presence of a long stretch of glutamic acid (Fig. 1A). When the protein-immobilized resin was washed with 0.5 M potassium acetate, the domain B polypeptide remained bound to the resin of full-length Tfa1 (Fig. 1B, lane 14) or of GST1/N417 (data not shown). In contrast, neither GST1/200C nor GST1/N304 could retain the domain B polypeptide under these conditions (lanes 15 and 16). These results led us to conclude that both the N-terminal hydrophobic and internal glutamic acid-rich regions of Tfa1 are required for the normal interaction with domain B of Gal11 and that either one is capable of mediating a weak binding.

Region of Tfa2 Involved in Binding to Domain A of Gal11—Next we analyzed Tfa2 for the region involved in the interaction with domain A of Gal11. Various fusion derivatives of GST/Tfa2 and a bacterial extract containing the domain A polypeptide of Gal11 (amino acids 716–929) (Fig. 2A) were used for the binding assay as described above. As shown in Fig. 2B, the domain A polypeptide was retained on the GST/Tfa2 resin, but not on the control resin (lanes 2 and 3). The results of the N- and C-terminal deletion analyses indicated that the C-terminal 51 amino acids (GST2/278C) were sufficient for interaction with domain A (lanes 4–9). The fusion GST2/215–316, but neither GST2/215–290 nor GST2/215–277, captured the domain A polypeptide (lanes 10–12). We thus conclude that the minimal region required for interaction with domain A of Gal11 resides between amino acids 278 and 316 of Tfa2, a domain rich in basic amino acids (Fig. 2A).

Effect of C-terminal Deletion of Tfa2 in gal11-AB Mutant—Deletion in domain B of Gal11 (from amino acids 48 to 326), which mediates the interaction with TFII E at subunit Tfa1, is known to cause a partial loss of the Gal11 function in the cell...
Gal11-TFIIE Interaction

Fig. 2. Interaction between domain A of Gal11 and Tfa2. A, schematic representation of deletion derivatives of Tfa2. The upper open rectangle and thick black bar represent Gal11 and the domain A polypeptide, respectively. The lower open rectangle represents Tfa2, which has acidic (residues 86–93) and basic (residues 294–311) regions (26). Deleted forms of Tfa2 are indicated by thin black bars. Fusion proteins GST/Tfa2, GST2/N163, and GST2/N70 contain a HAHis tag between GST and Tfa2 derivatives. The results of protein affinity chromatography are summarized to the right; + and – indicate successful and unsuccessful binding to the domain A polypeptide of Gal11, respectively. B, binding of the domain A polypeptide to Tfa2 derivatives. Affinity chromatography was performed as described under "Experimental Procedures." Proteins retained on the resin were separated by SDS-PAGE and stained with Coomassie Brilliant Blue (upper panel) or analyzed by immunoblotting with anti-Gal11 antibody (lower panel). Input extract (10%) was co-electrophoresed (lane 1). GST and fusion proteins are indicated with dots. Molecular masses are given in kilodaltons to the left.

A

B

Fig. 3. Construction of mutant Tfa2 that fails to bind Gal11. A, phenotypes of cells containing mutations in GAL11 and/or TFA2. TRP1-marked plasmids bearing TFA2 derivatives (pSK490, pSK708, pSK709, and pSK710) were introduced into strains HS256, HS26, and HS27. Transformants were plated on 5-fluoroorotic acid-containing medium to lose the URA3-marked plasmid bearing TFA2. Cells containing the indicated mutations (10^6 cells) were tested for growth on YPD medium at 30 °C or at 37 °C and on EBGal medium at 30 °C. Production of the α-factor was tested by the halo assay (29). B, interaction between domain A of Gal11 and C-terminal deletion derivatives of Tfa2. Fusion proteins of GST with the indicated Tfa2 derivatives were used for the binding assay. Bound proteins and input extract (10%) were analyzed by Coomassie Brilliant Blue staining (upper panel) or by immunoblotting with anti-Gal11 antibody (lower panel). GST and fusion proteins are indicated with dots. Molecular masses are given in kilodaltons to the left. wt, wild type; Δ, gal11-ΔB; Δ, gal11-Δ.

In a strain containing such a deletion (gal11-ΔB), the interaction between Gal11 and TFIIE should be mediated through domain A and Tfa2. If the domain A-binding region of Tfa2 was also deleted in the gal11-ΔB mutant, the interaction between Gal11 and TFIIE should be totally abolished. Such a yeast mutant should exhibit phenotypes similar to those of a strain containing the gal11-null mutations (26). Cells carrying a gal11^A mutation were able to grow on rich glucose medium (YPD) at 30 °C, but not at 37 °C or on galactose medium (EBGal) at 30 °C. The gal11^A strains could not produce normal amounts of α-factor judged by the halo formation due to growth inhibition on a lawn of an α-factor-sensitive strain (Fig. 3A) (18, 20). Phenotypes of a strain containing the gal11-ΔB mutation were less remarkable than those of gal11^A mutations, exhibiting a slow growth on YPD medium at 37 °C or on EBGal medium at 30 °C and formation of a subnormal size of a halo (Fig. 3A). To obtain Tfa2 that fails to interact with domain A of Gal11, we constructed C-terminal deletion derivatives from wild-type Tfa2. Three derivatives containing the N-terminal 288, 302, and 318 amino acids were constructed, fused to GST, and tested for binding to domain A. As shown in Fig. 3B, GST fusion protein GST2/N318 captured the domain A polypeptide as efficiently as full-length Tfa2 (compare lanes 3 and 4). No trace of the domain A polypeptide was seen in the bound fraction from GST2/N288, whereas it was marginally detectable in the bound fraction from GST2/N302 (lanes 5 and 6). Next, these C-terminal deletion derivatives were introduced into gal11-ΔB cells, and transformants expressing the deleted form of Tfa2 were selected by the plasmid shuffling technique (Fig. 3A). Yeast expressing the N-terminal 318 amino acids of Tfa2 (tfa2-N318) showed phenotypes indistinguishable from those of gal11-ΔB cells harboring full-length TFA2 (wild-type). In contrast, tfa2-N302 and tfa2-N288 supported growth neither on YPD medium at 37 °C nor on EBGal medium at 30 °C. Furthermore, these deletions did not produce normal amounts of α-phorone in the gal11-ΔB mutant. The GAL11 wild-type cells harboring tfa2-N302 or tfa2-N288 showed no significant growth defects and produced normal amounts of α-phorone. We conclude therefore that the sequence comprising amino acids 302–318 of Tfa2 is important for interaction with domain A of Gal11.

Construction and characterization of TFIIE mutant that fails to interact with Gal11—We then constructed cells expressing C-terminal deletion derivatives of Tfa1 and Tfa2 in various combinations and examined their phenotypes (Fig. 4A). As shown previously (32), the TFA2 wild-type cells expressing the N-terminal 417 (tfa1-N417) and 304 (tfa1-N304) amino acids of Tfa1 exhibited no significant phenotypic alterations. Combinations of the tfa1-N417 mutation and each of the TFA2 deletion mutations tfa2-N318, tfa2-N304, and tfa2-N288 caused no noticeable abnormality of cells. Cells containing both...
the tfa1-N304 and tfa2-N318 mutations showed normal phenotypes. In contrast, cells containing the tfa1-N304 tfa2-N302 or tfa1-N304 tfa2-N288 double mutation showed pleiotropic effects. These mutant cells were unable to grow either at 37 °C on YPD medium or at 30 °C on EBGal medium or to produce effects. These mutant cells were unable to grow either at 37 °C on YPD medium or at 30 °C on EBGal medium or to produce

The results of these experiments are consistent with the idea that the Gal11-binding domain of TFA1 and TFA2 is involved in the efficient binding of the Gal4-a-factor.

Next we analyzed the effect of the TFIIE-ΔC mutation on constitutive transcription of the genes encoding actin (ACT1), endochitinase (CTS1), ribosomal protein (CYH2), the transcriptional activator for galactose-inducible genes (GAL4), the antagonist of Gal4 (GAL80), histidine synthetase (HIS4), and the α-factor (MFα1). Total RNA was prepared from TFIIE-ΔC-expressing cells grown at 30 °C or at 37 °C for 5 h and subjected to primer extension analysis (Fig. 5A). When the TFIIE-ΔC mutant was grown at 30 °C, the mRNA level of ACT1 was comparable to the wild-type control (lanes 1 and 3). The ACT1 mRNA level in the TFIIE-ΔC mutant decreased to 30% of the wild-type level by the temperature up-shift to 37 °C (lanes 6 and 8). The TFIIE-ΔC mutation caused a reduction in the mRNA level of CTS1, CYH2, or HIS4 to 50% of the wild-type level at 30 °C, and the temperature up-shift resulted in a further decrease in these transcripts (<20% of the wild-type levels). The MFα1 mRNA level was more severely affected by TFIIE-ΔC and was reduced to 10% (at 30 °C) and 5% (at 37 °C) of the wild-type level. In contrast, the TFIIE-ΔC mutation did not affect the mRNA level of GAL4 or GAL80 either at 30 °C or at 37 °C. Transcription of the same set of genes was affected by the gal11Δ mutation (lanes 4 and 9). The ACT1 mRNA level was slightly reduced by the gal11Δ mutation at 37 °C, but not at 30 °C. When gal11Δ cells were grown at 30 °C, the mRNA level of CTS1, CYH2, HIS4, or MFα1 was reduced in comparison to the wild-type level, and the reduction was enhanced at...
37 °C. In contrast, the mRNA level of GAL4 or GAL80 was not significantly affected by the gal11Δ mutation. Furthermore, the mRNA levels of these genes in the TFIIE-ΔC mutant were similar to those in gal11Δ (compare lanes 3 and 4 and lanes 8 and 9). The observation that transcription of the tested genes was inhibited in both TFIIE-ΔC and gal11Δ at 37 °C more severely than at 30 °C may be related to the temperature-sensitive growth of these mutant yeasts.

Phenotypes Caused by Combination of TFIIE-ΔC and gal11Δ Mutations—If Gal11 exerts its function exclusively through interaction with TFIIE, the effect of TFIIE-ΔC gal11Δ double mutations should be identical to that of a single mutation. As shown in Fig. 5B, a strain carrying TFIIE-ΔC gal11Δ mutations was viable, and its phenotypes were similar to those in TFIIE-ΔC or gal11Δ mutants. The mRNA levels of the tested genes (except GAL4 and GAL80) were reduced by TFIIE-ΔC gal11Δ mutations (Fig. 5A, lanes 5 and 10) to an extent comparable to that caused by either single mutation. In other words, the effect of either the TFIIE-ΔC or gal11Δ mutation on transcription was not additive. These results suggest that both Gal11 and TFIIE function in the same regulatory pathway of transcription.

In parallel, we analyzed mRNA levels in a previously isolated yeast strain that contains a temperature-sensitive mutation in TFA1 (tfa1-21) (32). The temperature up-shift of tfa1-21 cells to 37 °C is known to result in degradation of both the Tfa1-21 and Tfa2 proteins and thereby in a remarkable decrease in the steady-state level of bulk poly(A)⁺ RNA as well as of various specific transcripts (32). When tfa1-21 mutant cells were grown at 30 °C, the mRNA levels of the tested genes were similar to those in wild-type cells (Fig. 5A, lane 2). However, the temperature up-shift to 37 °C resulted in the disappearance of these transcripts, except for GAL4 and GAL80 (lane 7). Such a gene-specific transcriptional effect of the tfa1-21 mutation has been observed previously (32). Thus, the mRNA levels of GAL4 and GAL80 were affected, but less remarkably (within 2-fold of the wild-type levels) than those of the other genes tested either by gal11Δ or by the mutations in TFIIE (see GAL4 and GAL80 rows).

Interaction between Gal11 and TFIIE in Cell-free Transcription Reaction—To assess the functional interaction between Gal11 and TFIIE further, in vitro transcription analyses were performed. The reaction mixtures contained recombinant TBP, recombinant TFIIB, highly purified TFIIF, and RNA polymerase II holoenzyme prepared from a gal11Δ strain (the holoenzyme used contained TFIIF). Recombinant full-length TFEIE, TFIIE-ΔC, and Gal11 were also purified (Fig. 6A) and added to the reaction mixtures. The template DNA contained the core promoter of GAL7, and the transcripts were analyzed by primer extension. As shown in Fig. 6B, addition of wild-type TFIIE yielded the expected size transcript (compare lanes 1 and 4). Further addition of Gal11 resulted in an ~4-fold increase in the amount of transcript (compare lanes 4 and 5), a result in good agreement with our previous study (24), in which an in vitro system consisting of highly purified core RNA polymerase II, TFIIF, and TFIIE from yeast and the recombinant proteins TBP, TFIIB, and TFIIE was used. When a reaction mixture received TFIIE-ΔC instead of wild-type TFIIE, the amount of transcript was reduced ~2.5-fold (compare lanes 2 and 4), and under these conditions, Gal11 showed no effect on the amount of transcript (compare lanes 2 and 3). These results indicate that the Gal11-binding domains of TFIIE are required for the stimulatory function of Gal11 and further suggest that Gal11 stimulates basal transcription by interacting with TFIIE in the reconstituted systems.

DISCUSSION

In this work, we have dissected the general transcription factor TFIIE with respect to its domains responsible for interaction with Gal11, a global transcription regulator and a constituent subunit of RNA polymerase II holoenzyme. These experiments have revealed that a major role of the C-terminal regions of the two subunits of TFIIE, although dispensable for viability, is to regulate transcription by interacting with Gal11 and that these two factors (Gal11 and TFIIE) appear to function in a common pathway. The evidence supporting this hypothesis is as follows. First, regions required for binding to the two essential domains of Gal11 were identified in the respective subunits of TFIIE. Second, TFIIE-ΔC, which has a deletion in each of the two subunits in the interaction domain with Gal11, and gal11Δ mutations caused similar effects on the phenotypes as well as on the in vivo transcription of several genes. Third, the effects of TFIIE-ΔC or gal11Δ on the phenotypes and transcription were not enhanced by coexistence of both mutations. Finally, stimulation of basal transcription by Gal11 was observed in an in vitro transcription system composed of the holoenzyme of RNA polymerase II, TBP, TFIIB, TFIIF, and TFIIE. Such a stimulation was not seen if TFIIE was replaced with TFIIE-ΔC. Since the TFIIE-ΔC mutation contains a relatively large deletion in each of the subunits, it is possible that the deletions cause a loss of interaction with another transcription factor(s). The fact that TFIIE-ΔC functioned less efficiently than wild-type TFIIE in the reconstituted transcription system suggests the presence of interaction between the C-terminal regions of TFIIE and the general transcription factors or RNA polymerase II. Nevertheless, we still believe that the major role of the C-terminal regions of the TFIIE subunits in vivo is interaction with Gal11 for the following reasons. First, neither tfa1-2-N304 (32) nor tfa2-2-N302 (see Fig. 3A) alone caused any phenotypic alterations. Second, a combination of these two mutations resulted not only in gal11Δ-like phenotypes, but also in inhibition of transcription of the genes whose maximal expression requires GAL11.

We previously suggested that GAL11 is required for maximal transcription of various genes containing the TATA box (27). Here we have shown that transcription from TATA-containing
genes including ACT1 (36), CTS1 (37), CYH2 (38), HIS4 (39), and MFA1 (40) is reduced not only by gal11Δ, but also by TFIIE-ΔC. We had reported that expression of MEL1 (encoding α-galactosidase) was not significantly affected by gal11Δ as judged by the α-galactosidase assay (18) despite the fact that MEL1 has the canonical TATA box. To the contrary, Long et al. (41) reported that the α-galactosidase activity decreased in gal11Δ yeast to 25% of the wild-type level. We have recently reinvigorated this problem and found that the mRNA level of MEL1 is in fact lowered in gal11Δ to 25–35% of the wild-type level,2 supporting the latter report. In addition, expression of PHOS (encoding acid phosphatase), which also bears the typical TATA sequence in its core promoter region, has recently been shown to be reduced in gal11Δ yeast to one-third of the wild-type level (16). Thus, expression of all of the TATA-bearing genes so far tested is significantly affected by gal11Δ, although further studies are needed before concluding that all TATA-bearing genes require Gal11 for their full expression. On the other hand, we have shown that Gal11 is dispensable for the genes containing the initiator or non-consensus TATA sequences in the core promoter (TATA-less genes) (27). Most recently, we have demonstrated, using a temperature-sensitive tfa1-21 mutant, that TFIIE also exhibits a similar type of core promoter dependence as Gal11 for its function (32). Transcription from TATA-less promoters, such as those of GAL4 (42) and GAL80 (43), is affected less remarkably than that from the TATA-containing promoters in TFIIE-ΔC cells, as is observed in gal11Δ cells or in tfa1-21 cells at the restrictive temperature. These results are all in conformity with the idea that the promoter structure dependence of Gal11 function is determined by the requirement of TFIIE for transcription and that transcription of several TATA-less promoters is independent of the promoter dependence as Gal11 for its function (32). Transcription from TATA-less promoters, such as those of GAL4 (42) and GAL80 (43), is affected less remarkably than that from the TATA-containing promoters in TFIIE-ΔC cells, as is observed in gal11Δ cells or in tfa1-21 cells at the restrictive temperature. These results are all in conformity with the idea that the promoter structure dependence of Gal11 function is determined by the requirement of TFIIE for transcription and that transcription of several TATA-less promoters is independent of the function of TFIIE and Gal11. In this context, it may be noteworthy that yeast TBP-associated factors have been reported to function of TFIIE and Gal11. In this context, it may be note-

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3 T. Ohishi, Y. Suzuki, and T. Fukasawa, unpublished data.
4 H. Sakuragi and T. Fukasawa, unpublished data.