Promoter Structure-dependent Functioning of the General Transcription Factor IIE in Saccharomyces cerevisiae*

Hiroshi Sakurai‡, Tomoko Ohishi§, and Toshio Fukasawa

From the §School of Health Sciences, Faculty of Medicine, Kanazawa University, 5–11–80 Kodatsuno, Kanazawa, Ishikawa 920, Japan, the ‡Laboratory of Molecular Genetics, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan, and the ¶Kazusa DNA Research Institute, 1532-3 Yana, Kusarazui, Chiba 292, Japan

General transcription factor (TF) IIE is an essential component of the basal transcription complex for protein-encoding genes, which is widely conserved in eukaryotes. Here we analyzed requirement for TFIIE for transcription in vivo by using yeast Saccharomyces cerevisiae cells harboring mutations in the TFA1 gene encoding the larger one of the two subunits of TFIIE. Deletion analysis indicated that the N-terminal half of Tfa1 protein has an essential function to support the cell growth. In a temperature-sensitive tfa1 mutant cell, the steady-state level of bulk poly(A)* RNA decreased rapidly at the restrictive temperature. Surprisingly, levels of several mRNAs, whose transcription is directed by the promoters lacking the typical TATA sequence, were not affected in the mutant cells at that temperature. This promoter-specific functioning of TFIIE was reproduced in a cell-free system composed of TFIIE-depleted nuclear extracts. These results strongly suggest that requirement for TFIIE varies in each gene depending on the promoter structures in vivo.

Recent biochemical studies in yeast, fruit fly, and mammalian cells have led to reconstitution of the basal transcription machinery in vitro with RNA polymerase II and so-called general transcription factors, namely TATA-binding protein (TBP),1 TFIIA, TFIIE, TFIIF, and TFIIH (1–4). These components are highly conserved, and in some cases, functionally interchangeable among the above organisms. However, exact in vivo functioning of some of the components under real physiological conditions remains to be elucidated. These issues can be best addressed in yeast, the organism that offers powerful genetic approaches that are not available in other eukaryotes. For this reason, in vivo requirement for some of the general transcription factors have been studied by genetic methods in the yeast Saccharomyces cerevisiae. TBP has been concluded to be required for transcription by all three nuclear RNA polymerases (5). Two subunits of TFIIH, which is also involved in nucleotide excision repair processes (for review, see Ref. 6), are highly conserved, and in some cases, functionally interchangeable among the above organisms. Indeed, as we describe here, TFIIE shows differential effects on different promoters in S. cerevisiae. Implications of these findings in our understandings of the molecular mechanism for transcriptional initiation are discussed.

EXPERIMENTAL PROCEDURES

Plasmids—Plasmid pSK495, a URA3-marked centromeric plasmid expressing hemagglutinin epitope (HA)-tagged TFA1 open reading frame (ORF) under the control of the ADH1 promoter, was constructed by replacing the TFA2 ORF of pSK461 (16) with the blunt-ended NdeI-BamHI fragment of PET-TFA1 (18). Plasmid pSK492 was constructed by subcloning the blunt-ended SphI fragment of pSK495 into the PvuII sites of pRS314, a TRP1-marked centromeric plasmid (19). Plasmids bearing 3’ end deletion derivatives of TFA1 were constructed from pSK492 by digestion with exonuclease III from the 3’ end of the TFA1 ORF.

Yeast Strains and Media—Yeast strain HS30 (MATα ade2-1 leu2-1, 112 trp1-1 ura3-1 can1-100 tfa1::ADE2 (pSK495)) was a chromosomal tfa1 null strain harboring pSK495. Strain HS33 was identical to HS30 except for the presence of pSK492 instead of pSK495. Strain HS34 was a chromosomal tfa2 null derivative (tfa2::LEU2) of HS33 harboring pSK461 in addition to pSK492.

Rich media consisted of 2% polypeptone and 1% yeast extract containing 2% glucose (YPD), 2% galactose (YPGal), 2% sucrose (YPSuc), 2% glycerol (YPGly), or 2% sodium lactate plus 3% glycerol (YPGly/Lac) as carbon sources. Minimal medium consisted of 2% glucose and 0.67% yeast nitrogen base. Minimal medium containing 5-fluoroorotic acid (5-FOA) was prepared as described (20).

Plasmid Shuffling and Isolation of Mutant—Plasmid bearing full-length (pSK492) or 3’end deletion derivatives of TFA1 was introduced into yeast strain HS30. Transformsants were selected for Trp+ and then suggested that TFIIB and TFIIF are involved in the accurate start site selection (10, 11).

The general transcription factors together with RNA polymerase II assemble on the core promoter, which consists of both or either the TATA box and the initiator (12), to generate the preinitiation complex. The formation of the complex is supposed to be amenable for regulation by sequence-specific DNA-binding transcription factors (2, 3). Recently, the preinitiation complex has been suggested to be formed from a pre-formed subcomplex called holoenzyme of RNA polymerase II (for review, see Refs. 13 and 14). The S. cerevisiae RNA polymerase II holoenzyme contains some of the general transcription factors and multifunctional transcription regulators such as Srb proteins, Swi/Snf proteins, Gal11, Sin4, Rgr1, and more than a dozen as yet unidentified proteins (13, 14). By using temperature-sensitive (ts) mutations in the SRB4 and SRB6 genes, it has been indicated that the holoenzyme is involved in transcription of most RNA polymerase II transcribed genes (15).

We have recently demonstrated that the yeast Gal11 protein makes functional contact with TFIIIE in vivo as well as in vitro (16). We have further found that Gal11 is required for full expression of certain genes bearing the TATA box but is dispensable for initiator- or nonconsensus TATA-directed transcription in vivo (17). From these results, we hypothesized that the yeast TFII E could also exhibit such a promoter elementspecific functioning. Indeed, as we describe here, TFII E shows differential effects on different promoters in S. cerevisiae. Implications of these findings in our understandings of the molecular mechanism for transcriptional initiation are discussed.

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‡ To whom correspondence should be addressed. Tel.: 81-762-65-2558; Fax: 81-762-34-4360; E-mail: sakura@kenrokui.ipc.kanazawa-u.ac.jp

§ The abbreviations used are: TBP, TATA binding protein; TF, transcription factor; ts, temperature-sensitive; 5-FOA, 5-fluoroorotic acid; HA, hemagglutinin epitope; ORF, open reading frame; GST, glutathione S-transferase.
Temperature-sensitive mutations in TFA1 were isolated as follows. The TFA1 ORF in pSK492 was mutagenized by a primer chain reaction technique (15). Mutagenized fragments were isolated by digestion with NheI (in the HA epitope sequence) and NspI (nucleotide position +597) (18) and were recloned into pSK492 vector at the NheI-NspI site. The mutant library thus prepared was introduced into HS30, and the resultant Trp+ transformants were grown on 5-FOA plates at 30 °C to eliminate the preexisting URA3-marked plasmid bearing the wild-type TFA1. Clones that failed to grow on YPD at 37 °C were selected by replica-plating as the candidates.

**RNA Analyses—**Total RNA was isolated as described (17, 21) and quantified by absorbance at 260 nm. The integrity of the RNA preparations was confirmed by methylene blue staining of RNA after agarose gel electrophoresis under denaturing conditions. The amounts of poly(A)+ RNA were determined by hybridization with a labeled poly(T) probe as described (15). The relative poly(A)+ RNA levels were determined by scintillation counting of the dot-blotted filter. Primer extension analyses were carried out as described (17). The sequences of primers are as follows: ACT1, ACCGGTACATATACACAAACCGGC; ADH1 (10); CYC1 (10); CYH2, CTTGTGTTACTGTTCC; DEDI, TCTGAGGCATCTCTGTTTCTTCT; GAL4, CCGTTTCTCCCTT-GGACACCTTGGAGCT; GAL7, GTAAGCTTTATGAGGATGGC; GAL80, GCCCATCCTTTGGCTGCGTTGAGACCGACG; HIS3, GTCGGATCTTCTTTGTGACCAAGGCGTT. Primary transcripts of the rRNA and trypophan tRNA (tRNA\textsuperscript{Trp}) genes were analyzed by S1 nuclease mapping technique. The probe oligonucleotides used were labeled with \textsuperscript{32}P at processed RNA regions to detect nascent transcripts as described (5). The relative RNA levels were determined by densitometric analysis of the autoradiograms (Shimadzu, CS-9000).

**Protein Affinity Chromatography—**The ORFs of the wild-type and mutant TFA1 genes were cloned into pGEX-3X (Pharmacia Biotech Inc.) and expressed in Escherichia coli cells, from which proteins tagged with glutathione S-transferase (GST) were prepared. Each of the fusion proteins GST-Tfa1 and GST-tfa1-21 was immobilized on glutathione-agarose and incubated with a bacterial extract containing Tfa2 (18) in buffer E containing 0.3 M potassium acetate. After extensive washing with the same buffer, proteins retained on the resin were eluted with SDS sample buffer, subjected to SDS-polyacrylamide gel electrophoresis, and visualized by Coomassie Brilliant Blue staining.

**Immunoblot Analysis—**Whole cell extracts were prepared by disruption of cells with glass beads. Nuclear extracts were prepared as described (16). Equivalent amounts of protein from each sample were subjected to SDS-polyacrylamide gel electrophoresis and analyzed by immunoblotting with an ECL detection system (Amersham Life Science, Inc.).

**In Vitro Transcription Assays—**One mg of nuclear protein prepared from HS34 was incubated with control or anti-HA antibody immobilized protein A-Sepharose (Pharmacia) in buffer F containing 0.3 M potassium acetate for 5 h on a rotating wheel. After centrifugation at 12,000 \times g for 5 min, the resultant supernatant was recovered for in vitro transcription reaction, which was carried out as described respectively (21, 22). Template DNAs (20 μg/ml) used were pG80–72 (GAL80) and pSK164 (GAL7), and the transcripts were analyzed by S1 nuclease mapping and primer extension, respectively (21, 22).

**RESULTS**

C-terminal Deletion Analysis of Tfa1—Yeast TFIIIE consists of the 66- and 43-kDa subunits, which are encoded by the TFA1 and TFA2 genes, respectively (18, 23). Both genes are essential for cell viability (18). We first determined the Tfa1 region essential for the in vivo function. Several 3' end deletion derivatives of TFA1 were constructed (Fig. 1A) and introduced into yeast cells by a plasmid shuffling technique (20). As shown in Fig. 1B, the transformants harboring N-189, which produces a peptide of amino acid residues from 1 to 189 of Tfa1, grew in the absence of wild-type TFA1. By contrast, derivatives with further deletions toward the N terminus (i.e. N-185 and N-186H) could not support cell growth. The cells harboring N-189 grew normally on rich media containing 2% glucose, 2% galactose, 2% sucrose, or 2% glycerol as a sole carbon source at 30 °C.

These cells could also grow at 37 °C. However, N-242 but not N-189 could support cell growth at 16 °C (Fig. 1C). These results show that the N-terminal peptide of 189 amino acids is sufficient to support cell growth and that the region from 190 to 242 has an important function for growth of yeast at 16 °C.

Next, we analyzed effect of the C-terminal deletion in N-189 on the transcription of various genes. Total RNA prepared from cells harboring wild-type or N-189 TFA1 was subjected to primer extension analysis. As shown in Fig. 2A, quantitation of the autoradiograms by densitometric analysis showed that the level of constitutive transcription of ACT1, ADH1, CYH2, or DEDI in cells harboring N-189 was practically equal to the level in cells harboring wild-type TFA1. To know whether the N-terminal domain of 189 amino acids is sufficient for activated transcription, the levels of uninduced or induced transcription (for review, see Ref. 24) of GAL7, GAL80 (both activated by Gal4), TRP5, HIS3 (both activated by Gcn4), and CYC1 (activated by Hap complex) were analyzed (Fig. 2B).

Under the uninducing conditions, the mRNA levels of these genes were not affected by the C-terminal deletion. Activated transcription of GAL7, TRP5, or CYC1 was observed in cells harboring N-189 to levels indistinguishable from those in the wild-type cells. The N-terminal fragment of 189 amino acids could also support activated transcription of GAL80 and HIS3,
but the levels reduced to one-third of the wild-type levels. These results together with the above observations on the capability to support the cell growth suggest that the N-terminal domain of 189 amino acids have an essential function of Tfa1 but may not be sufficient for full expression of certain genes.

Isolation and Characterization of a Temperature-sensitive Mutation in TFA1—To investigate the requirement for TFIIE for transcription in vitro, we isolated ts mutations in TFA1. The region containing the N-terminal domain of TFA1 of essential function (amino acid residues from 1 to 200) was mutagenized by a primer chain reaction technique in vitro, and the mutant library was introduced into yeast cells. We isolated a recessive mutant, tfa1–21, that failed to grow at 37 °C but grew normally at 30 °C (see “Experimental Procedures”). After the temperature up-shift, the wild-type (HS33) and tfa1–21 cells grown in YPD medium. At the indicated times, aliquots of cells were removed, and total RNA was prepared. The integrity of the RNA samples was confirmed by methylene blue staining of RNA (Total RNA). Equal amounts of RNA (2 μg) were dot-blotted on a filter and hybridized with radioactively labeled probe DNAs labeled with 32P at processed RNA regions. For determination of the relative levels of rRNA (rRNA) and tRNAW transcripts, total RNA was subjected to S1 nuclease mapping with probe DNAs labeled with 32P at processed RNA regions. The radioactive intensity of each band on the autoradiograms was measured by scintillation counting and was normalized to the 0 time value as 100%. For determination of the relative levels of rRNA and tRNAW transcripts, the intensity of each band on the autoradiograms was measured with a densitometer and was normalized to the 0 time value as 100%.

To analyze the effect of tfa1–21 mutation on transcription directed by RNA polymerase II, the amount of total poly(A)+ RNA was determined by hybridization with a radioactively labeled-poly(T) probe. Although this approach measures the steady-state level of mRNAs in average, the amount may reflect an average level of newly synthesized mRNAs because half-lives of S. cerevisiae mRNAs are relatively short (2.5–45 min) (25). As shown in Fig. 3, B and C, the level of bulk poly(A)+ RNA declined sharply in tfa1–21 cells after the shift to 37 °C. Such a drastic change in the poly(A)+ RNA level was not seen in cells harboring the wild-type TFA1.

We also investigated effect of tfa1–21 mutation on rRNA synthesis by RNA polymerase I and tRNA synthesis by RNA polymerase III. The amounts of primary transcripts of rRNA and tRNAW were determined by S1 nuclease mapping with the respective probes labeled with 32P at processed regions (5). Within 1 h after the temperature up-shift, the amount of the rRNA and tRNAW transcripts did not change significantly in both wild-type and tfa1–21 cells but then gradually decreased more remarkably in tfa1–21 than in wild-type cells (Fig. 3, B and C). This synthetic pattern of the rRNA or tRNAW transcripts was similar to that previously reported in yeast bearing a ts mutation in RPB1 encoding the largest subunit of RNA polymerase II (5, 15, 26). We assume that the observed effect of the tfa1–21 mutation may be an indirect consequence of defective transcription by RNA polymerase II rather than direct involvement of Tfa1 in transcription directed by RNA polymerase I or RNA polymerase III.

Effect of tfa1–21 Mutation on Transcription of Individual...
Genes—To determine the amount of specific transcripts from individual promoters, total RNA used in the above experiments was subjected to primer extension analysis with specific primers (Fig. 4). The mRNA levels of ACT1, ADH1, CYH2, DED1, MFa1, and TRP5 (left) and HIS3, GAL80, and GAL4 (right) in tfa1–21 cells. The intensity of each band on an autoradiogram was measured with a densitometer and was normalized to the 0 time value as 100%. The half-lives of mRNA encoded by ACT1, CYH2, DED1, MFa1, and HIS3, were determined by Herrick et al. (25) to be 30, 43, 4, 5, and 7 min, respectively. The half-lives of mRNA of ADH1, TRP5, GAL80, and GAL4 were determined in this work to be 43, 52, 20, and 18 min, respectively, by using rpb1–1 mutant cells (RY262; also see Ref. 26) according to the published method (25) except that mRNA was analyzed by primer extension instead of blot hybridization (data not shown).

In contrast to the transcripts studied above, synthesis of certain transcripts appeared rather refractory to the loss of TFIIE function (Fig. 4). 1) The amount of one of the two species of HIS3 mRNA, which starts at +1, did not significantly change in the mutant cells grown at the restrictive temperature, whereas the amount of the other species, which starts at +13, rapidly decreased to an undetectable level within 3 h after the temperature up-shift. The former is directed by a nonconsensus TATA element that is used when the transcription is inefficient, for example, in histidine-abundant environment while the other is directed by the canonical TATA box, which is used when the transcription proceeds efficiently, for example, in histidine-deficient conditions (27, 28). 2) The constitutive transcription of GAL80 is known to be initiated at the +1 site, which is dependent on the initiator element (21). The level of +1 transcript reduced to 60% the original level within the initial 0.5 h, and then remained unchanged at least for 4 h. 3) The level of two species of mRNA from GAL4, which contains neither the TATA or TATA-like sequences nor any other known core promoter elements (29), slightly decreased but was detectable even at 4 h after the temperature up-shift.

To study the effect of tfa1–21 mutation on the basal in comparison with the activated transcription in HIS3 and GAL80, we prepared total RNA from the wild-type or tfa1–21 yeast grown in uninducing as well as inducing conditions before and after the temperature shift (Fig. 5). Transcription of HIS3 is preferentially initiated at the +13 site in the induced (or derepressed) state (27, 28). In agreement with the experiment of Fig. 4, the temperature up-shift of tfa1–21 cells resulted in a rapid disappearance of +13 transcript but no change in +1 transcript. In galactose-grown cells, transcription of GAL80 is initiated at a set of downstream sites that depends on the typical TATA element in addition to the +1 site (21). At the restrictive temperature, tfa1–21 cells showed a rapid reduction in the level of the downstream transcripts. By contrast, the amount of +1 transcript was slightly reduced by the temperature up-shift and then remained unchanged at least for 4 h. Under the identical conditions, the level of GAL7 mRNA, whose synthesis is directed exclusively by the canonical TATA sequence (30), decreased rapidly after the temperature up-
shift. Considering the half-life of the respective mRNA described in the figure legends, we conclude that the amount of the transcripts studied above represented its rate of synthesis.

Interaction of Mutant tfa1 Protein and Small Subunit of TFIIE—The mutant Tfa1 protein contains two amino acid substitutions, Ser at 68 to Pro and His at 103 to Leu. Both alterations cooperatively contribute to the ts phenotype of tfa1–21 (31). We then analyzed interaction as indicated. Transcription initiation sites are indicated on the right. The asterisk indicates an artifact that appeared in all the lanes.

that Tfa1–21 protein failed to bind to Tfa2.

We next studied the intracellular fate of the wild-type and mutant Tfa1 proteins as well as of Tfa2 protein after the temperature up-shift (Fig. 6B). In this experiment, we used a chromosomal tfa1 tfa2 null strain harboring HA-tagged TFA2 in addition to either HA-TFA1 (TFA1, lanes 1–5) or HA-tfa1–21 (tfa1–21, lanes 6–10). As a control, extracts were also prepared from a TFA1 TFA2 wild-type strain lacking HA sequence (lane 13). Equivalent amounts of protein were analyzed by immunoblotting with an anti-HA antibody (12CA5, top) or anti-Rpb1 (SWG16, bottom). 10 and 5% of the extract from wild-type cells before the temperature up-shift were co-electrophoresed as the standard (lanes 11 and 12). Positions of HA-Tfa1, HA-Tfa2, and Rpb1 are indicated on the left.

FIG. 6. Interaction of mutant Tfa1 with Tfa2. A, protein affinity chromatography. Each of fusion proteins GST-Tfa1 and GST-tfa1–21 was immobilized on glutathione-agarose and incubated with a bacterial extract containing Tfa2. Input and bound proteins were electrophoresed in an SDS-polyacrylamide gel and visualized by Coomassie Brilliant Blue staining. Positions of GST, GST-fusions, and Tfa2 are indicated on the left. B, intracellular fate of the wild-type or tfa1–21 protein after the temperature up-shift. Whole cell extracts were prepared from a chromosomal tfa1 tfa2 null strain harboring HA-tagged TFA2 in addition to either HA-TFA1 (TFA1, lanes 1–5) or HA-tfa1–21 (tfa1–21, lanes 6–10). As a control, extracts were also prepared from a TFA1 TFA2 wild-type strain lacking HA sequence (lane 13). Equivalent amounts of protein were analyzed by immunoblotting with an anti-HA antibody (12CA5, top) or anti-Rpb1 (SWG16, bottom). 10 and 5% of the extract from wild-type cells before the temperature up-shift were co-electrophoresed as the standard (lanes 11 and 12). Positions of HA-Tfa1, HA-Tfa2, and Rpb1 are indicated on the left.

FIG. 7. In vitro transcription analyses of GAL80 and GAL7 in the TFIIE-depleted extract. A, immunoblot analysis of HA-Tfa1 and HA-Tfa2 in nuclear extracts. A nuclear extract was prepared from strain HS34, which expresses both HA-TFA1 and HA-TFA2. The extract was incubated with control (α-con) or anti-HA (α-HA) antibody-immobilized resin, and input and unbound proteins were subjected to immunoblot analysis with anti-HA antibody. Positions of HA-Tfa1 and HA-Tfa2 are indicated on the right. B, in vitro transcription analyses of GAL80 and GAL7. The reaction was performed using the indicated extract with GAL80 (top) or GAL7 (bottom) as the template DNA. The transcripts were analyzed by S1 nuclease mapping (GAL80 transcripts) or primer extension (GAL7 transcripts). α-Amanitin (20 μg/ml) and purified TFIIE (40 ng of protein) (18) were added to the reaction mixture as indicated. Transcription initiation sites are indicated on the right. The asterisk indicates an artifact that appeared in all the lanes.

In Vitro Analysis of Promoter-specific Function of TFIIE—To examine whether the promoter-specific functioning of TFIIE in \( \text{in vivo} \) could be reproduced in \( \text{in vitro} \), we constructed a cell-free transcription system composed of a TFIIE-depleted nuclear extract. Thus, whole nuclear extracts from cells expressing HA-tagged Tfa1 and Tfa2 was incubated with an anti-HA antibody to deplete TFIIE. Immunoblot analysis of the nuclear extracts showed that more than 90% of Tfa2 was lost from the extract (Fig. 7A, compare lanes α-con and α-HA). Although the signal of Tfa1 protein was originally weaker than that of Tfa2 (see lanes input and α-con), Tfa1 was not detected in the antibody-treated extract (lane α-HA). Then, \( \text{in vitro} \) transcription reaction was
Reconstituted by using these extracts and GAL80 or GAL7 template. As the promoter sequence, GAL80 template contains from −72 to +84, and GAL7 template contains from −69 to +43, with respect to the most upstream initiation site. As shown in Fig. 7B, transcription of GAL80 (top panel) was initiated at the +1 as well as downstream four sites (+37, +47, +56, and +67). The synthesis of these transcripts was concluded to be catalyzed by RNA polymerase II judged by α-amanitin sensitivity (lanes under Input). In the TFIIE-depleted extract (lanes under α-HA), transcription from +1 was initiated as efficiently as in the control extract (lanes under α-con). Densitometric analysis indicated that +1 transcript in the TFIIE-depleted extract was 75% of the corresponding transcript in the control extract in amount. By contrast, the levels of the +37, +47, +56, and +67 transcripts were reduced to 18, 10, 13, and 16% of the respective control, indicating that transcription from the downstream sites was severely impaired by the loss of TFIIE. Addition of purified TFIIE to the reaction mixture resulted in a restoration of transcription from the downstream sites but exerted no appreciable effect on transcription from +1 (lanes under α-HA). The transcription of GAL7 was also inhibited in the TFIIE-depleted extract (reduced to 7% of the control level), which was restored by the addition of TFIIE to the control level (bottom panel). These results, in accordance with the in vivo experiments, fortify the conclusion that TFIIE exhibits differential effects on different genes and that the gene specificity appeared to be correlated with the structure of the core promoter.

DISCUSSION

Based on the genetic and biochemical experiments described above, we conclude that the requirement for TFIIE for transcription varies in each gene depending on the promoter structures in S. cerevisiae. The transcription of ACT1, ADH1, CYH2, DED1, MFα1, TRP5, and GAL7, which ceased in tfa1–21 cells at the restrictive temperature, is mediated by the canonical TATA sequence (30, 32–37). The TATA-dependent transcription from the +13 site of HIS3 and from the downstream sites of GAL80 was also severely affected by the tfa1–21 mutation. By contrast, transcription mediated by a nonconsensus TATA sequence of HIS3 was not significantly affected in the ts mutant under our experimental conditions. The initiator-directed transcription of GAL80 or unknown element-directed transcription of GAL4 were moderately reduced in the mutant cells at the restrictive temperature. Under these conditions, more than 90% of both subunits of TFIIE, if not 100%, was depleted (see Fig. 6B). Although all the TFIIE-dependent genes tested here contain the canonical TATA sequence, this does not necessarily mean that TFIIE is involved in all the transcriptions from the TATA-containing promoters. Although we have demonstrated that three promoters lacking canonical TATA sequence initiated transcription more or less indifferently of the loss of TFIIE function, it is still possible that promoter elements other than the TATA box are involved in the requirement for TFIIE. Further analysis is required to clarify the relationship between the requirement for TFIIE and the promoter structure. Nevertheless, the present results support the notion that the preinitiation complex varies depending on the structure of core promoters (see below).

It has been known that TFIIE makes functional contact with TFIIH and recruits it to the preinitiation complex in yeast and human (6, 31, 38–44). In both organisms, TFIIH is the only general transcription factor that possesses enzymatic activities, namely helicase, ATPase, and protein kinase (for review, see Refs. 6 and 44). The helicase activity of TFIIH is essential for transcription in yeast (9). Human TFIIIE stimulates the kinase and ATPase activities and, under certain conditions, either stimulates or inhibits the helicase activity (6, 31, 40–44). These results strongly suggest that eukaryotic TFIIIE is involved in transcription reaction through regulating the enzymatic activities of TFIIH. In vitro transcription analyses in mammalian systems suggested that TFIIIE is not always required for accurate transcription initiation. Superciling circular templates or creating a region of heteroduplex DNA around the start site circumvents the requirement for TFIIIE (and TFIIH), suggesting that the TFIIE-dependence is correlated to the helical stability of the promoter region (45–49). In addition, the small subunit of TFIIIE interacts with the sequence upstream of the initiation site in the presence of the large subunit (50). Recent analysis of yeast TFIIIE-RNA polymerase II co-crystals suggests participation of TFIIIE in a key conformational switch occurring at the active center upon polymerase-DNA interaction (51). These observations imply functional correlation between TFIIE and the structure of the start site region, and further suggest involvement of the TFIIE function in the open complex formation. It is also suggested, by using abortive initiation assay, that TFIIIE and TFIIH are involved in the step of promoter clearance after the open complex formation (46). Taking these observations into account, we suggest that the promoter elements of HIS3, GAL80, and GAL4 may have an intrinsic activity to open the initiation site region and thereby to circumvent the requirement for TFIIE in yeast. In the case of the initiator element of GAL80, we have identified an initiator-binding protein in the nucleus (21), which is required for accurate transcription from the initiator.2 It is, therefore, possible that binding of the protein may facilitate the melting of DNA around the start site region. In this context, it may be noteworthy that transcription from the adenovirus-associated virus P5 promoter is mediated by binding of YY1 to the initiator element without participation of any of TBP, TFIIE, TFIIH, and TFIIH (52). Since the sequence encompassing the initiation site is shown to be important for function of TFIIH in a mammalian cell-free system (48), it is interesting to know whether the TFIIE requirement is also affected by the structure of the start site region in yeast.

Although TFIIIE seems not always required for transcription, TFIIH is required for most, if not all, of the genes in yeast (7, 8). A question arises then as to how TFIIH is recruited to the promoters that show a low dependence on TFIIE. Since yeast RNA polymerase II holoenzyme contains TFIIH but not TFIIIE (13, 14), TFIIH can be recruited to any promoter as a component of the holoenzyme without mediation of TFIIH. In the present report, we show that a C-terminal region of Tfa1 was dispensable for normal growth of yeast as well as for transcription of several genes. By contrast, the corresponding region of the human homolog is involved in interaction with TFIIH (31). It is tempting to speculate, therefore, that the function of the C-terminal region of Tfa1 could be substituted for by another general transcription factor and/or a component of the holoenzyme in yeast. An increasing number of reports have suggested that the core promoter is a critical component of specific enhancer-promoter interactions (53–57). TFIIIE in other eukaryotes than yeast has been shown to be a target of several regulatory proteins, such as the Drosophila Kruppel protein (58), Fos/Jun dimer (59), and a putative coactivator p100 for Epstein-Barr virus nuclear antigen 2 (60). These findings may be interpreted to imply that the dependence of TFIIH on the promoter structure is an essential determinant for the enhancer-promoter interaction in various genes. The exact relationship between the promoter structure and the TFIIH/TFIIH interaction remains to be elucidated in the future.

2T. Ohishi, H. Sakurai, and T. Fukasawa, unpublished data.
Requirement for TFIIE for Transcription in Yeast

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