The Yeast *TFB1* and *SSL1* Genes, Which Encode Subunits of Transcription Factor IIH, Are Required for Nucleotide Excision Repair and RNA Polymerase II Transcription

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The essential *TFB1* and *SSL1* genes of the yeast *Saccharomyces cerevisiae* encode two subunits of the RNA polymerase II transcription factor TFIIH (factor b). Here we show that extracts of temperature-sensitive mutants carrying mutations in both genes (*tfb1-101* and *ssl1-1*) are defective in nucleotide excision repair (NER) and RNA polymerase II transcription but are proficient for base excision repair. RNA polymerase II-dependent transcription at the *CYC1* promoter was normal at permissive temperatures but defective in extracts preincubated at a restrictive temperature. In contrast, defective NER was observed at temperatures that are permissive for growth. Additionally, both mutants manifested increased sensitivity to UV radiation at permissive temperatures. The extent of this sensitivity was not increased in a *tfb1-101* strain and was only slightly increased in a *ssl1-1* strain at temperatures that are semipermissive for growth. Purified factor TFIIH complemented defective NER in both *tfb1-101* and *ssl1-1* mutant extracts. These results define *TFB1* and *SSL1* as bona fide NER genes and indicate that, as is the case with the yeast Rad3 and Ssl2 (Rad25) proteins, Tfb1 and Ssl1 are required for both RNA polymerase II basal transcription and NER. Our results also suggest that the repair and transcription functions of Tfb1 and Ssl1 are separable.

Transcription from mammalian or *Saccharomyces cerevisiae* promoters by RNA polymerase II requires multiple initiation factors, including TFIIB (factor e), TFIID (factor d), TFIIE (factor a), TFIIF (factor g), and TFIIH (factor b) (2, 6). The transcriptionally active form of factor b in a yeast reconstituted system in vitro is designated holo-TFIIH, comprising core TFIIH, Ssl2 protein, and the three-subunit kinase TFIIK (22). Core TFIIH with associated Ssl2 (core TFIIH-Ssl2) consists of six subunits of ~105, 85, 70, 50, 55, and 38 kDa (6, 22). The first four subunits have been identified as the products of the *SSL2* (*RAD25*), *RAD3*, *TFB1*, and *SSL1* genes, respectively (6, 10, 22). Homologous genes designated *XPB*, *XPD*, *p62*, and *p44*, respectively, encode subunits of TFIIH(BTF2) in human cells (4, 7, 13, 19, 20). Mutations in the *XPB* and *XPD* genes are associated with several human hereditary diseases, including xeroderma pigmentosum, xeroderma pigmentosum with Cockayne's syndrome, and trichothiodystrophy (1, 27).

Core TFIIH-Ssl2 is required for both transcription by RNA polymerase II and nucleotide excision repair (NER) in *S. cerevisiae* and humans (4, 23, 25, 28). Consistent with their requirement for transcription, *SSL2*, *RAD3*, *TFB1*, and *SSL1* are essential yeast genes (10–12, 17, 18, 34). The same presumably holds true for the homologous human genes, though the essentiality of gene function is difficult to demonstrate directly for mammalian cells.

Recent studies have shown that cell extracts of viable yeast *rad3* and *ssl2* mutants are defective in NER (28). These defects can be corrected by complementation of the extracts with purified core TFIIH or core TFIIH-Ssl2 complexes, respectively, but not by complementation with purified recombinant Rad3

or Ssl2 proteins alone. These observations provide direct evidence that the yeast Rad3 and Ssl2 proteins function in NER as components of the TFIIH-Ssl2 complex. It remains to be experimentally determined whether the remaining four subunits of core TFIIH are required for NER. Until proven otherwise it is possible that a subunit tightly bound in TFIIH is specifically required for transcription but not for repair. Unlike the case with the *XPD* and *XPB* genes, no human diseases have been linked to genes encoding the p62 and p44 subunits of TFIIH. Conditional-lethal *tfb1* and *ssl1* mutants have been identified in *S. cerevisiae* and have been shown to be hypersensitive to killing by UV radiation (16a, 34). However, this phenotype can result from defects in any of multiple cellular responses to DNA damage other than NER. Indeed, the extent of the UV-radiation sensitivity of the available *tfb1* and *ssl1* mutant strains is significantly less than that observed for strains carrying mutations in the *RAD3* gene or other genes that are indispensable for NER (3, 9, 21). Here we demonstrate that *tfb1* and *ssl1* mutants are defective in both transcription and NER in vitro and that defective repair is corrected by the addition of purified core TFIIH. In contrast, extracts of both mutants are competent for base excision repair (BER).

MATERIALS AND METHODS

Strains. The yeast strains used are YSB207 (*TFB1 MAT*a *ura3-52 leu2-3,112 his3Δ200 tfb1Δ1::LEU2*)(pRS316-TFB1) and the otherwise isogenic *tfb1* mutant YSB260 (same as YSB207 except that the plasmid is pRS313-tfb1-101) (16a), JJ567 (*SSL1*) and the otherwise isogenic *ssl1* mutant JJ636 (34), SX46A (*MAT*a *ade2 his3-532 trp1-289 ura3-52*), SF657-2D*rad1Δ* (29), and BJ2168*rad10Δ* (29). The latter two strains have the *RAD1* and *RAD10* genes deleted, respectively.

Yeast nuclear extracts. Nuclear extracts were prepared according to the method of Wang et al. (31, 32). Nuclear extracts of the temperature-sensitive *tfb1-101* and *ssl1-1* strains were prepared similarly with the following modifica-

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tions. Cells were grown in YPD medium (1% yeast extract, 2% Bacto Peptone, and 2% glucose) at 25°C to late logarithmic phase. Cells were harvested by centrifugation, washed once in water, and resuspended to 0.1 g/ml in 0.1 M EDTA-KOH (pH 8.0) plus 10 mM dithiothreitol. After incubation at 25°C for 10 min with shaking, cells were recovered by centrifugation and resuspended to 1 g/ml in YPS solution (1% yeast extract, 2% Bacto Peptone, and 1 M sorbitol). Spheroplasts were obtained by adding yeast lytic enzyme at a concentration of 1.4 mg per g of cells and incubating the cells at 25°C for 1.5 to 2 h. Spheroplast lysis, isolation of nuclei, and production of nuclear extracts were performed as described previously (31, 32).

Yeast whole-cell extracts. Whole-cell extracts of various strains containing overexpressed Rad2 protein were prepared as described previously (31, 32). Whole-cell extracts of the *tfb1* and *ssl1* temperature-sensitive mutants were prepared identically except that cells were grown at 28°C.

In vitro transcription. In vitro transcription in yeast nuclear extracts was performed as previously described with plasmid pCYC1G$^-$ as the DNA template (5, 15, 16). Reactions were stopped by the addition of 20 mM EDTA, and mixtures were treated with 8 U of RNase T1 at 37°C for 10 min. Sodium dodecyl sulfate and proteinase K were then added to 0.5% and 200 μg/ml, respectively. Incubation at 37°C was continued for 30 min. RNA was precipitated in ethanol in the presence of 10 μg of yeast carrier tRNA, washed in 70% ethanol, and dissolved in 12 μl of water. After the addition of 8 μl of a solution containing 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol, the reaction mixtures were heated at 90°C for 2 min and loaded on a 6% sequencing gel for electrophoresis. Transcripts were detected by autoradiography of dried gels.

DNA damage. To obtain *N*-acetyl-2-aminofluorene (AAF)-modified DNA, 100 μg of plasmid pUC18 was incubated at 37°C for 3 h in 1 ml of TE buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA) containing 3 μM *N*-acetoxy-2-acetylaminofluorene (AAAF [the activated form of AAF]) and 20% ethanol. DNA was then purified in a 5 to 20% sucrose gradient in 10 mM Tris-HCl (pH 7.5) plus 1 mM EDTA–0.5 M NaCl. Centrifugation, fraction collection, and DNA identification were performed as described previously (32). Fractions containing supercoiled plasmid DNA were pooled and recovered by ethanol precipitation. The DNA was dissolved in TE buffer and stored at −20°C. To prepare osmium tetroxide-damaged DNA, 100 μg of the plasmid pUC18 was incubated at 70°C for 90 min in 300 μl of a buffer containing 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 100 mM NaCl, and 300 μg of osmium tetroxide per ml. The damaged DNA was purified in a 5 to 20% sucrose gradient as described above.

In vitro assay of NER. Standard reaction mixtures (50 μl) contained 300 ng each of AAF-treated pUC18 DNA and undamaged plasmid DNA [pGEM3Zf(+)]; 45 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)-KOH (pH 7.8); 7.4 mM MgCl$_2$; 0.9 mM dithiothreitol; 0.4 mM EDTA; 2 mM ATP; 20 μM (each) dATP, dGTP, and TTP; 8 μM dCTP; 1 μCi of [α-^{32}P]dCTP (3,000 Ci/mmol); 40 mM phosphocreatine (disodium salt); 2.5 μg of creatine kinase; 4% glycerol; 5 μg of bovine serum albumin; 5% polyethylene glycol 8000; 50 μg of yeast whole-cell extract containing overexpressed Rad2 protein; and 250 μg of yeast nuclear extract. After incubation at 26°C for 2 h, plasmid DNA was purified, linearized with *Hin*dIII restriction endonuclease, resolved by 1% agarose gel electrophoresis, and autoradiographed as described previously (32).

In vitro assay of BER. Standard reaction mixtures (50 μl) contained 300 ng each of OsO$_4$-treated pUC18 DNA and undamaged pGEM3Zf(+) DNA; 45 mM HEPES-KOH (pH 7.8); 7.4 mM MgCl$_2$; 0.9 mM dithiothreitol; 0.4 mM EDTA; 2 mM ATP; 20 μM (each) dATP, dGTP, and dCTP; 8 μM TTP; 1 μCi of [α-^{32}P]TTP (3,000 Ci/mmol); 40 mM phosphocreatine (disodium salt); 2.5 μg of creatine kinase; 4% glycerol; 5 μg of bovine serum albumin; and 80 μg of yeast nuclear extract. Following incubation at 37°C for 2 h, DNA purification, electrophoresis, and autoradiography were performed as described above for the NER assay. To assay BER of uracil-containing DNA, plasmid DNA was replaced by a 30-mer duplex oligonucleotide containing a single uracil residue at a defined site. The uracil-containing strand has the sequence GGATGGCATGCA<u>U</u>TA ACCGGAGGCCGCGCG. Annealing of this oligonucleotide to its 30-mer complementary strand was performed as described previously (30). After incubation at 26°C for 2 h, the duplex DNA was purified by phenol extraction followed by chloroform extraction. DNA was precipitated with ethanol and dissolved in 7 μl of water. The DNA was mixed with 5 μl of a solution containing 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol. The mixture was heated at 60°C for 2 min and loaded onto a 20% sequencing gel for electrophoresis. Repair synthesis was visualized by autoradiography on the wet gel.

RESULTS

Growth characteristics of the *tfb1-101* and *ssl1-1* mutants. As shown in Fig. 1A, both the *tfb1-101* and *ssl1-1* mutant strains were viable at temperatures below 33°C. However, when these cells were grown in liquid medium at 23°C and then plated and incubated at 37°C, less than 10% of the cells, relative to the otherwise isogenic parent strains, survived 2 to 4
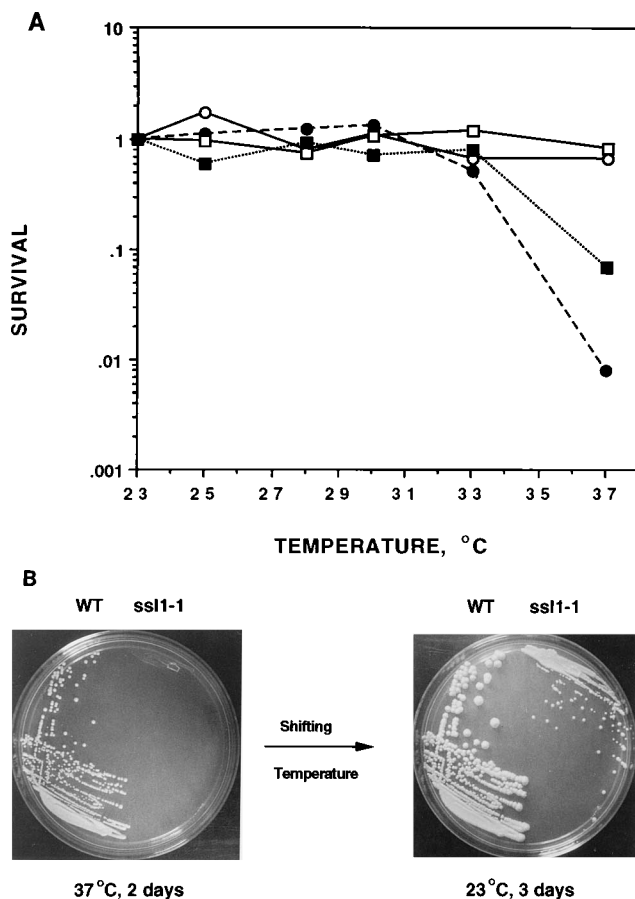

FIG. 1. Effect of temperature on the growth of *tfb1-101* and *ssl1-1* cells. (A) Cells grown in YPD medium at 23°C to the logarithmic phase of growth were diluted in YPD medium and plated onto YPD plates that had been prewarmed to the indicated temperature. Plates were immediately incubated at the indicated temperatures, and colonies were counted after incubation for 2 to 4 days. Results are the averages for triplicate experiments. ○, YSB207; ●, YSB260 (*tfb1-101*), isogenic with YSB207; □, JJ567; ■, JJ636 (*ssl1-1*), isogenic with JJ567. (B) Cultures of JJ567 (wild type [WT]) and JJ636 (*ssl1-1*) in YPD medium at 23°C were streaked onto a YPD plate. After incubation at 37°C for 2 days, the temperature was changed to 23°C and incubation was continued for 3 more days.

days later. The *tfb1-101* strain suffered a permanent loss of viability under these experimental conditions (data not shown). In contrast, the *ssl1-1* strain regained viability and growth capacity when the temperature of the plates was shifted down to 23°C (Fig. 1B).

Transcription activities of *tfb1-101* and *ssl1-1* extracts. Since Tfb1 and Ssl1 proteins are components of core TFIIH (10, 22) we examined the effects of the *tfb1-101* and *ssl1-1* mutations on transcription activity in vitro. Transcription by RNA polymerase II was performed with nuclear extracts prepared from cells grown at 23°C, using plasmid pCYC1G$^-$ as the DNA template. Transcription is initiated by RNA polymerase II from the yeast *CYC1* promoter and elongated through a G-depleted cassette immediately downstream of the promoter (5, 16). The digestion of RNAs by RNase T1 at G residues results in two transcripts of 350 and 375 nucleotides and an occasional third transcript of 400 nucleotides (5, 15, 33). As shown in Fig. 2A, at the permissive temperature (23°C) both *tfb1-101* and *ssl1-1* extracts are proficient for RNA polymerase II transcription. However, pretreatment of the mutant extracts at the restrictive temperature (37°C) for 5 min inactivated RNA poly-
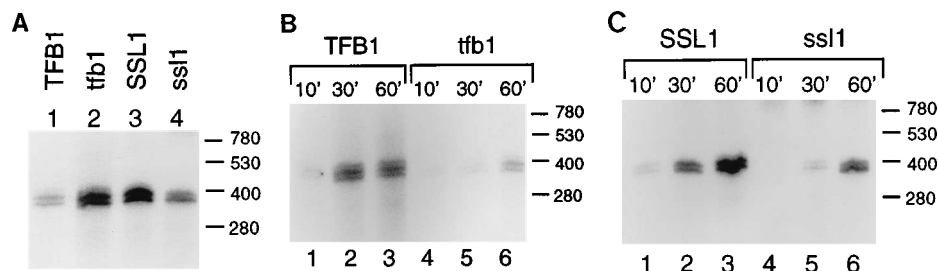
FIG. 2. RNA polymerase II transcription in *tfb1-101* and *ssl1* nuclear extracts. In vitro transcription by RNA polymerase II was performed with 100 μg of yeast nuclear extract as described in Materials and Methods. The G-depleted transcripts were labeled with [α-^{32}P]UMP and detected in gels by autoradiography. RNA size markers (in nucleotides) are on the right. (A) Transcription was performed at 23°C for 60 min. (B) Transcription mixtures were preincubated at 37°C for 5 min without DNA template or [^{32}P]UTP. Transcription was then performed at 23°C for 10 to 60 min as indicated, after the addition of 300 ng of pCYC1G$^-$ DNA and 10 μCi of [α-^{32}P]UTP (3,000 Ci/mmol). (C) Transcription was performed as described for panel B but with the indicated nuclear extracts. TFB1, YSB207 (wild type); tfb1, YSB260 (*tfb1-101*); SSL1, JJ567 (wild type); ssl1, JJ636 (*ssl1-1*).

merase II transcription activity (Fig. 2B and C). In contrast, identical treatment did not affect transcription in isogenic wild-type cell extracts (Fig. 2B and C). Consistent with the reversible effect of heat on the growth of *ssl1-1* cells (Fig. 1B), in vitro mRNA transcription was initially inactivated by heat treatment (compare lanes 4 and 5 of Fig. 2C with lanes 1 and 2 of Fig. 2B and C) but recovered to normal levels after extended incubation at 23°C (compare lane 4 of Fig. 2A with lane 6 of Fig. 2C). This result indicates that *ssl1-1* mutation results in a reversible heat-labile protein for mRNA transcription. This phenomenon was not observed with *tfb1-101* (compare lane 2 of Fig. 2A with lane 6 of Fig. 2B). These results demonstrate that Tfb1 and Ssl1 proteins are required for RNA polymerase II transcription and suggest that the temperature-sensitive phenotype of *tfb1-101* and *ssl1-1* cells is a result of conditionally defective transcription.

NER in *tfb1-101* and *ssl1-1* extracts. NER was monitored with a previously described cell-free system in which repair synthesis of plasmid DNA treated with AAAF was compared with that observed in an undamaged control plasmid (31, 32). Thus, damage-specific incorporation of [^{32}P]dCMP in the treated plasmid DNA catalyzed by the yeast cell extracts is indicative of NER. The experimental conditions used in this cell-free system do not support coupled in vitro transcription and translation (28). Hence, the system exclusively reflects the action of proteins that participate directly in the biochemistry

of NER. As shown in Fig. 3, both *tfb1* and *ssl1* mutant extracts were defective in NER compared with the isogenic parental controls. In both cases, defective repair was observed at 26°C, a temperature that, as noted above, is fully permissive for growth in the absence of DNA damage and for RNA polymerase II-mediated transcription. Consistent with this result, both *tfb1-101* and *ssl1-1* cells showed increased sensitivity to UV radiation at 23°C (Fig. 4). The extent of this sensitivity did not increase in *tfb1-101* cells and increased only slightly in *ssl1-1* cells at the elevated semipermissive temperature (Fig. 4). Neither strain was as sensitive as the *rad3-2* mutant included for comparison (Fig. 4). These results show that whereas the *tfb1-101* and *ssl1-1* mutations result in conditional defects in transcription, they result in constitutive defects in NER. Hence, we conclude that the transcription and NER functions of Tfb1 and Ssl1 proteins are separable.

Complementation of defective NER in *tfb1-101* and *ssl1-1* extracts. Defective NER in extracts of *tfb1-101* and *ssl1-1* cells was complemented to wild-type levels by the addition of a purified multiprotein complex containing core TFIIH, Ssl2, Rad2, and Rad14 proteins (23) (Fig. 5). Complementation was also observed following the addition of purified core TFIIH (factor b) (data not shown). These results demonstrate that Tfb1 and Ssl1 proteins are directly involved in the biochemistry of NER. Hence, like the *RAD3* and *SSL2* genes, *TFB1* and *SSL1* encode polypeptides which participate both in RNA polymerase II transcription and in NER.

As mentioned above, a large excess of purified Rad3 protein does not correct defective NER in *rad3* extracts, suggesting that endogenous mutant Rad3 protein is tightly associated with other components of core TFIIH (28). An excess of purified Ssl2 protein can partially replace the resident mutant form in *ssl2* extracts (28). This and other observations (6, 22, 28) indicate that the Ssl2 protein is less tightly bound than Rad3 in the core TFIIH-Ssl2 complex in vitro. In order to evaluate the stability of Tfb1 and Ssl1 proteins in core TFIIH we attempted to complement *tfb1-101* extracts by the addition of *ssl1-1* extracts and vice versa. As shown in Fig. 6, *tfb1-101* and *ssl1-1* extracts were unable to complement each other for the defective NER. In contrast, *rad10* mutant extracts corrected defective NER in *tfb1*, *ssl1*, and *rad1* extracts. These results suggest that the mutant Tfb1 and Ssl1 subunits are tightly associated in core TFIIH and are unable to exchange with exogenous functional proteins, consistent with previous observations that this complex remains intact following extensive purification (6, 22). These results also suggest that Tfb1 and Ssl1 proteins are required for NER as components of the core TFIIH, further
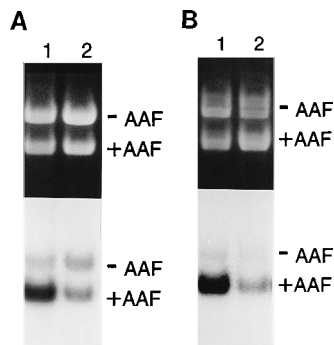

FIG. 3. NER in *tfb1-101* and *ssl1-1* mutant extracts. NER assays in cell extracts were performed at 26°C for 2 h as described in Materials and Methods. (A) NER in YSB207 extract (lane 1) and the isogenic *tfb1-101* mutant extract (lane 2); (B) NER in JJ567 extract (lane 1) and the isogenic *ssl1-1* mutant extract (lane 2). +AAF, pUC18 DNA containing AAF adduct; −AAF, undamaged pGEM3Zf(+) DNA. Top, ethidium bromide-stained gel; bottom, autoradiogram.
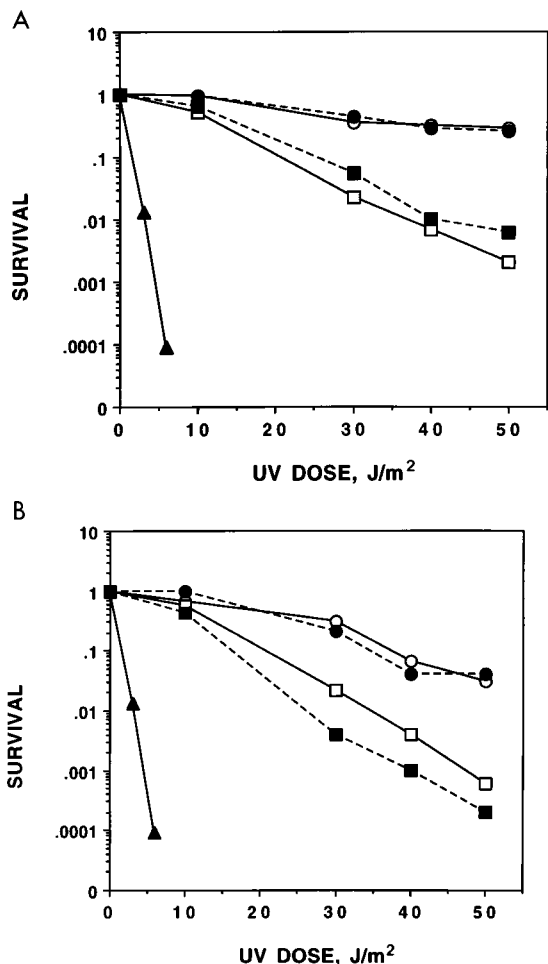
FIG. 4. UV-radiation sensitivity of yeast TFIIH mutants. YPD cultures at 23°C in logarithmic phase of growth were diluted in YPD medium, plated onto YPD plates, and incubated at 23, 30, or 33°C for 30 min. The uncovered plates were then irradiated with UV light at the indicated doses. After incubation at 23, 30, or 33°C for 2 to 4 days, the surviving colonies were counted. Results are the averages for duplicate experiments. Survival rates are expressed relative to the survival rate of nonirradiated cells. (A) UV-radiation sensitivity of YSB207 at 23°C (○) or 33°C (●) and its isogenic *tfb1-101* mutant YSB260 at 23°C (□) or 33°C (■). (B) UV-radiation sensitivity of JJ567 at 23°C (○) or 33°C (●) and its isogenic *ssl1-1* mutant JJ636 at 23°C (□) or 33°C (■). UV-radiation sensitivity of *rad3-2* mutant cells at 30°C (▲) is also shown in panels A and B.

FIG. 5. Complementation of NER in *tfb1-101* (A) and *ssl1-1* (B) mutant extracts. NER assays in cell extracts supplemented with 2 μl (2.8 μg) of yeast TFIIH complex were performed at 26°C for 2 h as described in Materials and Methods. The TFIIH complex was from fraction 36 of the Ni^{2+}-nitrilotriacetic acid-agarose column chromatography, which was preceded by purification on Bio-Rex 70 and phosphocellulose (22, 23). The fraction contained core TFIIH, Ssl2, Rad2, and Rad14 proteins (22, 23). In vitro NER in wild-type cell extracts was not affected by the yeast TFIIH complex. Lanes 1, wild-type NER; lanes 2, complemented NER in mutant extracts. Top, ethidium bromide-stained gel; bottom, autoradiogram.

DISCUSSION

It was previously shown that the Rad3 and Ssl2 subunits of the yeast RNA polymerase II transcription factor TFIIH are required for NER and that these proteins participate directly in this process (28). We now demonstrate that the same is true of two other subunits of yeast TFIIH, the Tfb1 and Ssl1 proteins. These results are presumed to account for the UV-radiation sensitivity of the *tfb1-101* and *ssl1-1* mutants. The relatively modest levels of sensitivity to UV radiation suggest that the *tfb1-101* and *ssl1-1* alleles are leaky in vivo.

It is now evident that all four components of the yeast TFIIH core-Ssl2 complex for which genes have been identified (Rad3, Ssl2, Ssl1, and Tfb1) participate in both NER and transcrip-
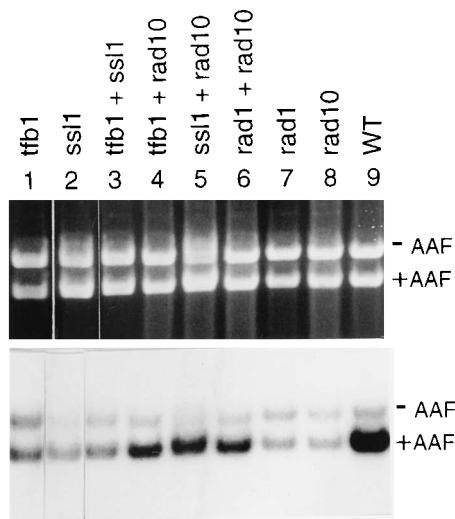
FIG. 6. Complementation of NER with *tfb1*, *ssl1*, and *rad* mutant extracts. Assays of NER in the indicated cell extracts were performed at 26°C for 2 h as described in Materials and Methods. NER of individual extract was in 250 μg of nuclear extract and 50 μg of whole-cell extract containing overexpressed Rad2. Complementation of two different extracts was performed with 150 μg of each nuclear extract plus 50 μg of whole-cell extract from one of the complementing strains overexpressing Rad2. WT, wild-type extract. −AAF, undamaged pGEM3Zf(+) DNA; +AAF, pUC18 DNA containing AAF adduct. Top, ethidium bromide-stained gel; bottom, autoradiogram.

supporting the conclusion that the entire complex of core TFIIH-Ssl2 participates in NER (28).

BER in *tfb1-101* and *ssl1-1* extracts. BER of DNA is a process whereby damaged bases are excised as free bases (8). The biochemical events which precede repair synthesis during BER and NER are distinct. Hence, mutants defective in the latter process are expected to be proficient in the former. To confirm the specificities of defective NER observed in *tfb1-101* and *ssl1-1* extracts, we examined the ability of the extracts to support the repair of uracil-containing DNA and of osmium tetroxide-damaged DNA, both of which require enzymes which operate in the BER pathway (8, 30). As shown in Fig. 7, BER was proficient in both mutant extracts. BER is also proficient in *rad3* and *ssl2-xp* mutant extracts (29, 32). Hence, yeast TFIIH is apparently specifically required for the NER pathway.
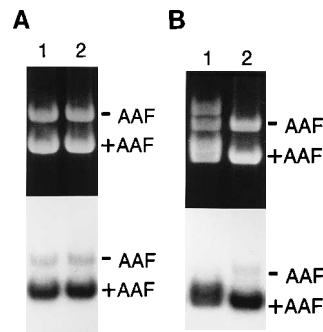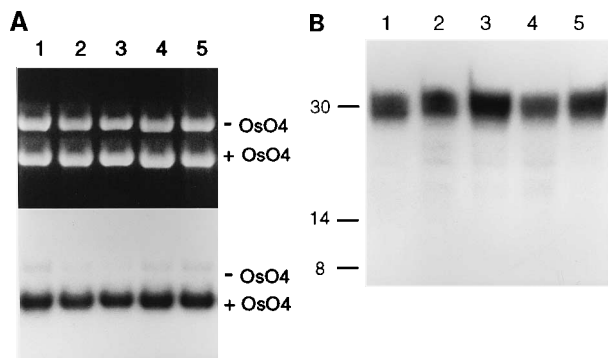
FIG. 7. BER in *tfb1-101* and *ssl1-1* mutant extracts. (A) BER of OsO$_4$-damaged plasmid pUC18 DNA was performed at 37°C for 2 h as described in Materials and Methods. Lane 1, *TFB1*; lane 2, *tfb1-101*; lane 3, *SSL1*; lane 4, *ssl1-1*; lane 5, *rad10*. +OsO$_4$, damaged pUC18 DNA; −OsO$_4$, undamaged pGEM3Zf(+) DNA. Top, ethidium bromide-stained gel; bottom, autoradiogram. (B) BER of a 30-mer duplex DNA containing one uracil residue at position 13 was performed at 26°C for 2 h as described in Materials and Methods. Lane 1, wild type (SX46A); lane 2, *rad10*; lane 3, *TFB1*; lane 4, *tfb1-101*; lane 5, *ssl1-1*. Oligonucleotide size markers are indicated on the left.

tion. Consistent with the latter function, the genes that encode these proteins are all essential for viability (10–12, 17, 18, 34). The repair and transcription functions of these four gene products are apparently distinct, since at least some mutations in the genes differentially inactivate repair and transcription (6, 9, 11, 28). We predict that the remaining two subunits of core TFIIH, the 55- and 38-kDa polypeptides, are also bifunctional and are encoded by essential genes. By implication, the human genes that encode p62 protein (the Tfb1 homolog) and p44 protein (the Ssl1 homolog) are likely also required for NER. Immunodepletion studies with antibodies against p62 and p44 proteins inhibit in vitro NER in human cell extracts (13, 25). However, the entire TFIIH complex was also depleted from the extracts in these studies. Thus, definitive conclusions regarding the role of p62 and p44 in NER await mutational studies of the genes.

Several lines of evidence indicate that core TFIIH-Ssl2 is a stable complex in cells. First, the intact complex can be isolated after extensive purification (22). Second, a vast molar excess of pure protein is unable (in the case of Rad3) or only partially able (in the case of Ssl2) to functionally replace mutant forms of these proteins by mass action in *rad3* and *ssl2* mutant extracts, respectively (28). Third, the present studies show that *tfb1* and *ssl1* extracts are unable to complement each other, whereas they can correct defective NER in extracts containing mutant proteins which are not components of core TFIIH, e.g., *rad10* extracts. These observations support the notion that core TFIIH-Ssl2 is common to two different complexes in yeast cells. While the association of core TFIIH-Ssl2 with TFIIK (22) forms a holo-TFIIH for RNA polymerase II transcription initiation, the association of core TFIIH-Ssl2 with repair proteins forms a repairosome (23) for NER. We have previously noted that this model leads to interesting regulatory considerations (23, 28). Specifically, when cells are exposed to certain forms of DNA damage, core TFIIH-Ssl2 may be preferentially assembled into a larger complex (the repairosome) which is dedicated to NER. This event, coupled with an attendant reduction in the rate of transcription initiation (due to a limitation of core TFIIH-Ssl2 complex), could optimize the potential for NER at sites of stalled transcription that initiated prior to the genetic insult, and at other sites in the genome.

It would appear that the role of the TFIIH-Ssl2 complex in

DNA repair in *S. cerevisiae* is limited to the process of NER. This conclusion derives from the following observations. (i) All the subunits of yeast core TFIIH-Ssl2 thus far examined are required for NER. (ii) None of these proteins are required for BER. (iii) Epistasis analysis has placed the *SSL2* gene exclusively in the *RAD3* epistasis group (which includes genes for NER), with no epistatic relationship to the *RAD52* and *RAD6* epistasis groups, which include genes for recombinational repair and postreplication repair, respectively (18).

In eukaryotes NER appears to be characterized structurally by an extremely large (>800-kDa) multiprotein complex (the repairosome) (23). The size of this complex may severely limit its accessibility to sites of base damage in chromatin. This problem may have been solved during eukaryotic evolution by the direct coupling of the NER and transcription processes. Other DNA repair processes (such as BER) may not be limited by this accessibility problem and hence are not directly coupled to transcription. This hypothesis, which seeks to explain the coupling of the transcription and DNA repair processes, does not necessarily contradict reports in the literature which suggest that forms of base damage which are not usually processed by the NER pathway are nonetheless repaired more efficiently in the transcribed strands of active genes (14, 24). In the latter situation other factors may play a role in targeting various DNA repair modes to sites of damage in the transcribed strand of active genes. For example, in *Escherichia coli* none of the known components of the transcription machinery are required for NER. However, when *E. coli* cells are exposed to UV radiation, the NER machinery is apparently preferentially directed to sites of base damage where transcription is stalled, by a factor designated the transcription repair coupling factor. This factor has not yet been identified in eukaryotes. However, the phenotypes of cells from patients with pure Cockayne's syndrome, i.e., Cockayne's syndrome which is not accompanied by xeroderma pigmentosum, suggest that the products of at least two Cockayne's syndrome genes may function as transcription repair coupling factors (26). Future biochemical analyses of DNA repair during transcription in cell-free systems may shed light on how repair of active genes in preference to inactive genes and of the transcribed strands in preference to the nontranscribed strands is achieved in eukaryotes.

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