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DNA repair deficiencies associated with mutations in genes encoding subunits of transcription initiation factor TFIIH in yeast

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ABSTRACT

Several proteins, including Rad3 and Rad25(Ssl2), are essential for nucleotide excision repair (NER) and function in the RNA polymerase II transcription initiation complex TFIIH. Mutations in genes encoding two other subunits of TFIIH, *TFB1* and *SSL1*, result in UV sensitivity and have been shown to take part in NER in an *in vitro* system. However, a deficiency in global NER does not exclude the possibility that such repair-deficient mutants can perform transcription-coupled repair (TCR), as shown for xeroderma pigmentosum group C. To date, temperature-sensitive C-terminal truncations of Tfb1 are the only TFIIH mutations that result in intermediate UV sensitivity, which might indicate a deficiency in either the global NER or TCR pathways. We have directly analyzed both TCR and global NER in these mutants. We found that *ssl1*, *rad3* and *tfb1* mutants, like *rad25(ssl2-xp)* mutants, are deficient in both the global NER and TCR pathways. Our results support the view that the mutations in any one of the genes encoding subunits of TFIIH result in deficiencies in both global and TCR pathways of NER. We suggest that when subunits of TFIIH are in limiting amounts, TCR may preclude global NER.

INTRODUCTION

DNA damage sensitive mutants of yeast have been assigned to three epistasis groups by complementation analyses which were originally based upon survival studies of cells responding to UV light or ionizing radiation (1,2). The classification of mutants into epistasis groups has been largely supported by biochemical evidence from investigations into the repair of one or more DNA lesions. These three epistasis groups contain mutants with defects in three generally non-overlapping biochemical pathways: nucleotide excision repair (*RAD3* group), spontaneous and DNA-damage induced mutagenesis (*RAD6* group), and recombinogenic mechanisms for damage tolerance (*RAD52* group).

Nucleotide excision repair (NER) is a multistep process initiated by the recognition of DNA damage. Dual single-strand incisions 3′ and 5′ to the damage in the strand containing the damage are then introduced and the damage is excised as part of an oligonucleotide. DNA polymerases, DNA ligase, and additional proteins fill in the resulting gap to regenerate intact duplex DNA. NER has been reconstituted *in vitro* using *Escherichia coli*, human, and yeast cell-free systems. NER occurs via similar biochemical mechanisms in all three systems (3,4).

There are at least two classes of NER: a global repair pathway that deals with lesions throughout the genome and a repair pathway specific for the transcribed strands of active genes termed transcription-coupled repair (TCR). The consequence of TCR *in vivo* is a preferential repair of the transcribed strands of active genes over the non-transcribed strands and the genome overall. *Escherichia coli mfd*$^{-}$ mutants (5), yeast *rad26*Δ mutants (6) and Cockayne's syndrome cells (7), exhibit a deficiency of TCR that results in a reduction of the rate of repair of the transcribed strands of active genes to the same rate as that of the non-transcribed strands and the overall genome. The converse situation exists in xeroderma pigmentosum group C cells and yeast *rad7*Δ and *rad16*Δ mutants. UV-induced DNA damage in these cells is not repaired in the genome overall but the transcribed strands of active genes are repaired very rapidly (8,9). These two classes of NER have been demonstrated in *E.coli*, *Saccharomyces cerevisiae* and mammalian cells (10).

Several yeast proteins required for NER have been shown to be components of the transcription initiation factor TFIIH (11,12). As has been demonstrated for their mammalian homologs (13–15), these repair proteins are either integral parts of the TFIIH protein complex or are accessory factors which co-purify with TFIIH through multiple chromatographic steps. Currently, there are five proteins which associate to make up the heart of the TFIIH complex, which is often referred to as core TFIIH. Core TFIIH consists of the Rad3 (89 kDa), Ssl1 (62 kDa), Tfb1 (73 kDa) and two additional proteins (p55 and p38). In addition, Ssl2(Rad25) (85 kDa) co-purifies with TFIIH (11). Association of additional proteins with core TFIIH/Ssl2 results in formation of holo-TFIIH. Holo-TFIIH possesses protein kinase activity and

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is the transcriptionally active form of TFIIH (36). Yeast strains harboring mutations in either of two genes, *rad3* and *rad25*(*ssl2*), exhibit a complete lack of nucleotide excision repair throughout the genome as well as within expressed genes under control of class II promoters (16–20, this report). Furthermore, temperature sensitive *rad3* and *rad25* mutants are completely deficient in RNA polymerase II transcription at the non-permissive temperature (21,22). The similarities in the repair and transcription deficiencies in the *rad3* and *rad25* mutants suggested that their protein products are required both in repair and in transcription. Protein–protein interactions between Rad3 and Rad25, as part of transcription initiation factor TFIIH, have been demonstrated *in vivo* using the two-hybrid system (23) and *in vitro* (11,12).

It appears that core TFIIH/Ssl2 functions as an intact complex in repair and in transcription. Therefore, it is possible that mutations in genes encoding each of the remaining subunits of TFIIH might be deficient in either TCR or global NER (or both), as in *rad3* and *rad25* mutants. Ssl1 and Tfb1 proteins have been shown to be integral components of TFIIH. Strains with mutations in the genes encoding Ssl1 and Tfb1 are very UV sensitive and the proteins they encode are required in an *in vitro* repair system (24–27). While cell-free systems have been developed for both transcription and NER *in vitro*, there is as yet no eukaryotic *in vitro* system capable of TCR. Thus, investigation of the role of TFIIH and its subunits in TCR requires determination of DNA repair deficiencies *in vivo*. We therefore examined the fine structure of DNA repair in yeast *ssl1*, *tfb1* and *rad3* mutants. We found that mutations in *ssl1* and *rad3*, like the *ssl2-xp* mutation (20), result in severe deficiency in NER, both in global and transcription-coupled pathways.

Various *tfb1* mutants have been used to study the role of Tfb1 in transcription initiation (26). The mutants are temperature-sensitive for growth due to their inability to transcribe at the non-permissive temperature. At the permissive temperature, these temperature-sensitive mutants also exhibit a UV sensitivity intermediate between that of repair-proficient strains and that of *rad3* and *ssl2-xp* mutants (26). The *tfb1* mutants produce different truncated versions of the protein, and the extent of the truncations correlated well with the degree of the UV sensitivity, i.e., the greater the truncation the more UV sensitive the strain. Tfb1 was recently demonstrated to be essential for nucleotide excision repair in an *in vitro* system (27). We found that these truncations of Tfb1 also correlate well with the DNA repair deficiencies of the resultant mutant strains at the permissive temperature.

The temperature-sensitive *tfb1* strains used in this study (mentioned above) all contain a disrupted chromsomal *TFB1* gene. Each strain, including the parent strain, carries a single allele of *TFB1* on a centromeric *ARS* plasmid. The level of protein expression in these temperature-sensitive strains relative to expression from the chromosomal locus is not yet known. Expression of *TFB1* on the plasmid may be higher or lower than the expression of *TFB1* at its chromosomal locus. We found that TCR and NER are deficient in these temperature-sensitive mutants at the non-permissive temperature. Furthermore, the 'parent' strain containing a plasmid-borne *TFB1*$^+$ gene displayed little repair of the non-transcribed strand but retained good repair of the transcribed strand of RPB2 at the non-permissive temperature.

MATERIALS AND METHODS

Media, plasmids and strains

YPD medium is 1% yeast extract/2% Bacto-peptone (Difco)/2% glucose (28). Synthetic glucose medium (SD) is 2% glucose/0.67% bacto-yeast nitrogen base without vitamins (Difco) supplemented with the appropriate amino acids and bases (28). Agar (1.5%) was added to media for plates. Yeast strains used in this study are listed in Table 1. Plasmid pKS212 is a Bluescript vector (Stratagene) which contains the internal 1.0 kb *Eco*RI–*Xho*I from *RPB2* (29). Strand-specific RNA probes for *RPB2* were synthesized by cleaving pKS212 with *Xho*I or *Eco*RI and incubating the linearized plasmid with rNTPs and T7 RNA polymerase or T3 RNA polymerase, respectively, under conditions recommended by the manufacturer.

Growth and UV irradiation of yeast cells

All strains were grown and irradiated as described previously (20). Briefly, cells were grown to log phase in YPD or minimal SD media supplemented with the appropriate amino acids. Cells were collected by centrifugation and resuspended in ice-cold phosphate-buffered saline (PBS) at 1×10^7 cells/ml. The cell suspensions were transferred to Pyrex dishes ($25 \times 15 \times 4$ cm^3) such that the depth of the suspension was ~0.2 cm to ensure a uniform UV dose to all cells. Shaking cell suspensions were irradiated with 30 J/m^2 or 40 J/m^2 of predominantly 254nm UV light at 0.33 J/m^2/s using a Westinghouse L782-30 germicidal lamp. Initial dimer frequencies were 0.73 ± 0.08 or 0.97 ± 0.17 per fragment following exposure to 30 or 40 J/m^2, respectively. The cells were collected by centrifugation after irradiation and either lysed immediately or resuspended in their original growth media. Cells were incubated for various times to allow DNA repair and then lysed. All manipulations were performed under yellow light to preclude photoreactivation.

Table 1. Strains used in this study

Strain	Genotype	Source
JJ565	*MATa, ura3-52, his4-316, ino1-1, SSL2*$^+$*, SSL1*$^+$	T. Donahue
JJ636	*MATa, ura3-52, his4-316, ino1-1, SSL2*$^+$*, ssl1-1*	T. Donahue
JJ638	*MATa, ura3-52, his4-316, ino1-1, SSL2*$^+$*, ssl1-3*	T. Donahue
802-7A	*MATa, ura3-52, his4-316, ino1-1, ssl2-1, ssl1-1*	T. Donahue
LP2649-1A	*MATα, rad3-2, leu2-3,112, ura3-52, can1*	Yeast Genetic Stock Center
YSB207	*MATa, ura3-52, leu2-3,112, his3Δ200, tfb1Δ::LEU2*$^+$*/pRS316-TFB1*$^+$	S. Buratowski
YSB251	*MATa, ura3-52, leu2-3,112, his3Δ200, tfb1Δ::LEU2*$^+$*/pRS313-tfb1-6*	S. Buratowski
YSB151	*MATa, ura3-52, leu2-3,112, his3Δ200, tfb1Δ::LEU2*$^+$*/pRS313-tfb1-1*	S. Buratowski
YSB260	*MATa, ura3-52, leu2-3,112, his3Δ200, tfb1Δ::LEU2*$^+$*/pRS313-tfb1-101[tfbΔSal]*	S. Buratowski

Isolation of yeast DNA

Cells were digested with Zymolyase 100T as described (29). After digestion, spheroplasts were collected by centrifugation and resuspended in 0.2 ml of Zymolyase buffer lacking Zymolyase. Spheroplasts were then diluted with 2.8 ml of 0.05 M Tris–HCl (pH 8.5)/0.05 M EDTA and lysed by the addition of 0.2 ml of 20% Sarkosyl (30). The mixture was incubated at 70°C for 10 min and then chilled on ice. Cellular debris and Sarkosyl were precipitated by the addition of 0.64 ml of 5 M potassium acetate. Mixtures were incubated at 4°C overnight and centrifuged at 6000 r.p.m. in a Sorvall HS-4 rotor at 4°C for 20 min. Supernatants containing chromosomal DNA were transferred to fresh tubes and precipitated by the addition of 2 vol ice-cold ethanol (31). Pellets were washed once with ice-cold 70% ethanol. If necessary, samples were incubated in the presence of RNaseA (final concentration 50 µg/ml) to digest RNA and the DNA was precipitated with ethanol and washed as before. Purified DNA was resuspended in 10 mM Tris–HCl (pH 7.5)/1 mM EDTA (TE) and stored at 4°C.

Strand-specific analysis of frequency of cyclobutane pyrimidine dimers (CPDs)

The incidence of CPDs in a particular restriction fragment was determined by methods developed in this laboratory (32,33). Briefly, 1 µg of purified and restricted DNA in 10 mM Tris–HCl (pH 7.5)/0.1 M NaCl/10 mM EDTA/1 mg/ml BSA was digested with T4 endonuclease V, a CPD-specific DNA glycosylase/AP lyase, in 40 µl for 30 min at 37°C. The specific activity of the T4 endonuclease V, prepared by B. Donahue from an overproducing *E.coli* strain, was ~9×10^{14} U/µg of protein on irradiated DNA and ~1.4×10^{11} U/µg of protein on unirradiated DNA (A. Ganesan and P. Hanawalt, unpublished observation). One unit (U) is defined as 1 nick/min on 250 ng of pSV2gpt DNA irradiated with 32 J/m^2 (~1 CPD/plasmid). T4 endonuclease V digestion of yeast DNA samples was stopped by the addition of 10 µl 12.5% Ficoll/5 mM EDTA/ 0.125% bromophenol blue/0.25 M NaOH. Samples were immediately loaded into 1.0% alkaline agarose gels under 30 mM NaOH/1 mM EDTA and electrophoresed at 1.7 V/cm overnight with recirculating buffer. DNA was transferred to Hybond N$^+$ membrane (Amersham). Membranes were prehybridized for at least 2 h, then hybridized with strand-specific RNA probes made from pKS212. Autoradiographic signal intensities were quantified using a Helena Quick Scan R & D Densitometer.

Genomic analysis of frequency of CPDs

Monoclonal antibodies against CPDs were used to determine the frequency of CPDs in genomic DNA in a modification of the technique of Stapleton *et al.* (34). Antibodies were generated by a mouse immunized with UV-irradiated DNA (35). Denatured genomic DNA (~0.5 µg) in 10× SSPE was applied to Hybond N+ membranes using a slot blot apparatus after which the wells were rinsed with 20× SSPE. DNA was fixed to the Hybond N+ membranes with 0.4 N NaOH as per the manufacturer's instructions and the membranes were rinsed for 1 min in 5× SSPE. Non-specific binding of antibodies to the membranes was prevented by incubation of the membranes in 5% dried non-fat milk in PBS-Tween20 (PBS-T) for at least 1 h at 24°C after which the membranes were washed several times in PBS-T and incubated with the primary mouse monoclonal antibody to CPDs [1:2000 dilution of TDM-2 in PBS (35)] for 4 h at 24°C. Membranes were washed several times in PBS-T and incubated with the secondary goat anti-mouse monoclonal antibody (1:3000 dilution in PBS) radioactively labelled with ^{35}S (Amersham) for 4 h at 24°C, then washed several times in PBS-T and wrapped in Saran wrap. Radioactivity was detected by exposure of X-ray film or phophor screen to the membranes. The amount of DNA bound to the membranes was then determined by hybridization with radioactive RNA or DNA probes specific for the *RPB2* gene. Antibody binding was then corrected for the amount of DNA bound to the membrane.

RESULTS

Suppressor mutations *ssl1-1* and *ssl1-3* result in complete deficiency in removal of CPDs

We measured removal of CPDs from the *RPB2* gene in yeast strains which possess the wild type *SSL1* gene (JJ565) or suppressor alleles *ssl1-1*(JJ636) and *ssl1-3*(JJ638). Exponentially growing cultures at 30°C were irradiated with 30 J/m^2 UV. Cells were either harvested and lysed immediately or returned to their original medium and allowed to repair their DNA before lysis. DNA was isolated as described in Materials and Methods and digested with the appropriate restriction endonucleases. DNA samples were divided into halves, one of which was digested with the CPD-specific enzyme T4 endonuclease V while the other was mock-treated. The DNA was then denatured and electrophoresed through alkaline agarose gels, transferred to nylon membranes and hybridized with radioactive RNA probes specific for either the transcribed or non-transcribed strand of the *RPB2* gene. Membranes that were hybridized with probes for the transcribed strand of *RPB2* in *SSL1*$^+$ and *ssl1-1* are shown in Figure 1. Restoration of hybridization signal at the size corresponding to the full-length restriction fragment in the T4 endonuclease-treated lanes is indicative of repair. It is apparent (Fig. 1) that repair of the transcribed strand of *RPB2* was very rapid in the repair-proficient parent strain *SSL1*$^+$. By 5 min after UV irradiation ~30% of the CPDs had been removed from the transcribed strand and within 30 min, 70% of CPDs had been removed. In contrast, repair of the transcribed strand of *RPB2* was completely absent in the *ssl1-1* strain. There was no restoration of full length restriction fragments which hybridize to the RNA probe for the transcribed strand (Fig. 1).

These membranes were deprobed and hybridized with radioactive RNA probes specific for the non-transcribed strand of *RPB2*. Quantitative analyses of repair levels were obtained by densitometric scanning of autoradiographs (Fig. 2). Repair of the non-transcribed strand in the repair-proficient parent strain was similar to that of several other repair-proficient strains reported previously (20,29). Yeast strains containing suppressor mutations in *SSL1*, *ssl1-1* and *ssl1-3*, showed little or no repair of the non-transcribed strand. Thus, the repair deficiency observed for *ssl1* suppressor mutants is similar to that previously reported for *ssl2-xp* mutants (Fig. 2; 20).

Repair-deficiency associated with a *rad3* mutant

The Rad3 protein, as part of TFIIH, is essential for both transcription initiation (22) and nucleotide excision repair (16–18). The *rad3-2* allele contains a missense mutation leading to incorporation of arginine instead of glycine at position 461.

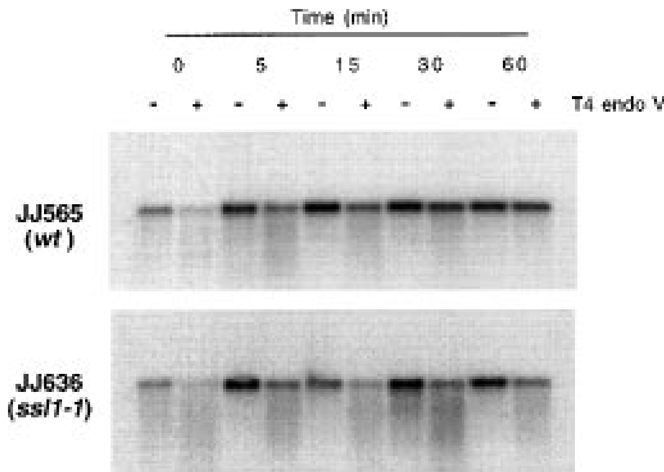

Figure 1. Removal of CPDs from the transcribed strand of the *RPB2* gene is absent in an *ssl1-1* mutant. Exponentially growing cultures of JJ565 (*SSL1*+) and JJ636 (*ssl1-1*) at 30°C were irradiated with 30 J/m^2 of UV and incubated in minimal medium supplemented with the appropriate amino acids for the times indicated. DNA was prepared, digested with restriction endonucleases, treated with T4 endonuclease V or mock treated, electrophoresed and detected with RNA probes specific for the transcribed strand of the *RPB2* gene. The autoradiograms show the 5.3 kb *Pvu*I–*Pvu*II restriction fragment. (**A**) JJ565 (*SSL1*+); (**B**) JJ636 (*ssl1-1*).

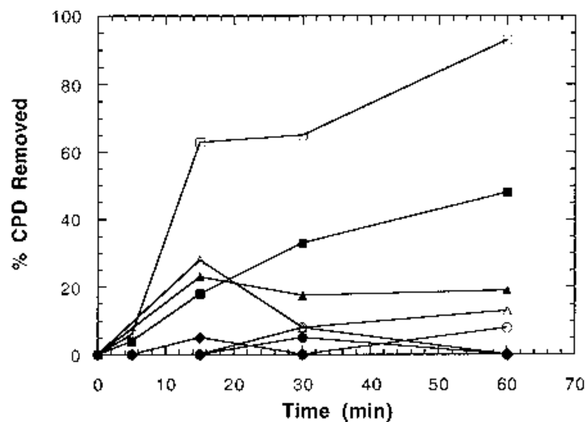

Figure 2. Time course for removal of CPDs from both strands of *RPB2* in JJ565 (*SSL1*+), JJ636 (*ssl1-1*), JJ638 (*ssl1-3*), and LP2649-1A (*rad3-2*). Repair of transcribed strands was determined from the measured incidences of CPDs in the *Pvu*I–*Pvu*II restriction fragments from *RPB2*. Each point represents the average of two experimental determinations with the exception of JJ638 (*ssl1-3*). JJ565 (*SSL1*+) transcribed strand, □, non-transcribed strand, ■; JJ636 (*ssl1-1*) transcribed strand, ○, non-transcribed strand, ●; JJ638 (*ssl1-3*) transcribed strand, ◊, non-transcribed strand, ♦; LP2649-1A (*rad3-2*) transcribed strand, Δ, non-transcribed strand, ▲.

Glycine at postion 461 is conserved among the Rad3 homologues of fission yeast and humans. A repair deficient *rad3-2* mutant is completely deficient in global NER. However, the capacity of *rad3-2* mutants for TCR had not previously been determined.

We measured removal of CPDs from the *RPB2* gene in yeast *rad3-2* mutants. These mutants displayed almost no repair of either the transcribed strand or the non-transcribed strand (Fig. 2). The small amount of repair observed in these experiments is not significantly different from the *ssl1* mutants. The repair deficiency observed for the *rad3-2* mutant is similar to that of *ssl1* and *ssl2-xp* mutants (Fig. 2; 20).

Effect of mutations in the TFB1 subunit of TFIIH on DNA repair

TFB1 is another protein essential for the transcription initiation function by TFIIH. Although originally isolated as a factor associated with a kinase activity which phosphorylated the C-terminal domain (CTD) of RNA polymerase II, it has since been demonstrated that Tfb1 itself does not possess this kinase activity (36).

Mutations which result in truncations of Tfb1 protein at the C-terminus yield strains that display both UV sensitivity and temperature-sensitivity for growth. The extent of the truncations correlates well with the UV sensitivity of the mutant strains at the permissive temperature. At the permissive temperature, the UV sensitivity observed in these temperature-sensitive mutants is intermediate between that of repair-proficient strains and that of *rad3* and *ssl2-xp* mutants (26). We found that these truncations of Tfb1 also correlate well with the DNA repair deficiencies of the resultant mutant strains at the permissive temperature.

We examined the removal of CPDs from the *RPB2* gene in these yeast strains and observed decreased repair of the transcribed strand at the permissive temperature (24°C). The least UV sensitive mutant, *tfb1-6*, exhibited a substantial decrease in the rate of removal of CPDs from the transcribed strand when compared with repair observed for the *TFB1*+ parent strain (Fig. 3). The *tfb1-6* strain removed ~50% of the CPDs within the first 15 min after UV irradiation while the parent strain removed 80–90% of the CPDs in this period. The rapid repair of the transcribed strand observed for the parent strain, relative to repair observed for repair-proficient JJ565 grown in supplemented minimal medium, may be due to the rapid growth (and transcription) that occurs when strains are grown in rich medium. Within 30 min after UV irradiation, *tfb1-6* removed ~65% of the CPDs while *TFB1*+ removed 80–90% of the CPDs in this period. Yeast strains *tfb1-1* and *tfb1-101*, containing greater truncations of the Tfb1 protein, were correspondingly more deficient in repair of the transcribed strand of *RPB2* (Fig. 3). In summary, we found that at the permissive temperature the deficiencies in NER for the temperature-sensitive truncation mutants reflected the degree of UV sensitivity.

Repair of the non-transcribed strand was also determined for the *tfb1* mutants (Fig. 3). Increasingly greater truncations of the C-terminus of Tfb1 resulted in increasingly greater DNA repair deficiencies. For example, *tfb1-6* exhibited reduced repair of the non-transcribed strand of RPB2 compared with the parental strain *TFB1*+. This is similar to the reduction in repair observed for the transcribed strand in *tfb1-6* compared with the *TFB1*+. Thus, the repair of both the transcribed and non-transcribed strands was greatly reduced in *tfb1-6* compared with *TFB1*+. The strains with the largest C-terminus truncations of Tfb1, *tfb1-1* and *tfb1-101*, exhibited the greatest reduction in repair of both the transcribed strand and non-transcribed strands of RPB2, 90 and 100%, respectively.

Repair assays with *tfb1* mutants were repeated at the non-permissive temperature (37°C), at which transcription is abolished (26,27). We observed a decrease in repair of the transcribed strand of *RPB2* in all *tfb1* mutants at 37°C (Fig. 4) compared with the repair observed in these same strains at 24°C (Fig. 3). The

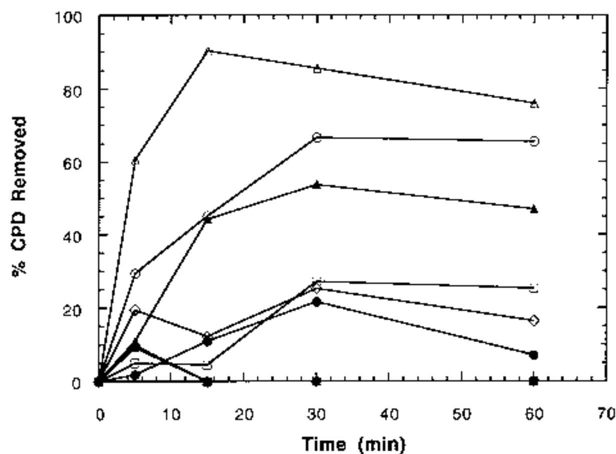

Figure 3. TFB1 is essential for nucleotide excision repair *in vivo*. Time course for removal of CPDs from the transcribed strand of *RPB2* at 24°C in YSB207 (*TFB1*+), YSB251 (*tfb1-6*), YSB151 (*tfb1-1*) and YSB260 (*tfb1-101*). Exponentially growing cultures were irradiated with 40 J/m^2 of UV and incubated in rich medium (YPD) for the times indicated. Each point represents one experimental determination. YSB207 (*TFB1*+) transcribed strand, Δ, non-transcribed strand, ▲; YSB251 (*tfb1-6*) transcribed strand, ○, non-transcribed strand, ●; YSB151 (*tfb1-1*) transcribed strand, □, non-transcribed strand, ■; YSB260 (*tfb1-101*) transcribed strand, ◊, non-transcribed strand, ◆.

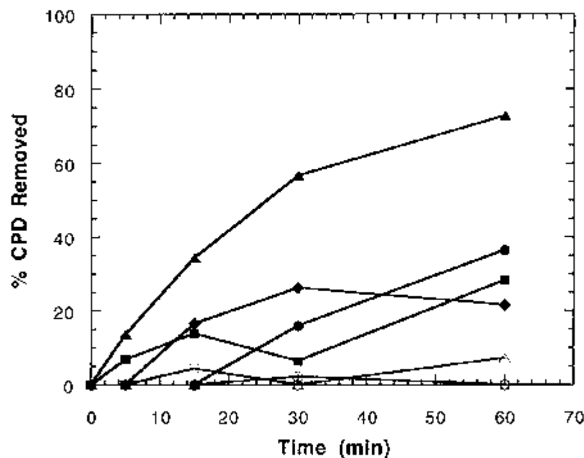

Figure 4. Time course for removal of CPDs from *RPB2* at 37°C in YSB207 (*TFB1*+), YSB251 (*tfb1-6*), YSB151 (*tfb1-1*) and YSB260 (*tfb1-101*). Exponentially growing cultures were irradiated with 40 J/m^2 of UV and incubated in rich medium (YPD) for the times indicated. Each point represents one experimental determination. YSB207 (*TFB1*+) transcribed strand ▲, non-transcribed strand Δ; YSB251 (*tfb1-6*) transcribed strand ●, non-transcribed strand ○; YSB151 (*tfb1-1*) transcribed strand ■, non-transcribed strand □; YSB260 (*tfb1-101*) transcribed strand ◆, non-transcribed strand ◊.

repair-proficient parent strain (*TFB1*+) also showed a significant decrease in repair of the transcribed strand of *RPB2* (Figs 3 and 4). At the non-permissive temperature, *TFB1*+ removed ~30–40% of CPDs from the transcribed strand of *RPB2* during the first 15 min following UV irradiation. In contrast, *TFB1*+ strain removed 80–90% of CPDs by 15 min following UV irradiation at the permissive temperature. Repair of the non-transcribed strand of *RPB2* was completely deficient at the nonpermissive temperature in all *tfb1* strains as well as the parent strain. As shown in Figure 4, all strains examined showed no significant repair of the non-transcribed strand of RPB2 in the 60 min following UV irradiation. This repair deficiency is in marked contrast to the repair of the non-transcribed strand observed in *TFB1*+ and *tfb1-6* at the permissive temperature (Fig. 3). For example, *TFB1*+ removed 40–50% of the CPDs from the non-transcribed strand of RPB2 during the first 15 min following UV irradiation at the permissive temperature.

Repair deficiencies in genomic DNA

We analyzed the rates and extent of removal of CPDs from genomic DNA in *tfb1*, *ssl1* and *rad3* mutants using an assay that utilizes monoclonal antibodies against CPDs (35). We observed repair of CPDs in genomic DNA of the repair-proficient strains *TFB1*+ and *SSL1*+ (Fig. 5). This genomic DNA repair was similar to the repair observed for the non-transcribed strand of *RPB2* in these same mutants (Figs 2 and 3). Genomic repair was also proficient in the least UV sensitive *tfb1* mutant, *tfb1-6* (Fig. 5). Strains containing larger deletions of TFB1 (*tfb1-1*, Fig. 5, and *tfb1-101*, data not shown) exhibited little or no repair of genomic DNA. Strains containing *ssl1-1* (Fig. 5) and *ssl1-3* (data not shown) alleles also exhibited little or no repair of genomic DNA.

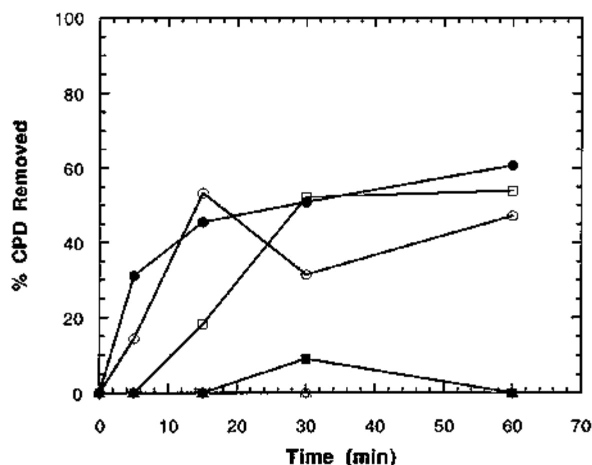

Figure 5. Time course for removal of CPDs from genomic DNA. Monoclonal antibodies against CPDs were used to determine the presence of CPDs in genomic DNA affixed to Hybond N+ membranes. Radioactively-labelled secondary antibodies were used to detect the primary antibodies. Membranes were exposed to X-ray film and autoradiographic signal intensities were quantified using a Hewlett-Packard ScanJet IIp with DeskScan II and Image 1.44 applications. Each point represents the average of two experimental determinations. YSB207 (*TFB1*+) ○; YSB251 (*tfb1-6*) □; YSB151 (*tfb1-1*) ◊; 802-7A (*ssl1-1ssl2-1*) Δ; JJ565 (*SSL1*+) ●; LP2649-1A (*rad3-2*) ■.

DISCUSSION

Previously, we examined the DNA repair deficiency of a yeast strain harboring a mutation in the *RAD25(SSL2)* gene, which encodes for a subunit of the transcription initiation factor TFIIH (11,20). This mutation resulted in a total lack of repair throughout the genome regardless of the transcriptional status of the DNA. We concluded that TFIIH, including Ssl2, must not function

solely in the recognition of DNA damage in the transcribed strand, i.e., transcription-coupled repair. Rather, we proposed TFIIH forms an integral part of the DNA incision complex responsible for DNA cleavage at the site of DNA damage (20,37).

In the present study, we examined DNA repair in yeast strains with mutations in *RAD3*, *SSL1* and *TFB1*, genes encoding other subunits of core TFIIH. Our expectation was that the repair deficiencies in *rad3* and *ssl1* mutants would be similar to those in *ssl2-xp* mutants. We found that the mutations in the *RAD3* and *SSL1* genes resulted in strains with a complete deficiency in the removal of CPDs from the entire genome, not just from transcriptionally active regions of the genome. Our results are consistent with the lack of global repair reported for yeast *rad3* mutants (16,17) and a yeast cell-free repair system made from *rad3* or *ssl1* mutants (36). We extend those results by demonstrating that *rad3* and *ssl1* mutants are defective for preferential repair of the transcribed strand of *RPB2*.

To date, the C-terminal truncations of Tfb1 are the only mutations in a subunit of TFIIH which result in UV survival that is intermediate between that observed for repair-proficient strains and *rad3* or *ssl2-xp* mutants. We examined DNA repair in *tfb1* mutants to test whether they might be deficient in global NER, but not in TCR. We found that increasingly greater truncations of the C-terminus of the Tfb1 protein resulted in strains with diminishing repair capabilities in both TCR and global NER at the permissive temperature. These results strengthen our previous conclusion that TFIIH functions in overall NER as an integral component of the DNA incision complex.

The truncation of Tfb1 might destabilize the protein–protein interactions within TFIIH. It has already been demonstrated that the C-terminus of Tfb1 is required for interactions between Tfb1 and Ssl1 in the TFIIH complex (26). Weakening of the interactions holding TFIIH together would likely result in diminution or total loss of function of TFIIH in NER and/or transcription initiation. When C-terminal truncated Tfb1 protein was used in a reconstituted *in vitro* transcription system, transcription was diminished (27), consistent with the idea of a destabilized TFIIH complex. The repair deficiencies we observed for *tfb1* truncation mutants at the permissive temperature support this idea.

The repair deficiencies observed in *tfb1* mutants and the parent strain (*TFB1*$^+$) at the non-permissive temperature were very different from the deficiencies observed at the permissive temperature. All C-terminal truncation mutants, *tfb1-1*, *tfb1-6* and *tfb1-101*, and the parent strain were deficient in repair of the transcribed strand of *RPB2*. All strains completely lacked repair of the non-transcribed strand of *RPB2* at the non-permissive temperature.

The deficiency of repair in *TFB1*$^+$ at 37°C (the non-permissive temperature) is at odds with results previously obtained with repair-proficient strains (20,29,38; unpublished observations). Repair of both the transcribed and non-transcribed strands of *RPB2* or *GAL7* was faster at 37°C than at 24°C in repair-proficient strains. The repair defect in *TFB1*$^+$ at 37°C resembles the repair deficiency of yeast *rad7*Δ and *rad16*Δ mutants and the human autosomal recessive disease xeroderma pigmentosum group C (XP-C). However, *rad7*Δ, *rad16*Δ mutants and XP-C exhibit no significant deficiency in repair of the transcribed strand of actively transcribing genes compared to repair-proficent cells (8,9). The possibility exists that the unusual repair deficiency of YSB207(*TFB1*$^+$) strains is due to abnormal expression of the plasmid-borne *TFB1* gene. If expression of *TFB1* on the plasmid were lower than the expression of *TFB1* at its chromosomal locus, then levels of Tfb1 protein in the cell would be in limiting amounts. Following exposure to UV radiation, a competition between TCR and global NER (i. e., two subpathways of NER) is established and TCR is favored. On the other hand, if expression of *TFB1* on the plasmid were higher than the expression of *TFB1* at its chromosomal locus, then an excess of Tfb1 protein might lead to the formation of incomplete TFIIH complexes by titrating out the remaining available constituents of core TFIIH (i. e., Rad3, Ssl2, Rad25 and Ssl1 proteins). To test our proposal, the expression of Tfb1 in the strains we examined can be determined at both the permissive and non-permissive temperatures.

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