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An interaction between the Tfb1 and Ssl1 subunits of yeast TFIIH correlates with DNA repair activity

Paul Matsui, Jacqueline DePaulo and Stephen Buratowski*

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115, USA

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ABSTRACT

Yeast transcription factor TFIIH (also known as factor b) is a component of the RNA polymerase II initiation complex. Several TFIIH subunits (*RAD3, SSL2* and *SSL1*) have also been implicated in DNA repair. Ssl1 interacts directly with another TFIIH subunit, Tfb1, which has not previously been shown to have a role in DNA repair. We isolated mutations in *TFB1* that lead to a temperature sensitive phenotype. These mutations result in C-terminal truncations of the Tfb1 protein and disrupt its interaction with Ssl1. The C-terminal 114 amino acids of Tfb1 are necessary and sufficient for this interaction. Interestingly, cells carrying these truncations in Tfb1 cause sensitivity to ultraviolet (UV) light induced DNA damage, as previously observed for mutations in *RAD3, SSL1* and *SSL2*. Many other mutations in RNA polymerase II basal factors were tested and found not to cause an increase in UV sensitivity, indicating that this phenotype is not due to a general defect in transcription.

INTRODUCTION

Transcription by eukaryotic RNA polymerase II requires the assembly of a multi-component initiation complex on the promoter DNA (1,2). One factor required for transcription initiation is TFIIH (also known as BTF2 and δ in the mammalian systems, and factor b in the yeast system). TFIIH is a multi-subunit complex (3–8), and is thought to be one of the last factors incorporated into the initiation complex.

In yeast, highly purified TFIIH preparations contain proteins with molecular masses of 38, 50, 55, 73 and 85 kDa (8). The 73 kDa subunit is encoded by the *TFB1* gene (4), and the 50 and 85 kDa subunits are the products of the *SSL1* and *RAD3* genes, respectively (8). In addition, the Ssl2 protein can associate with the TFIIH complex (8). The structure and function of TFIIH appears to be highly conserved over eukaryotic evolution. The 62 and 44 kDa subunits of mammalian TFIIH are the respective homologues of Tfb1 (3,5) and Ssl1 (9). The 90 kDa subunit of human TFIIH is the product of the *ERCC3* gene (10), which encodes a protein 54% identical in amino acid sequence to Ssl2 (11,12). An 80 kDa protein that associates with human TFIIH is the Rad3 homologue ERCC2 (13).

TFIIH fractions exhibit several enzymatic activities. Consistent with amino acid sequence predictions, TFIIH has an ATP-dependent DNA helicase activity (10,14). TFIIH also exhibits a protein kinase activity that can phosphorylate the C-terminal domain of RNA polymerase II largest subunit (7,15,16). Some mutations in *SSL1, SSL2* and *RAD3* confer sensitivity to ultraviolet (UV) light (11,17,18), suggesting that these genes are necessary for DNA repair activity. Like their yeast counterparts, *ERCC2* and *ERCC3* are both involved in DNA repair (19). It is unclear how this DNA repair activity might be related to the role of these proteins in transcription.

In light of the complicated phenotypic and biochemical behavior of TFIIH, it is important to identify individual subunits, their interactions and their associated functions. It is of particular interest to determine whether each subunit functions in transcription, in DNA repair, or in both processes. In this report, we describe mutations in *TFB1* that confer a temperature sensitive phenotype. These mutations lead to a C-terminal truncation of Tfb1 and disrupt its ability to interact with Ssl1. The *TFB1* mutants also result in hypersensitivity to UV light, suggesting that the interaction between Tfb1 and Ssl1 may be important for DNA repair.

MATERIALS AND METHODS

Isolation of temperature sensitive TFB1 alleles

Mutant yeast strains were derived from YSB208 [*MATα, ura3-52, leu2-3, 112, his3Δ200, tfb1Δ::LEU2, (pRS316-TFB1)*] by plasmid shuffling (20). The *HIS3* plasmid pRS313-TFB1 was created by inserting an *Spe*I fragment from pTFB1 [4] into the *Spe*I site of pRS313 (21). Ten μg of plasmid DNA was mutagenized with hydroxylamine and transformed into YSB208. Approximately 8000 His^+ transformants were replica plated to media containing 5-flouro-orotic acid to select against the *URA3* gene on the unmutagenized plasmid. Colonies were then tested for the ability to grow at 15, 30 and 37°C. Plasmids were recovered from colonies that were temperature sensitive for growth and re-tested. Two plasmids (pRS313-tfb1-1 and pRS313-tfb1-6) were found that supported normal growth at

* To whom correspondence should be addressed

30°C but only very slow growth at 37°C. The *tfb1* coding regions were sequenced using the Sequenase v2.0 kit (US Biochemicals).

Various deletion alleles of TFB1 were tested for complementation of the TFB1 deletion by plasmid shuffling into YSB208. Three C-terminal deletions [pRS313-TFB1ΔSal (carrying amino acids 1–528), pRS313-TFB1ΔCla (amino acids 1–422), pRS313-TFB1ΔR1 (amino acids 1–214)] were created by restriction digest and re-ligation of pRS313-TFB1 (amino acids 1–642).

Mapping the Tfb1–Ssl1 interaction by the two-hybrid system

The original identification of the TFB1–SSL1 interaction by two-hybrid system has previously been described (8). Constructs encoding different regions of Tfb1 fused to the Gal4 binding domain (see below) were tested in combination with a Gal4 activation domain–Ssl1 fusion. Positive interactions were indicated by the ability to grow on histidine deficient media containing 25 mM aminotriazole and interactions were confirmed by the secondary β-galactosidase assay (22).

Gal4–TFB1 fusions were constructed by restriction digestion and re-ligation of the original pGAL4bd-TFB1 to produce the following deletion series: pG4bd-TFB1ΔSal (amino acids 5–528), pG4bd-TFB1ΔH3 (amino acids 5–269), pG4bd-TFB1ΔPst (amino acids 5–141), pG4bd-TFB1-R1 (amino acids 214–642). Additionally, a *Hin*dIII fragment from pG4bd-TFB1-R1 and a *Sal*I fragment from pG4bd-TFB1 were ligated into pY2 (23) to produce pG4bd-TFB1-H3 (amino acids 269–642) and pG4bd-TFB1-Sal (amino acids 528–624), respectively.

Testing for sensitivity to UV light

The viability of various yeast strains after exposure to UV light was determined as previously described (11,17). Strains within each set are isogenic except for the indicated mutation. Strains used to test a *TFB1* mutation are YSB208 (*TFB1+*) and YSB151 (*tfb1-1*), YSB251 (*tfb1-6*), and YSB260 (*tfb1-101*) (this paper). SPT15 testing was done on strains FY23 (*SPT15+*), YSB66 [*spt15(Y94C)*] and YSB67[*spt15(K133, 138L)*] (24; SB, unpublished data). TFIIB mutants were tested in strains YSB141 (*SUA7*), YSB170 (*sua7-4*) and YSB176 (*sua7-35*) (SB, unpublished data). *TDS4* was tested with strains YSB109 (*TDS4+*), YSB121 (*tds4-142*) and YSB123 (*tds4-144*) [25; SB, unpublished data]. *RPB1* was tested with strains DB1033 (*RPB1*) and RY262 (*rpb1-1*) described in (26) as well as Z26 (*RPB1*), C1 (*rpb1-101*) and C3 (*rpb1-103*) described in (27). *SSL1* was tested with strains JJ567 (*SSL1+*), JJ636 (*SSL1-1*) and JJ638 (*SSL1-3*) (17).

RESULTS

The C-terminal region of Tfb1 is necessary for protein stability at high temperature

In order to define the regions of Tfb1 necessary for particular functions, random mutagenesis was performed and conditional alleles of *TFB1* were isolated by plasmid shuffling (20). Two alleles (*tfb1-1* and *tfb1-6*) were identified that grew at 30°C but not at 37°C (Fig. 1). Sequence analysis revealed that both mutations resulted in a premature stop codon. *tfb1-1* carries a deletion of the C-terminal 111 amino acids while *tfb1-6* results in the deletion of only the final 34 amino acids.

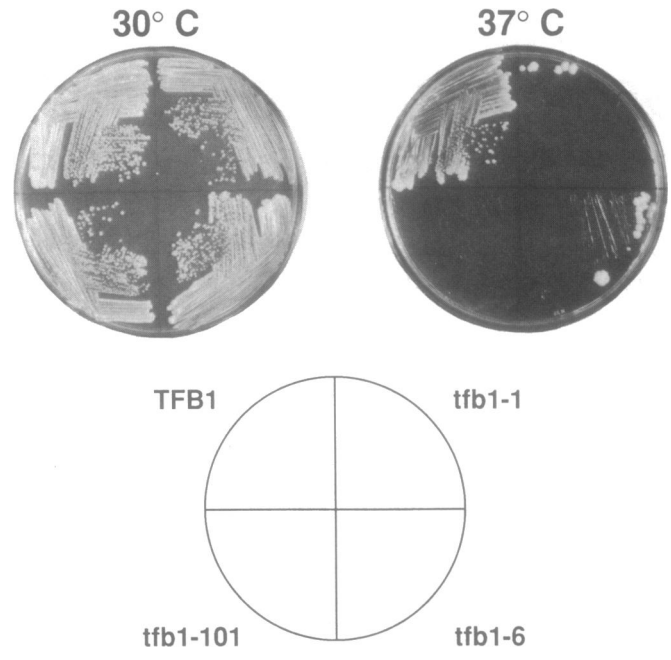

Figure 1. Temperature sensitive growth of strains carrying *TFB1* mutations. Yeast strains carrying the wild-type or the indicated mutant allele of *TFB1* were streaked for single colonies at permissive (30°C) or non-permissive (37°C) temperatures.

In order to characterize the *TFB1* mutants, protein extracts from wild-type and mutant strains were immunoblotted (Fig. 2). At 30°C, the Tfb1-1 mutant protein was produced at levels comparable to wild type protein (lanes 1 and 2). Upon shifting cells to 37°C, the wild-type protein levels were unaffected (lanes 3 and 5), whereas the level of mutant protein greatly decreased (lanes 4 and 6). Similar results were observed with the Tfb1-6 mutant protein (data not shown). Therefore, although the C-terminal residues are not required for viability at 30°C, their absence causes the protein to be unstable at 37°C.

Concurrent with the random mutagenesis of *TFB1*, several deletion alleles were constructed and tested for the ability to support viability. Sequences 3′ to the open reading frame were not essential for *TFB1* function, as they could be deleted with no effect (data not shown). A *TFB1* gene encoding amino acids 1–528 (a deletion of the C-terminal 114 amino acids) caused a temperature sensitive phenotype and was designated *tfb1-101*. Like Tfb1-1 and Tfb1-6, this protein was found by immunoblotting to be degraded at 37°C (data not shown). In contrast to the other deletion mutants, the Tfb1-101 was produced at reduced levels relative to the wild-type protein (data not shown). Further C-terminal deletions of Tfb1 were unable to support viability (see below).

A *TFB1* mutation causes sensitivity to UV light

Some temperature sensitive *SSL1* mutants exhibit increased sensitivity to UV radiation, leading to the suggestion that Ssl1 functions in the repair of DNA (17). Some *SSL2(RAD25)* mutants also exhibit UV sensitivity (11,12). Since Tfb1 is associated

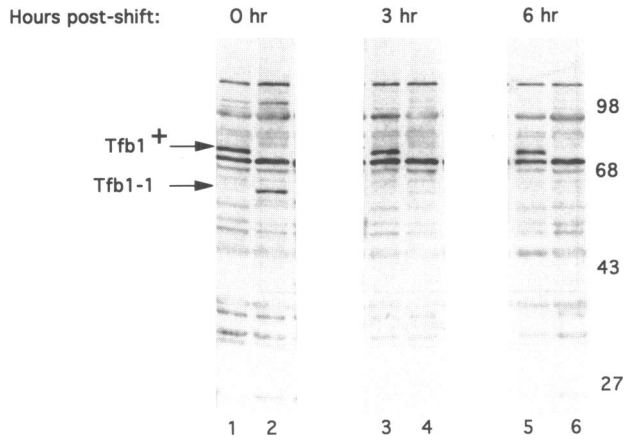

Figure 2. C-terminal deletions of Tfb1 cause the protein to be unstable at high temperatures. Western blot analysis of Tfb1 protein in wild-type *TFB1* and *tfb1-1* strains. Cells were harvested at 0 h (lanes 1 and 2), 3 h (lanes 3 and 4) or 6 h (lanes 5 and 6) after shifting cultures to the non-permissive temperature. Cells were lysed with glass beads and soluble proteins were analyzed by SDS–PAGE and Western blotting with anti-Tfb1 serum. Positions of the wild-type (Tfb1$^+$) and mutant (Tfb1-1) proteins are indicated by arrows. Positions of molecular mass standards are indicated in kDa at the right of the gel.

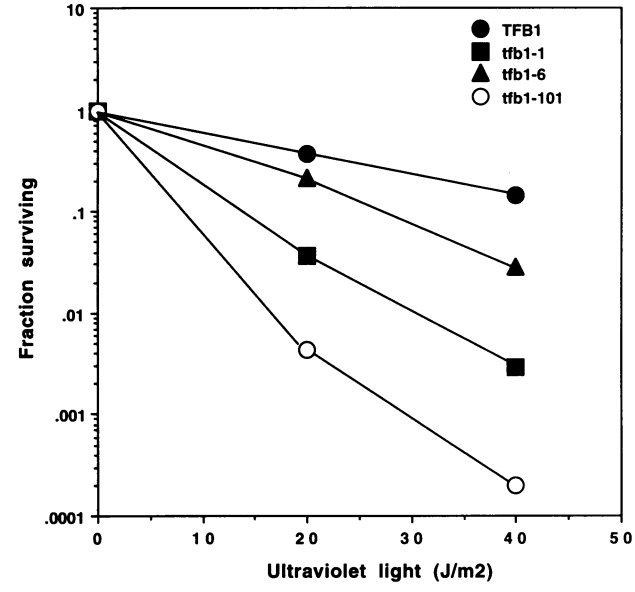

Figure 3. Tfb1 mutations cause increased sensitivity to UV light. Survival curves were plotted for wild-type and mutant cells. The x-axis shows the dose of UV light to which cells were exposed in Joules per square meter (J/m^2). The y-axis shows the fraction of surviving cells (normalized to the number of viable cells in the sample not exposed to UV light). The wild type strain (TFB1, strain YSB208) is indicated by black circles. The isogenic mutant strains are represented by black squares (tfb1-1, strain YSB151), black triangles (tfb1-6, YSB251) and white circles (tfb1-101, YSB260).

within the TFIIH complex with these proteins, we reasoned that *TFB1* mutants might also confer UV sensitivity. Yeast strains carrying temperature sensitive alleles of *TFB1* were therefore tested for resistance to UV light exposure. A survival curve (Fig. 3) indicates that while a wild-type strain was relatively insensitive to the levels of UV light used, the three strains carrying mutations in *TFB1* exhibited increased sensitivity. The severity of sensitivity roughly correlated with the extent of the deletion. The smallest deletion, *tfb1-6*, was ~10-fold more sensitive to 40 J/m^2 UV light than wild type, while *tfb1-101* was >1000-fold more sensitive. Therefore, it is likely that the C-terminal region of Tfb1 is required for repair of UV damaged DNA.

UV sensitivity is not a general property of transcription factor mutations

To test whether any defect in RNA polymerase II transcription might lead to UV sensitivity, several strains carrying mutations in other transcription factor genes were compared with their wild-type parental strains (Fig. 4). All of the mutant strains are temperature sensitive for growth, and many of them exhibit a slow growth phenotype at the permissive temperature.

As previously observed (17), strains carrying mutations in the *SSL1* gene were hypersensitive to UV light (Fig. 4). In contrast, mutations in the TATA binding protein gene (*SPT15*) that affect its DNA binding (Y94C) or protein–protein interactions (K133,138L) did not cause increased sensitivity to UV light (Fig. 4). Similarly, no effect was seen with TFIIB mutants (Fig. 4) bearing substitution in either the zinc finger region (*sua7-35*) or the repeat region (*sua7-4*). Strains carrying mutations in the RNA polymerase III factor TFIIIB were also found to have no increased sensitivity to UV light (Fig. 4).

Yeast strains carrying mutations in the largest subunit of RNA polymerase II were of particular interest. Since TFIIH has been shown to possess a kinase that can phosphorylate the C-terminal domain (CTD) of Rpb1, it was possible that the TFIIH–CTD connection was important for DNA repair. However, no increase in UV sensitivity was caused by partial deletions of the CTD (Fig. 4). A point mutation in the largest subunit of RNA polymerase II that causes the enzyme to be unstable at high temperatures (*rpb1-1*, Fig. 4) led to a very small increase (~3-fold) in sensitivity, but this increase was much less than that seen with mutations in TFIIH subunits. Therefore, UV sensitivity has been observed for each of the cloned TFIIH subunits, but is not a general effect of defects in transcription.

Ssl1 associates with the C-terminus of Tfb1

Ssl1 was previously shown to interact with Tfb1 in the 'two-hybrid' assay (8). This *in vivo* assay is based on the ability of two proteins to bring together the GAL4 DNA binding and transcription activation domains and thereby activate a reporter gene. To map the regions of Tfb1 responsible for interacting with Ssl1, various fusions between Tfb1 and the Gal4 DNA binding domain were made and tested in combination with a Gal4 activation domain–Ssl1 fusion (Fig. 5). The resulting yeast strains were tested for the ability to express a *HIS3* gene under control of a *GAL4* responsive promoter. An interaction is indicated by the ability of the cells to grow on media lacking histidine.

Surprisingly, only the C-terminal region of the Tfb1 protein was essential for interaction with Ssl1. Deletion of the N-terminal 528 amino acids did not disrupt the Ssl1 interaction, whereas a fusion protein carrying only the C-terminal 114 amino acids of Tfb1 (528–642) was still able to interact with Ssl1. Deletions of the C-terminal residues disrupted the interaction with Ssl1. Gal4 binding domain fusions made with the mutant proteins Tfb1-1 or

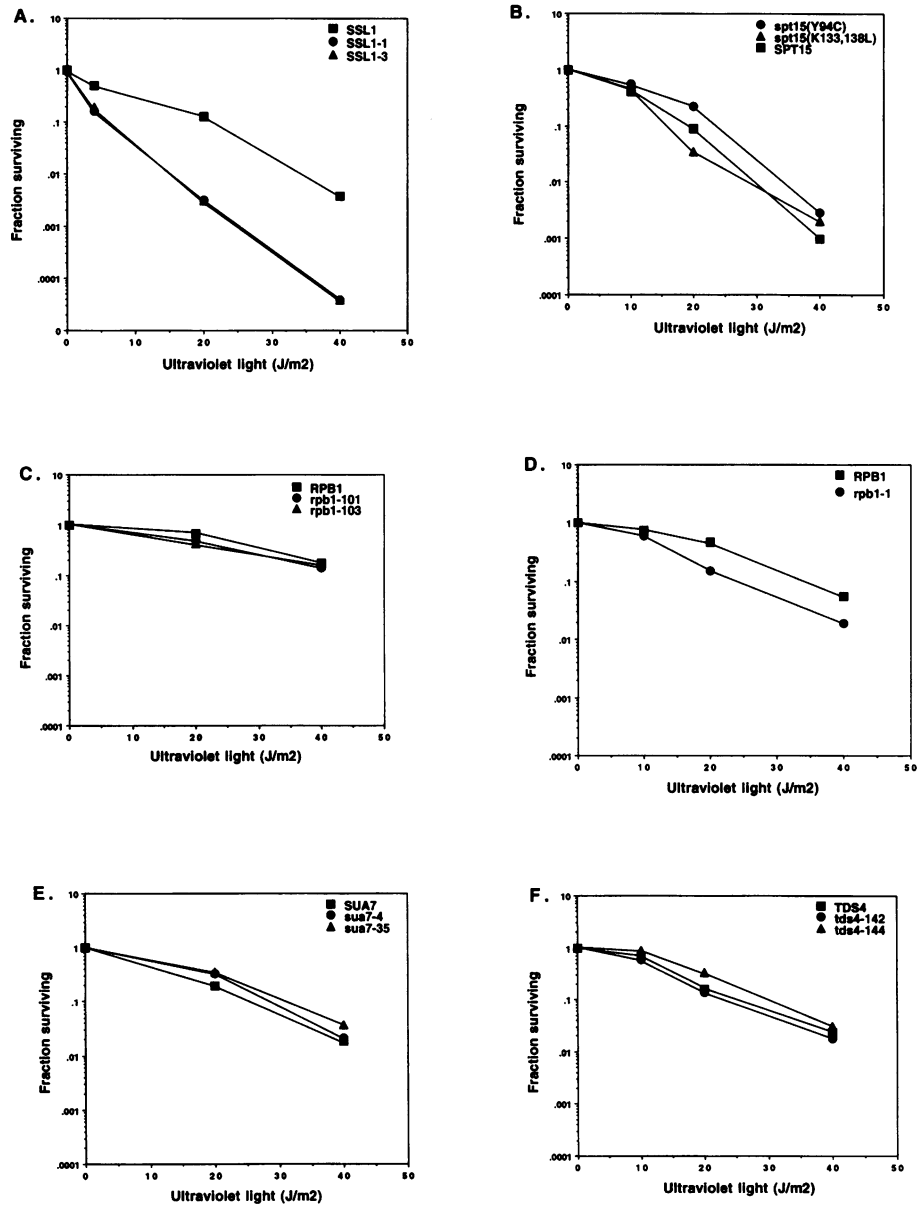

Figure 4. Comparison of UV light sensitivity caused by various transcription factor mutants. Survival curves were plotted as in Figure 3 for yeast strains carrying the indicated mutant alleles, as well as their wild-type isogenic parents. **(A)** *SSL1-1* and *SSL1-3* are temperature sensitive alleles of the 50 kDa subunit of yTFIIH (17). **(B)** The two *spt15* alleles are conditional alleles of the yeast TATA binding protein (TBP) gene [25; SB, unpublished data]. **(C)** *rpb1-101* and *rpb1-103* are temperature sensitive alleles of the largest subunit of RNA polymerase II (26) with deletions of the C-terminal domain ($11^{2/7}$ and $10^{5/7}$ repeats, respectively). **(D)** *rpb1-1* is a temperature sensitive mutant allele of the largest subunit of RNA polymerase II (26). **(E)** *sua7-4* is a mutation in the repeat region of TFIIB and *sua7-35* carries a mutation in the zinc finger region (SB, unpublished data). **(F)** The two *tds4* alleles are temperature sensitive variants of the 70 kDa subunit of the RNA polymerase III transcription factor TFIIIB [25; SB, unpublished data].

Tfb1-6 (lacking 111 or 34 of the C-terminal amino acids) were also unable to interact with Ssl1 (data not shown). Therefore, we have defined a C-terminal region of Tfb1 that is both necessary and sufficient for the Ssl1 interaction.

DISCUSSION

Four subunits of the yeast TFIIH complex have been identified: *SSL1*, *TFB1*, *RAD3* and *SSL2*. The products of these genes are implicated in transcription by their co-purification or association with TFIIH activity. Yet it is striking that mutations in any of these essential genes, including *TFB1*, can cause increased sensitivity to DNA damage by UV light. We show here that UV sensitivity is not a general property of RNA polymerase II transcription factor mutants. These findings suggest that components of the TFIIH complex specifically act in both transcription initiation and DNA repair. It is currently unclear whether both processes utilize the same complex, or different complexes that share some subunits.

It will be important to determine the roles of individual TFIIH subunits in both repair and transcription and whether these

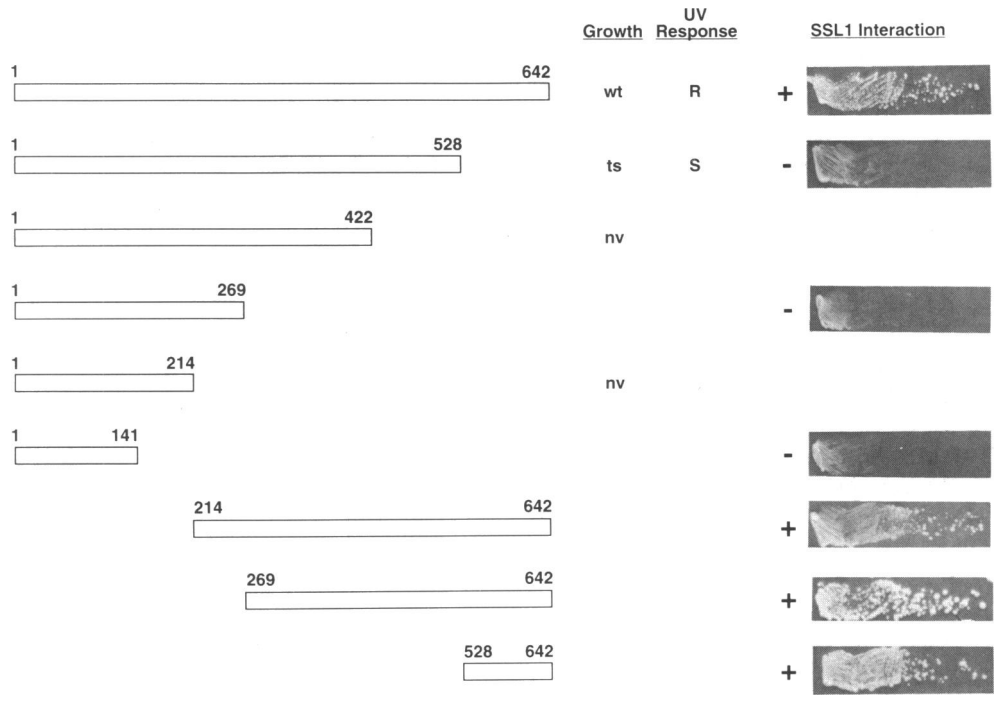

Figure 5. The C-terminal domain of Tfb1 is necessary and sufficient for interaction with Ssl1. Various deletions of the TFB1 gene were created and the positions of the amino acids remaining are indicated. C-terminal deletions were tested for the ability to support viability. Results are indicated in the 'Growth' column. Cells were characterized as wild-type (wt), temperature sensitive (ts) or non-viable (nv). Viable cells were also tested for response to UV light (UV response). Cells carrying full length Tfb1 were resistant (R), while cells carrying the temperature sensitive deletion (1–528) were hypersensitive (S). Tfb1 fusions to the Gal4 DNA binding domain were also created and tested by two-hybrid assay for interaction with an Ssl1–Gal4 activation domain protein (SSL1 interaction). A positive interaction (+) is indicated by the ability of the cells to grow under selective conditions (22).

functions are carried out independently. There is considerable evidence demonstrating that transcribed DNA is repaired at a greater rate than non-transcribed DNA, and that the template strand is preferentially repaired [reviewed in (28)]. Since several TFIIH subunits have also been identified as DNA repair proteins, it seems likely that TFIIH participates in transcription/repair coupling (29). However, at least some of the TFIIH subunits are also important for general nucleotide excision repair (19,30). It is likely that transcription-coupled repair is a specialized form of the repair mechanism used throughout the genome. Therefore, studies of TFIIH will be relevant to the mechanisms of both overall and transcription-coupled repair as well as transcription, and any findings unique to either system will be informative.

The biochemical and phenotypic behavior of TFIIH is complicated. In addition to ATPase and helicase activities, the complex also exhibits a kinase activity that can phosphorylate the unusual C-terminal domain of the RNA polymerase II largest subunit (7,15,16). Recent experiments suggest that the helicase activity, but not the kinase, may be essential for transcription (10,14,31). Both *RAD3* and *SSL2* encode helicase proteins. The helicase activity of Ssl2 is essential for viability (12). The Rad3 helicase activity is not essential for viability, but is required for DNA repair (18). One aspect of TFIIH not yet understood is the observation that *SSL1* and *SSL2* mutants display abnormalities in mRNA translation (11,17).

In this report, we characterize mutant alleles of *TFB1* that are sensitive to both high temperature and UV light. The temperature sensitivity apparently results from the rapid degradation of the proteins at the non-permissive temperature. Biochemical characterization of the mutant alleles has shown that cells with *TFB1* mutations are defective specifically in nucleotide excision repair, the same pathway affected by *RAD3* and *SSL1* mutations (32).

Sequence analysis and immunoblotting show that the mutations all result in partial truncations of the carboxyl terminus of the protein. The C-terminal region of Tfb1 is also necessary and sufficient for interaction with Ssl1. Surprisingly, the primary sequence of the Tfb1 C-terminal region is not highly conserved with its human homologue p62 (4,5). However, there may be structural similarity not apparent from the primary amino acid sequence. Structural studies and analysis of point mutants within this region may help to determine which amino acids are likely to mediate direct protein–protein interactions.

The correlation of Tfb1–Ssl1 interaction with UV and temperature sensitivity suggests several molecular explanations for the observed phenotypes. One possibility is that weakened interactions between TFIIH subunits at the permissive temperature cause a reduction in the amount of active complex in the cell, leading to a deficiency in DNA repair. At the non-permissive temperature, Tfb1 may dissociate completely from the TFIIH complex and then rapidly be degraded. A second possibility is that Tfb1 forms normal amounts of TFIIH complex (presumably through its interactions with TFIIH subunits other than Ssl1) but that the Tfb1–Ssl1 interaction is necessary for the function of TFIIH in DNA repair specifically.

It should be noted that some TFIIH subunits may not be directly involved in DNA repair, yet could produce a UV sensitive phenotype. This could occur if a mutation in the subunit disrupts the entire complex and prevents another subunit from carrying

out its role in nucleotide excision repair. Conversely, some subunits may function only during DNA repair, yet be necessary for the integrity of TFIIH and thereby essential for gene expression and viability. One example may be Rad3. Point mutations that destroy helicase activity cause UV sensitivity, but are otherwise functional for transcription *in vivo* and *in vitro* (18). Further genetic and biochemical analyses will be required to explore the roles of individual subunits in transcription/DNA repair factor TFIIH.

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