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Tfb5 Is Partially Dispensable for Rad26 Mediated Transcription Coupled Nucleotide Excision Repair in Yeast

Baojin Ding, **Christine Ruggiero**, **Xuefeng Chen**, and **Shisheng Li***
Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803

Abstract

Nucleotide excision repair (NER) is a conserved DNA repair mechanism capable of removing a variety of helix-distorting DNA lesions. A specialized NER pathway, called transcription coupled NER (TC-NER), refers to preferential repair in the transcribed strand of an actively transcribed gene. To be distinguished from TCR-NER, the genome-wide NER process is termed as global genomic NER (GG-NER). In *Saccharomyces cerevisiae*, GG-NER is dependent on Rad7, whereas TC-NER is mediated by Rad26, the homolog of the human Cockayne syndrome group B protein, and by Rpb9, a nonessential subunit of RNA polymerase II. Tfb5, the tenth subunit of the transcription/repair factor TFIIH, is implicated in one group of the human syndrome trichothiodystrophy. Here, we show that Tfb5 plays different roles in different NER pathways in yeast. No repair takes place in the nontranscribed strand of a gene in *tfb5* cells, or in both strands of a gene in *rad26 rpb9 tfb5* cells, indicating that Tfb5 is essential for GG-NER. However, residual repair occurs in the transcribed strand of a gene in *tfb5* cells, suggesting that Tfb5 is important, but not absolutely required for TC-NER. Interestingly, substantial repair occurs in the transcribed strand of a gene in *rad7 tfb5* and *rad7 rpb9 tfb5* cells, indicating that, in the absence of GG-NER, Tfb5 is largely dispensable for Rad26 mediated TC-NER. Furthermore, we show that no repair takes place in the transcribed strand of a gene in *rad7 rad26 tfb5 cells*, suggesting that Tfb5 is required for Rpb9 mediated TC-NER. Taken together, our results indicate that Tfb5 is partially dispensable for Rad26 mediated TC-NER, especially in GG-NER deficient cells. However, this TFIIH subunit is required for other NER pathways.

Keywords

Rad7; Rad26; Rpb9; nucleotide excision repair; *Saccharomyces cerevisiae*; Tfb5; global genomic repair; transcription coupled repair

1. Introduction

Nucleotide excision repair (NER) is a ubiquitous DNA repair mechanism capable of removing a variety of helix-distorting lesions, including UV-induced cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts [1]. NER is a multistep reaction and requires the coordinated action of about 30 proteins implicated in damage recognition, helix opening, lesion verification, dual incision of the damaged strand bracketing the lesion, removal of an oligonucleotide containing the lesion, gap-filling DNA synthesis, and ligation.

*Corresponding Author [225-578-9102(Phone)/225-578-9895(FAX)/ shli@vetmed.lsu.edu].

A specialized NER pathway, called transcription coupled NER (TC-NER), refers to preferential repair in the transcribed strand (TS) of an actively transcribed gene. A transcribing RNA polymerase complex stalled at a DNA lesion on the TS may serve as a signal for rapidly recruiting NER machinery [1]. Factors that are specifically required for TC-NER, such as Mfd, Rad26, and Cockayne syndrome complementation group A (CSA) and B (CSB) proteins, have been identified in *Escherichia coli*, yeast and human, respectively [2–7]. Rpb9, a nonessential subunit of RNA polymerase II (Pol II), has been shown to mediate a TC-NER mechanism that is independent of Rad26 in yeast [8].

The genome-wide NER process is sometimes termed as global genomic NER (GG-NER), to be distinguished from the process of TC-NER. In mammalian cells, Xeroderma pigmentosum complementation group C (XPC) protein has been shown to be specifically required for GG-NER, but dispensable for TCR-NER [9,10]. In *Saccharomyces cerevisiae*, Rad7 and Rad16, which do not have significant sequence or structural similarity to XPC, are specifically required for GG-NER, but dispensable for TC-NER [11]. Rad7 and Rad16 are components of a complex that binds specifically to UV damaged DNA in an ATP-dependent manner, and the complex has DNA-dependent ATPase activity [12]. Besides Rad7 and Rad16, the complex may also contain several other factors such as replicating sequence binding factor 1 (Abf1) [13,14]. Recently, it was found that Rad7 and Rad16 are also components of an E3 ubiquitin ligase complex [15,16].

In eukaryotic cells, most DNA sequences that were previously thought to be transcriptionally inert are actually transcribed [17]. Recent high resolution mapping of transcription in *S. cerevisiae* showed that a total of 85% of the genome is transcribed [18]. Therefore, some genes or genomic sequences, which were previously supposed to be transcriptionally “silent” and were assumed to be repaired by GG-NER, may actually be repaired by both GG-NER and TC-NER mechanisms.

Transcription factor IIH (TFIIH) is a multi-protein complex required for both Pol II transcription and NER [19–22]. The role of TFIIH in transcription is mainly at the initiation stage, as it dissociates from the Pol II complex early in the transcription elongation process [23]. The role of TFIIH in NER can be independent of transcription, as the complex is required in reconstituted cell free reactions in the complete absence of transcription [24,25]. It has been shown that the formation of an open DNA structure around a lesion during NER requires the ATP-dependent helicase activities of TFIIH [26–28]. TFIIH may play a unique role in TC-NER. In a short region around the transcription start site, the TS is preferentially repaired in the absence of Rad26 in yeast [29], or CSA and CSB in human cells [30,31]. Preferential repair in the more down stream regions of the TS, however, requires the TC-NER factors Rad26 in yeast [29], and CSA and CSB in human cells [30,31]. It is therefore proposed that TFIIH engaged in transcription initiation may play a direct role in TC-NER. Furthermore, recent studies suggest that a TC-NER complex may be formed without the displacement of Pol II from the DNA [32–35]. TFIIH can be recruited to the TC-NER complex and remodel Pol II to let NER machinery gain access to the lesion on the TS [32,33,36]. Therefore, TFIIH may also play a special role in TC-NER when Pol II is in transcription elongation mode.

Trichothiodystrophy (TTD) is a premature aging syndrome, characterized by sulfur-deficient brittle hair and nails resulting from a reduced level of cysteine-rich matrix proteins. Associated features include progressive mental and physical retardation, ichthyosis, β-thalassaemia trait, unusual facial features, and in many cases photosensitivity [1]. TTD can be caused by mutations in XPB and XPD, two TFIIH subunits that have ATP-dependent DNA helicase activities. The third group of TTD (TTD-A) has recently been found to be caused by mutations in Tfb5, the tenth subunit of TFIIH [37,38]. Tfb5 is highly conserved, with a sequence identity of 28% and a sequence similarity of 56% between human and yeast [37]. In humans, the absence of Tfb5

seems to affect TFIIH stability because the steady-state level of TFIIH in TTD-A cells is about 25–30% of its wild type counterpart [37,39,40]. TTD-A cells are mildly UV sensitive. A UV induced DNA synthesis assay, which measures overall NER synthesis, suggested that NER capacity in TTD-A cells is ~ 10% of that in wild type cells [37,39,40]. In yeast, Tfb5 does not seem to affect TFIIH stability [38]. However, deletion of Tfb5 also causes mild UV sensitivity [38,41]. Furthermore, it was recently shown that whole cell extracts from yeast *tfb5* cells is deficient in overall NER [41]. However, how Tfb5 affects NER in intact yeast cells is still not well known.

In view of the observations that Tfb5 is not absolutely required for NER [37,39–41] and that TFIIH may work on different architectural complexes during different NER processes in the cells [1], it is possible that Tfb5 plays a different role in different NER pathways. We attempted to address this issue by analyzing the roles of Tfb5 in NER in different well-defined yeast NER mutants. We found that Tfb5 is partially dispensable for Rad26 mediated TC-NER, especially in GG-NER deficient cells. However, this TFIIH subunit is required for other NER pathways.

2. Materials and Methods

2.1. Yeast strains

Wild type yeast strain BJ5465 (*MATa ura3-52 trp1 leu2Δ1 his3Δ200 pep4::HIS3 prb1Δ1.6R can1 GAL*) was obtained from the American Type Culture Collection. All deletion mutants were made in BJ5465 background. The cells were transformed with linearized plasmids bearing the respective genes to be deleted, with a portion of their genes replaced by the yeast *URA3* or *LEU2* gene as described previously [8]. The transformed cells were selected on SD plates containing no uracil or leucine at 30°C. In order to introduce a second deletion using a plasmid bearing the gene of interest replaced by the *URA3* gene, the previously introduced *URA3* gene that had replaced the first gene was knocked out. The *URA3* knockout was done by transforming the cells with a linearized plasmid bearing a truncated (with the sequence between the sites of *Stu*I and *Eco*RV removed) *URA3* gene, and selecting the cells on SD plates containing 5-fluoro-orotic acid [42]. All the deletions were confirmed by PCR analysis. Nucleotides (with respect to the starting codon ATG) +214 to +1454, +58 to +2297, +11 to +366, and +15 to + 202 were deleted for the *RAD7*, *RAD26*, *RPB9* and *TFB5* genes, respectively.

2.2. UV irradiation, repair incubation and DNA isolation

Yeast cells were grown at 30°C in minimal medium containing 2% galactose to late log phase (A_{600} ~1.0), harvested, and washed twice with ice-cold water. The washed cells were resuspended in 2% galactose and irradiated with 100 J/m^2 of 254 nm UV light. One-tenth volume of a stock solution containing 10% yeast extract and 20% peptone was immediately added to the irradiated cell suspension, and the cells were incubated for various times in the dark at 30°C before being pelleted. The pelleted cells were broken with glass beads and the genomic DNA was isolated using a hot SDS procedure as described previously [8].

2.3. NER analysis of UV induced CPDs

The gene fragments of interest were 3′ end labeled with [α-^{32}P]dATP using a procedure described previously [43,44]. Briefly, ~ 1 μg of total genomic DNA was digested with restriction enzyme(s) to release the fragments of interest and incised at CPD sites with an excess amount of purified T4 endonuclease V (Epicentre). Excess copies of biotinylated oligonucleotides, which are complementary to the 3′ end of the fragments to be labeled, were mixed with the sample. The mixture was heated at 95°C for 5 minutes to denature the DNA and then cooled to an annealing temperature of around 50°C. The annealed fragments were attached to streptavidin magnetic beads (Invitrogen) and the other fragments were removed by washing the beads at the annealing temperature. The attached fragments were labeled with

[α-^{32}P]dATP (Perkin Elmer), and resolved on sequencing gels. The gels were dried and exposed to a Phosphorimager screen (Bio-Rad).

The signal intensities at gel bands corresponding to CPD sites were quantified using Quantity One software (Bio-Rad). The total signal intensity in a lane of a gel was obtained after the gel background signal was subtracted. The total signal intensity in a lane was used to normalize the loading of different lanes in a gel. The percent CPDs remaining at individual sites following different times of repair incubation were then calculated.

3. Results

3.1. Tfb5 is required for GG-NER

We examined the roles of Tfb5 in NER in yeast cells using a high resolution (nucleotide level) technique. One of the most obvious advantages for using this technique is that repair rates in different regions of a fragment can be resolved on the same gel, and therefore a small difference of repair among different regions can usually be unambiguously identified. Wild type and different NER deficient cells were cultured to late log phase, UV irradiated and incubated for different periods of time. Genomic DNA was isolated, digested with restriction enzymes to release the gene fragment of interest and incised at CPD sites with an excess amount of T4 endonuclease V [45]. The incised fragments were strand specifically end labeled, resolved on DNA sequencing gels and exposed to phosphorimager screens, as previously described [43, 44].

To investigate the role of Tfb5 in GG-NER, we first analyzed repair in the NTS of the constitutively expressed *RPB2* gene and the galactose induced *GAL1* genes. The reason for choosing these two genes for analysis is that transcriptions of these genes are well characterized. Furthermore, many studies [8,11,46,47] have shown that NER in the NTS of the two actively transcribed genes is absolutely dependent on Rad7/Rad16, indicating that repair in the NTS of these genes is entirely accomplished by GG-NER. As expected, apparent repair occurred in wild type cells (Fig. 1A, C, E and F). However, no repair can be seen in the NTS of the genes in *tfb5* cells (Fig. 1B, D, E and F), indicating Tfb5 is required for GG-NER. To confirm this result, we analyzed the role of Tfb5 in *rad26 rpb9* cells, where TC-NER is completely abolished, so that GG-NER can be unambiguously examined [8,46]. Apparent repair occurred in both strands of the *RPB2* gene in *rad26 rpb9* cells (Fig. 2A, C, E and F). In fact, GG-NER in some sites of the gene was very efficient in the TC-NER deficient cells (Fig. 2A and C). However, no repair can be seen in any sites of the gene in *rad26 rpb9 tfb5* cells (Fig. 2B, D, E and F). Taken together, our results indicate that Tfb5 is required for GG-NER.

3.2. Tfb5 is required for Rpb9 mediated TC-NER, but partially dispensable for Rad26 mediated TC-NER in the constitutively expressed RPB2 gene

It has been shown that deletion of *TFB5* in yeast does not significantly affect transcription of most genes [38]. In agreement with the previous report, we found that the levels of *RPB2* transcription are similar between wild type and *tfb5* cells (not shown). Rapid repair can be seen in the TS of the *RPB2* gene in wild-type cells (Figs. 3A and 4A). However, only residual (~ 15 %) repair can be seen in the coding region of the TS in *tfb5* cells during the repair incubation of 4 hours (Figs. 3B and 4A). As mentioned above, no repair can be detected in the NTS of the *RPB2* gene in *tfb5* cells. These results indicate that Tfb5 is important, but not absolutely required for TC-NER.

It has been shown that Rad7 and Rad16 are required for repairing the NTS of transcriptionally active genes [11]. Therefore, *rad7*/*rad16* cells have been repeatedly used to unambiguously examine TC-NER (e.g., see ref. [8,29,46,48]). In agreement with previous reports [11,47], no

repair can be seen in the NTS (not shown), or in the region of the TS that is over 40 nucleotides upstream of the transcription start site (Fig. 3C). Interestingly, substantial (~ 60 %) repair occurred in the coding region of the TS in *rad7 tfb5* cells during the repair incubation of 4 hours (Figs. 3D and 4B). This result indicates that Tfb5 can be largely dispensable for TC-NER in GG-NER deficient *rad7* cells.

In agreement with our previous studies [8,46,49], simultaneous elimination of Rad7 (or Rad16), Rad26 and Rpb9 completely abolishes NER in yeast (Fig. 3I). In *rad7 rad26* cells, where only Rpb9 mediated TC-NER is operative (Fig. 3, compare panels E and I), obvious repair can be seen in the coding region of the TS, especially in the short region immediately downstream the transcription start site (Fig. 3E, marked by the bracket; Fig. 4C). It has been suggested that the rapid repair in the short region immediately downstream of the transcription start site is accomplished by TFIIH, which is associated with Pol II during transcription initiation [29–31]. However, no repair can be seen in the *RPB2* gene of *rad7 rad26 tfb5* cells, including the short region immediately downstream of the transcription start site (Figs. 3F, and 4C). These results indicate that Tfb5 is required for Rpb9 mediated TC-NER throughout the *RPB2* gene.

In *rad7 rpb9* cells, where only Rad26 mediated TC-NER operates (Fig. 3, compare panels G and I), very fast repair can be seen in the coding region of the TS (Figs. 3G and 4D). Substantial repair can also be seen in the coding region of the TS in *rad7 rpb9 tfb5* cells (Figs. 3H and 4D), indicating that Tfb5 is partially dispensable for Rad26 mediated TC-NER in GG-NER deficient cells.

3.3. Tfb5 plays a similar role in TC-NER in the induced GAL1 gene

To see how general the TC-NER trends we observed in the *RPB2* gene is, we also analyzed TC-NER in the *GAL1* gene, which is highly induced by galactose [50,51]. In contrast to the *RPB2* gene, whose transcription level is not significantly affected by Tfb5 (see above) [38], the *GAL1* gene showed ~ 5–10 fold reduction of transcription in *tfb5* cells (not shown). This level of transcriptional reduction in the *GAL1* gene agrees well with a previous report [38]. As the transcription level of the induced *GAL1* gene in wild type cells is extremely high [51], we reasoned that a relatively high level of transcription still occurred in the induced *GAL1* gene in *tfb5* cells.

The trends of Rad26 and Rpb9 mediated TC-NER are similar between the *RPB2* and *GAL1* genes, except that TC-NER in *rad7 rad26* (or *rad16 rad26*) cells is slower in the *RPB2* gene (compare Figs. 3E and 5E, and 4C and 6C) [8]. Also, the TC-NER initiation site in the *GAL1* gene is at about nucleotide -180 (relative to the transcription start site) (Fig. 5), which is more upstream than that of the *RPB2* gene (at about nucleotide -40) (Fig. 3). Similar to the TS of the *RPB2* gene, the TS of the *GAL1* gene showed substantial repair in *rad7 tfb5* (Figs. 5D and 6B) and *rad7 rpb9 tfb5* cells (Figs. 5H and 6D), but no apparent repair in *rad7 rad26 tfb5* cells (Figs. 5F and 6C). These results indicate that, in the induced *GAL1* gene, Tfb5 is required for Rpb9 mediated TC-NER, but partially dispensable for Rad26 mediated TC-NER, especially in GG-NER deficient cells.

4. Discussion

In human cells, the role of Tfb5 in NER may be achieved at least partially by stabilizing TFIIH [40]. In addition, the human Tfb5 may play a direct role in NER. A recent *in vitro* study showed that human Tfb5 (p8/TTD-A) triggers DNA opening by stimulating XPB ATPase activity together with the damage recognition factor XPC-hHR23B [52]. In yeast, the role of Tfb5 in NER may be accomplished primarily by a direct action. It has been shown that the steady-state levels of other TFIIH subunits are not changed in *tfb5* cells [38]. We also observed that the steady-state levels of TFIIH subunits are similar between wild type and *tfb5* strains we used

(not shown). Yeast Tfb5 interacts with Tfb2, another subunit of TFIIH [41]. It was proposed that the yeast Tfb5 acts as an architectural stabilizer conferring structural rigidity to the core TFIIH such that the complex is maintained in its functional architecture [41]

Although it plays a stimulatory role [52], Tfb5 is not absolutely required for NER *in vitro*, as the core TFIIH complex (without Tfb5 and the CTD kinase subunits) is able to open DNA around a lesion [24,53]. Our data presented here indicates that Tfb5 is essential for GG-NER *in vivo* (Figs. 1 and 2). One possibility is that the XPB ATPase activity of TFIIH needs to be stimulated by Tfb5 [52] to efficiently open the DNA structure around a lesion in the chromatin environment *in vivo*. Alternatively, the Tfb5-lacking TFIIH may not be efficiently recruited to a GG-NER complex in the cells. The role of Tfb5 in GG-NER may be similar to that of the Rad7/Rad16 complex, which is also not absolutely required for NER *in vitro*, although it dramatically stimulates the process [42]. However, the Rad7/Rad16 complex is essential for GG-NER *in vivo* [11,47].

We observed that Tfb5 is partially dispensable for Rad26 mediated TC-NER. During TC-NER, TFIIH is recruited to damaged DNA only in the presence of Rad26 [54]. One possibility is that Tfb5-lacking TFIIH can still be efficiently recruited to a TC-NER complex by Rad26. The recruited Tfb5-lacking TFIIH may, to some extent, be able to open the DNA around a lesion. However, this scenario can not explain the Rad26-independent TC-NER close to the transcription start site. TFIIH is essential for transcription initiation, and is obligatorily loaded to the transcription initiation complex [23]. TC-NER in the yeast *URA3* gene becomes Rad26 dependent 30 – 40 nucleotides downstream from the transcription start site [29]. In the human *JUN* gene, TC-NER becomes CSA and CSB dependent at about + 20 nucleotides into the coding region [30,31]. It has been proposed that this TC-NER factor-independent TC-NER close to the transcription start site may be due to the association of TFIIH with Pol II, as TFIIH is believed to be released from Pol II 30 – 60 nucleotides downstream from the start site [55]. In this study, we also observed efficient TC-NER close to the transcription start site in the *RPB2* gene of *rad7 rad26* cells (Fig. 3E, marked with a bracket). However, simultaneous deletion of *TFB5* and *RAD26* genes completely abolished TC-NER in this region of the gene (Fig. 3F), indicating that the Rad26-independent TC-NER in this region is Tfb5 dependent. Therefore, an alternative scenario would be that Rad26 may be able to facilitate the opening of lesion-containing DNA at the transcription bubble without the participation of a fully functional (Tfb5-containing) TFIIH. Rad26 is a DNA-dependent ATPase [56]. It also contains a DNA helicase motif, although no helicase activity can be detected [57]. Recent studies in human cells or purified NER factors suggested that a TC-NER complex may be formed without the displacement or degradation of Pol II [32–35]. Our recent results in yeast also suggest that ubiquitylation and subsequent degradation of Rpb1, the largest subunit of Pol II, is unrelated to TC-NER [58].

We constantly observed that TC-NER is faster in GG-NER deficient *rad7*/*rad16* cells than in wild type cells (compare Fig. 3A and C, and Fig. 5A and C; see also Fig. 1 in ref. [8]). Interestingly, we also observed that TC-NER is more efficient in *rad7 tfb5* cells than in *tfb5* cells (Figs. 3–6). The reason(s) for the observations remain(s) to be understood. One explanation is that the Rad7/Rad16 complex and Rad26 may compete for NER factors that are shared by different NER pathways, and elimination of Rad7/Rad16 may make more NER factors available to be recruited by Rad26. An alternative explanation is that Rad7/Rad16 may play a role in inhibiting TC-NER.

In contrast to Rad26 mediated TC-NER, Rpb9 mediated TC-NER appears to be dependent on Tfb5. Our recent data suggests that the transcription elongation function of Rpb9 is involved in TC-NER, and impairment of transcription elongation abolishes Rpb9 mediated TC-NER [49]. However, the requirement of Tfb5 for Rpb9 mediated TC-NER does not seem to be due

to a role for Tfb5 in transcription elongation. Cells with a deficiency in transcription elongation are generally sensitive to nucleotide-depleting drugs, such as 6-azauracil and mycophenolic acid [59]. However, *tfb5* cells are not sensitive to these drugs (not shown). Therefore, without the involvement of Rad26, the Tfb5-lacking TFIIH may not be able to remodel the Pol II complex stalled at a lesion or to open the DNA round the lesion at the transcription bubble *in vivo*, resulting in deficiency in Rad26-independent TC-NER.

In view of the fact that Tfb5 [37] and the NER process [1] are highly conserved between yeast and humans, it is reasonable to speculate that Tfb5 plays similar roles in different NER pathways in human cells. It would be very interesting to test this idea.

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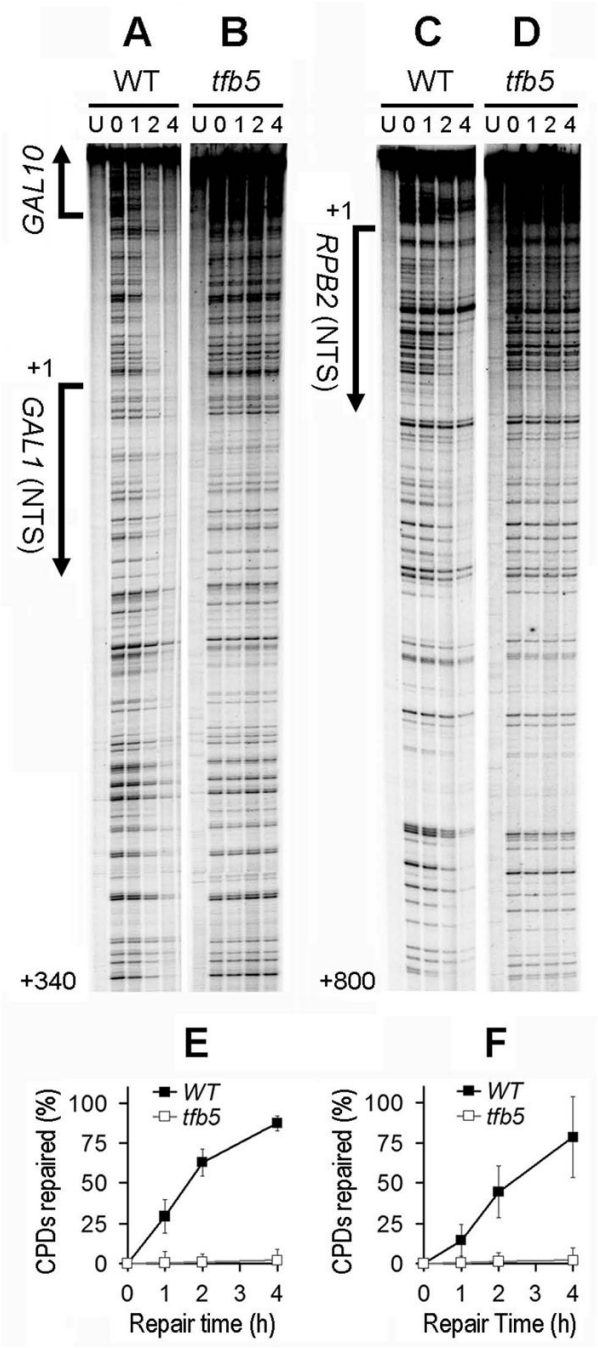

Fig. 1. NER in the NTS of the induced *GAL1* gene and the constitutively expressed *RPB2* gene in wild type (WT) and *tfb5* cells

(A) – (D) Gels showing NER in the NTS of the two genes. (E) Plot showing percent CPDs repaired (mean ± standard deviation) in the NTS (+1 to + 340) of the *GAL1* gene. The data were obtained by quantification of the gels shown in panels A and B. (F) Plot showing percent CPDs repaired (mean ± standard deviation) in the NTS (+1 to + 800) of the *RPB2* gene. The data were obtained by quantification of the gels shown in panels C and D.

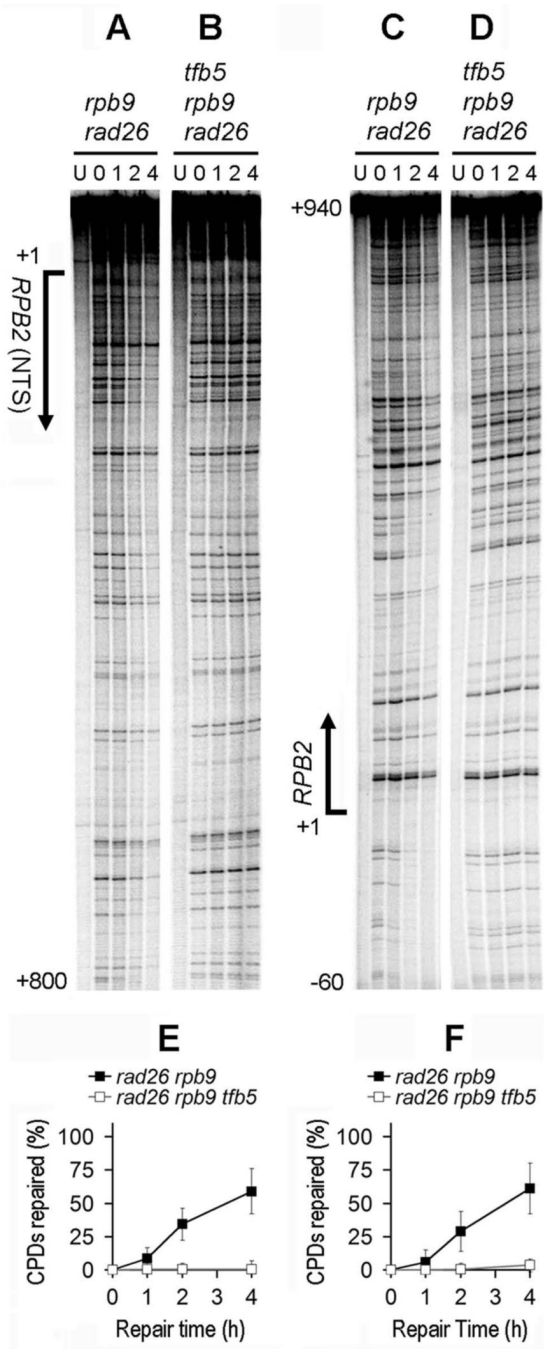

Fig. 2. NER in the *RPB2* gene of TC-NER deficient *rad26 rpb9* cells

(A) – (D) Gels showing NER in the *RPB2* gene in *rad26 rpb9* and *rad26 rpb9 tfb5* cells. (E) Plot showing percent CPDs repaired (mean ± standard deviation) in the NTS (+1 to + 800) of the *RPB2* gene. The data were obtained by quantification of the gels shown in panels A and B. (F) Plot showing percent CPDs repaired (mean ± standard deviation) in the TS (+1 to + 940) of the *RPB2* gene. The data were obtained by quantification of the gels shown in panels C and D.

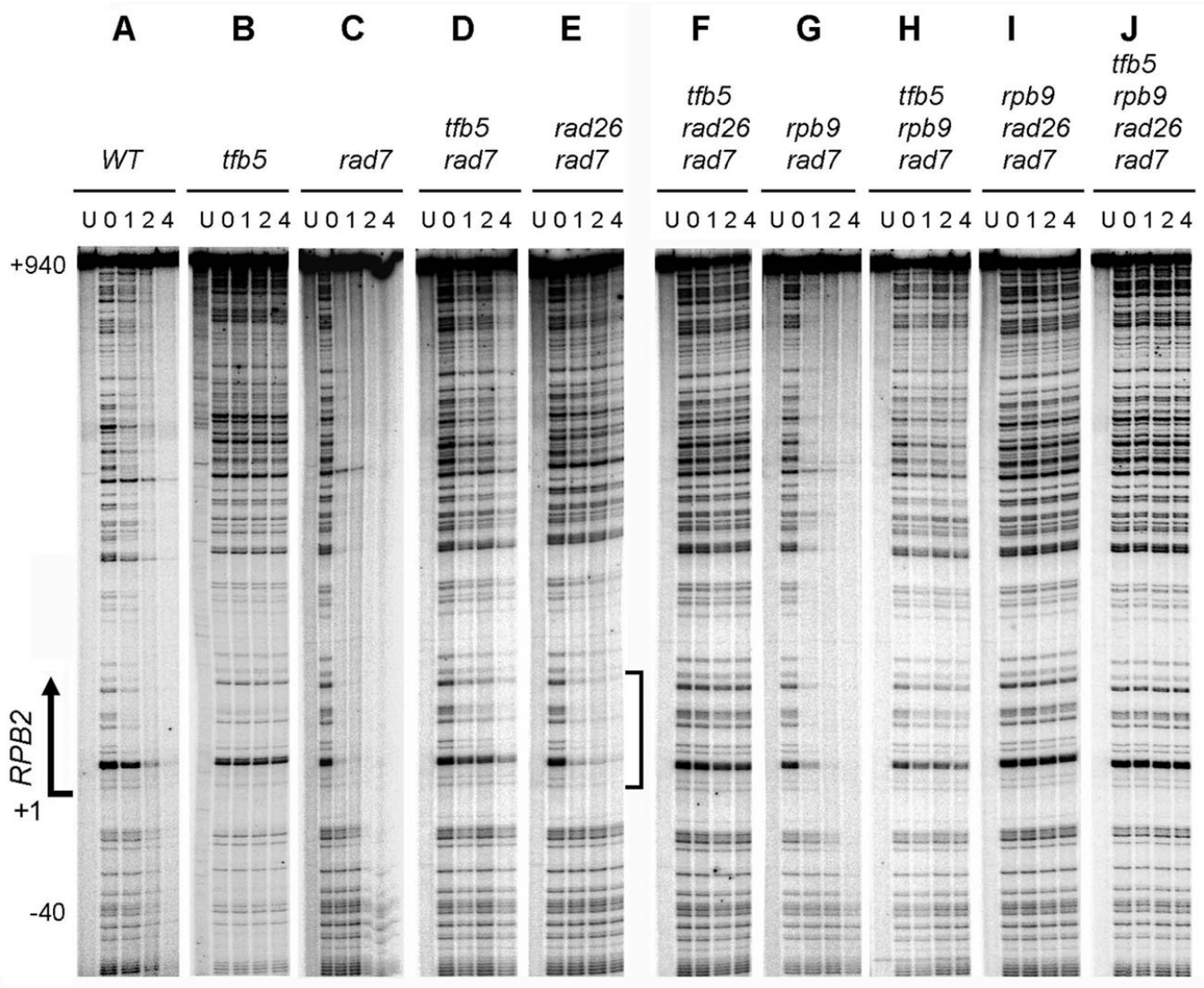

Fig. 3. TC-NER in the constitutively transcribed *RPB2* gene

The lanes are DNA samples from unirradiated (U) and UV irradiated cells following 0, 1, 2 and 4 h repair incubation. The arrow on the left of the gels indicates the transcription start site. The bracket on the right of panel E marks the region where robust Rad26-independent TC-NER occurs.

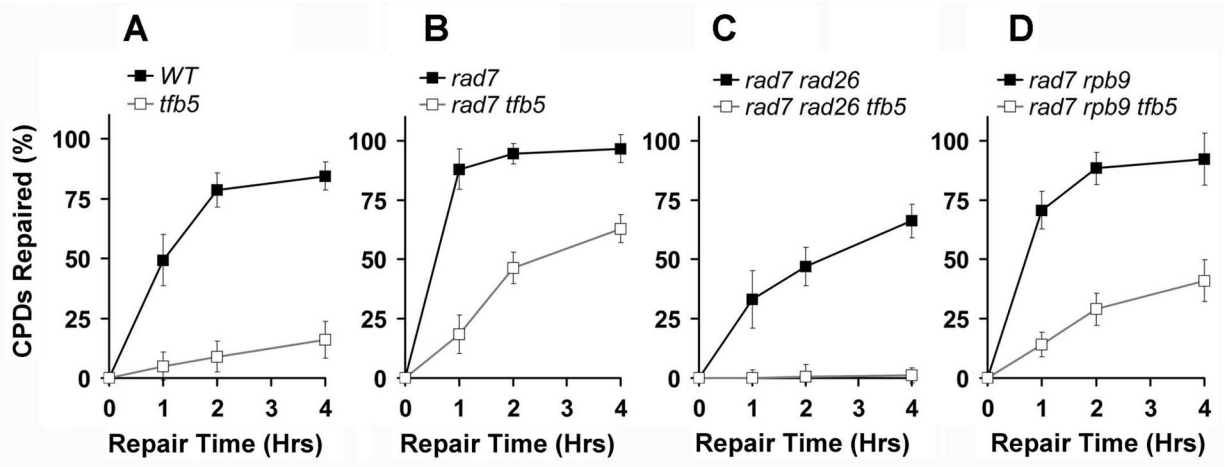

Fig. 4. Plots showing repair of CPDs in the TS (+1 to + 940) of the *RPB2* gene

The data were obtained by quantification of the gels shown in panels A – H of Fig. 3. The values are shown as average (mean ± standard deviation) of the percent CPDs repaired at different times of repair incubation.

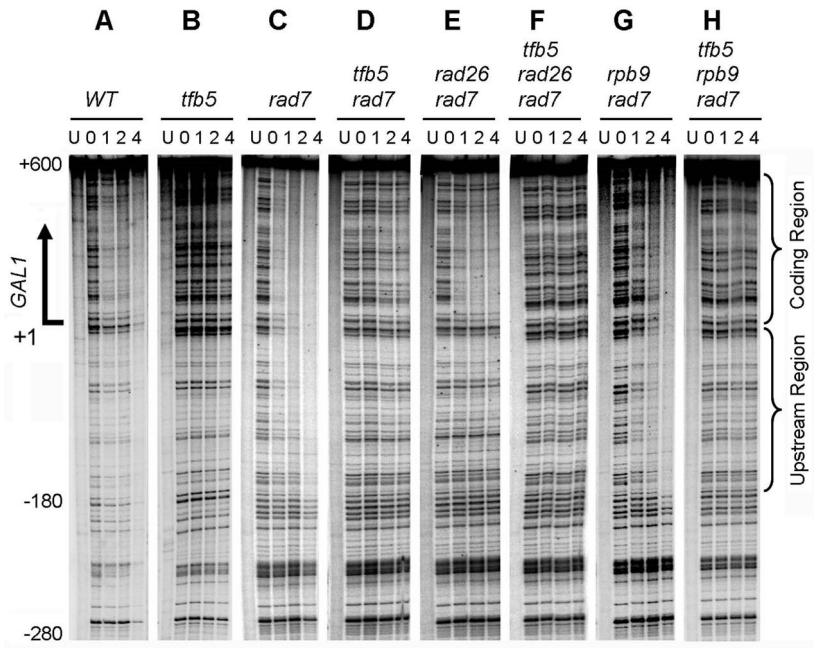

Fig. 5. TC-NER in the galactose induced *GAL1* gene

The lanes are DNA samples from unirradiated (U) and UV irradiated cells following 0, 1, 2 and 4 h repair incubation. The arrow on the left of the gels indicates the transcription start site.

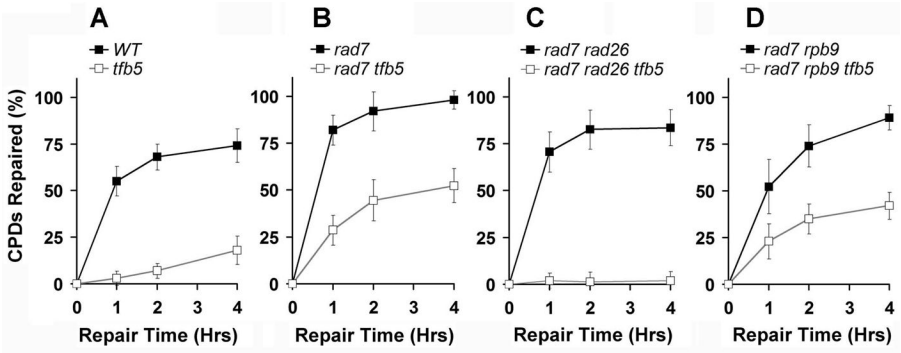

Fig. 6. Plots showing repair of CPDs in the TS (+1 to + 600) of the *GAL1* gene

The data were obtained by quantification of the gels shown in panels A – H of Fig. 5. The values are shown as average (mean ± standard deviation) of the percent CPDs repaired at different times of repair incubation.