Deletion of the *CSB* homolog, *RAD26*, yields Spt⁻ strains with proficient transcription-coupled repair

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ABSTRACT

It has been previously shown that disruption of *RAD26* in yeast strain W303-1B results in a strain that is deficient in transcription-coupled repair (TCR), the preferential repair of the transcribed strand of an expressed gene over the non-transcribed strand and the rest of the genome. *RAD26* encodes a protein that is homologous to Cockayne syndrome group B protein (CSB) and is a member of the SWI2/SNF2 family of DNA-dependent ATPases involved in chromatin remodeling. Like the *rad26* mutant, cells from Cockayne syndrome patients are defective in TCR. We examined the role of Rad26 in TCR by disrupting *RAD26* in two repair-proficient laboratory strains and, remarkably, observed no effect upon TCR. Our results indicate that disruption of *RAD26* alone is insufficient to impair TCR. Thus, W303-1B must already possess a mutation that, together with disruption of *RAD26*, causes a deficiency in TCR. We suggest that other genes are mutated in Cockayne syndrome cells that contribute to the deficiency in TCR. Surprisingly, deletion of *RAD26* results in expression of genes that are repressed by flanking transposon δ elements, an Spt⁻ phenotype. The δ elements appear to perturb local chromatin structure. Expression of genes flanked by δ elements in *rad26*Δ mutants is consistent with a role for Rad26 in chromatin remodeling.

INTRODUCTION

Nucleotide excision repair is a major pathway dealing with the removal of DNA damage found in all organisms examined thus far. The biochemical mechanism of nucleotide excision repair has emerged for prokaryotes and eukaryotes. Identification and cloning of genes involved in nucleotide excision repair, as well as expression of their products, has enabled the reconstitution of repair *in vitro* (1–4). In a multistep process, eukaryotic nucleotide excision repair proteins interact with the DNA damage, make dual single-strand incisions 3′ and 5′ to the damage in the strand containing the damage, followed by removal of the damage as part of a 24–26 bp oligonucleotide in yeast and a 27–29 bp oligonucleotide in humans (5).

Subsequently, DNA polymerases, DNA ligase and additional proteins fill in the resulting gap to regenerate intact duplex DNA.

Nucleotide excision repair does not occur uniformly throughout the genome of prokaryotes and eukaryotes (6,7). The transcribed strands of expressed genes are repaired faster than the non-transcribed strands and the genome overall. This faster repair of the transcribed strand is called transcription-coupled repair (TCR). It has been suggested that repair of the transcribed strand is directly coupled to transcription and that recognition of the DNA lesion by repair proteins may be facilitated by a stalled RNA polymerase complex at the site of DNA damage. While referred to as TCR, it has not been definitively shown that repair is directly coupled to transcription or, rather, the increased repair is a consequence of disrupted chromatin (facilitated by Rad26) preceding or subsequent to transcription through a DNA sequence.

The human disease Cockayne syndrome (CS) is associated with a defect in TCR (8–10). The *CSA* and *CSB* genes have been identified as uniquely affecting TCR in mammalian cells. *CSB*, also known as *ERCC6*, complements the deficiency in TCR in rodent cells of complementation group 6 and is mutated in CS cells from complementation group B (CS-B) (11,12). A yeast gene with homology to the human *CSB* gene (*RAD26*) was isolated, sequenced (13,14) and a *rad26* deletion mutant created (14). Consistent with the DNA repair deficiency observed in cultured cells from CS patients, the yeast *rad26* mutant lacks TCR (14). When grown in the presence of glucose, a *rad26* mutant shows approximately the same rate and extent of removal of cyclobutane pyrimidine dimers (CPDs) from the transcribed strands as from the non-transcribed strands of the repressed *GAL7* gene and the constitutive *RPB2* gene, i.e. there is no TCR (14–16). However, when grown in the presence of galactose, this same *rad26* mutant is proficient for TCR in the induced *GAL7* (15) and the constitutive *RPB2* genes (16). Therefore, there appear to be Rad26-dependent and Rad26-independent TCR pathways.

To date, repair rates for the *rad26* mutant have all been determined in one genetic background, W303-1B. We deleted *rad26* in three other strains of different genetic backgrounds and examined their ability to carry out TCR. Surprisingly, we observed no defect in TCR of an expressed gene in these new *rad26* mutants. Importantly, deletion of *RAD26* enabled expression from genes flanked by transposon δ elements, i.e. an Spt⁻ phenotype. The δ elements alter local chromatin structure and gene expression. Suppression of δ element

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Table 1. Strains used in this study

Strain	Genotype	Source
LNY1	MATa *lys2, ura3, leu2::hisG, trp1::hisG, ho::LYS2*	L. Neigeborn
LNY2	MATα *lys2, ura3, leu2::hisG, trp1::hisG, ho::LYS2*	L. Neigeborn
LNY3	MATa/α *lys2/lys2, ura3/ura3, leu2::hisG/leu2::hisG, trp1::hisG/trp1::hisG, ho::LYS2/ho::LYS2*	L. Neigeborn
LNY40	MATa *ade6 mating type testers*	L. Neigeborn
LNY41	MATα *ade6 mating type testers*	L. Neigeborn
SGY1	MATa *lys2, ura3, leu2::hisG, trp1::hisG, ho::LYS2*	This study
SGY2	MATa *lys2, ura3, leu2::hisG, trp1::hisG, ho::LYS2, rad26Δ::TRP1*	This study
SGY5	MATa/α *lys2/lys2, ura3/ura3, leu2::hisG/leu2::hisG, trp1::hisG/trp1::hisG, ho::LYS2/ho::LYS2, rad26Δ::TRP1/RAD26*	This study
SGY6	MATa/α *lys2/lys2, ura3/ura3, leu2::hisG/leu2::hisG, trp1::hisG/trp1::hisG, ho::LYS2/ho::LYS2, rad26Δ::TRP1/rad26Δ::TRP1*	This study
W303-1B	MATα *ho, ade2-1, trp1-1, leu2-3,112, can1-100, his3-11,15, ura3-1*	A. van Gool
MGSC102	MATα *ho, ade2-1, trp1-1, leu2-3,112, can1-100, his3-11,15, ura3-1, rad26::HIS3*	A. van Gool
SGY7	MATα *ho, ade2-1, trp1-1, leu2-3,112, can1-100, his3-11,15, ura3-1, rad26Δ::TRP1*	This study
FY56	MATα *his4-912δ, lys2-128δ, ura3-52*	J. Huibregtse
SGY8	MATα *his4-912δ, lys2-128δ, ura3-52, rad26::HIS3*	This study
FY58	MATa *his4-912δ, lys2-128δ, ura3-52, trp1Δ63*	M. Hampsey
FY111	MATa *his4-912δ, lys2-128δ, ura3-52, trp1Δ63, spt6-140*	M. Hampsey
FY114	MATa *his4-912δ, lys2-128δ, ura3-52, trp1Δ63, leu2Δ1, his3Δ200, spt4Δ2::HIS3*	M. Hampsey
L735	MATa *his4-912δ, lys2-128δ, ura3-52, trp5, leu1, spt4Δ1::URA3*	M. Hampsey
SGY9	MATa *his4-912δ, lys2-128δ, ura3-52, trp1Δ63, rad26Δ::TRP1*	This study

phenotypes suggests a role for Rad26 in chromatin remodeling or transcription elongation. Our results lead us to suggest that Rad26 (or CSB) is necessary but not sufficient to enable TCR.

MATERIALS AND METHODS

Deletion of *RAD26*, PCR, Southern analyses and RNA probes

Chimeric PCR primers were designed to delete the *RAD26* gene. The primers were used to amplify the *TRP1* gene and the PCR product was used to transform yeast and delete the *RAD26* locus. The *rad26Δ::TRP1* deletion mutants were selected on synthetic complete medium (6.7% yeast nitrogen base, 2.0% dextrose and the appropriate bases and amino acids) lacking the amino acid tryptophan (17). Haploid mutant strains were grown in YPD (1.0% yeast extract, 2.0% peptone, 2.0% dextrose; Gibco BRL) and genomic DNA was isolated and purified as described before (18). Genomic DNA was used for PCR reactions to screen mutants for the deletion allele using two oligonucleotides designed to amplify either wild-type or the deleted *RAD26* locus (Fig. 2). After PCR, the presence of the deletion allele was confirmed by Southern analysis of the *RAD26* locus (data not shown). To make radioactive RNA probes, plasmid pKS212, carrying the 1 kb *Eco*RI–*Xho*I fragment of the large subunit of RNA polymerase II (*RPB2*) in pBluescript (Stratagene), was linearized by digestion with either *Xho*I for the non-transcribed strand- or *Eco*RI for the transcribed strand-specific RNA probe of the gene. Transcribed strand and non-transcribed strand RNA probes were synthesized with either T7 RNA polymerase from the T7

promoter or T3 RNA polymerase from the T3 promoter (Boehringer Mannheim), respectively, and incubation with rNTPs and [α-^{32}P]CTP (Amersham Corp.) as recommended by the manufacturer.

Strains, meiotic induction and tetrad analysis

Strains used in the current study are described in Table 1. Wild-type strains and heterozygous and homozygous diploid *rad26Δ* mutants were sporulated in medium containing 1.0% potassium acetate, 0.05% glucose, 0.1% yeast extract and the appropriate amino acids (18). Asci were digested with a β-glucuronidase preparation from *Helix pomatia* (Sigma Corp.) in suspension in sterile water for 10 min at room temperature. Ten microliters of the suspension was spread across YPD plates and allowed to dry, after which asci were broken apart and spores separated using a Narishige micromanipulator mounted on a CK2 Olympus microscope.

UV irradiation and isolation and restriction of yeast DNA

Growth and irradiation of strains and purification of DNA were as previously described (19). Cell walls were digested with Zymolyase 100T (ICN Biochemicals) and lysed by addition of sarcosine (20). Chromosomal DNA was purified as described (21). The DNA samples were then restricted to completion with *Hinc*II. Restricted DNA was then ethanol precipitated and resuspended in TE (10 mM Tris–HCl, pH 8.0, 1 mM EDTA, pH 8.0).

Repair analysis

The incidence of CPDs in each strand of a specific restriction fragment was determined by previously developed methods

(6,19,22). Equal amounts of purified, restricted DNA were either mock treated (10 mM Tris–HCl, pH 7.5, 0.1 M NaCl, 10 mM EDTA, 1 mg/ml BSA) or treated with the enzyme T4 endonuclease V in mock buffer for 30 min at 37°C. T4 endonuclease V is a CPD-specific DNA glycosylase/AP lyase which produces a single-strand break specifically at each CPD. DNA was denatured and electrophoresed through 0.5% alkaline agarose gels to separate full-length restriction fragments from the digestion products of T4 endonuclease V, transferred to Hybond N$^+$ (Amersham) membranes and hybridized with strand-specific ^{32}P-labeled RNA probes generated by *in vitro* transcription of linearized pKS212. Autoradiograms were generated and signal intensities determined using a Hewlett Packard Scanjet II and the application NIH Image v.1.62. The Poisson expression was applied to calculate the number of CPDs per fragment from the ratio of intensities of bands from T4 endonuclease V-treated and mock-treated DNA samples.

RESULTS

Deletion of *RAD26* is insufficient to abolish TCR

To date, repair rates for *rad26* mutants have all been determined in one genetic background, W303-1B. A portion of the *RAD26* gene was previously deleted and replaced with the *HIS3* gene in W303-1B (14). These strains are defective for TCR, as demonstrated previously (14) and shown in Figure 1A and B. The transcribed strand has 40–45% CPDs removed 30 min following UV irradiation. Repair of the non-transcribed strand is slightly slower than the transcribed strand, reaching ~25% CPDs removed by 30 min post-irradiation. Despite being referred to as TCR-deficient, we observed significantly faster repair of the transcribed strand over the non-transcribed strand with this *rad26* mutant (14,15). Consistent with our data, others also noted faster repair of the transcribed strands relative to repair of the non-transcribed strands in the same *rad26* mutant (14,15). We disrupted *RAD26* in three other strains with different genetic backgrounds and examined their ability to carry out TCR. The entire *RAD26* open reading frame was deleted and replaced with the *TRP1* gene in W303-1B, FY58 and SK1. The *RAD26* gene was replaced with the original *rad26::HIS3* allele in strain FY56. Deletions were confirmed using PCR (Fig. 2) and/or hybridization of gene-specific probes to restriction enzyme-digested genomic DNA immobilized on nylon membranes (Southern blots) (data not shown). As has been previously shown for *rad26::HIS3* mutants in W303-1B, *rad26Δ::TRP1* mutants in W303-1B are also deficient in TCR (Fig. 3A and B). The transcribed strand has 45% CPDs removed 30 min following UV irradiation while repair of the non-transcribed strand is slightly slower, reaching 25–30% CPDs removed by 30 min post-irradiation. Thus, both alleles of *RAD26* result in a deficiency in TCR in the W303-1B strain. However, introducing either the original *rad26::HIS3* or the new *rad26Δ::TRP1* allele into other strain backgrounds gave markedly different results.

SK1 strains containing a deletion allele of *RAD26* were examined for their ability to perform TCR. Surprisingly, we observed no defect in TCR of an expressed gene in these new *rad26Δ* mutants. As is apparent in Figure 4A, restoration of hybridization signal in the T4 endonuclease V-treated samples
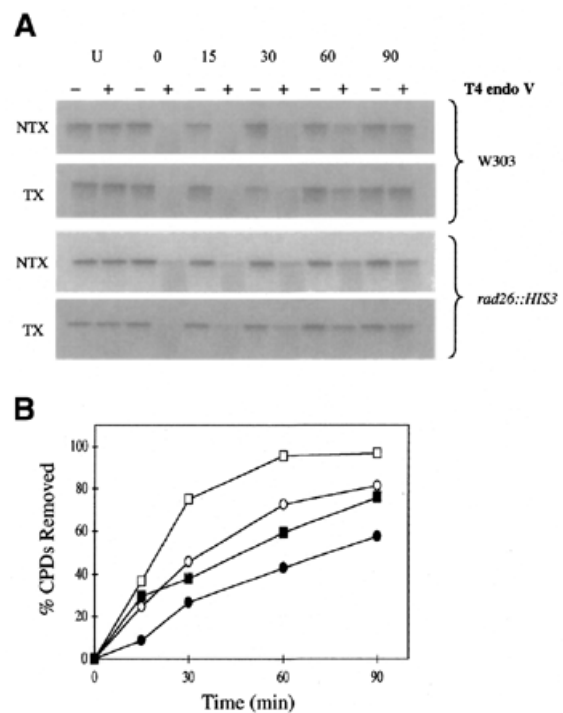

Figure 1. TCR assay for a *rad26::HIS3* mutant and the parent strain W303-1B. (**A**) Exponentially growing cultures of *rad26::HIS3* and the parent strain (W303-1B) at 30°C were irradiated with 65 J/m^2 UV radiation and incubated in growth medium for the times indicated. DNA was prepared from the cells as described in Materials and Methods. U, unirradiated. (**B**) Removal of CPDs from the *RPB2* gene in *rad26::HIS3* and the parent strain W303-1B. Repair of transcribed and non-transcribed strands was determined from the measured incidences of CPDs in the *Hinc*II restriction fragments from *RPB2*. A graphical representation of repair at the times indicated is shown. Data points represent the average of four independent biological experiments. Open square, W303-1B transcribed strand; filled square, W303-1B non-transcribed strand; open circle, *rad26::HIS3* transcribed strand; filled circle, *rad26::HIS3* non-transcribed strand.

indicates rapid repair of the transcribed strand in both strains. Repair of the non-transcribed strand was slower than repair of the transcribed strand. Data are presented graphically in Figure 4B. The transcribed strand is largely free of CPDs by 60 min following UV irradiation (~90% removed). Repair of the non-transcribed strand is slower than the transcribed strand, reaching ~60% CPDs removed by 60 min post-irradiation. Clearly there is no defect in TCR in *rad26* mutants in the SK1 background. We note that repair of the non-transcribed strand in *rad26* mutants in the SK1 background is significantly elevated at 90 min following UV irradiation relative to the repair observed in the parent strain (>90% versus ~65% CPDs removed).

We examined the ability of the *rad26Δ* mutants in the FY56 and FY58 backgrounds to perform TCR and global genomic repair. As shown in Figure 5A, repair of the transcribed strand in the *rad26Δ::TRP1* mutant was faster than repair of the non-transcribed strand in the *rad26Δ::TRP1* mutant and the parent strain FY58. Similar repair was observed in a *rad26::HIS3* mutant and its parent strain FY56 (data not shown). Repair in both *rad26Δ::TRP1* and *rad26::HIS3* mutants and the parent strains is shown graphically in Figure 5B. Greater than 70% of the CPDs were removed from the transcribed strand by 30 min
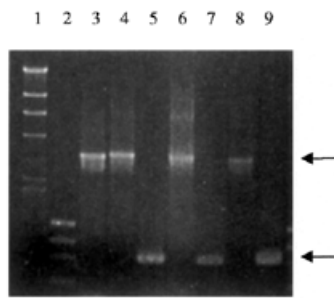
Figure 2. Confirmation of deletion of *RAD26* in the SK1 background. PCR primers complementary to sequences immediately upstream and downstream of the *RAD26* gene were used to amplify the intervening sequences from the chromosomal locus of haploid wild-type and *rad26Δ::TRP1* cells. The wild-type locus yields a product of 3.2 kb (top arrow). The *rad26::HIS3* disruption allele yields a product of 3.4 kb and migrates slightly slower than the PCR product from the wild-type locus. The *rad26Δ::TRP1* mutant yields a product of 936 bp (bottom arrow), indicative of the deleted allele. Lane 1, λ DNA cut with *Hin*dIII; lane 2, φχ DNA cut with *Hae*III; lane 3, W303-1B; lane 4, *rad26::HIS3* (W303-1B); lane 5, *rad26Δ::TRP1* (W303-1B); lane 6, SK1; lane 7, *rad26Δ::TRP1* (SK1); lane 8, FY58; lane 9, *rad26Δ::TRP1* (FY58).
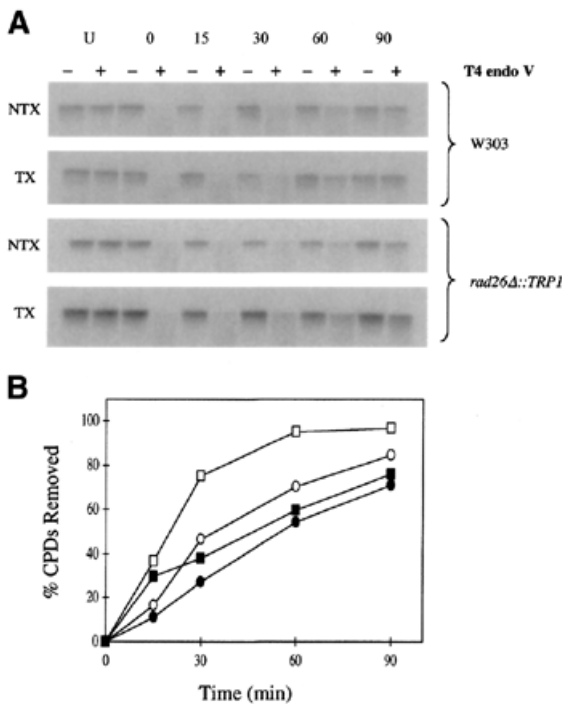

Figure 3. TCR assay for a new *rad26Δ::TRP1* mutant and the parent strain W303-1B. (**A**) Exponentially growing cultures of *rad26Δ::TRP1* and the parent strain (W303-1B) at 30°C were irradiated with 65 J/m² UV radiation and incubated in growth medium for the times indicated. DNA was prepared from the cells as described in Materials and Methods. (**B**) Removal of CPDs from the *RPB2* gene in *rad26Δ::TRP1* and the parent strain W303-1B. Repair of transcribed and non-transcribed strands was determined from the measured incidences of CPDs in the *Hin*cII restriction fragments from *RPB2*. A graphical representation of repair at the times indicated is shown. Data points represent the average of four independent biological experiments. Open square, W303-1B transcribed strand; filled square, W303-1B non-transcribed strand; open circle, *rad26Δ::TRP1* transcribed strand; filled circle, *rad26Δ::TRP1* non-transcribed strand.
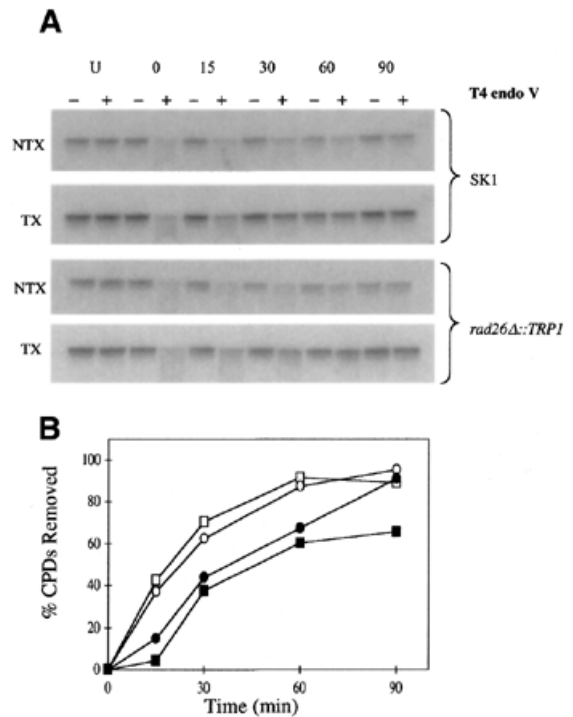

Figure 4. Deletion of *RAD26* does not abolish TCR of UV-induced damage in an SK1 strain. (**A**) TCR assay for a *rad26Δ* mutant in an SK1 genetic background. Exponentially growing cultures of *rad26Δ* and the parent strain at 30°C were irradiated with 65 J/m² UV radiation and incubated in growth medium for the times indicated. DNA was prepared from the cells as described in Materials and Methods. (**B**) Removal of CPDs from the *RPB2* gene in *rad26Δ::TRP1* and the parent strain SK1. Repair of transcribed and non-transcribed strands was determined from the measured incidences of CPDs in the *Hin*cII restriction fragments from *RPB2*. A graphical representation of repair at the times indicated is shown. Data points represent the average of four independent biological experiments. Open square, SK1 transcribed strand; filled square, SK1 non-transcribed strand; open circle, *rad26Δ::TRP1* transcribed strand; filled circle, *rad26Δ::TRP1* non-transcribed strand.

following UV irradiation. The non-transcribed strand showed ~35% CPDs removed by 30 min following UV irradiation (Fig. 5B). The repair observed is indicative of proficient TCR in these *rad26Δ* strains.

Deletion of *RAD26* yields an Spt⁻ phenotype

The original *rad26::HIS3* deletion allele (14) was amplified by PCR and the linear PCR product was used to transform yeast strain FY56. Confirmation of the *rad26::HIS3* allele in FY56 was by PCR (data not shown). The *HIS3* gene should not complement the histidine auxotrophy of cells carrying the *his4-912δ* allele in FY56 unless deletion of *RAD26* yields the Spt⁻ phenotype (23–25). Similarly, expression of Lys2 from *lys2-128δ* enables growth on synthetic complete medium lacking lysine only if deletion of *RAD26* gives the Spt⁻ phenotype. The Spt⁻ phenotype appears to be dependent upon changes in the cellular capacity to remodel chromatin. Rad26 is a member of the Swi2/Snf2 family of DNA-dependent ATPases that are thought to function in chromatin remodeling. We examined both FY56 *rad26::HIS3* and FY58 *rad26Δ::TRP1* mutants for ability to grow on synthetic
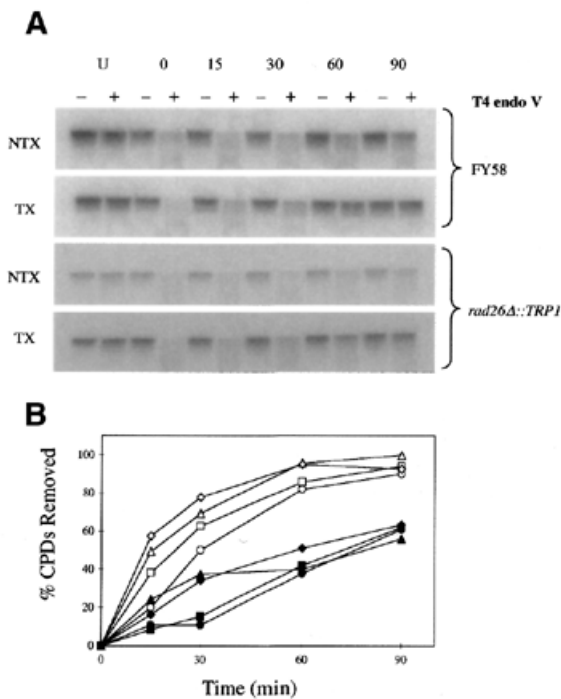
Figure 5. Deletion of *RAD26* does not abolish TCR of UV-induced damage in FY58 and FY56 strains. (**A**) TCR assay for *rad26Δ* mutants in an FY58 strain. Exponentially growing cultures of a *rad26Δ::TRP1* mutant and the parent strain FY58 at 30°C were irradiated with 65 J/m² UV radiation and incubated in growth medium for the times indicated. DNA was prepared from the cells as described in Materials and Methods. (**B**) Removal of CPDs from the *RPB2* gene in *rad26* mutants and the parent strains. Repair of transcribed and non-transcribed strands was determined from the measured incidences of CPDs in the *Hin*cII restriction fragments from *RPB2*. A graphical representation of repair at the times indicated is shown. Data points represent the average of four independent biological experiments, except for *rad26Δ::TRP1*, which was performed three times. Open square, FY58 transcribed strand; filled square, FY58 non-transcribed strand; open circle, *rad26Δ::TRP1* transcribed strand; filled circle, *rad26Δ::TRP1* non-transcribed strand; open triangle, FY56 transcribed strand; filled triangle, FY56 non-transcribed strand; open diamond, *rad26::HIS3* transcribed strand; filled diamond, *rad26::HIS3* non-transcribed strand.

complete medium lacking lysine and histidine, i.e. exhibit a Spt⁻ phenotype. Both the FY56 *rad26::HIS3* and FY58 *rad26Δ::TRP1* mutants were able to grow on medium lacking histidine and lysine. The parent strains were unable to grow on medium lacking either histidine or lysine (Fig. 6). Thus, deletion of *RAD26* results in a Spt⁻ phenotype.

Strains carrying mutations in either *SPT4* or *SPT6* are repair proficient

Given the Spt⁻ phenotype we observed for *rad26* mutants, we wished to see if *spt* mutants, thought to be involved in chromatin remodeling and/or transcription elongation, might be defective in TCR or global genome repair. Repair experiments with *spt* mutants derived from strain FY58 were performed as above. Repair of the transcribed strand of *RPB2* in *spt4Δ1::URA3* and *spt4Δ2::HIS3* was as rapid as in the parent strains (Fig. 7). Likewise, repair of the non-transcribed strand of *RPB2* was at the same rate as repair in the parent strains. Therefore, mutations in the *SPT4* or *SPT6* gene do not singly affect the ability of these strains to efficiently repair the
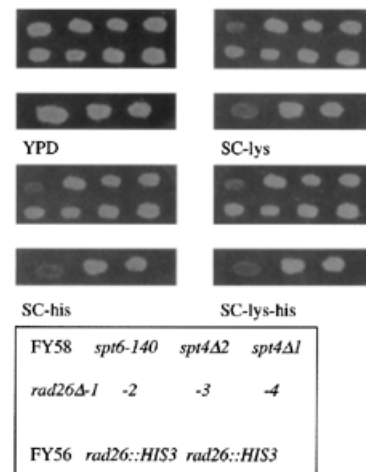
transcribed and non-transcribed strands of an expressed gene (Fig. 7).

Deletion of *RAD26* causes no defects in mating or conditional growth phenotypes

Haploid *rad26Δ::TRP1* mutants were mated with wild-type and *rad26Δ::TRP1* strains. Mating efficiencies were the same for wild-type and *rad26Δ::TRP1* strains as compared to mating of wild-type to wild-type strains. Thus, there does not appear to be significant derepression of the silent mating loci in the *rad26Δ::TRP1* mutants relative to the wild-type strains.

We also observed no cold-sensitive or heat-sensitive phenotypes at 15 or 37°C, respectively, for the *rad26Δ* strains, as has been reported previously for *spt* mutations. We conclude that Rad26 may not form an integral part of protein complexes involved in chromatin remodeling and/or transcription, as reported for the Spt4, Spt5 and Spt6 proteins (26).

DISCUSSION

The *RAD26* gene, the homolog of the human CS group B gene, was reported to enable TCR in yeast. Like cells from CS patients, yeast *rad26::HIS3* mutants lack TCR. To date, all repair studies with yeast carrying the *rad26::HIS3* deletion were in the W303 background. We have examined TCR in *rad26* deletion alleles in different genetic backgrounds and found that the defect in TCR was strain dependent. We found that the *rad26::HIS3* mutant and a new deletion mutant, *rad26Δ::TRP1*, in the W303-1B background exhibit a deficiency in TCR relative to other repair-proficient laboratory strains. No defect was observed in these strains for repair of the non-transcribed strands of expressed genes. Thus, repair of the genome overall is proficient based on the strong correlation between repair of the non-transcribed strand and overall genomic repair (27). Deletion of *RAD26* in the three other strains had no effect upon TCR. Based on our results, we
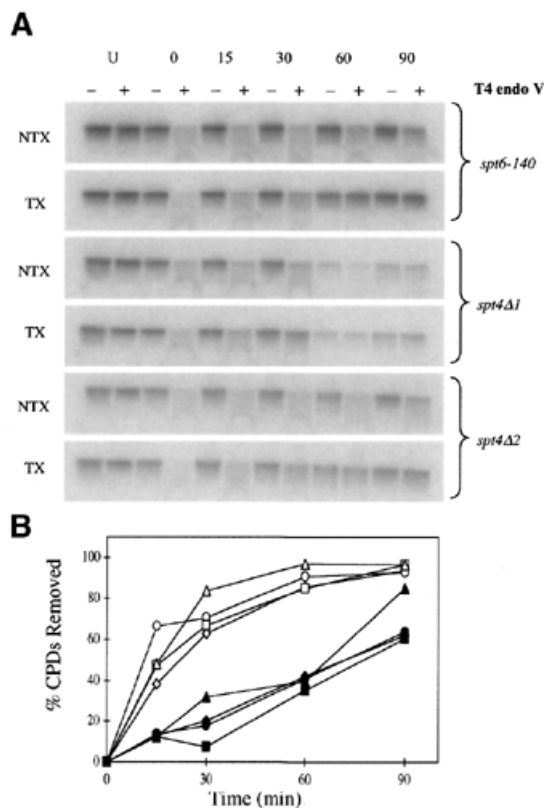

Figure 6. Deletion of *RAD26* results in a Spt⁻ phenotype, indicative of chromatin alterations. Two different *rad26Δ* mutants and their respective wild-type parent strains were grown on YPD plates and then replica-plated onto YPD, SC-his, SC-lys and SC-his,lys plates. Plates were incubated at 30°C for 3–5 days.

Figure 7. Mutation of *SPT4* or *SPT6* does not affect TCR of UV-induced damage in the FY58 background. (**A**) TCR assay for *spt4* and *spt6* mutants in an FY58 strain. Exponentially growing cultures of *spt4* or *spt6* mutants and the parent strain FY58 at 30°C were irradiated with 65 J/m² UV radiation and incubated in growth medium for the times indicated. DNA was prepared from the cells as described in Materials and Methods. (**B**) Removal of CPDs from the *RPB2* gene in *spt* mutants and the parent strain. Repair of transcribed and non-transcribed strands was determined from the measured incidences of CPDs in the *Hinc*II restriction fragments from *RPB2*. A graphical representation of repair at the times indicated is shown. Data points represent the average of four independent biological experiments. Open diamond, FY58 transcribed strand; filled diamond, FY58 non-transcribed strand; open circle, *spt6-140* transcribed strand; filled circle, *spt6-140* non-transcribed strand; open square, *spt4Δ2* transcribed strand; filled square, *spt4Δ2* non-transcribed strand; open triangle, *spt4Δ1* transcribed strand; filled triangle, *spt4Δ1* non-transcribed strand.

suggest that another protein is required to enable TCR, along with Rad26. Deficiency in either activity is insufficient to abolish TCR. Deficiency in both activities leads to a loss of TCR. It may be that W303 is already defective for this second activity. Our results suggest that suppressors of a TCR defect in the W303 background might not suppress *rad26* (28), but may be suppressing another mutation already present in W303-1B. Such a possibility remains to be demonstrated.

A role for Rad26 and CSB in TCR was suggested based on their homology to members of the Swi/Snf family of DNA-dependent ATPases (29). That model stated that Rad26 and CSB might be responsible for displacing the RNA polymerase II stalled at the site of DNA damage, enabling repair of the newly uncovered damage. Several members of the Swi/Snf family are proteins with demonstrated roles in chromatin remodeling and removal of proteins bound to chromatin (30–33).

Remodeling of chromatin enhances the binding of transcription factors and presumably DNA repair proteins to nucleosomal DNA. Such properties suggest that Rad26 also functions in chromatin remodeling and/or protein displacement. Does Rad26 act as a DNA-dependent ATPase which is activated by binding to damaged DNA in the relatively nucleosome-free template strand that is undergoing transcription? Recently, Vermeulen and colleagues demonstrated that CSB protein could remodel nucleosomes *in vitro* (34). Furthermore, can other members of the Swi/Snf family fulfill the role of Rad26 in TCR?

Consistent with a role in chromatin remodeling is our observation that deletion of *RAD26* results in suppression of δ insertion mutations in the 5′-non-coding region of the *HIS4* and *LYS2* genes (Spt⁻ phenotype) (23–25). The δ element is a copy of the Ty transposon long terminal repeat. In wild-type cells insertion of Ty or solo δ elements upstream of genes leads to altered transcription initiation start sites and non-functional transcripts. Transcription can begin at the normal transcription start site and terminate within the δ element or transcription can begin within the δ element and terminate at the normal termination site. The result is either a truncated mRNA or an mRNA that is not read in the normal reading frame, respectively. Thus, wild-type cells containing *his4-912δ* or *lys2-128δ* alleles cannot grow in the absence of exogenous histidine or lysine, i.e. they have a His⁻ or Lys⁻ phenotype. Mutations in a number of genes encoding proteins involved in chromatin structure or remodeling alter the transcription start site such that mRNAs encoding functional His4 and Lys2 proteins are produced. For example, mutations in the *SWI2/SNF2* or *SPT6* genes result in *his4-912δ* or *lys2-128δ* strains that are able to grow on selective media lacking histidine or lysine, respectively. They have His⁺ and Lys⁺ phenotypes. We found that partial deletion of *RAD26* with *HIS3* or complete deletion of *RAD26* with *TRP1* in an FY56 or FY58 strain, respectively, enables growth on selective medium lacking histidine or lysine, or both. Thus, *rad26* mutants have an Spt⁻ phenotype. However, the fact that deletion of *RAD26* does not result in a TCR defect suggests that another protein, perhaps another member of the Swi/Snf family, is able to substitute for Rad26 and enable TCR.

ACKNOWLEDGEMENTS

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