Spt4 modulates Rad26 requirement in transcription-coupled nucleotide excision repair

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The nucleotide excision repair machinery can be targeted preferentially to lesions in transcribed sequences. This mode of DNA repair is referred to as transcription-coupled repair (TCR). In yeast, the Rad26 protein, which is the counterpart of the human Cockayne syndrome B protein, is implicated specifically in TCR. In a yeast strain genetically deprived of global genome repair, a deletion of RAD26 renders cells UV sensitive and displays a defect in TCR. Using a genome-wide mutagenesis approach, we found that deletion of the SPT4 gene suppresses the rad26 defect. We show that suppression by the absence of Spt4 is specific for a rad26 defect and is caused by reactivation of TCR in a Rad26-independent manner. Spt4 is involved in the regulation of transcription elongation. The absence of this regulation leads to transcription that is intrinsically competent for TCR. Our findings suggest that Rad26 acts as an elongation factor rendering transcription TCR competent and that its requirement can be modulated by Spt4.

Keywords: nucleotide excision repair/RAD26/SPT4/transcription-coupled repair

Introduction

Nucleotide excision repair (NER) is an evolutionarily conserved DNA damage repair pathway that protects the genome from deleterious effects of DNA lesions. Repair is accomplished by excision of a single-stranded oligonucleotide containing a lesion from the DNA molecule, allowing DNA resynthesis using the undamaged strand as a template (de Laat et al., 1999; Batty et al., 2000). NER is a versatile repair pathway capable of removing a wide range of structurally distinct DNA lesions.

The genome is repaired with heterogeneous kinetics. This phenomenon is exemplified by the fact that transcribed sequences are repaired preferentially by NER, which seems to be the result of a direct link between the NER machinery and the transcription apparatus. This transcription-coupled repair (TCR) mode leads to the removal of lesions from the transcribed strand of active genes only. Recognition of DNA damage may proceed directly via RNA polymerase. In Escherichia coli, a factor was identified that is thought to couple the repair machinery physically to transcription. This transcription-repair coupling factor (TRCF) interacts directly with the UvrA protein, a component of bacterial NER, and is capable of displacing a stalled RNA polymerase, allowing repair to take place (Selby and Sancar, 1993).

In human cells, TCR requires the CSB gene product. The hereditary recessive disorder Cockayne syndrome can be caused by a mutation in the CSB gene (Troelstra et al., 1992), and cells derived from Cockayne syndrome patients show a defect specifically in the TCR pathway (Venema et al., 1990). Previously, we cloned RAD26, the Saccharomyces cerevisiae orthologue of CSB (van Gool et al., 1994). Like human CSB+ cells, rad26 cells show a defect in TCR. Sequence analysis revealed that both the CSB and the Rad26 protein are SWI/SNF-type putative helicases (Troelstra et al., 1992; van Gool et al., 1994). Biochemical studies showed that both proteins have a DNA-stimulated ATPase activity (Guzder et al., 1996; Selby and Sancar, 1997; Citterio et al., 1998). In addition, in human cells, a fraction of RNA polymerase II was found to be associated with CSB (Tantin et al., 1997; van Gool et al., 1997). Whether CSB/Rad26 act as eukaryotic TRCFs or function in a different manner is currently not known.

DNA lesions can be repaired independently of transcription through the global genome repair (GGR) pathway. GGR removes lesions throughout the genome in both transcribed and non-transcribed DNA. During GGR, lesion recognition is not mediated by the DNA-scanning capacity of a polymerase but proceeds through a different mechanism. In yeast, the Rad7 and Rad16 proteins are involved specifically in GGR (Verhage et al., 1994) and have a presumed function in the recognition of lesions (Guzder et al., 1997). The yeast S. cerevisiae GGR pathway is exceptionally efficient. When TCR is absent, lesions in transcribed sequences are repaired readily by GGR (van Gool et al., 1994; Verhage et al., 1996). This is underscored by the fact that disruption of TCR does not lead to any detectable decrease in survival after UV irradiation (van Gool et al., 1994), indicating that the defect is masked by GGR. Mutants in either the RAD7 or RAD16 genes are completely devoid of GGR (Verhage et al., 1994). The availability of these mutants enables us to determine the specific contribution of TCR to damage removal in transcribed sequences. We have previously analysed the role of Rad26 in TCR (Verhage et al., 1996; Tijsterman et al., 1997). TCR of UV damage is absolutely dependent on transcription and on NER (Verhage et al., 1996), but Rad26 is only partially required. Analysis of gene-specific repair showed that mutants genetically deprived of both Rad16 and Rad26 still show considerable repair of the transcribed strand of the RPB2 gene (Verhage et al., 1996). The Rad26 requirement for TCR is different for different genes. Closer examination of the repair
profile within the RPB2 and URA3 genes revealed, in addition to intergenic variations, a distinct region of Rad26-independent TCR, localized in a small region directly downstream of the transcription start site (Tijsterman et al., 1997). This region of Rad26-independent TCR ranges from +1 to about +40 with respect to the transcription start site. Downstream of these sequences, TCR suddenly switches to a Rad26-dependent mode, which suggests that the Rad26 requirement in TCR is dependent on the type of transcription, which differs from site to site within a single gene.

These conditions may be generated by the mode of transcription taking place at that particular position. Transcription is a tightly regulated process that is fine-tuned at different levels. Regulation at the level of transcription initiation has been studied most extensively. Today the elongation phase of transcription is also widely recognized as a genuine target for regulation, by both positive and negative regulators (Uptain et al., 1997).

The transition of initiation to elongation appears to be a critical step in transcription. In this phase, the RNA polymerase passes through a distinct physical transition. Hypophosphorylated RNA polymerase is assembled in a pre-initiation complex with general transcription factors (Orphanides et al., 1996). To enter processive elongation, the C-terminal domain (CTD) of the RNA polymerase is phosphorylated, which is thought to proceed via the positive transcription elongation factor P-TEFb, which harbours kinase activity and can trigger the transition of transcription initiation to elongation (Wada et al., 2000). The transition from the hypophosphorylated form of the RNA polymerase II CTD to the hyperphosphorylated form coincides with a number of events. The mediator complex is thought to be substituted by elongator within the transition region (Otero et al., 1999). Additional factors, described in human cells, such as 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) sensitivity-inducing factor (DSIF), negative elongation factor (NELF) and FACT (facilitates chromatin transcription), play a role in the progression of transcription from early to processive elongation (Wada et al., 1998, 2000; Yamaguchi et al., 1999). In addition, it has been shown in vitro that the basal transcription factor TFIIH leaves the complex during early elongation (Zawel et al., 1995; Dvir et al., 1997). TFIIH is also part of the NER complex that is recruited to damaged sites. We suggested previously that Rad26-independent TCR might be explained by the presence of TFIIH in the transcription complex triggering repair and obviating the need for Rad26. The role of Rad26 further downstream might be to recruit TFIIH to the stalled transcription elongation complex to allow transcription resumption and repair to occur (Tijsterman et al., 1997).

TCR has not been reconstituted in vitro. The absence of an in vitro assay severely hampers the molecular dissection of the transcription–repair coupling mechanism. This prompted us to study the mechanism of TCR using a genetic approach. Analysis of genetic interactants with Rad26 may shed light on the mechanism of TCR and the function of Rad26 in this process.

Here we describe the identification and characterization of an extragenic mutation suppressing a rad26 defect. We show that suppression is caused by the absence of Spt4 protein. It has been shown previously that Spt4 is in complex with Spt5 and possibly with Spt6 (Swanson and Winston, 1992; Hartzog et al., 1998). Cells carrying mutations in these genes display phenotypes associated with defects in transcription elongation (Swanson and Winston, 1992), and the gene products are thought to be involved directly in transcription elongation (Hartzog et al., 1998). The Spt4/5 heterodimeric complex was found to be homologous to the DSIF complex in human cells that confers in vitro transcription sensitivity to DRB. Biochemical work on DSIF has provided a detailed model for the molecular action of Spt4 and Spt5 (Wada et al., 1998). The complex is involved in repression of transcription elongation at the early elongation–processive elongation transition, and repression is modulated by CTD phosphorylation (Yamaguchi et al., 1999; Wada et al., 2000).

We found that abolishing Spt4 activity suppresses a rad26 defect, suggesting that Rad26-independent repair is activated in spt4 cells. In spt4 cells deprived of GGR, Rad26 no longer contributes significantly to survival after UV irradiation. We show that suppression of the UV sensitivity conferred by rad26 is indeed the result of damage removal in transcribed sequences by transcription-coupled NER. Apparently Rad26 is dispensable for TCR when regulation of transcription elongation is disturbed. A model explaining the Rad26–Spt4 interplay will be discussed.

Results

Isolation of suppressors of rad26 UV sensitivity

To study the role of Rad26 in TCR, we set out to screen the yeast genome for modifiers of the rad26 phenotype. To this end, we used UV sensitivity as a selectable phenotype and employed a disruption library in which random yeast genomic fragments were mutagenized by insertion of an mTn transposable element (Burns et al., 1994; Ross-Macdonald et al., 1999).

Using these libraries, we generated ~25 000 S.cerevisiae transformants and screened them for increased survival after UV irradiation. A rad26 mutation contributes to survival after UV irradiation only in a global genome-deficient background, hence transformants were generated in a rad16rad26 background. From isolated survivors, mTn-flanking sequences were rescued by inverse PCR and sequenced. Using this procedure, we identified a transposon insertion in the SPT4 open reading frame (ORF) that rendered rad16rad26 cells more UV resistant.

To show that the UV-resistant phenotype was due to the mTn insertion in SPT4, we constructed a complete gene disruption of SPT4 in a rad16rad26 background. These rad16rad26spt4 cells have a phenotype indistinguishable from that of the transposon-generated mutation, showing that the obtained phenotype is indeed linked to the SPT4 gene (data not shown). A rad16rad26spt4 mutant has a UV sensitivity comparable to that of a rad16 single mutant, suggesting that the UV sensitivity conferred by rad26 is completely suppressed or that the UV sensitivity conferred by the rad16 mutation is partially suppressed (Figure 1A).

The SPT4 ORF partially overlaps with a small ORF of unknown function, YGR064W. By deleting SPT4, this hypothetical gene is also largely deleted. Therefore, the
Cells were UV irradiated at 70 J/m² and allowed to repair. Suppression of UV sensitivity caused by the spt4 mutation was not observed (Figure 1B). From these data, we conclude that suppression of rad16rad26 UV sensitivity is indeed caused by the spt4 mutation.

**Characterization of the suppression by spt4**

To characterize further the UV resistance conferred by spt4, we performed epistasis analysis to pinpoint the phenotype in a distinct pathway. We deleted SPT4 in a number of completely or partially NER-deficient strains and tested their UV sensitivity (Figure 1C). The spt4 mutation is not more resistant to UV than its parental strain, showing that UV resistance is not a general property of spt4 cells. In the NER-deficient rad14 strain, as well as in a rad4 strain (data not shown), the spt4 mutation has no effect. This indicates that the spt4 mutation specifically affects the NER pathway and does not affect a DNA repair or tolerance pathway other than NER. In addition, deletion of SPT4 in a rad16 background does not alter the UV sensitivity compared with a rad16 single mutant, which shows that spt4 has no effect on global genome NER. These findings lead us to conclude that suppression by the absence of Spt4 is specific for a RAD26 defect since no other pathways seem to be affected by the spt4 mutation. GGR is dependent on both Rad16 and Rad7. Deleting either one knocks out GGR activity. Indeed, the suppression of rad26 by spt4 is observed in rad16rad26spt4 as well as in rad7rad26spt4 mutants (data not shown).

**Repair analysis of mutants lacking the SPT4 gene**

Epistasis analysis showed that spt4 directly affects the UV sensitivity caused by a rad26 defect. Since RAD26 is implicated specifically in TCR, this suggests that in these cells TCR is reactivated. To address this question directly, we analysed removal of UV photoproducts at the molecular level in vivo and determined the effect of the spt4 mutation. Damage removal was determined by two distinct methods, which we described previously (Verhage et al., 1994; Tijsterman et al., 1996). As a target for repair analysis, we used the RPB2 and URA3 genes, which have both been studied extensively in our laboratory (Verhage et al., 1994, 1996; Tijsterman et al., 1996, 1997). Cells were UV irradiated at 70 J/m² and allowed to repair photoproducts for various lengths of time. Subsequently, genomic DNA was isolated and the presence of DNA damage was determined.

First we determined the level of repair in a 3.4 kb fragment consisting of almost the entire ORF of the RPB2 gene. DNA was treated with T4endoV, which cuts the damaged strand 5′ of UV-induced cyclobutane pyrimidine dimers (CPDs), run on an alkaline agarose gel and was blotted onto a nylon membrane. The transcribed and non-transcribed strands were probed using M13-derived single-stranded probes, and blots were visualized by autoradiography. Loss of a T4endoV digestion pattern over time is indicative of NER taking place during post-irradiation incubation.

In rad16rad26 cells, GGR is absent. Consistent with our previous findings, no repair could be observed in the non-transcribed strand of the RPB2 gene in a strain deprived of Rad16 protein (Figure 2; Verhage et al., 1994). As we reported previously, the transcribed strand was repaired to a large extent even in the absence of Rad26, indicating that there is a significant level of Rad26-independent TCR taking place at the RPB2 locus (Figure 2A and C). Rad26, however, does contribute to repair since TCR in rad16 cells is more pronounced compared with that in rad16rad26 cells (Figure 2C and D). Deletion of SPT4
in \textit{rad16\textit{rad26}} cells affects the repair of the transcribed strand, whereas no significant repair could be detected on the non-transcribed strand (Figure 2). In addition, the repair of the transcribed strand in the presence of Rad26 is not affected by \textit{sp4} (Figure 2B and D). This indicates that the absence of \textit{Sp4} indeed affects Rad26-independent TCR specifically. TCR at the \textit{RPB2} locus in \textit{rad16\textit{rad26spt4}} cells is typically faster than that in \textit{rad16\textit{rad26}} cells, although the overall repair after longer periods of time leads to an equal level of damage removal. These data point to the molecular events underlying the genetic interaction between Rad26 and Spt4. The activation of TCR by \textit{sp4} explains the increased survival of \textit{rad16\textit{rad26spt4}} cells.

Next, we assessed repair in \textit{sp4} cells more closely by analysing damage removal at the nucleotide level. Isolated DNA was digested with appropriate restriction enzymes, after which an internal fragment of the \textit{URA3} gene was purified using biotinylated primers immobilized on solid-phase streptavidin. Subsequently, the fragment was oligonucleotide-directed 3′ end-labelled and treated with T4 endoV. The latter treatment gives rise to a damage-dependent digestion pattern indicative of the presence and removal of CPDs, as previously described (Tijsterman et al., 1996).

TCR at the \textit{URA3} locus is highly dependent on Rad26. In \textit{rad16\textit{rad26}} cells, no significant repair could be detected in the ORF, as we have reported previously (Tijsterman et al., 1997; Figure 3). Deletion of \textit{SPT4} leads to a marked increase in TCR capacity. This Rad26-independent TCR acts throughout the entire fragment studied, comprising a 330 bp internal fragment of the \textit{URA3} ORF. Rad26-independent repair in the presence of \textit{Sp4} is confined to the first 40 nucleotides downstream of the transcription start site (Tijsterman et al., 1997). Here we show that deletion of \textit{SPT4} leads to TCR that extends throughout the ORF until at least 500 bases downstream of the transcriptional start. In the same mutants and with the same target, repair of non-transcribed DNA remains unaffected by the \textit{sp4} mutation, again emphasizing its specificity for TCR only.

We show that the activation of TCR by the absence of \textit{Sp4} is a general phenomenon for RNA polymerase II-transcribed genes and that Rad26-independent TCR takes place throughout transcribed DNA. These findings are consistent with the reduced UV sensitivity observed in these cells. \textit{rad14} cells are completely defective in NER. Survival experiments showed that the absence of \textit{Sp4} does not suppress a \textit{rad14} defect (Figure 1). Repair analysis corroborates this observation. We found no evidence that damage is removed in \textit{rad14\textit{sp4}} cells, indicating that the repair observed in \textit{rad16\textit{rad26sp4}} cells is NER (data not shown).

**Transcription level in \textit{spt4} mutants**

Since TCR is dependent on transcription, it is conceivable that the rate of transcription may influence TCR efficiency. We have suggested previously that high transcription rates may render TCR Rad26 independent (Verhage et al., 1996). To determine whether the transcription rate is altered in \textit{sp4} cells, we analysed transcript levels using poly(dT) to probe mRNA. To check whether the probe allows detection of differences in transcript levels, we employed an \textit{rbp1-1} mutation rendering RNA polymerase II transcription thermosensitive. At the non-permissive temperature, transcription is completely blocked and mRNA levels rapidly decrease with time (Nonet et al., 1987; Holstege et al., 1998). Using the poly(dT) probe, we indeed observed a dramatic drop in signal when RNA was isolated from \textit{rbp1-1} cells kept at 37°C for various lengths of time, while the signal was

\begin{figure}[h]
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\includegraphics[width=\textwidth]{Fig2}
\caption{The absence of \textit{Sp4} activates transcription-coupled NER. Gene-specific repair assay showing the repair kinetics of the indicated strains at a 3.4 kb \textit{RPB2} fragment. Cells were grown in YPD, UV irradiated and allowed to remove lesions for the indicated times. Genomic DNA was extracted, digested with the appropriate restriction enzymes and either treated or mock treated with T4 endoV. Samples were run on an alkaline agarose gel, blotted onto a nylon membrane and probed for either the transcribed (TS) or non-transcribed strand (NTS) of the \textit{RPB2} gene using a 32P-labelled single-stranded probe. Fragments were visualized by autoradiography. (A) An experiment showing repair in \textit{rad16rad26} and \textit{rad16rad26sp4} cells. (B) As (A), but \textit{rad16} and \textit{rad16sp4} cells were analysed. (C and D) Graphical representation of repair analysis as described in (A) and (B), respectively. Southern blots were quantified using a BioRad Molecular Imager and the percentage of repair was determined from signal intensities as described in Materials and methods. At least two independent experiments were carried out for each data series.}
\end{figure}
unaffected in \( \text{RPB1} \) cells (Figure 4B). In all cases, rRNA levels remained unaffected, which served as the loading control (Figure 4C).

Next, we determined steady-state mRNA levels in \( \text{SPT4} \) and \( \text{spt4} \) cells under conditions identical to those used for repair experiments. We observed no significant effect on the level of transcription due to \( \text{spt4} \) after DNA damage had been inflicted (Figure 4A), although UV irradiation itself seems to affect transcript levels. Irradiation induced a slight drop in the level of transcription, after which the mRNA level increased with time and peaked at ~40 min after irradiation. This transcription profile was observed in all strains analysed, regardless of Spt4 activity.

**spt5-194 does not suppress rad26 UV sensitivity**

The yeast Spt4 and Spt5 proteins, and their human orthologues, form a heterodimeric complex and have been shown to function in transcription elongation (Hartzog et al., 1998; Wada et al., 1998). To determine whether a defect in Spt5 also results in suppression of the \( \text{rad26} \) UV sensitivity, we tested a specific \( \text{spt5} \) mutation documented to display phenotypes similar to those of a \( \text{spt4} \) deletion. Like \( \text{spt4} \), the \( \text{spt5-194} \) mutant displays an SPT phenotype and is 6-azauracil (6-AU) sensitive (Swanson and Winston, 1992; Hartzog et al., 1998). In addition, \( \text{spt5-194} \) combined with a \( \text{spt4} \) mutation leads to synthetic lethality (Swanson and Winston, 1992). Introduction of the \( \text{spt5-194} \) mutation into \( \text{rad16rad26} \) cells does not, however, result in suppression of the UV sensitivity (Figure 5). This finding indicates that Spt5 may not play a role in TCR similar to that of Spt4 or that, despite the shared phenotypes with \( \text{spt4} \), the specific \( \text{spt5-194} \) mutation does not lead to a defect in TCR-modulating activity.
suppressed by a defect in RAD14 on MMS is suppressed by and grown at either 30 or 38.5 °C for 3±5 days. The indicated strains were grown in YPD, streaked onto YPD plates in equal quantities. Droplets were UV irradiated in increments of 3 J/m² and grown at 30°C for 3 days.

**Fig. 5.** sp5-194 does not suppress rad16rad26 UV sensitivity. The indicated strains were grown in YPD and diluted in water. Approximately 1000 cells were spotted onto YPD plates containing 0 or 0.0075% MMS and incubated at 30°C for 3±5 days. (A) Suppression of sp4 temperature sensitivity by rad14. The indicated strains were grown in YPD, streaked onto YPD plates and grown at either 30 or 38.5°C for 3–5 days. (B) Slow growth of sp4 on MMS is suppressed by rad14. The indicated strains were grown in YPD, diluted in water in a 3-fold serial dilution series, spotted onto YPD plates containing 0 or 0.0075% MMS and incubated at 30°C for 3–5 days.

**Discussion**

We have identified the transcription elongation factor Sp4 as an important factor in regulating TCR activity in yeast. A disruption of SPT4 was found to suppress a rad26 defect by specifically activating TCR, independently of Rad26. Deletion of SPT4 has no effect on GGR.

TCR at the RPB2 locus is, to a large extent, intrinsically Rad26 independent. Deletion of SPT4, however, leads to enhanced Rad26-independent TCR resembling a Rad26 repair profile. TCR in URA3 is strongly dependent on Rad26. No repair could be detected within the transcribed strand of URA3 in rad16rad26 cells. Here, deletion of SPT4 has an even more striking effect on TCR. In the absence of Sp4, removal of lesions is no longer confined to the transcription initiation/elongation transition zone only but takes place throughout the ORF. These data show that the absence of Sp4 renders TCR Rad26 independent and potent throughout the transcribed DNA of RNA polymerase II-transcribed genes. We find no obvious involvement of Spt5 in the regulation of TCR, which would imply that Sp4 possibly acts independently of Spt5, at least at the level of regulating the Rad26 requirement. However, it is feasible that a possible function of Spt5 in TCR is not disturbed in the specific mutant used in this study.

**Rad26-independent TCR**

The results presented above show that: (i) as we previously suggested (Verhage et al., 1996; Tijsterman et al., 1997), Rad26 is not required for TCR per se, indicating that transcription–repair coupling can take place independently of the Rad26 intermediate; (ii) Rad26-independent TCR can take place throughout the ORF of an RNA polymerase II-transcribed gene; (iii) active TCR can be virtually uncoupled from Rad26 function; and (iv) the requirement for Rad26 in TCR can be modulated by altering the mode of transcription.

We previously suggested that Rad26-independent TCR may correlate with the transcription rate, based on the observation that TCR in a highly transcribed GAL7 gene does not require Rad26 (Verhage et al., 1996). Here we show that Rad26-independent TCR due to the absence of Sp4 is most probably not the result of an increased rate of transcription. This indicates that the kinetics of transcript synthesis alone cannot explain the loss of Rad26 requirement in TCR.

**Sp4 and transcription elongation**

Sp4 is known to be involved in the regulation of transcription elongation. Cells carrying the sp4 mutation display a variety of phenotypes, suggesting a defect in transcription. Cells defective in sp4 display a hyper-rec
phenotype, which can be interpreted in terms of a transcription elongation defect, as has been shown for other elongation factors (Malagon and Aguilera, 1996; Chavez and Aguilera, 1997; Prado et al., 1997; Piruat and Aguilera, 1998). sp4 mutants are thermosensitive and hypersensitive to 6-AU, indicative of a defect in transcription elongation (Exinger and Lacroute, 1992; Hartzog et al., 1998). Interestingly, ppr2 mutants that are defective in the transcription elongation factor SII are also 6-AU sensitive. A combination of sp4 and ppr2 mutations leads to a strongly enhanced thermosensitivity (Hartzog et al., 1998). This points to a genetic link between SPT4 and SII, again suggesting a role for Sp4 in transcript elongation and indicating that the transcription taking place in sp4 mutants requires SII while normally no effect of an SII mutation can be observed.

Recently, DSIF, a novel transcriptional regulator, was identified based on its ability to confer DRB sensitivity to in vitro transcription. This complex, comprised of a 160 and a 14 kDa subunit, turned out to be the human counterpart of the yeast Spt5–Sp4 complex (Wada et al., 1999). Recently, Yamaguchi et al. (1999) have fully reconstituted the DRB-sensitive transcriptional system, which includes DSIF as well as a five-subunit complex named NELF. DSIF and NELF cooperate to repress transcription elongation by direct contacts to the RNA polymerase and the transcript. DSIF and NELF only associate with hypophosphorylated RNA polymerase II and are released upon CTD phosphorylation, thereby alleviating transcriptional blockage. The requirement for an unphosphorylated RNA polymerase II CTD and a transcript suggests that DSIF and NELF act upon early elongation complexes.

**Spt4 and TCR-competent transcription**

The biochemical studies on DSIF provide an attractive model for Rad26-independent TCR. We have previously shown that, directly downstream of the transcription start site at the RPB2 and URA3 loci, TCR is Rad26 independent, which can be explained by the presence of TFIH in transcription complexes at these positions (Zawel et al., 1995; Dvir et al., 1997). TFIH is required for TCR and possibly for transcription resumption at the damaged site. Compelling evidence for the latter function of TFIH was provided recently by Le Page et al. (2000), who showed that TFIH is required for transcription-coupled base excision repair (BER) of oxidative damage. TFIH is not required for BER in general, suggesting that it is recruited to stalled RNA polymerase for transcription resumption rather than repair.

Based on our previous observation that TCR in early elongation is Rad26 independent, in conjunction with our findings in this study and the general requirement for TFIH in TCR, we suggest a model for the role of Rad26 in TCR (Figure 7). Sp4 is believed to act as a gate keeper at the early elongation–processive elongation transition. In this way, Sp4 may force transcription to commit to a processive elongation mode. Complexes in this mode depend on Rad26 to allow TCR to take place. It is conceivable that different forms of transcription elongation exist. Both Sp4-mediated, processive elongation and Sp4-independent transcription may be active even within a single gene. Sp4-independent transcription leads to TCR that is intrinsically Rad26 independent, as is the case during early elongation when transcription is not affected by Sp4.

Some genes may be unaffected by Sp4, and TCR in these genes may therefore be largely Rad26 independent. Possibly high rates of transcription allow early transcription elongation complexes to escape Sp4 suppression and render TCR Rad26 independent, as we have observed previously (Verhage et al., 1996). The fact that Sp4 plays a non-essential role in transcription in yeast may explain the relatively high level of Rad26-independent TCR.

During normal growth, Sp4, Rad26 and possibly other factors as well may modulate the ratio between these different modes of transcription depending on local requirements. The presence of DNA damage may lead to a transiently biased mode of transcription, possibly less processive, but better suited to transcribe a bumpy template. As we have shown in this study, the ratio between the transcription modes regulated by Sp4 can be biased genetically by depleting cells of Sp4, rendering TCR generally Rad26 independent. A very recent study in Cockayne syndrome cells has shown that DNA damage is not the sole condition leading to a requirement for CSB. Loci carrying highly transcribed repetitive DNA producing highly structured RNAs display a defect in chromatin condensation during metaphase (Yu et al., 2000). This so-called metaphase instability is thought to arise from a defect in transcription leading to blocked polymerases that prevent mitotic shutdown of transcription. This suggests that CSB is required for transcription of these loci, indicating a function distinct from a primary role in repair. Our observations, in conjunction with these recent findings in Cockayne syndrome cells, provide new evidence that the requirements for TCR are largely transcriptional and can be brought about by an interplay between Rad26 and Sp4.

The physical identity of a transcription complex that is TCR competent and independent of Sp4 remains to be determined. Phosphorylation status may be a factor discriminating between such complexes since this is known to be a key determinant in the transition from early to processive elongation (Dahmus et al., 1994). Another attractive possibility would be the presence of TFIH in elongating RNA polymerase machinery as a determining factor, since this complex appears to be required for transcription resumption as well as for TCR. During early elongation, where TFIH is present, TCR is intrinsically Rad26 independent. Possibly, during processive elongation, Rad26 directly recruits TFIH to the site of transcription. In this respect, it is interesting to note the recent finding that elongating human RNA polymerase can
interact with TFIIH but only in association with CSB (Tantin et al., 1998). Our study corroborates these data and indicates that possibly via TFIIH, transcription itself is a major determinant in transcription–repair coupling. Spt4 may, in fact, act as a gate keeper for transcription elongation by regulating the presence of TFIIH in RNA polymerase complexes.

Transcription elongation is likely to be regulated by several elongation factors that act upon processive hyperphosphorylated RNA polymerases. During TCR, the transcription complex presumably has to be displaced from the site of the lesion, which is achieved by Rad26, or, alternatively, the transcription complex can relocate unailed when it is intrinsically less processive. The latter may be the case for Spt4-independent transcription. It is conceivable that elongation factors counteracting transcriptional arrest and promoting processivity render transcription dependent on Rad26 for TCR. This would predict that a defect in such elongation factors renders transcription TCR competent.

Materials and methods

Strains and media

Experiments were conducted in the S. cerevisiae W303-1B background, genotype: MATa can1-100 ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-1. Strains are listed in Table I. All strains were kept on selective medium: yeast nitrogen base (YNB) (0.67% YNB, 2% glucose, 2% bacto agar) supplemented with appropriate markers. For UV survival, repair analysis and RNA isolation, cells were grown in complete medium (YPD) (1% yeast extract, 2% bacto peptone, 2% glucose) at 30°C under vigorous shaking.

Table I. Yeast strains

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</tr>
<tr>
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<td>Y262</td>
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</tr>
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*The remainder of the genotype is identical to W303-1B. *van Gool et al. (1994). *Verhage et al. (1996). *This rad16Δ::URA3 mutation described by Verhage et al. (1994) was subsequently rendered rad26Δ::HIS3 using the construct described (van Gool et al., 1994).
UV dose–response tests

Cells were grown in YPD, or YNB when harbouring plasmids, diluted in water to OD_{600} = 0.03. A 1.5 µl aliquot (~1000 cells) was spotted onto YPD plates irradiated with UV light at a 0.5 J/m²/s. Different doses were inflicted on individual droplets by masking cells for different periods of time. Cells were incubated at 30°C for 3 days in the dark to allow formation of colonies.

UV irradiation and DNA isolation

UV irradiation of yeast was performed as described (Verhage et al., 1994). DNA was isolated by washing cells and incubating them in 5 ml of SF buffer (1 M sorbitol, 60 mM NaPO₄, pH 7, 2.5 mM EDTA, 6.6 mM β-mercaptoethanol) and 25 U of zymolyase (ICN) at 4°C for 16 h. Spheroplasts were spun down and resuspended in 5 ml of 50 mM Tris pH 8, 20 mM EDTA, 150 mM NaCl. SDS was added to 1% and incubated for 10 min at room temperature. The suspension was extracted with phenol:chloroform 1:1 and chloroform, and precipitated with 3 ml of 2-propanol. High molecular weight DNA was obtained by centrifugation and the pellet was washed in 70% ethanol. DNA was resuspended in 1 ml of 2-propanol. DNA pellet was washed in 80% ethanol, dried and resuspended in 0.5 ml of 10 mM Tris±HCl, 1 mM EDTA pH 8.0. DNA concentration was determined by measuring the optical density at 260 nm.

Gene-specific repair analysis

Repair analysis was performed essentially as described (Verhage et al., 1994), except that a 3.4 kb Nud fragment containing bases 117–3518 of the 3675 bp RPB2 ORF was used as a target. Quantification of the Southern blots obtained was carried out using a Bio-Rad Molecular Imager and Image Quant software. The level of repair was calculated in terms of the amount of dimers per fragment according to the Poisson distribution, as described previously (Verhage et al., 1994).

Repair analysis at nucleotide resolution

Nucleotide-specific repair analysis was performed essentially as described (Tjisterman et al., 1996). DNA was digested with EcoRV and MspI, and an internal 412 bp URA3 fragment was isolated using a biotinylated primer and streptavidin-coated paramagnetic beads (Dynal). The isolated fragment was 3'-[32P]dCTP end-labelled using U2TB (5'-gattttggggggATCTTGACTGATTTTTCCATG) for the transcribed strand and U5MSN5 (5'-tttccggggggGGTGTCATAATCAACCAATCG) for the non-transcribed strand. Sequences shown in upper case are for the non-transcribed strand, is an ATP-dependent DNA damage sensor. J. Biol. Chem., 272, 21665–21668.


Swanson,M.S. and Winston,F. (1992) SPT4, SPTS and SPT6

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References


interactions: effects on transcription and viability in *Saccharomyces cerevisiae*. Genetics, 132, 325–336.


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