The Rad4 homologue YDR314C is essential for strand-specific repair of RNA polymerase I-transcribed rDNA in *Saccharomyces cerevisiae*
The Rad4 homologue YDR314C is essential for strand-specific repair of RNA polymerase I-transcribed rDNA in *Saccharomyces cerevisiae*

Ben den Dulk, Jourica A. Brandsma and Jaap Brouwer
Summary

The *Saccharomyces cerevisiae* protein Rad4 is involved in damage recognition in Nucleotide Excision Repair (NER). In RNA polymerase II transcribed regions Rad4 is essential for both NER subpathways Global Genome Repair (GGR) and Transcription Coupled Repair (TCR). In ribosomal DNA (rDNA), however, the RNA polymerase I transcribed strand can be repaired in the absence of Rad4. In *Saccharomyces cerevisiae* the YDR314C protein shows homology to Rad4. The possible involvement of YDR314C in NER was studied by analyzing strand specific CPD removal in both RNA pol I and RNA pol II transcribed genes. Here we show that the Rad4-independent repair of rDNA is dependent on YDR314C. Moreover, in Rad4 proficient cells preferential repair of the transcribed strand of RNA pol I transcribed genes was lost after deletion of *YDR314C*, demonstrating that Rad4 cannot replace YDR314C. CPD removal from the RNA pol II transcribed *RPB2* gene was unaffected in *ydr314c* mutants. We conclude that the two homologous proteins Rad4 and YDR314C are both involved in NER and probably have a similar function, but operate at different loci in the genome and are unable to replace each other.
1 Introduction

Nucleotide Excision Repair (NER) is a DNA repair process capable of recognizing and removing a wide variety of helix distorting lesions, like the UV induced 6-4 photoproducts (6-4PP) and cyclobutane pyrimidine dimers (CPD). After recognition of the damage, a single strand DNA fragment containing the lesion is excised, allowing DNA synthesis using the undamaged strand as a template (de Laat et al., 1999; Prakash and Prakash, 2000). The basic mechanism of NER is present in organisms ranging from *Escherichia coli* to man. The core NER proteins have been identified using an *in vitro* reconstituted system with purified proteins (Guzder et al., 1995; He et al., 1996; Mu et al., 1996). One of the essential components of the NER reaction in *Saccharomyces cerevisiae* is the damage recognition protein Rad4. Binding of the Rad4-Rad23 complex to the damaged site initiates the recruitment of the other NER proteins that cooperatively complete the repair of the damaged DNA (Guzder et al., 1998; Jansen et al., 1998).

*In vivo*, additional proteins are required to facilitate efficient removal of lesions. Extensive studies in various organisms revealed that certain NER proteins are specifically involved in preferential repair of the transcribed strand of transcriptionally active DNA. This process is designated Transcription Coupled Repair (TCR) and, in yeast, requires Rad26, Rpb4 and Rpb9 (van Gool et al., 1994; Li and Smerdon, 2002). Other proteins, like Rad7 and Rad16, are specifically involved in removal of lesions throughout the entire genome, a process referred to as Global Genome Repair (GGR). The core NER proteins, like Rad4, are essential for both GGR and TCR (Bang et al., 1992; Verhage et al., 1994). Previously, however, we showed that Rad4 is not essential for strand specific repair of RNA pol I transcribed rDNA, whereas all other core NER proteins, including Rad23, are indispensable (Verhage et al., 1996a).

In human cells the XPC-hHR23B complex is homologous to the Rad4-Rad23 complex in *Saccharomyces cerevisiae* (Legerski and Peterson, 1992; Masutani et al., 1994). In contrast to *rad4* mutants, cells devoid of XPC are completely defective in repair of RNA pol I transcribed rDNA (Christians and Hanawalt, 1994). Moreover, Rad4 and XPC differ in their contributions to GGR and TCR in RNA pol II transcribed genes. XPC cells are only defective in GGR (Venema et al., 1991) whereas *rad4* cells lack both GGR and TCR (Verhage et al., 1994).

The yet uncharacterized *Saccharomyces cerevisiae* protein YDR314C displays homology with established Rad4 homologues (Anantharaman et al., 2001; Marti et al., 2003). Moreover, analogous to Rad4, YDR314C is reported to co-immunoprecipitate with Rad23 in a large scale interaction study (Gavin et al., 2002). These similarities suggest that the *YDR314C* gene product could be a functional Rad4 homologue.

In the fission yeast *Schizosaccharomyces pombe* two Rad4 sequence homologues were identified as well. Both homologues, designated Rhp41 and Rhp42, have been shown to be involved in NER (Fukumoto et al., 2002; Marti et al., 2003). Strand specific repair analysis indicated that Rhp42 is involved in GGR whereas Rhp41 has a role in both TCR and GGR (Fukumoto et al., 2002). Epistasis studies confirmed the role of Rhp41 in both NER subpathways (Marti et al., 2003). However, deletion of *rhp42* in cells lacking GGR due to a mutation in the *rhp7* gene, resulted in increased UV sensitivity, whereas deletion of *rhp42* in TCR deficient *rhp26* mutants did not,
suggesting that Rhp42 is involved in TCR rather than GGR. On the other hand, transcription recovery, indicative for the efficiency of repair in transcribed DNA, was affected in \textit{rhp41} cells but not in \textit{rhp42} cells, contradicting the results from the epistasis analysis. Rhp41 and Rhp42 are apparently both involved in NER, but their relative contribution to GGR and TCR is not yet clear.

In \textit{Saccharomyces cerevisiae} no function has yet been assigned to the \textit{YDR314C} gene product. In this paper the involvement of \textit{YDR314C} in NER is described. We show that \textit{YDR314C} cannot substitute for Rad4 in RNA pol II transcribed regions but is essential for preferential repair of RNA pol I transcribed rDNA.
2 Results

A Rad4 homologue in Saccharomyces cerevisiae
Recently, an open reading frame in Saccharomyces cerevisiae was identified that shows substantial resemblance to Rad4 (Anantharaman et al., 2001; Marti et al., 2003). The homology between all functional Rad4 proteins is limited to the carboxyl terminal region referred to as a Rad4 protein family A (Rad4pfam-A) domain (Bateman et al., 2004) (Fig. 1A). The exclusive conservation of the carboxyl terminal region suggests that the characteristics essential for NER are embedded within this domain. Indeed, for the human Rad4 homologue it was shown that the carboxyl terminal region is essential for the interactions with TFIIH, hHR23B and damaged DNA (Uchida et al., 2002). The carboxyl terminal region of the yeast Rad4 homologues contains, partially overlapping the pfam-A domain, an ancient transglutaminase fold (Anantharaman et al., 2001), which is also present in peptide-N-glycanases. In the Rad4 family members, however, the predicted catalytic residue is absent, suggesting that the transglutaminase fold is inactive. In contrast to the carboxyl termini, considerable diversity exists among the amino terminal regions of the Rad4 homologues. This indicates that apart from the shared function, additional functions might be present.

Interestingly, in Saccharomyces cerevisiae the yet uncharacterized ORF YDR314C encodes a protein containing a carboxyl terminal Rad4pfam-A domain (Marti et al., 2003) (Fig. 1A,B). In addition to the sequence homology, the YDR314C gene product was, like Rad4, found to co-immunoprecipitate with Rad23 in a large-scale tandem-affinity purification (TAP) experiment (Gavin et al., 2002). The sequence homology and the interaction with Rad23 indicate that YDR314C could be a genuine Rad4 homologue and consequently may have a similar function in NER. On the other hand, the UV sensitivity of rad4 mutants is comparable to that of the other core NER mutants. Indeed, deletion of YDR314C, even in rad4 and rad16 mutants, does not affect sensitivity towards UV irradiation (Fig. 2A,B) or other DNA damaging and stress inducing agents (data not shown).

Figure 1
(A) Schematic representation of Rad4 homologues. The gray shaded boxes represent the conserved region that is categorized as a Rad4pfamA domain (Bateman et al., 2004). The amino acid position is represented at the bottom of the figure.
Figure 1
(B) Alignment of the Rad4pfam-A domains of Rad4, Rhp41, Rhp42, XPC and YDR314C. Protein sequences were aligned with the clustalW program version 1.82. Similar and identical residues are boxed light and dark gray respectively.
Previously we showed that the ribosomal DNA (rDNA) locus can be repaired in the absence of Rad4 (Verhage et al., 1996a). The rRNA genes are present in ~150 tandemly repeated units of 9.1 kb. The densely packed rDNA is localized in the nucleolus, a membrane-free intranuclear compartment. The rRNA genes are highly transcribed, yet, depending on the growth rate, no more than 40% to 60% of the repeats is transcriptionally active (Dammann et al., 1993). Each repeat consists of a 5S and 35S unit that is transcribed by pol III or pol I respectively. UV induced lesions in the rDNA locus are repaired by NER and it was shown that preferential repair of the transcribed strand occurs (Verhage et al., 1996a; Conconi et al., 2002; Meier et al., 2002). Cells deleted for RAD4 are still capable of repairing the RNA pol I transcribed strand of rDNA whereas repair is completely abrogated in cells lacking one of the other core NER proteins.

A plausible explanation for the Rad4-independent repair in rDNA could be that another protein fulfils the damage recognition role in NER in the RNA pol I transcribed regions. Considering the similarities of YDR314C and Rad4, we investigated the role of YDR314C in Rad4-independent repair. CPD removal from RNA pol I transcribed rDNA

Figure 2
UV survival test. Cells were grown for 3 days in YPD, diluted in water to OD600 values that resulted in 100-200 colonies for each of the 3 administered UV doses and for the non irradiated sample. The diluted cells were plated on YPD and irradiated with the doses indicated. The irradiated cells were grown for 3 days in the dark at 30°C, colonies were counted and survival was calculated. Survival after UV was determined and plotted as a function of the applied UV dose.

(A) UV survival of W1588 and ydr314c mutants (black and open circles respectively) and of rad16 and rad16ydr314c mutants (black and open triangles respectively).

(B) Survival of rad4 and rad4ydr314c mutants (black and open squares respectively). The values depicted in the graphs are averages of at least 3 independent experiments, error bars represent standard deviations.
rDNA was analyzed in rad4 and rad4ydr314c mutants using strand specific probes. Cells lacking Rad4 are defective in CPD removal except for lesions in the RNA pol I transcribed strand, which can be repaired to approximately 50% (Fig. 3A,B) (Verhage et al., 1996a). Interestingly, the Rad4-independent repair is completely abrogated when YDR314C is deleted (Fig. 3A,B), demonstrating that YDR314C is indeed responsible for the repair of RNA pol I transcribed rDNA in rad4 mutants.

We subsequently examined the role of YDR314C in rDNA repair in cells containing functional Rad4. Single ydr314c mutants were analyzed for CPD removal in RNA pol I transcribed rDNA. Figures 3C and 3D show that in NER+ cells the non-transcribed strand is repaired slightly slower than the transcribed strand and that the overall repair of both strands is significantly lower compared to CPD removal in RNA pol II transcribed regions (compare Fig. 3C,D and 4A,B). After two hours, 70% of the lesions is removed from the transcribed strand and 65% from the non-transcribed strand, corresponding to our results reported earlier (Verhage et al., 1996a).

In ydr314c mutants the percentage of removed lesions after two hours is reduced to 55% in the non-transcribed strand and 50% in the transcribed strand (Fig. 3C,D). Thus, in the absence of YDR314C a substantial amount of lesions can still be removed, albeit with lower efficiency. The slight decrease in dimer removal observed in the non-transcribed strand of rDNA might indicate that YDR314C is involved in GGR. However, the fact that GGR is completely defective in rad4 mutants shows that YDR314C can not replace Rad4 in GGR, implying that YDR314C is not directly involved in GGR of pol I transcribed rDNA.

To investigate a possible role of YDR314C in strand specific repair, we measured the effect of a YDR314C deletion in GGR defective rad16 cells. Due to the impaired GGR, the difference in repair-efficiency between the transcribed and non-transcribed strand is more pronounced in a rad16 background (Verhage et al., 1996b). For RNA pol I transcribed rDNA, deletion of RAD16 does not lead to a complete defect in GGR like in RNA pol II transcribed genes, but lesion removal from the non-transcribed strand is reduced to 30%. A clear strand bias can be observed since the transcribed strand is repaired to 70% (Fig. 3E,F) (Verhage et al., 1996a). Interestingly, preferential repair of the transcribed strand is completely absent after deletion of YDR314C in rad16 mutants (Fig. 3E,F), even when lesion removal was analyzed after 4 hours of incubation (Fig. 3G,H). These results demonstrate that YDR314C is essential for the preferential repair of the RNA pol I transcribed strand in rDNA.

### CPD removal in RNA pol II transcribed DNA

The experiments above show that Rad4 is unable to function in strand specific repair of RNA pol I transcribed rDNA, whereas YDR314C is essential for this mode of repair. Thus, Rad4 cannot replace YDR314C in rDNA repair. In RNA pol II transcribed genes on the other hand, NER is dependent on Rad4. To examine whether YDR314C can substitute for Rad4 in NER of RNA pol II transcribed genes, CPD removal from both strands of the RPB2 gene was measured in ydr314c mutants. We show that the YDR314C deletion has no effect on the repair-efficiency (Fig. 4A,B), even when YDR314C is deleted in a rad16 mutant, in which TCR is the sole mode of repair (Fig. 4C,D). These results demonstrate that YDR314C has no role in NER of the RPB2 gene, suggesting that YDR314C is not involved in repair of RNA pol II transcribed genes in general.
Figure 3
Gene specific repair assay. Cells were grown in YPD, irradiated and allowed to remove lesions for the times indicated. Genomic DNA was extracted, digested with HindIII and either mock-treated or treated with T4endoV. Samples were run on an alkaline agarose gel, blotted on a nylon membrane and probed with an EcoRI-Mrul rDNA fragment for either the transcribed strand (TS) or the non-transcribed strand (NTS). Fragments were visualized using a Bio-Rad Molecular Imager and fragment intensities were quantified with Quantity One (Bio-Rad). (A) Southern blots showing the removal of dimers from rDNA at various time points in rad4 and rad4ydr314c mutants respectively. Time points after UV irradiation are indicated, samples mock-treated or treated with the dimer-specific enzyme T4endoV are denoted - and +, respectively. TS, transcribed strand; NTS, non-transcribed strand. (B) Graphical representation of quantified Southern blots. The percentage removed dimers as a function of time. rad4 TS and NTS (black and open triangles respectively) and rad4ydr314c TS and NTS (black and open circles respectively). Values are the mean of at least three independent experiments. Error-bars indicate standard deviations. (C) As (A), but for W1588 and ydr314c cells. (D) As (B) but for W1588 and ydr314c cells. (E) As (A), but for rad16 and rad16ydr314c mutants. (F) As (B) but for rad16 and rad16ydr314c mutants. (G) As (E) but samples taken after 0, 120 and 240 minutes respectively. (H) As (F) but samples taken after 0, 120 and 240 minutes respectively.
YDR314C is essential for strand-specific repair of rDNA

Figure 4
Gene specific repair assay. Cells were grown in YPD, irradiated and allowed to remove lesions for the times indicated. Genomic DNA was extracted, digested with HindIII and either mock-treated or treated with T4endoV. Samples were run on an alkaline agarose gel, blotted on a nylon membrane and probed with an EcoRI-Mrul rDNA fragment for either the transcribed strand (TS) or the non-transcribed strand (NTS). Fragments were visualized using a Bio-Rad Molecular Imager and fragment intensities were quantified with Quantity One (Bio-Rad).

(A) Southern blots showing the removal of dimers from rDNA at various time points in wildtype cells (W1588) and the ydr314c mutant. Time points after UV irradiation are indicated, samples mock-treated or treated with the dimer-specific enzyme T4endoV are denoted - and +, respectively. TS, transcribed strand; NTS, non-transcribed strand.

(B) Graphical representation of quantified Southern blots. The percentage removed dimers as a function of time. W1588 TS and NTS (black and open triangles respectively) and ydr314c TS and NTS (black and open circles respectively). Values are the mean of at least three independent experiments. Error-bars indicate standard deviations.

(C) As (A), but for rad16 and rad16ydr314c mutants.

(D) As (B) but for rad16 and rad16ydr314c mutants.
3 Discussion

The YDR314C gene product shows homology to the members of the Rad4 family (Anantharaman et al., 2001; Martí et al., 2003) and interaction with Rad23 has been reported (Gavin et al., 2002), suggesting a role for YDR314C in NER. In genome wide screens ydr314c mutants exhibit poor growth in medium containing nystatin or sorbitol (Giaever et al., 2002). Furthermore, a synthetic lethal interaction of YDR314C and CHS1 was reported (Tong et al., 2004). These phenotypes might indicate involvement in processes like amino acid synthesis, osmoregulation and cell wall maintenance.

Here we show that the YDR314C gene product is responsible for Rad4-independent repair in the RNA pol I transcribed rDNA locus. Moreover, we demonstrate that YDR314C is not merely acting as a substitute when Rad4 is absent, but that preferential repair of the RNA pol I transcribed strand specifically requires YDR314C. The effect is especially evident in the GGR deficient rad16 background, in which there is a clear difference in repair of the transcribed and non-transcribed strand. This strand bias is completely absent in rad16ydr314c double mutants, demonstrating that YDR314C, despite the presence of Rad4, is essential for preferential repair of the transcribed strand. The specific decrease in repair of the transcribed strand suggests that YDR314C is involved in TCR, however, we have not shown that in RNA pol I transcribed rDNA the preferential repair of the transcribed strand is dependent on active transcription. We therefore can not exclude the possibility that the YDR314C dependent repair in rad16 cells is independent of transcription, but only occurring in the template strand.

Deletion of YDR314C has no effect on dimer removal from both strands of the RNA pol II transcribed RPB2 gene. This suggests that YDR314C solely acts on RNA pol I transcribed regions and is unable to substitute for Rad4 in TCR of RNA pol II transcribed genes. The absence of UV sensitivity of ydr314c cells shows that removal of lesions from rDNA does not significantly contribute to survival. Considering that YDR314C was reported to co-immunoprecipitate with Rad23 and the fact that repair of rDNA is defective in rad23 but not in rad4 mutants, we assume that YDR314C functions, like Rad4, in complex with Rad23.

The two homologues Rad4 and YDR314C appear to have non-overlapping roles. Rad4 is essential for repair of both strands of RNA pol II transcribed genes and is unable to act in strand specific repair of genes transcribed by RNA pol I. YDR314C on the other hand is essential for preferential repair in RNA pol I transcribed rDNA and can not replace Rad4 in repair of RNA pol II transcribed regions. A simple explanation for the non-overlapping functions could be that Rad4 and YDR314C are prevented from travelling in and out the nucleolus respectively. However, the requirement of Rad4 for GGR of rDNA demonstrates that the inability of Rad4 to act in preferential repair of the transcribed strand of rDNA is not due to exclusion of Rad4 from the rDNA locus. Moreover, YDR314C appears not to be restricted to the nucleolus, since proteome-wide GFP localization experiments show that YDR314C is present throughout the nucleus (Huh et al., 2003). Given that Rad4 and YDR314C are not spatially confined, we conclude that although Rad4 and YDR314C have homologous functions in analogous processes, they are unable to substitute for each other.

In Schizosaccharomyces pombe two Rad4 homologues are present as well. Involve-
YDR314C is essential for strand-specific repair of rDNA

ment of these proteins in repair of RNA pol I transcribed rDNA has not yet been studied. In contrast to Rad4 and YDR314C, Rhp41 and Rhp42 both seem to function, to different degrees, in GGR and TCR of RNA pol II transcribed genes (Fukumoto et al., 2002; Marti et al., 2003). Moreover, rhp41rhp42 double mutants exhibit enhanced UV sensitivity compared to either single mutant, showing that the *Schizosaccharomyces pombe* Rad4 homologues have redundant functions. In addition to their role in NER, Rhp41 and Rhp42 are involved in NER dependent short-patch mismatch repair during meiosis (Marti et al., 2003). A possible involvement of YDR314C and Rad4 in this type of DNA repair in *Saccharomyces cerevisiae* has not yet been investigated.

In human cells, XPC appears to be the only homologue of Rad4 since a second gene encoding a Rad4pfam-A domain containing protein is not present in the human genome (Bateman et al., 2004). There are marked differences between the roles of XPC and Rad4 in NER. In rad4 cells, repair of RNA pol II transcribed genes is completely defective whereas lesions in the RNA pol I transcribed strand of rDNA can still be removed. In human cells on the other hand, XPC is essential for repair of both strands of RNA pol I transcribed rDNA (Christians and Hanawalt, 1994) but not required for TCR in RNA pol II transcribed regions (Venema et al., 1991). Here we show that in *Saccharomyces cerevisiae*, the Rad4-independent repair is explained by the involvement of YDR314C. It remains unclear how NER in humans can process lesions in the transcribed strand without XPC.

The reason why Rad4 and YDR314C are unable to replace each other at different loci in the genome is yet unknown. Possibly, differences in chromatin structure at different chromosomal positions determine the requirement for either Rad4 or YDR314C. The poorly conserved N-terminal region might harbor the properties that are necessary to perform NER at different loci in the genome. The difference in the N-termini among the Rad4 family members could also reflect additional functions of the Rad4 homologues, apart from their role in the NER reaction. Further studies are necessary to identify the factors that influence the requirement of either YDR314C or Rad4 to facilitate NER.
4 Experimental procedures

Strains and media

All experiments were conducted in the *Saccharomyces cerevisiae* W1588-4a background. The strains used in this study are listed in table 1. W1588-4a (Mortensen *et al.*, 2002) was kindly provided by R. Rothstein. Strain MGSC 471 (*rad16::hisG*) and MGSC 479 (*rad4::HisGURA3HisG*) were constructed analogous to the previously described MGSC 268 and MGSC 283 respectively (Jansen *et al.*, 2000), using a W1588-4a instead of a W303-1B background. YDR314C deletions were constructed by transforming target strains with a loxLEU2lox disruption cassette, created by ligating a loxLEU2lox fragment to PCR generated YDR314C flanking regions, using the following primers:

5’-TGGAACAGTGCTGAAAATGCGT, 5’-TTCGGTGACC
GGTTTCAAGGTTTGACCCTTCG, 5’-CATGGTTACC
GATTCGACGCTGTTTCGCAGAG and 5’-GGAGGCGATT
CCACGTCGCTAT. Underlined sequences contain a BstEII restriction site by which the flanking regions were ligated to the loxLEU2lox sequence. Correct integration of the constructs was confirmed by Southern blot analysis. Strains MGSC 471, 537, W1588-4a and MGSC 517 were transformed with an URA3 fragment to obtain the URA3+ strains MGSC 578-581 respectively.

UV survival

Cells were grown for 3 days in YPD and diluted in water to appropriate OD 600 values. The diluted cells were plated on YPD. NER+ cells were irradiated with 0, 20, 40 and 80 J/m², *rad16* cells with 0, 5, 20 and 35 J/m² and *rad4* cells with 0, 1, 2.5 and 4 J/m² respectively. Cells were grown for 3 days in the dark at 30°C, colonies were counted and survival was calculated. The values depicted in the graphs are averages of at least 3 independent experiments; error-bars represent standard deviations.

Table 1. Yeast strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1588-4a</td>
<td>MATa leu2-3,112 ade2-1 can1-100 his3-11,15</td>
<td>R. Rothstein</td>
</tr>
<tr>
<td>MGSC 471</td>
<td><em>rad16::hisG</em></td>
<td>This study</td>
</tr>
<tr>
<td>MGSC 479</td>
<td><em>rad4::HisGURA3HisG</em></td>
<td>This study</td>
</tr>
<tr>
<td>MGSC 517</td>
<td>ydr314c::loxLEU2lox*</td>
<td>This study</td>
</tr>
<tr>
<td>MGSC 518</td>
<td><em>rad4::HisGURA3HisG ydr314c::loxLEU2lox</em></td>
<td>This study</td>
</tr>
<tr>
<td>MGSC 537</td>
<td><em>rad16::HisG ydr314c::loxLEU2lox URA3</em></td>
<td>This study</td>
</tr>
<tr>
<td>MGSC 578</td>
<td><em>rad16::HisG URA3</em></td>
<td>This study</td>
</tr>
<tr>
<td>MGSC 579</td>
<td><em>rad16::HisG ydr314c::loxLEU2lox URA3</em></td>
<td>This study</td>
</tr>
<tr>
<td>MGSC 580</td>
<td>URA3*</td>
<td>This study</td>
</tr>
<tr>
<td>MGSC 581</td>
<td>ydr314c::loxLEU2lox URA3*</td>
<td>This study</td>
</tr>
</tbody>
</table>

*The remainder of the genotype is identical to that of W1588-4a*
Sensitivity towards various chemical agents
Serial dilutions of stationary cells were made in water. Of each dilution 2ml was spotted on YPD or YNB plates with a concentration varying from 0 to 0.03% methyl methanesulfonate (MMS), 0 to 15 mg/ml cisplatin, 0 to 3% dimethylsulfoxide (DMSO), 0 to 6 mM H2O2, 0 to 6 mM caffeine and 0 to 100 mg/ml 6-aza-uracil respectively. For the 6-aza-uracil test URA3+ cells were used. Cells were grown for 2 days at 30°C.

Repair analysis
Cells were grown in YPD to an OD600 of 4.0, pelleted, and resuspended in ice-cold PBS at an OD600 of 1.4. The cells were irradiated to 84 J/m2 at a rate of 2.9 J/m2/s. The irradiated cells were pelleted, resuspended in YPD and kept at 30°C to allow repair. After 0, 30, 60 and 120 minutes cells were pelleted, resuspended in ice-cold water to stop repair, pelleted and frozen at -20°C prior to DNA isolation. DNA was isolated as described by Li and Smerdon (2002), with the following modifications. After the RNase A+T treatment, ammonium acetate was added to a final concentration of 2.5M. The solution was kept on ice for 30 minutes. Following the removal of insoluble components by centrifugation the DNA was precipitated with ethanol. Repair of rDNA was measured as described previously (Verhage et al., 1996a). Analysis of RPB2 repair was performed as described previously (Jansen et al., 2000). The Southern blots were quantified using a Bio-Rad Molecular Imager and Quantity One software. The values depicted in the graphs are the average of 3 independent experiments and the error-bars indicate standard deviations.


References


