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Nucleotide Excision Repair in the Yeast Saccharomyces cerevisiae: Its Relationship to Specialized Mitotic Recombination and RNA Polymerase II Basal Transcription

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Nucleotide excision repair in the yeast *Saccharomyces cerevisiae*: its relationship to specialized mitotic recombination and RNA polymerase II basal transcription

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SUMMARY

Nucleotide excision repair (NER) in eukaryotes is a biochemically complex process involving multiple gene products. The budding yeast *Saccharomyces cerevisiae* is an informative model for this process. Multiple genes and in some cases gene products that are indispensable for NER have been isolated from this organism. Homologues of many of these yeast genes are structurally and functionally conserved in higher organisms, including humans. The yeast Rad1/Rad10 heterodimeric protein complex is an endonuclease that is believed to participate in damage-specific incision of DNA during NER. This endonuclease is also required for specialized types of recombination. The products of the *RAD3*, *SSL2*(*RAD25*) *SSL1* and *TFB1* genes have dual roles in NER and in RNA polymerase II-dependent basal transcription.

1. INTRODUCTION

The genetic versatility of the yeast *Saccharomyces cerevisiae* has facilitated the isolation and characterization of multiple mutants which are defective in nucleotide excision repair (NER) (Friedberg *et al.* 1991; Prakash *et al.* 1993). The genetic complexity of NER revealed by these mutants provided early clues that the biochemistry of this process involves a large number of gene products. Many of the yeast genes involved in NER have since been cloned and sequenced and shown to have functional homologues in humans (see later discussion) (Friedberg *et al.* 1991; Hoeijmakers 1993; Prakash *et al.* 1993). Hence, the biochemical complexity of this process is apparently general in eukaryotes. At present nine genes are known to be indispensable for NER in yeast (table 1) and there is indirect evidence for at least two other genes in this class. Additionally, at least three other genes are known to be involved in NER, but are not absolutely required for this process (table 1). This paper reviews the known functions of the polypeptides encoded by several of these genes, with an emphasis on their participation in other aspects of DNA metabolism, notably mitotic recombination and transcription.

2. DAMAGE-SPECIFIC INCISION DURING NER

The specific recognition of base damage and the incision of the affected polynucleotide strand at such sites are unique and distinctive hallmarks of NER. Over a decade ago it was first demonstrated that damage-specific incision of DNA during NER in the prokaryote *E. coli* involves cutting of the affected DNA strand on each side of a damaged base, thereby generating an oligonucleotide fragment ~ 12 nucleotides in length that includes the base damage, which is ultimately excised (Grossman & Thiagalingam 1993; Sancar & Tang 1993; Van Houten & Snowden 1993). This bimodal incision paradigm appears to be universal, since studies have provided indirect evidence for such a mechanism in human cells (Svoboda *et al.* 1993), though the size of the oligonucleotide fragment generated in such cells is more than twice that in *E. coli*. Genetic and biochemical studies have identified two endonucleases that operate during NER in *S. cerevisiae*, consistent with a bimodal damage-specific mechanism in this organism as well.

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Table 1. *Nucleotide excision repair genes from* S. cerevisiae

cloned genes that are indispensable for NER	
RAD1	non-essential for viability
RAD2	non-essential for viability
RAD4	non-essential for viability
RAD10	non-essential for viability
RAD14	non-essential for viability
RAD3	essential for viability
SSL2(*RAD25*)	essential for viability
SSL1	essential for viability
TFB1	essential for viability
suspected genes that are indispensable for NER	
TFB2	probably essential for viability
TFB3	probably essential for viability
Genes that are not absolutely required for NER	
RAD7	non-essential for viability
RAD16	non-essential for viability
RAD23	non-essential for viability

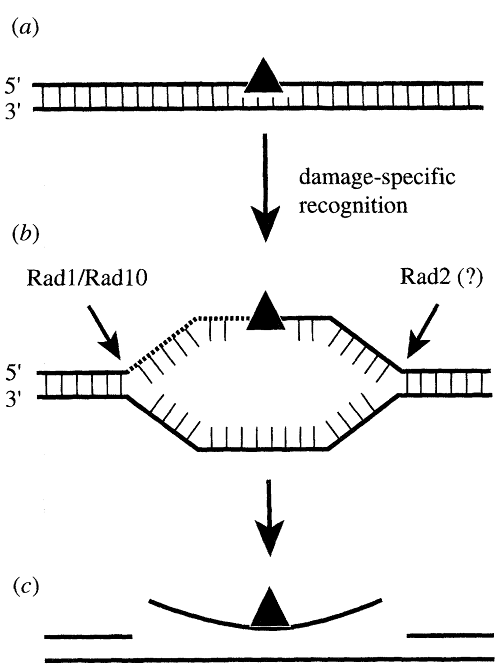

Figure 1. Possible roles of the Rad1/Rad10 and Rad2 endonucleases in bimodal incision during nucleotide excision repair in yeast. Biochemical events associated with damage-specific recognition are believed to result in a localized region of denaturation, incorporating the site of base damage. The (known) Rad3 and Ssl2 DNA helicases may well participate in this denaturation process. The properties of the purified Rad1/Rad10 endonuclease *in vitro* suggest that it could specifically recognize the duplex/3′ single strand junction on the damaged strand, thereby generating a nick 5′ to the site of base damage. The duplex/3′ single strand junction on the opposite DNA strand must be protected from such cleavage. If the Rad2 endonuclease is endowed with the ability to recognize duplex/5′-single strand junctions such specificity could account for cleavage of the damaged strand 3′ to the site of damage.

(*a*) *The Rad1/Rad10 endonuclease*

Complete mutational inactivation of the *RAD1* or *RAD10* genes results in extreme sensitivity to ultraviolet (UV) radiation and a total defect in NER in cell-free extracts (Wang *et al.* 1993). Such mutants are also defective in specialized forms of mitotic recombination (Friedberg *et al.* 1991; Prakash *et al.* 1993). The *RAD1* and *RAD10* genes encode polypeptides with apparent molecular masses of ~ 24 and ~ 130 kDa respectively (Friedberg *et al.* 1991; Prakash *et al.* 1993). Both polypeptides have been purified to physical homogeneity (Friedberg *et al.* 1991; Prakash *et al.* 1993) and form a stable and specific heterodimeric complex with 1:1 stoichiometry (Tomkinson *et al.* 1994). The Rad1/Rad10 complex, but not either protein alone catalyses the Mg^{2+}-dependent nicking of supercoiled DNA and the degradation of M13 circular single-stranded DNA, but does not cleave double-stranded linear DNA (Sung *et al.* 1993; Tomkinson *et al.* 1993). More refined analyses using polymer substrates of defined length and sequence have demonstrated that the Rad1/Rad10 endonuclease specifically recognizes the junction between double-stranded DNA and 3′ single stand tails (A. J. Bardwell *et al.* 1994*b*). Duplex polymers and partially duplex polymers with duplex/5′-single strand tails are not recognized by the enzyme. Single-stranded 49 mer polymers with no secondary structure are also not degraded by the Rad1/Rad10 endonuclease. Hence, it is likely that the degradation of M13 circular single-stranded DNA reflects the recognition of duplex-3′-single strand junctions in regions of DNA with secondary structure, rather than single-stranded DNA per se. Supercoiled DNA is a dynamic structure that contains transient single-stranded loops or bubbles. Presumably the Rad1/Rad10 endonuclease cleaves at the duplex/single strand junctions of these loops.

(*b*) *The role of the Rad1/Rad10 endonuclease in* NER *and recombination*

The specificity of the Rad1/Rad10 endonuclease for duplex/3′single strand junctions suggests a plausible model for its participation in DNA damage-specific incision during NER. The model requires the elaboration of Y-shaped duplex-single strand junctions flanking sites of base damage (figure 1). Such junctions would mark the limits of a region of localized unwinding of the DNA duplex conceptually similar to the denaturation 'bubbles' postulated during transcription and replication. We will return to a consideration of how such a NER 'bubble' may be generated in a later section of the paper. The specific recognition of the duplex/3′-single strand junction 5′ to a site of base damage (figure 1) is expected to constitute a substrate for the Rad1/Rad10 endonuclease. The polypeptide product of the *RAD2* gene is also a single-stranded endonuclease (Habraken *et al.* 1993) and is an attractive candidate for a second junction-specific endonuclease, possibly endowed with duplex/5′-single strand polarity. As such this enzyme might recognize the duplex/5′-single strand junction 3′ to a site of base damage (figure 1). To the extent that *rad1*, *rad10* and *rad2* mutants have been studied, there is no evidence that they manifest a residual capacity for incision of DNA. Hence, the mechanism of NER *in vivo* presumably provides for coordinated incisions at both

Y junctions in the model substrate shown in figure 1, such that Rad1/Rad10-mediated incisions do not occur in the absence of functional Rad2 protein and vice versa. Such coordinated catalysis is consistent with the evidence suggesting that the Rad1, Rad10 and Rad2 proteins are part of a multiprotein complex (repairosome) (see later discussion).

The junction-specific endonuclease activity of the Rad1/Rad10 protein complex also accommodates its known role in mitotic recombination between repeated sequences. Fishman-Lobell and Haber (1992) generated a model plasmid substrate containing two copies of the *E. coli lacZ* gene, one of which contains a 117 base pair (b.p.) cutting site for the HO mating-type endonuclease. Upon induction of the HO endonuclease a sequence-specific double-strand break is introduced in the copy of the *lacZ* gene containing the 117 b.p. cutting site. This cleavage introduces about 60 b.p. of 3′ terminal DNA in one of the *lacZ* sequences which is not present in the other. Mutants defective in the *RAD1* gene were not able to effect recombination between these repeated *lacZ* sequences. However, when both *lacZ* sequences contained HO endonuclease cutting sites, i.e., when complete homology between the repeated sequences was restored, recombination was effected in both wild-type and *rad1* mutants. Physical analysis of recombination intermediates suggests that *rad1* (and *rad10*) mutants are unable to remove the 3′ non-homologous 60 b.p. region, which is consequently trapped as a duplex/3′-single strand junction structure that stalls the completion of recombination. In wild-type cells this junction is presumably recognized by the Rad1/Rad10 endonuclease, resulting in the removal of non-homologous DNA. In contrast to the *RAD1* and *RAD10* genes, the *RAD2* gene is not required for mitotic recombination between repeated sequences (Friedberg *et al.* 1991; Prakash *et al.* 1993).

3. THE COUPLING OF NER AND TRANSCRIPTION

RNA polymerase II transcription in yeast requires the participation of multiple proteins designated factors a, b, d, e and g, corresponding to the mammalian transcription factors TFIIE, TFIIH, TFIID, TFIIB and TFIIF respectively (Conaway & Conaway 1993; Feaver *et al.* 1994). All of these proteins combine with RNA polymerase II to form a large transcription complex at the promoter prior to the initiation of transcription. The fate of this complex during transcript elongation is unclear, aside from evidence that factor d (TFIID) remains at the promoter and factor g (TFIIF) plays a role in elongation.

Factor b (TFIIH) holoenzyme is comprised of multiple subunits, some of which are required for a protein kinase that phosphorylates the C-terminal domain of the β subunit of RNA polymerase II (Svejstrup *et al.* 1994*a*). Four polypeptides of 95, 89, 70, and 50 kDa are encoded by genes designated *SSL2*(*RAD25*) *RAD3*, *TFB1* and *SSL1* respectively. All four of these genes have been shown to be essential for viability in haploid yeast cells (Friedberg *et al.* 1991; Gileadi *et al.* 1992; Gulyas & Donahue 1992; Yoon *et al.* 1992; Prakash *et al.* 1993), and conditional–lethal *rad3* and *ssl2* mutants have been shown to be defective in RNA polymerase II-dependent transcription under restrictive conditions (Qiu *et al.* 1993; Guzder *et al.* 1994). Two other genes designated *TFB2* and *TFB3* encode polypeptides of ~ 55 and ~ 38 kDa that are tightly associated with the Rad3, Tfb1 and Ssl1 polypeptides, resulting in a stable core factor b complex of five polypeptides *in vitro* (Feaver *et al.* 1993). Studies on the interactions between these five polypeptides have demonstrated that Rad3 protein specifically interacts with Ssl1 (L. Bardwell *et al.* 1994) and Ssl1 protein also interacts with Tfb1 protein (Feaver *et al.* 1993; L. Bardwell *et al.* 1994). Rad3 protein has also been shown to interact with Ssl2 protein (L. Bardwell *et al.* 1994).

In addition to their requirement for RNA polymerase II basal transcription, the Ssl2, Rad3, Tfb1 and Ssl1 polypeptides are required for NER. This conclusion stems from the demonstration that purified core factor b complex corrects defective NER in cell-free extracts of *rad3*, *tfb1* and *ssl1* mutants (Wang *et al.* 1994; Z. Wang *et al.*, unpublished observations). Similarly, core factor b with bound Ssl2 protein corrects defective NER in extracts of an *ssl2* mutant (Wang *et al.* 1994). It has been independently demonstrated that an *ssl2* mutant is defective in the removal of pyrimidine dimers from DNA *in vivo* (Sweder & Hanawalt 1994). Purified Rad3 protein alone does not complement defective NER in extracts of *rad3* mutants, and purified Ssl2 protein only partially corrects the defect in *ssl2* extracts (Wang *et al.* 1994). Hence, Rad3 and Ssl2 proteins (and presumably Ssl1, Tfb1 and the 55 and 38 kDa polypeptides) participate in NER as components of a multiprotein complex.

(*a*) *The role of factor b polypeptides in* NER

The specific biochemical role(s) of the factor b subunits in NER is unknown. A biochemical function has been identified for Rad3 protein, which is a DNA–DNA and DNA–RNA helicase with strict 5′→3′ polarity with respect to the strand to which it is bound (Friedberg *et al.* 1991; Prakash *et al.* 1993). The Rad3 DNA–DNA helicase activity is retained in factor b (Z. Wang *et al.* unpublished observations). A mutant *rad3* allele that encodes a helicase-defective form of Rad3 protein supports the viability of cells, but confers a NER-defective phenotype (Friedberg *et al.* 1991; Prakash *et al.* 1993). Similarly, factor b purified from a different strain carrying a similar mutation (which also renders defective NER) supports normal transcription *in vitro* (Feaver *et al.* 1993). Hence, it appears that the helicase function of Rad3 protein is required for NER but not for its role in transcription.

The translated sequence of the yeast *SSL2* gene suggests that it also encodes a DNA helicase (Gulyas & Donahue 1992). The highly conserved human homologue of *SSL2* designated *XPB* (*ERCC3*) has in fact been shown to encode a protein with 3′→5′ DNA helicase activity (Schaeffer *et al.* 1994). While it remains to be directly demonstrated that purified yeast Ssl2 protein is also a 3′→5′ DNA helicase, such is likely to be the case. A mutation in a conserved helicase motif of *SSL2* is lethal (Prakash *et al.* 1993), suggesting that the (presumed) Ssl2 yeast helicase is required for transcription. At present the possibility that this catalytic activity is additionally required for NER cannot be excluded.

It is not obvious precisely what role(s) the helicase activity of the Rad3 (and possibly Ssl2) protein plays in NER. Earlier we discussed a hypothetical substrate generated during NER with 3′ and 5′ duplex-single strand junctions, as an appropriate substrate for endonucleolytic cleavage by the Rad1/Rad10 (and Rad2) endonuclease. Conceivably such a NER 'bubble' is produced through the action of one or other (or both) of these helicases.

4. A MULTIPROTEIN NER COMPLEX (REPAIROSOME) IN YEAST

Core factor b interacts with Rad2 and Rad4 proteins (A.J. Bardwell *et al.* 1994*a*). Furthermore, *in vitro*-translated Rad2 protein co-immunoprecipitates with *in vitro*-translated Tfb1 and Ssl2 proteins (A.J. Bardwell *et al.* 1994*a*). Neither Rad1, Rad10 or Rad14 proteins have been shown to interact with factor b or its individual subunits. Nonetheless, extensive purification of yeast extracts for RNA polymerase II-dependent transcription activity *in vitro* has yielded a fraction that corrects defective NER in *rad1*, *rad10*, *rad2*, *rad3*, *rad14*, *ssl2* and to a lesser extent *rad4* mutants (Svejstrup *et al.*, 1994*b*). These results provide evidence for a multiprotein complex in yeast cells comprising at least the five core subunits of factor b plus Ssl2, Rad2, Rad14, Rad1/Rad10 complex and Rad4 proteins.

The structural and functional relationships between this complex, which we designate the *NER repairosome*, and the factor b holoenzyme required for transcription initiation remain to be elucidated. Conceivably core factor b is incorporated into two multiprotein complexes, one of which participates in transcription initiation and the other in NER. It also remains to be determined whether the repairosome alluded to here participates exclusively in NER that is coupled to transcription, repair in transcriptionally silent regions of DNA, or both. Recent studies suggest that TFIIH (yeast factor b) and TFIIE (yeast factor a) are required for the initiation of transcription but not for transcript elongation in a mammalian cell-free system (Goodrich & Tjian 1994). Hence, the transcription apparatus may be devoid of NER proteins during the latter process. Following arrest at sites of base damage the stalled transcription complex may require the presence of factor b for transcription to 'reinitiate' after repair of base damage is completed. Under these conditions factor b may be provided in a preassembled repairosome that facilitates both the repair of the template strand and continued transcription. The human equivalent of the *E. coli* transcription repair coupling factor(s) (TRCF) (Hanawalt 1992) may play a crucial role in this recoupling of transcription and repair proteins. A similar model has been suggested for human cells (Drabkin *et al.* 1994). As an alternative to its dissociation and reassociation with the transcription machinery, factor b(TFIIH) (though not specifically required for transcript elongation), may remain physically associated with the elongation complex and repairosome assembly at sites of arrested transcription may be completed by exchanging CTD kinase subunits for those of the repairosome and TRCF.

A preassembled repairosome may also participate in NER that is not coupled to transcription. If so, among the many interesting questions that remain to be answered are how such a complex loads onto DNA and searches and finds base damage.

5. WHY IS NER COUPLED TO TRANSCRIPTION?

The finding that components of the RNA polymerase II basal transcription machinery also participate in a highly specialized and occasional (following DNA damage) metabolic transaction of DNA provides a rational biochemical basis for the long-standing observation that NER occurs more rapidly in transcriptionally active regions of the genome than in transcriptionally silent regions (Hanawalt 1993; Bohr 1993). Such coupling might have provided several selective advantages during eukaryotic evolution. The direct coupling of NER to transcription possibly provides a mechanism for solving the problem of the accessibility of a large repairosome to sites of base damage in chromatin in extensive regions of the genome. Additionally, if the specific recognition of base damage in the template strand during transcription is indeed effected by arrested transcription as suggested (Hanawalt 1993), the rapid positioning of NER proteins at such sites provides for efficient repair of the informationally relevant strand of transcriptionally active genes, which by dint of their expression are presumably important for cellular metabolism. The observation that the template strand is indeed typically repaired more rapidly than the coding strand during transcription (Hanawalt 1993; Bohr 1993) is consistent with this prediction. Finally, the participation of core factor b complex in different multiprotein complexes required for the initiation of basal transcription and for NER provides a potential mechanism for limiting the rate of basal transcription in the presence of NER; a useful response of cells that have sustained DNA damage.

6. IMPLICATIONS OF NER IN YEAST FOR HUMAN HEREDITARY DISEASE

The *RAD1*, *RAD10*, *RAD2*, *RAD4*, *RAD14*, *RAD3*, *SSL2*, *SSL1* and *TFB1* genes are conserved in the human genome (Hoeijmakers & Bootsma 1990; Hoeijmakers 1993; Prakash *et al.* 1993; Weeda 1993; Humbert *et al.* 1994). With the current exception of the human *RAD10* homologue, mutational inactivation of any of these human genes is associated with the cancerprone hereditary disease xeroderma pigmentosum (XP). Additionally, mutations in the human *XPD*, *XPB* and *XPG* genes (the homologues of the yeast *RAD3*, *SSL2* and *RAD2* genes) can confer the phenotype of XP together with features of a second hereditary disease called Cockayne syndrome, and some mutations in the *XPD* gene can confer a disease called trichothiodystrophy (TTD) (Hoeijmakers & Bootsma 1990; Hoeijmakers 1993; Weeda 1993). The molecular basis of the relationship between these multiple diverse syndromes is a challenging conundrum. The genetic and molecular versatility of *S. cerevisiae* offers the promise of important insights into these and other complexities of NER in eukaryotes.

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