

# Nucleotide Excision Repair in Yeast: Recent Progress and Implications

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Nucleotide excision repair (NER) is a ubiquitous process by which damaged bases are excised from the genome of living cells as oligonucleotide fragments (reviewed in Friedberg et al. 1995). Recent years have witnessed significant progress in our understanding of the biochemistry of NER in the yeast *Saccharomyces cerevisiae* (reviewed in Prakash et al. 1993; Friedberg et al. 1995; Friedberg 1996a). The establishment of a cell-free system that monitors NER of damaged plasmid DNA in vitro (Wang et al. 1995a,1996) has facilitated the systematic screening of yeast strains carrying mutations in multiple genes known to be required for or involved in NER. Additionally, the polypeptides encoded by many of these genes have been purified and the early steps in the NER process have been reconstituted in vitro (Guzder et al. 1995a). At least 26 gene products are believed to be required for or involved in NER (Table 1). The Rad1/Rad10 heterodimeric complex and the Rad2 protein are now known to function as junction-specific endonucleases which specifically cleave DNA at single-strand/duplex junctions with opposite single-strand polarity (Habraken et al. 1993; Bardwell et al. 1994; Fig. 1). Incisions (nicks) generated by these two endonucleases result in the formation of oligonucleotide fragments ~24–27 nucleotides in size, which include damaged bases (Guzder et al. 1995a). Displacement of the oligonucleotides generates single-strand gaps that are filled in by repair synthesis and sealed by ligation. The fundamental paradigm of bimodal incision flanking sites of base damage by junction-specific endonucleases has been demonstrated in both yeast and human (Sancar 1996; Wood 1996) cells and is likely conserved in all eukaryotes.

This impressive progress notwithstanding, a detailed description of the biochemistry and molecular biology of NER in yeast is far from complete. In this chapter we will attempt to address several unresolved issues, including the following:

1. How are single-strand/duplex junctions separated by ~24–27 nucleotides generated during NER?

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**Table 1.** Proteins known or believed to be required for NER in *S. cerevisiae*

Protein	Activity	Function in NER
Rad14	DNA-binding protein	? Damage recognition
Rpa1	DNA-binding protein	? Damage recognition
Rpa2	DNA-binding protein	? Damage recognition
Rpa3	DNA-binding protein	? Damage recognition
Rad3	Subunit of core TFIIH; DNA helicase	DNA unwinding for DNA incision
Ss12 (Rad25)	Subunit of core TFIIH; DNA helicase	DNA unwinding for DNA incision
Ss11	Subunit of core TFIIH	DNA unwinding for DNA incision
Tfb1	Subunit of core TFIIH	DNA unwinding for DNA incision
Tfb2	Subunit of core TFIIH	DNA unwinding for DNA incision
Tfb3	Subunit of core TFIIH	DNA unwinding for DNA incision
Tfb4	Subunit of core TFIIH	DNA unwinding for DNA incision
Rad1	Junction-specific endonuclease with Rad10	DNA incision 5' to sites of base damage
Rad10	Junction-specific endonuclease with Rad1	DNA incision 5' to sites of base damage
Rad2	Junction-specific endonuclease	DNA incision 3' to sites of base damage
Rad4	Binds to Rad23 protein	?
Rad23	Binds to Rad4 protein	?
Rad7	Binds to Rad16 protein	Required for NER of transcriptionally inactive DNA and coding strands of transcriptionally active genes
Rad16	Binds to Rad7 protein	Required for NER of transcriptionally inactive DNA and coding strands of transcriptionally active genes
Rad26	Unknown	Required for strand-specific NER
DNA Pol $\delta$ or $\epsilon$	DNA polymerase	Repair synthesis of DNA
Rfc1	DNA replication accessory factor	Repair synthesis of DNA
Rfc2	DNA replication accessory factor	Repair synthesis of DNA
Rfc3	DNA replication accessory factor	Repair synthesis of DNA
Rfc4	DNA replication accessory factor	Repair synthesis of DNA
PCNA	DNA replication accessory factor	Repair synthesis of DNA
Cdc9	DNA ligase	DNA ligation

Pol, polymerase; PCNA, proliferating cell nuclear antigen.

2. Since the RNA polymerase (RNAP) II transcription factor TFIIH is required for both transcription and NER, does active NER inhibit RNAP II transcription and vice versa by competing for TFIIH?