The Yeast RAD7 and RAD16 Genes Are Required for Postincision Events during Nucleotide Excision Repair

IN VITRO AND IN VIVO STUDIES WITH rad7 AND rad16 MUTANTS AND PURIFICATION OF A Rad7/Rad16-CONTAINING PROTEIN COMPLEX

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In eukaryotes, nucleotide excision repair (NER) is a complex reaction requiring multiple proteins. In the yeast Saccharomyces cerevisiae, two of these proteins, Rad7 and Rad16, are specifically involved in the repair of lesions from transcriptionally silent regions of the genome in vivo. Extracts prepared from rad7 or rad16 mutant cells are deficient, but not totally defective, in both oligonucleotide excision and repair synthesis of damaged plasmid DNA. We show that these extracts are, however, fully proficient in the incision step of the NER reaction in vitro. Furthermore, using a cdc9 mutant to trap incision intermediates, we demonstrate that rad7 and rad16 mutants are proficient in NER-dependent DNA incision in vivo. A purified protein complex containing both Rad7 and Rad16 proteins complements the oligonucleotide excision and repair synthesis defects in rad7 and rad16 mutant extracts. We conclude that the products of the RAD7 and RAD16 genes are involved in a postincision event(s) during NER in yeast.

The yeast RAD7 and RAD16 genes belong to the RAD3 epistasis group of DNA damage-responsive genes (1, 2). This epistasis group includes genes required for nucleotide excision repair (NER)1 of DNA, a process by which multiple types of base damage are excised from the genome as oligonucleotide fragments, and the resulting single strand gaps are repaired by DNA synthesis (repair synthesis) and ligation (3). In contrast to the extreme sensitivity to UV light by other DNA-damaging agents conferred by complete inactivation of NER genes, such as RAD1, RAD2, RAD3, RAD4, RAD10, and RAD14, deletion of the RAD7 or RAD16 gene confers partial UV radiation sensitivity (1, 2).

The precise role(s) of the RAD7 and RAD16 gene products in NER is not clear. The proteins are required for NER of transcriptionally repressed loci such as HMLa and HMRa in vivo (4), and for the nontranscribed (coding) strand of transcriptionally active genes. However, unlike the other RAD genes mentioned above, the RAD7 and RAD16 genes are not required for NER of the transcribed (template) strand of such genes (5).

Rad7 and Rad16 proteins have been shown to stably interact both in vivo and in vitro (6, 9). Rad16 is a member of the SWI2/SNF2 superfamily of proteins, several of which have been shown to be ATPases involved in chromatin remodeling (7). This observation, coupled with the requirement for the RAD7 and RAD16 genes for NER of transcriptionally repressed regions of the genome, has led to the notion that the Rad7 and Rad16 proteins may be subunits of a complex dedicated to the perturbation of chromatin structure in order to facilitate NER of transcriptionally silent regions of the genome and the nontranscribed strand of transcriptionally active genes (5).

It has been reported that a reconstituted in vitro system that supports damage-specific incision and oligonucleotide excision from purified plasmid DNA does not require the Rad7 and Rad16 proteins (8). Subsequent studies (9) reported that these proteins actually increase the efficiency of NER in vitro and offered the suggestion that the specific role of these proteins is to facilitate the recognition of damaged bases in nontranscribed regions of the genome in vivo (10). Contrary to the observation that the Rad7 and Rad16 proteins are not required for NER of plasmid DNA in a reconstituted in vitro system, we previously reported that they are indispensable for NER of plasmid DNA in yeast whole cell extracts (WCEs), using an assay that measures repair synthesis of DNA (6).

In view of the multiple and in some instances discordant roles proposed for the RAD7 and RAD16 products during NER of DNA, we have studied their requirement for specific events in this process in greater detail. We have investigated the possibility that when purified plasmid DNA is incubated with yeast WCEs, the DNA is assembled into nucleosomes. Such a result could explain the apparent contradiction between the requirement for Rad7 and Rad16 proteins for NER in WCEs and their dispensability in a reconstituted system using purified proteins, and it would also be consistent with the suggestion that the Rad7 and Rad16 proteins are required for the processing of nucleosome structures during NER. Additionally, we have utilized both in vitro and in vivo assays that allowed us to independently monitor the steps of damage-specific incision, oligonucleotide excision, and repair synthesis in wild-type and rad7 and rad16 mutants.

We have found no evidence for nucleosome formation in yeast WCEs that support robust NER of plasmid DNA. We show here that rad7 and rad16 mutants are fully proficient in damage-specific incision of DNA. In contrast, the postincision events of oligonucleotide excision and, as previously reported, repair synthesis (6) are severely deficient in rad7 and rad16 mutant extracts. Finally, we have purified a protein complex that includes Rad7 and Rad16 proteins, without overexpression of these proteins in yeast cells. This complex corrects both defective oligonucleotide excision and repair synthesis in vitro.

† The abbreviations used are: NER, nucleotide excision repair; WCE, whole cell extract; AAF, N-acetyl-2-aminofluorene; kb, kilobase(s); PCR, polymerase chain reaction.

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We conclude that the RAD7 and RAD16 gene products participate in postincision events during NER of transcriptionally silent DNA. The utility of such participation may be obviated in transcriptionally active DNA, perhaps as a direct consequence of transcription itself.

MATERIALS AND METHODS

Strains and Plasmids—The RAD7 strain W303-B (mata, ade2-1, trp1-1, leu2-3, 112, his3-11, 15, ural-3-1) and the isogenic strains MGC3104 (rad7::LEU2), MGC397 (rad7::URA3), and W303256 (rad7::LEU2) were generously supplied by Dr. Jaap Bronkhorst, University of Nijmegen, The Netherlands (5). Other strains used were SX46a (mata, ade2-3, his3-11, 15, ural-3-1) and its isogenic partners MGS139 (rad14::LEU2) and SX46a (rad7::URA3), rad4-10 (mata, rad4-10, ade2, his3-3, 32, trp1-298) and KG119 (saf2::TRP1, mata his7-2, leu2-3, 112, ura3-52, trp1-298). Strain LP2915-5D (cdc2-9, mata, his3-200, (s2-1, met15-3, trp1-298, ura3-52) was used to examine DNA incision in vivo. Strain LP2915-5D rad7::TRP1 was generated by transforming LP2915-5D with Sall-digested pWS21. This plasmid was derived from pWS19 containing the RAD7 gene and flanking sequences. Plasmid pWS19 was cut with HpaI-NdeI, thereby removing the entire RAD2 coding sequence. A BglII-EcoRI TRPI fragment was exchanged to generate pWS520. Strain LP2915-5D rad7::HIS3 was constructed by transformation of the parent strain with pWS520. Strain LP2915-5D prad7::HIS3, and strain LP2915-5D rad6::HIS3 was constructed by transformation of the parent strain with EcoRI-BamHI-digested prad6::HIS.

In Vitro NER—Yeast WCEs capable of supporting NER were prepared as described previously (11). NER was monitored by measuring DNA repair synthesis in pUC18 containing N-acetyl-2-aminofluorene (AAF) adducts. AAF-modified DNA was prepared by treating pUC18 with AAF and purifying the DNA on a 5–20% sucrose gradient as described (12). Plasmid DNA was recovered from extracts by restricting agarose without prior denaturing loading buffer (12.5% Ficoll, 5 mM EDTA, 0.125% bromophenol blue), and DNA was quantitated by phosphorimaging as described (6).

Excision Repair Assay—An oligonucleotide of known sequence (5'-TATGATTGGGGATAAGAT*TG-3') containing a single cyclobutane pyrimidine dimer near the 3'-end was kindly provided by Dr. John Stephen Taylor (Walter and Eliza Hall Institute, Australia). Primers of this sequence were used to generate a 0.6-kb internal fragment of RAD7 that encodes 192 amino acids (from amino acids 475–667) was generated by PCR using the following primers: upstream primer, 5'ATATGATCATTTAGTGATGTTGATGATACTGACTCGTCTCCCTAT3'; downstream primer, 5'ATATGATTCATTTAGTGATGTTGATGATACTGACTCGTCTCCCTAT3'. The 2.2-kb PCR product was cut with BamHI and cloned into the BamHI site of pRS314-TRP1. NER was initiated by irradiation of the recombinant yeast with a dose rate of 1 J/m²/s, using a germicidal lamp. Following irradiation, cell suspensions were harvested and treated with thrombin (24) to isolate each Rad protein. Purified full-length regions between the Rad and the glutathione agarose as described (24). After immobilizing fusion proteins from inclusion bodies by affinity chromatography on glutathione agarose as described (24). After immobilizing fusion proteins from inclusion bodies by affinity chromatography on glutathione agarose as described (24). After immobilizing fusion proteins from inclusion bodies by affinity chromatography on glutathione agarose as described (24). After immobilizing fusion proteins from inclusion bodies by affinity chromatography on glutathione agarose as described (24). After immobilizing fusion proteins from inclusion bodies by affinity chromatography on glutathione agarose as described (24). After immobilizing fusion proteins from inclusion bodies by affinity chromatography on glutathione agarose as described (24). After immobilizing fusion proteins from inclusion bodies by affinity chromatography on glutathione agarose as described (24). After immobilizing fusion proteins from inclusion bodies by affinity chromatography on glutathione agarose as described (24). After immobilizing fusion proteins from inclusion bodies by affinity chromatography on glutathione agarose as described (24). After immobilizing fusion proteins from inclusion bodies by affinity chromatography on glutathione agarose as described (24). After immobilizing fusion proteins from inclusion bodies by affinity chromatography on glutathione agarose as described (24). After immobilizing fusion proteins from inclusion bodies by affinity chromatography on glutathione agarose as described (24). After immobilizing fusion proteins from inclusion bodies by affinity chromatography on glutathione agarose as described (24). After immobilizing fusion proteins from inclusion bodies by affinity chromatography on glutathione agarose as described (24).
Nucleotide Excision Repair in rad7 and rad16 Mutants

RESULTS

Analysis of Nucleosome Formation during Incubation of Plasmid DNA in NER-proficient Extracts—In view of the notion that the RAD7 and RAD16 gene products may be required for the processing of chromatin during NER (4), we considered the possibility that chromatin or some other higher ordered DNA structure is generated during incubation of WCEs with purified plasmid DNA. We harvested plasmid DNA following incubation with yeast WCEs known to support robust NER and demonstrated efficient nucleosome formation following mild micrococcal nuclease digestion conditions (data not shown). Hence, there is no evidence of nucleosome formation on the input plasmid DNA in these yeast WCEs.

DNA Incision in RAD+ and rad Mutant Extracts—In an effort to identify a specific molecular defect in rad7 and rad16 mutants, we next examined individual steps in the NER reaction in yeast WCEs. We analyzed damage-specific incision of plasmid DNA in RAD+ and in rad7, rad16, rad14, rad4, and ss12 mutant extracts by monitoring the conversion of AAF-treated circular DNA in vitro (8). When covalently closed circular (form I) AAF-treated plasmid DNA (Fig. 3A, lane 1) was incubated with 250 μg of WCEs prepared from RAD+ strains, the DNA was converted to the relaxed state (form II) within 2 min of incubation (Fig. 3A, lanes 2–5), indicating incision of the DNA. When these experiments were repeated using a 10-fold reduced concentration of extract (25 μg of protein), subsaturating conditions were achieved, and only partial conversion of form I to form II DNA was observed at 2 min (Fig. 3B). As anticipated, when a damaged plasmid DNA was incubated with extracts from the incision-defective rad14, rad4 or ss12 mutants, the DNA remained in the form I state (Fig. 4A, lanes 4–6). In contrast, incubation with 250 μg of protein (Fig. 5A) or subsaturating amounts of protein (25 μg) (Fig. 5B) from a rad7 mutant resulted in identical kinetics of conversion of form I to form II DNA as observed with the RAD+ strain (Fig. 6).
This result was confirmed in a direct comparison of wild-type, rad7, rad16, and various NER-defective mutants (Fig. 4A). The same results were obtained with rad16 mutant extracts (data not shown). Thus, rad7 and rad16 mutant extracts are as active as wild-type extracts with respect to damage-specific incision of DNA.

Analysis of radiolabel incorporated by repair synthesis during incubation with wild-type (RAD+SED) extracts revealed increasing incorporation over a 2-h period in both form II DNA and in various topoisomers (Fig. 3C). The difference in the kinetics of repair synthesis and DNA incision reflects the fact that just a single nick in the form I substrate DNA converts it to the form II state, whereas the detection of radioactivity incorporated during repair synthesis is significantly less sensitive. Only background levels of repair synthesis were observed with extracts of rad4, rad14, and ssl2 mutants (Fig. 4B, lanes 4–6). Consistent with the results of previous studies (6), extracts of both rad7 and rad16 mutants supported markedly reduced (but clearly detectable) levels of repair synthesis in form II DNA (Figs. 4B and 5C). We have previously shown that the level of repair synthesis does not increase as a function of increasing the amount of WCEs (10). Hence, this observation is not the result of suboptimal incubation conditions. Furthermore, defective repair synthesis can be fully corrected by mixing rad7 or rad16 mutant extracts with extracts from other NER-defective mutants, such as rad14 or rad2 (10). Thus, the extracts are not nonspecifically inactivated for repair synthesis.

Conversion of form I to form II DNA was also observed when purified form I plasmid DNA not treated with AAF was incubated with extracts of RAD+SED strains (data not shown). Our conclusion that the conversion of AAF-treated DNA to form II reflects damage-specific incision derives from the observation that such conversion was not observed in extracts of rad4, rad14, or ssl2 NER-defective mutants (Fig. 4A). It is well documented that “untreated” plasmid DNA contains background levels of base damage, typically oxidative damage, generated during purification of the DNA (26, 27). Because “native” DNA prepared under the most careful conditions is subject to such
perturbations (26, 27), investigators wishing to avoid subtle topological changes in DNA and/or chromatin substrates during incubations in vitro should consider using extracts derived from cells that are defective in NER.

In summary, the results of the experiments described above indicate that rad7 and rad16 mutants are indeed defective in NER in yeast WCEs. However, this defect is not at the level of DNA incision, but in some postincision event(s).

rad7 and rad16 Mutants Are Proficient in Incision In Vivo—A number of in vivo studies have demonstrated that NER measured by the specific loss of pyrimidine dimers from the heterochromatic HMLα locus is defective in rad7 and rad16 mutant strains (4). The results of the experiments with WCEs described above invited the possibility that the failure to observe a loss of UV-induced lesions from the HMLα locus is similarly not the result of defective DNA incision. In wild-type living cells, once NER is initiated at any single lesion, the entire process is completed very rapidly. This precludes the facile detection of DNA incisions in the overall population of substrate DNA molecules. Hence, following UV irradiation of RAD+ cells, no significant change in the average molecular weight of genomic DNA is detected as a function of the time of incubation (28). Similar results were obtained when NER was examined at the level of individual genes by Southern hybridization with gene-specific probes (20). In such experiments, the presence of pyrimidine dimers in a defined DNA sequence can be detected by extracting the DNA and digesting it with dimer-specific enzymes, such as T4 endonuclease V (20). The persistence of dimers due to defective NER results in degradation of the DNA by the endonuclease and loss of a hybridization signal during subsequent Southern analysis (20). As NER approaches completion, substrate sites for the endonuclease probe (dimers) are lost, and the hybridization signal returns (20). However, in repair-proficient cells in which the DNA is not treated with a dimer-specific endonuclease, a hybridization signal of constant intensity is observed during the repair process (20). DNA incisions do not accumulate because the repair process at any given site is rapidly completed.

In order to facilitate the persistence of nicks associated with defective NER at a step following DNA incision, we utilized strains conditionally defective in the CDC9 gene, which encodes DNA ligase I (29). Fig. 6 shows the effect of the cdc9 ts mutation on NER at the MATα and HMLα loci in a RAD+ strain and in various rad mutants. When a UV-irradiated RAD+, cdc9 strain was incubated at the nonpermissive temperature (Fig. 6A), we observed a loss of both MATα and HMLα signals following Southern hybridization, as expected if incisions persisted at these loci due to a failure of DNA ligation. No loss of signal was detected in unirradiated cells (Fig. 6A). Similarly, no loss of signal was observed at either locus in an irradiated or unirradiated rad2, cdc9 strain, consistent with defective incision in such a mutant. In contrast, proficient incision was detected at both the MATα and HMLα loci in rad7, cdc9 and rad16, cdc9 strains. To demonstrate that DNA ligation is indeed defective at the restrictive temperature, experiments were repeated at the permissive temperature for the cdc9 mutation. No incisions were detected in the MATα and HMLα loci in any of the strains examined (Fig. 6B). These observations indicate that incisions are generated at UV radiation-induced photoproducts at the MATα and HMLα loci both in RAD+ and rad7/cdc9/rad16 mutant strains. These incisions are apparently rapidly religated under conditions permissive for DNA ligase I activity.

rad7 and rad16 Mutant Strains Are Defective in Oligonucleotide Excision in Vitro—Because rad7 and rad16 mutant extracts are proficient in DNA incision but defective in repair synthesis (6), we examined their ability to support the excision of oligonucleotide fragments generated by the bimodal incision reaction during NER. For these studies, we utilized a plasmid carrying a single pyrimidine dimer at a defined site on one of the DNA strands. The plasmid was radiolabeled close to the site of the dimer (see under “Materials and Methods”). Incubation of the plasmid with extracts of RAD+ cells resulted in the release of radiolabeled oligonucleotide fragments of the expected size (~30 nucleotides) (Fig. 7, lane 5). No excision was detected in extracts of rad1 and rad14 mutant strains (Fig. 7, lanes 1 and 2), consistent with the fact that these extracts do not support any damage-specific incision of DNA. Significantly reduced but clearly detectable oligonucleotide excision was observed in rad7 and rad16 mutant extracts (Fig. 7, lanes 3 and 4). Because these extracts are proficient for incision of damaged DNA, we conclude that as is the case in vivo (see above), incisions generated in vitro in rad7 and rad16 extracts are rapidly religated.

Complementation of Defective Repair Synthesis and Oligonucleotide Excision in rad7 and rad16 Mutant Extracts—Previous studies have demonstrated that the Rad7 and Rad16 proteins are stably associated in vivo and in vitro (6, 9). In order to avoid possible artifacts in the subunit composition of a complex that includes Rad7 and Rad16 proteins following overexpression of these proteins in yeast, we purified such a complex from a strain expressing endogenous Rad16 protein and His11-tagged Rad7 protein expressed from its normal promoter on a single copy plasmid. Rad7 and Rad16 proteins co-eluted through multiple distinct chromatographic steps (Fig. 2). We obtained ~30,000-fold enrichment of a Rad7/Rad16-containing complex with ~20% yield. Peak fractions from the final purification step (glycerol gradient sedimentation) (Fig. 2) coincided with a peak of activity that complemented defective repair synthesis in extracts of a rad7, rad16 double mutant (Figs. 2E and 8A). Addition of increasing amounts of this fraction resulted in increasing complementation of defective repair synthesis in rad7 and rad16 extracts (Fig. 8B, lanes 6–8 and 9–11, respec-
and markedly reduced levels of excision were observed in extracts of rad16 and rad7 mutants (lanes 3 and 4).

The observation that rad7 and rad16 mutants are defective in NER of the transcriptionally repressed HMLa locus but not the homologous transcriptionally active MATa locus led to the suggestion that the Rad7 and Rad16 proteins participate in global genome repair by contributing to disruption of heterochromatic regions in order to allow access of the repair machinery to sites of base damage (4). This model has been indirectly reinforced by the observation that Rad16 protein is a member of the SWI2/SNF2 superfamily of DNA-dependent ATPases (7), a number of which are known subunits of chromatin remodeling complexes (31).

We previously reported a requirement for the Rad7 and Rad16 proteins for normal levels of repair synthesis of naked plasmid DNA in WCEs under conditions that are independent of RNA polymerase II transcription (6). In contrast, it has been reported that in a reconstituted system using purified NER proteins (also independent of transcription), both DNA incision and oligonucleotide excision transpire in the absence of Rad7 and Rad16 proteins for normal levels of repair synthesis of naked plasmid DNA to yeast WCEs, the DNA is assembled into chromatin in vivo, whereas transcription-coupled repair operates normally in such strains (5). Similarly, the RAD26 gene product is implicated in transcription-coupled NER, because rad26 mutants are defective in this process but not in global genome repair (30).

The observation that rad7 and rad16 mutants are defective in NER of the transcriptionally repressed HMLa locus but not the homologous transcriptionally active MATa locus led to the suggestion that the Rad7 and Rad16 proteins participate in global genome repair by contributing to disruption of heterochromatic regions in order to allow access of the repair machinery to sites of base damage (4). This model has been indirectly reinforced by the observation that Rad16 protein is a member of the SWI2/SNF2 superfamily of DNA-dependent ATPases (7), a number of which are known subunits of chromatin remodeling complexes (31).

We previously reported a requirement for the Rad7 and Rad16 proteins for normal levels of repair synthesis of naked plasmid DNA in WCEs under conditions that are independent of RNA polymerase II transcription (6). In contrast, it has been reported that in a reconstituted system using purified NER proteins (also independent of transcription), both DNA incision and oligonucleotide excision transpire in the absence of Rad7 and Rad16 proteins (8), and that these proteins actually increase the efficiency of the recognition of base damage during NER (9). We were therefore led to examine NER in rad7 and rad16 mutant strains more closely. We investigated two possible explanations for defective repair synthesis in WCEs. First, we considered the possibility that following the addition of naked plasmid DNA to yeast WCEs, the DNA is assembled into chromatin, the processing of which specifically requires the Rad7 and Rad16 proteins during NER. Hence, defective repair synthesis in WCEs would reflect a defect in NER of chromatin that is obviated in a system reconstituted with purified proteins and naked DNA. However, the present studies provide no evidence for nucleosome assembly on plasmid DNA during incubation with yeast WCEs. This is not surprising, because it is well established that efficient nucleosome assembly is achieved with extracts from Xenopus oocytes or Drosophila embryos, which are highly enriched for histones (32), but not in yeast or human extracts. Indeed, this property of human extracts has been exploited to demonstrate a requirement for a distinct protein complex termed chromatin assembly factor 1 for efficient nucleosome assembly following repair synthesis in vitro (33). A similar protein complex encoded by the chromatin...
assembly complex genes has been identified in yeast (34).

A second possibility was that the Rad7 and Rad16 proteins may be involved in a specific stage of NER that precedes repair synthesis of DNA. We dissected the NER reaction using independent assays to examine individual events in this process. Extracts from rad7 or rad16 mutants are fully proficient in the incision of damaged DNA compared with wild-type strains. As already indicated, previous studies have shown that DNA incision (and oligonucleotide excision) can take place in a reconstituted in vitro system without added Rad7 and Rad16 protein (8). However, these studies did not exclude the possibility that such proteins may have been present as contaminants. Hence, the present studies provide a direct demonstration that rad7 and rad16 mutant extracts are proficient for DNA incision in vitro. This result was corroborated in vivo using a ts cdc9 mutant strain to trap incision intermediates during NER. Our studies additionally showed that rad7 and rad16 mutants are proficient for NER-dependent incision in regions such as HMLα, which were previously thought to be refractory to the NER machinery.

We confirmed an earlier observation (6) that rad7 and rad16 WCEs are defective in the repair synthesis step of the NER reaction. Additionally, we have provided a direct demonstration of a defect in oligonucleotide excision of base damage in these extracts. We have consistently observed that the defects in both oligonucleotide excision and repair synthesis in rad7 and rad16 mutants are not as complete as those observed in incision-defective mutants, such as rad1 or rad14. This is not the result of subsaturating reaction conditions, because previous studies have shown that deficient repair synthesis is independent of the protein concentration of WCEs (6). We estimate that the level of residual excision and resynthesis in rad7 and rad16 mutants to be ~10–20% of that of wild-type levels. This may explain the oligonucleotide excision observed in the reconstituted NER assay in the absence of added Rad7 and Rad16 proteins (8). Both defective repair synthesis and oligonucleotide excision can be complemented in vitro by a purified Rad7/Rad16-containing protein complex.

A Rad7/Rad16 complex was previously isolated from cells in which both proteins were simultaneously overexpressed from strong promoters on multicopy plasmids (9). In order to avoid stoichiometric artifacts that might arise from such overexpression, including the possible exclusion of other relevant subunits, we purified such a complex without overexpression of Rad7 or Rad16. Preliminary analysis shows that the complex has a molecular mass of ~250 kDa (considerably larger than that expected for a complex with a 1:1 stoichiometry of Rad7 and Rad16 protein) and that it includes at least one polypeptide other than Rad7 or Rad16.2 A more detailed characterization of this complex will be presented elsewhere.

In contrast to a previous suggestion (10) that the yeast Rad7 and Rad16 proteins participate in the recognition of base damage in transcriptionally silent DNA and stimulate DNA incision at such sites, we conclude that rad7 and rad16 mutants are proficient in the recognition and incision of sites of base damage in nontranscribed regions of DNA. However, such mutants cannot efficiently execute the subsequent steps of oligonucleotide excision and repair synthesis. Our results suggest that although the Rad7 and Rad16 proteins participate in postincision events during NER, they are not indispensable for this process. This may explain the fact that rad7 and rad16 mutants are only partially sensitive to DNA-damaging agents, such as UV radiation.

The specific role(s) of Rad7 and Rad16 in oligonucleotide excision (and possibly coupled repair synthesis) during NER of transcriptionally repressed regions of the genome and the nontranscribed strand of transcriptionally active genes, as well as the observation that such a role(s) is obviated during NER of the template strand of transcriptionally active genes, remains to be explained. All known members of the SWI/SNF superfamily of proteins are DNA-dependent ATPases for which the DNA dependence can vary. For example, the yeast and human SNF2 proteins (the ATPase subunits of the SWI/SNF complex) are stimulated by synthetic DNA with four-way junctions (31). This structure is believed to mimic crossover points where DNA enters and exits nucleosomes (31). On the other hand, imitation switch (ISWI) protein, the ATPase subunit of the nucleosome remodeling factor (NURF) complex (35), has a specific requirement for DNA in chromatin. Conceivably, the ATPase activity of Rad16 protein has a requirement for a particular DNA conformation generated by damage specific incision of the coding strand of transcriptionally active genes. Once this ATPase is activated, the Rad7/Rad16-containing complex may facilitate the remodeling of a protein-DNA structure (possibly including the NER machinery) that is uniquely associated with NER of transcriptionally repressed regions and the nontranscribed strand of transcriptionally active genes. This remodeling presumably facilitates oligonucleotide excision and repair synthesis. The dispensability of Rad7 and Rad16 for efficient oligonucleotide excision and repair synthesis of the template strand of transcriptionally active genes may be a consequence of a distinct DNA conformation that derives from transcription itself. Although our studies do not support the notion that purified plasmid DNA is organized into nucleosomes in yeast whole cell extracts, we cannot formally eliminate the possibility that a requirement for Rad7 and Rad16 proteins for oligonucleotide excision and repair synthesis during NER of plasmid DNA in such extracts reflects the presence of other protein-DNA complexes.

It is interesting to note that whereas the majority of the polypeptides involved in NER in yeast are highly conserved in humans, and the essential biochemistry of global genome repair in vitro appears to be identical in yeast and human extracts, human homologs of the yeast RAD7 and RAD16 genes have not been identified. It remains to be determined whether this reflects subtle differences in the biochemistry of NER and RNA polymerase II transcription in lower and higher eukaryotes, or whether the human homologs have simply eluded identification to date.

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