Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 9712–9716, November 1991
Biochemistry

DNA·RNA helicase activity of RAD3 protein of *Saccharomyces cerevisiae*

(DNA repair/transcription/xeroderma pigmentosum)

VÉRONIQUE BAILLY*, PATRICK SUNG†, LOUISE PRAKASH*, AND SATYA PRAKASH†‡

*Department of Biophysics, University of Rochester School of Medicine, Rochester, NY 14642; and †Department of Biology, University of Rochester, Rochester, NY 14627

Communicated by Philip C. Hanawalt, August 12, 1991 (received for review May 5, 1991)

ABSTRACT **The *RAD3* gene of *Saccharomyces cerevisiae* is required for excision repair of UV-damaged DNA and is essential for cell viability. The RAD3 protein exhibits a remarkable degree of sequence homology to the human excision repair protein ERCC2. The RAD3 protein is a single-stranded DNA-dependent ATPase and a DNA helicase capable of denaturing long regions of duplex DNA. Here, we demonstrate that RAD3 also possesses a potent DNA·RNA helicase activity similar in efficiency to its DNA helicase activity. The rad3 Arg-48 mutant protein, which binds but does not hydrolyze ATP, lacks the DNA·RNA unwinding activity, indicating a dependence on ATP hydrolysis. RAD3 does not show any RNA-dependent NTPase activity and, as expected, does not unwind duplex RNA. This observation suggests that RAD3 translocates on DNA in unwinding DNA·RNA duplexes. That the *rad3 Arg-48* mutation inactivates the DNA and DNA·RNA helicase activities and confers a substantial reduction in the incision of UV-damaged DNA suggests a role for these activities in incision. We discuss how RAD3 helicase activities could function in tracking of DNA in search of damage sites and effect enhanced excision repair of actively transcribed genes.**

The molecular mechanism of excision repair of DNA damaged by ultraviolet (UV) light is best understood in *Escherichia coli*, where the products of the *uvrA*, *uvrB*, and *uvrC* genes incise the damaged DNA (1, 2). In eukaryotes, excision repair of UV-damaged DNA requires a much larger number of genes and is poorly understood. In the yeast *Saccharomyces cerevisiae*, 10 genes are known to be involved in excision repair. Mutations in five of these genes, *RAD1*, *RAD2*, *RAD3*, *RAD4*, and *RAD10*, render cells highly sensitive to UV light and to other DNA-damaging agents. Mutants in any one of these five genes are totally defective in the incision step of excision repair (reviewed in ref. 3). In humans, seven complementation groups of the excision repair-deficient syndrome xeroderma pigmentosum (XP) have been identified by cell fusion studies (4, 5), and cells from these XP groups are highly defective in incision. XP patients are extremely sensitive to UV light and suffer from a high incidence of skin cancers and, frequently, also from neurological degeneration (6). In addition to the *XP* genes, eight complementation groups have been identified among UV-sensitive rodent cell lines (7). Mutants in five of these rodent complementation groups are defective in the incision step (8). The extent of overlap between the *XP* genes and the rodent excision-repair genes is not known.

Three human excision repair genes, *ERCC1*, *ERCC2*, and *ERCC3*, have been cloned by cross complementation of the excision-repair deficiency of rodent cell lines. All three *ERCC* genes show homology to the yeast excision-repair genes. The ERCC1 protein is homologous to the yeast RAD10 protein (9), and ERCC2 protein shows a high degree of homology to the yeast RAD3 protein (10). The *ERCC3* gene specifically complements the excision-repair defect of *XPB* mutant cells, and a homolog of *ERCC3* has been identified in *S. cerevisiae* (11). The existence of a high degree of homology in excision-repair proteins from yeast and human indicates evolutionary conservation of the excision-repair machinery in eukaryotes.

Among the excision-repair genes of *S. cerevisiae*, RAD3 is particularly interesting because it plays diverse roles in DNA repair and cell viability. The *rad3-2* missense mutation renders cells highly sensitive to UV light and DNA-crosslinking agents, and it causes complete defectiveness in the incision step of excision repair of DNA damaged by these agents (12, 13). Deletion mutations of *RAD3* result in a recessive lethal phenotype, indicating an essential role of *RAD3* in cell viability (14, 15). In our laboratory, RAD3 protein has been purified to homogeneity from yeast and shown to possess a single-stranded DNA-dependent ATPase activity (16), and a DNA helicase activity (17) that translocates 5′ → 3′ on single-stranded DNA and is capable of unwinding long regions of duplex DNA.

To evaluate the biological function of the RAD3 ATPase and helicase activities, we mutated the Lys-48 residue in the Walker type A nucleotide-binding motif, Gly-Xaa-Gly-Lys-Thr, in RAD3 to arginine. We purified the rad3 Arg-48 mutant protein and showed that it binds ATP but lacks ATPase and DNA helicase activities (18). The *rad3 Arg-48* mutation does not affect cell viability, but it confers an extreme sensitivity to UV light equivalent to that observed in the *rad3-2* mutant. Unlike the totally incision-defective *rad3-2* mutant, some nicking of UV-damaged DNA occurs in the *rad3 Arg-48* mutant; however, the level of incision is much reduced in the *rad3 Arg-48* mutant compared with wild type. In addition, the *rad3 Arg-48* mutant is defective in a postincision step of excision repair (18). The postincision role of RAD3 DNA helicase could be in the displacement of the nicked damage-containing DNA strand and in repair synthesis (18). The reduced incision proficiency in the *rad3 Arg-48* mutant could arise in various ways. One possibility is that ATP hydrolysis and DNA unwinding by RAD3 are important for the incision reaction *per se*. Alternatively, or in addition, ATP hydrolysis by RAD3 fuels the translocation on DNA of the damage recognition/incision machinery in search of lesions that distort the conformation of the DNA helix. Studies of Koo *et al.* (19) indicate such a DNA-tracking mechanism for the complex of the *E. coli* incision proteins UvrA and UvrB, which together exhibit a DNA helicase activity (20, 21) and bind damaged DNA tightly (2).

Regions of chromosomal DNA undergoing active transcription may present an impediment to the damage-searching machinery. Here, we demonstrate that RAD3

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: nt, nucleotide(s).

‡To whom reprint requests should be addressed.

protein, at the expense of ATP hydrolysis, catalyzes the efficient unwinding of long DNA·RNA duplexes. This activity distinguishes RAD3 from most other DNA helicases that have been examined (22). Our observation that the rad3 Arg-48 mutant protein lacks DNA·RNA helicase activity is consistent with a role of this activity in excision repair. We discuss how the RAD3 DNA and DNA·RNA helicase activities may be utilized to increase the efficiency of the excision-repair complex to locate and incise damaged DNA.

MATERIALS AND METHODS

Proteins. RAD3 and rad3 Arg-48 proteins were overproduced in *S. cerevisiae* by placing the coding frames for the two proteins under the control of the potent yeast *ADC1* promoter, as described (17, 18). To purify the proteins, cell extracts were treated with ammonium sulfate and chromatographed on columns of DEAE-Sephacel, DNA-agarose, hydroxyapatite, Bio-Rex 70, and Mono Q (17, 18). After volume reduction of pooled Mono Q fractions with Centricon-30 microconcentrators (Amicon), glycerol was added (50%, vol/vol) to the protein preparations before storage at −20°C. Both proteins were homogeneous as judged by the presence of only the RAD3 or rad3 Arg-48 protein band in gels stained with Coomassie blue or silver nitrate.

DNA·RNA Substrates. The DNA·RNA substrates shown in Fig. 1*A* were a gift from S. Matson (University of North Carolina, Chapel Hill). In addition, we constructed DNA and DNA·RNA substrates each containing a 69-bp duplex region and 23- and 33-nt single-stranded tails, as described (22). In the construction of DNA·RNA substrates, ^{32}P-labeled RNAs partially complementary to the (+)-strand of M13mp7 DNA were obtained by *in vitro* transcription utilizing the bacteriophage T7 RNA polymerase promoter and then were hybridized to the M13 single-stranded DNA.

RNA·RNA Substrate. The 69-bp *Hae* III fragment from M13mp18 DNA was introduced in both orientations into the *Sma* I site of the vector pGEM-3 (Promega), which contains the T7 RNA polymerase promoter upstream of the *Sma* I site. Plasmid DNA was digested with *Hin*dIII, and RNA molecules complementary to the inserts and radiolabeled using a mixture of [5,6-^{3}H]UTP and [α-^{32}P]UTP were obtained by *in vitro* "run-off" transcription using the T7 RNA polymerase, according to instructions supplied by Promega; the start site for transcription from the T7 RNA polymerase promoter and the *Hin*dIII "run-off" site lie 23 nt upstream and 33 nt downstream of the 69-bp *Hae* III insert, respectively. Following phenol/chloroform extraction, nucleic acids in reaction mixtures were precipitated by ethanol, redissolved in TBE buffer (89 mM Tris borate, pH 8.3/2 mM EDTA) containing 90% formamide, and heated at 95°C for 3 min. Samples were electrophoresed in 6% polyacrylamide gels made in TBE buffer and containing 8 M urea, and the radiolabeled transcripts were located by autoradiography and eluted from crushed gel slices into 0.3 M ammonium acetate/0.1% SDS at room temperature overnight. The eluates were extracted with phenol/chloroform and then treated with ethanol to precipitate RNA, which was redissolved in a small volume of water and stored frozen at −20°C until needed.

The RNA·RNA partial duplex (Fig. 1*B*) was obtained by incubating the transcripts (each at 3 ng/μl) complementary to either strand of the 69-bp *Hae* III fragment introduced into pGEM-3 (see above) in 10 mM potassium Mes (pH 5.6) containing 200 mM KCl and 0.5 mM EDTA for 5 min at 95°C and for 60 min at 65°C.

Helicase Assay. Unless stated otherwise, reaction mixtures (10 μl) were assembled in 40 mM potassium Mes (pH 5.6) and contained 30 mM KCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 1 mM ATP, 0.5 μg of bovine serum albumin, 4.5 units of RNasin (Promega), 30 ng of DNA·RNA or 1.5 ng of RNA·RNA substrate, and 200 ng of RAD3 or rad3 Arg-48 protein. After incubation for 30 min at 30°C, reaction mixtures were chilled on ice for 1 min and 5 μl of "stop" buffer (40% glycerol/1% SDS/50 mM EDTA/0.1% bromophenol blue/0.1% xylene cyanol) was added. Samples were electrophoresed at room temperature in 8% polyacrylamide gels (17, 23) and radioactive bands were revealed by autoradiography (^{32}P) or by fluorography (^{3}H) after treatment with En^{3}Hance (DuPont).

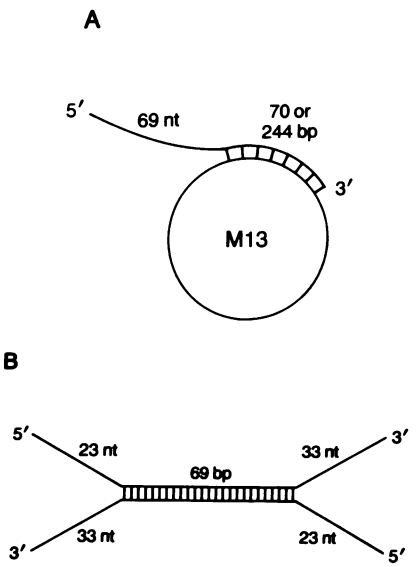

FIG. 1. Helicase substrates used. (*A*) DNA·RNA hybrids. ^{32}P-labeled RNAs of 139 nucleotides (nt) or 313 nt and partially complementary to the (+)-strand of M13mp7 were annealed to the latter, yielding substrates with a duplex region of 70 or 244 base pairs (bp) (22). (*B*) RNA·RNA hybrid. Two ^{3}H-labeled RNAs, both of 125 nt and sharing 69 bp of complementary region, were synthesized and hybridized as described in *Materials and Methods*.

RESULTS

RAD3 Protein Unwinds DNA·RNA Duplexes. Previously, we demonstrated the unwinding by RAD3 protein of DNA duplexes 70–850 bp in length (17). In the present study, for determining whether RAD3 possesses DNA·RNA helicase activity, hybrids of RNA and DNA were prepared by annealing ^{32}P-labeled RNAs, obtained from *in vitro* transcription reactions, onto the (+)-strand of M13mp7 phage DNA. The resulting substrates contained a DNA·RNA duplex region of 70 or 244 bp, a single-stranded RNA tail of 69 nt, and single-stranded circular M13 DNA (Fig. 1*A*).

Incubation of the DNA·RNA substrate containing a 70-bp duplex region with purified (17), homogeneous RAD3 protein in the presence of Mg^{2+} and ATP resulted in the displacement of the radiolabeled RNA, as analyzed by electrophoresis in polyacrylamide gels followed by autoradiography (Fig. 2*A*). Maximal unwinding of the DNA·RNA substrate occurred at pH 5.6. As reported previously, the single-stranded DNA-dependent ATPase and DNA helicase activities of RAD3 also exhibit a pH optimum of 5.6 (16, 17). The unwinding of RNA in 30 min of incubation increased linearly with the amount of RAD3 protein up to 100 ng (10 μg/ml), above which the availability of the DNA·RNA substrate became limiting for the incubation time (Fig. 2*B*). With RAD3 at 20 μg/ml, unwinding of the DNA·RNA substrate followed linear kinetics for 15 min (Fig. 2*C*).

A comparison of results obtained previously (17) revealed that the RAD3 DNA helicase activity, as studied with a DNA substrate containing a 71-bp duplex region, exhibits kinetic

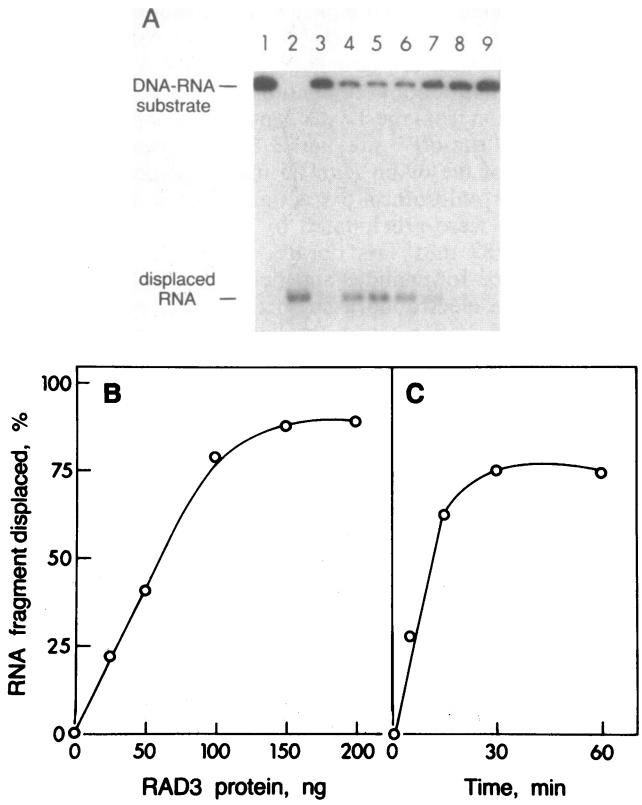

FIG. 2. RAD3 DNA·RNA helicase activity as a function of pH (*A*), enzyme concentration (*B*), and incubation time (*C*). The substrate containing a 70-bp duplex region was used in these experiments, and the extent of unwinding was determined by densitometric scanning of autoradiograms. (*A*) The substrate (lane 1) was heat-denatured (lane 2) or incubated for 30 min with 200 ng of RAD3 protein at pH 5.3, 5.5, 5.6, 5.7, 5.8, 5.9, or 6.1 (lanes 3–9, respectively). (*B*) Reaction mixtures containing various amounts of RAD3 protein were incubated for 30 min. (*C*) The substrate was incubated with 200 ng of RAD3 for various times.

parameters similar to those reported here for DNA·RNA unwinding. To directly compare the efficiency of RAD3 in unwinding DNA and DNA·RNA duplexes, we constructed substrates in which a 125-nt DNA or RNA fragment was hybridized to circular M13mp18 single-stranded DNA. These DNA·DNA and DNA·RNA substrates contained 69 bp of duplex region with single-stranded tails of 23 and 33 nt. Incubation of these DNA·DNA and DNA·RNA hybrid substrates with the RAD3 protein for varying time periods revealed that they were unwound with the same efficiency (results not shown).

RAD3 protein also unwound the DNA·RNA substrate with a 244-bp duplex region efficiently (Fig. 3*B*). Approximately 60% of this substrate was unwound by RAD3, while under the same conditions, ≈75% of 70-bp duplex DNA·RNA substrate was unwound (Fig. 2*C*). The ability to act on DNA·RNA duplexes is not a common property of all DNA helicases (22), as yeast RAD3 and *E. coli* UvrD protein are the only known DNA helicases that also possess a potent DNA·RNA helicase activity.

ATP Hydrolysis Fuels DNA·RNA Helicase Activity of RAD3. All helicases examined to date possess an NTPase activity that is activated upon binding of the helicase enzyme to single-stranded nucleic acid. Binding and hydrolysis of NTPs by helicases provide the energy for their translocation on the bound nucleic acid and for the denaturation of duplex regions (24, 25).

As expected, in the absence of ATP, RAD3 failed to displace the ^{32}P-labeled RNA from the DNA·RNA substrate containing the 70-bp or the 244-bp duplex region. Adenosine 5′-[γ-thio]triphosphate, a nonhydrolyzable analog of ATP, was ineffective in promoting the unwinding reaction (Fig. 3*A*, lane 7). The role of ATP hydrolysis in DNA·RNA helicase activity was further examined with the rad3 Arg-48 mutant protein, in which the Lys-48 residue in the Walker type A sequence of RAD3 has been changed to arginine (18). The rad3 Arg-48 mutant protein retains the ability to bind ATP but does not hydrolyze the nucleotide. The rad3 Arg-48 protein did not unwind the 70-bp (Fig. 3*A*, lane 8) or the 244-bp (Fig. 3*B*, lane 5) DNA·RNA duplex, thus confirming the requirement for ATP hydrolysis in the DNA·RNA helicase activity of RAD3.

Of various NTPs or dNTPs tested—dATP, CTP, GTP, dTTP, and UTP—only dATP replaced ATP in supporting the unwinding reaction (Fig. 3*A*, lanes 3 and 6). This result is consistent with our previous findings that ATP and dATP are the only nucleotides that are bound and hydrolyzed efficiently by RAD3 (16) and that support the DNA helicase activity of RAD3 (17). Omission of Mg^{2+} (data not shown) or addition of the metal-chelating agent EDTA (Fig. 3*A*, lane 9) inhibited the unwinding reaction. Since the single-stranded DNA-dependent ATPase activity of RAD3 is contingent upon the presence of Mg^{2+} (16), the requirement for the metal ion in the unwinding of DNA·RNA duplexes may simply reflect the dependence of the latter reaction on ATP hydrolysis.

RAD3 Does Not Unwind Duplex RNA. The simian virus 40 large tumor (T) antigen is required for the initiation and elongation phases of replication of the viral genome. In addition to possessing a DNA helicase activity (26), T antigen also unwinds duplex RNA (27). The RNA helicase activity of

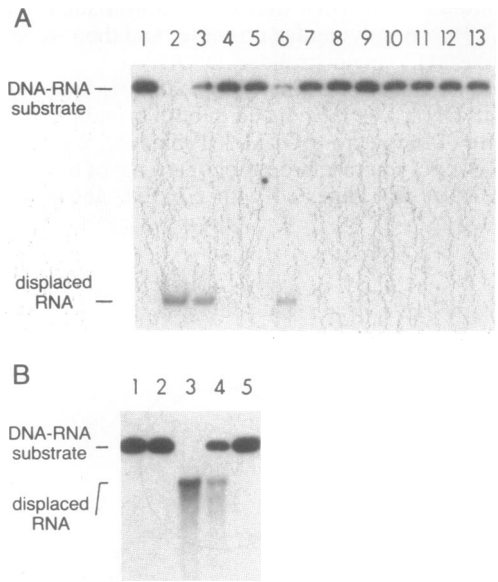

FIG. 3. Unwinding of DNA·RNA duplexes requires ATP hydrolysis. (*A*) Nucleotide requirement in DNA·RNA unwinding. The substrate containing a 70-bp duplex region (lane 1) was heat-denatured (lane 2) or incubated for 30 min with 200 ng of RAD3 protein (lanes 3–7 and 9–13) or rad3 Arg-48 protein (lane 8). The added nucleotides (1 mM) were as follows: lanes 1–3, ATP; lane 4, no added nucleotide; lane 5, ADP; lane 6, dATP; lane 7, adenosine 5′-[γ-thio]triphosphate; lane 8, ATP; lane 9, ATP and 20 mM EDTA; lane 10, CTP; lane 11, dTTP; lane 12, UTP; lane 13, GTP. (*B*) ATP-dependent unwinding of DNA·RNA substrate containing a 244-bp duplex region. The substrate (lane 1) was heat-denatured (lane 3) or incubated with 200 ng of RAD3 protein in the presence of ATP (lane 4) or its absence (lane 2). The substrate was also incubated with 200 ng of rad3 Arg-48 protein in the presence of ATP (lane 5). Smearing below the displaced RNA fragment is due to some heterogeneity in the length of RNA.

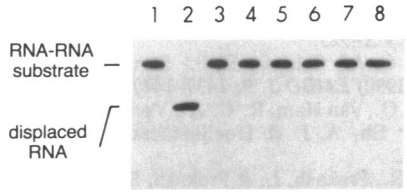

FIG. 4. RAD3 does not unwind RNA·RNA hybrid. The 69-bp ^{3}H-labeled RNA duplex (lane 1) was heat-denatured (lane 2); or incubated with 200 ng of RAD3 protein for 10, 20, 40, or 60 min (lanes 3–6, respectively); or incubated with 400 ng (lane 7) or 600 ng (lane 8) of RAD3 protein for 30 min.

T antigen may enhance the expression of certain viral as well as cellular genes by denaturing secondary structures in the mRNAs encoded by these genes, thereby facilitating translation of these mRNAs (27).

We examined whether RAD3 also possesses RNA·RNA unwinding activity. The duplex RNA substrate was prepared by hybridizing partially complementary RNA strands obtained from *in vitro* transcription reactions. This substrate consists of a duplex region of 69 bp and single-stranded tails of 23 or 33 nt (Fig. 1*B*). RAD3 did not unwind the RNA duplex, even after 60 min of incubation (Fig. 4, lane 6) or at a RAD3 concentration 3 times that used in the DNA·RNA unwinding reaction (lane 8). Since T antigen utilizes ATP in its DNA helicase function but requires UTP, CTP, or GTP in unwinding duplex RNA, we replaced ATP with the latter nucleotides in RNA unwinding assays with RAD3 protein. However, even then no RNA helicase activity was detected in the RAD3 protein (data not shown). The absence of RNA helicase activity from RAD3 is consistent with our observations that RAD3 does not hydrolyze any NTP in the presence of a variety of RNA cofactors (data not shown).

DISCUSSION

We have shown that RAD3 protein, a DNA helicase, also possesses a potent DNA·RNA helicase activity. The DNA·RNA unwinding activity of RAD3 resembles its DNA helicase activity in exhibiting the same pH optimum, in requiring ATP or dATP as nucleotide cofactor, and in possessing a very similar efficiency. Our observation that the rad3 Arg-48 mutant protein lacks the ability to unwind DNA·RNA duplexes confirms that the DNA·RNA helicase activity is intrinsic to RAD3. RAD3 does not hydrolyze any NTP in the presence of RNA alone, and it does not unwind duplex RNA, indicating that RAD3 translocates on the DNA strand in unwinding DNA·RNA duplexes. The dependence of RAD3 on single-stranded DNA for ATP hydrolysis would explain the similar requirements of RAD3 in unwinding DNA and DNA·RNA duplexes.

The capacity to unwind long DNA·RNA duplexes is not a property common to DNA helicases. Of the four *E. coli* DNA helicases examined—UvrD protein (helicase II), helicase I, Rep protein, and helicase IV—only the UvrD helicase catalyzes significant unwinding of DNA·RNA hybrids (22). The T antigen encoded by simian virus 40 unwinds DNA duplexes, RNA duplexes, and DNA·RNA hybrids (26, 27).

That RAD3 unwinds DNA·RNA duplexes as efficiently as DNA duplexes suggests that the DNA·RNA unwinding activity of RAD3 is physiologically important. The role of the DNA and DNA·RNA helicase activities of RAD3 in excision repair is indicated from our work with the rad3 Arg-48 mutant protein. Both the DNA and DNA·RNA helicase activities are absent from this mutant protein. In addition to a postincision defect, this mutation confers a much reduced level of incision ability. These observations implicate the DNA and DNA·RNA helicase activities of RAD3 in the incision and postincision steps of excision repair. We have suggested (18) a role for the DNA helicase activity of RAD3 in the displacement of the damage-containing strand following its incision. The RAD3 DNA and DNA·RNA helicase activities could be utilized in scanning DNA for damage sites, thereby facilitating the damage-recognition process and increasing the efficiency of the incision reaction. The *E. coli* UvrAB protein complex possesses a DNA helicase activity that can unwind short hybrid regions (20, 21). Recent studies of Koo *et al.* (19) suggest the involvement of UvrAB DNA helicase activity in tracking of the DNA helix in search of damaged DNA. The DNA helicase activity of RAD3 could similarly be involved in the movement of RAD3 and associated protein(s) along DNA in search of damage sites. The DNA·RNA helicase activity of RAD3 may enable the damage-recognition machinery to track through regions of DNA undergoing active transcription, thereby increasing the efficiency of the damage-searching process.

In mammals (28, 29) and yeast (30), bulky DNA damage present in actively transcribed regions is excised more efficiently than that in nontranscribed regions. While the relative lack of higher-order chromatin folding in transcribed regions may facilitate the damage-excision process, transcription itself may have an inhibitory effect on the rate of repair, as the presence of a stalled transcription complex at the damage site could interfere with damage recognition and/or subsequent steps of the repair process. In fact, transcription is severely reduced in UV-irradiated cells due to stoppage of RNA chain elongation at the damage sites in DNA (31), and a pyrimidine dimer in the transcribed strand is an absolute block to incision by the *E. coli* UvrABC enzyme, due to stalling of the RNA polymerase complex at the damage site (32). The bacteriophage T4 dda protein, a known DNA helicase, is highly effective in dislodging the stationary RNA polymerase molecules from the path of the moving replication fork (33). In addition to unwinding nucleic acids, RAD3 helicase may be instrumental in the displacement of the transcription complex stalled at the damage sites. A specific recognition of the stalled RNA polymerase complex by RAD3 might constitute the coupling mechanism that has been proposed to explain the phenomenon of preferential excision repair of the transcribed DNA strand (34, 35).

The DNA and DNA·RNA helicase activities of RAD3 are not essential for viability, since the rad3 Arg-48 mutant is viable. It remains to be determined whether the RAD3 DNA helicase and DNA·RNA helicase activities are dispensable in the viability function, which is likely to be DNA replication, because of the presence of another functionally equivalent helicase that can circumvent the need for the RAD3 helicase function. The human *ERCC3* (*XPB*) gene is an essential component of the excision-repair machinery and encodes a protein containing conserved sequences present in helicases (11). A homolog of *ERCC3* exists in yeast (11), and its putative helicase activity may substitute for the RAD3 helicase function in DNA replication.

Even though the UvrD DNA helicase of *E. coli* also possesses a DNA·RNA unwinding activity, this activity very likely has a different biological role than the RAD3 DNA·RNA helicase. In excision repair, *RAD3* is absolutely required for the incision of UV-damaged DNA and it also functions in a postincision reaction. Like the incision-defective *E. coli uvrA*, *uvrB*, or *uvrC* mutants, *rad3* mutants exhibit an extreme sensitivity to UV light. In contrast, *uvrD* has no role in incision, and *uvrD* mutants exhibit a much lower level of UV sensitivity relative to the incision-defective mutants. Subsequent to incision, UvrD acts with DNA polymerase I to facilitate the dissociation of the UvrABC enzyme complex from DNA (36, 37). The lack of involvement of UvrD in the incision step of excision repair suggests that the DNA and DNA·RNA helicase activities of UvrD are not

utilized in the incision process. Thus, UvrD would not be involved in tracking of DNA for damage sites or affect incision in other ways as we have suggested for the RAD3 protein. It has been proposed that the UvrD DNA·RNA helicase activity could function in the removal of RNA primers from the lagging strand synthesized during DNA replication (22). The 3′ → 5′ directionality of the UvrD helicase and the inviability of *uvrD polA* double mutants are consistent with this suggestion. The biological role of UvrD DNA and DNA·RNA helicase activities could be ascertained by examining mutants defective in these activities.

The human excision-repair protein ERCC2 shows a very high degree of homology to the RAD3 protein (10). The two proteins are of nearly identical length, with 778 and 760 amino acids in RAD3 and ERCC2, respectively. The alignment of sequences in the two proteins is colinear, except for differences toward the carboxyl terminus, where RAD3 contains residues absent from ERCC2. However, this carboxyl-terminal segment in RAD3 is functionally dispensable, since its deletion produces no biological effect (38). RAD3 and ERCC2 proteins share 52% identical residues, and if conservative amino acid substitutions are considered, the level of homology rises to 73%. ERCC2 contains all the domains identified in helicases and that are also present in RAD3; within these domains, the two proteins share 74% identical and 89% conserved residues. It is highly probable that ERCC2 possesses DNA helicase and DNA·RNA helicase activities, and that ERCC2 would function like RAD3 during excision repair and in other cellular processes.

We are grateful to S. Matson for his gift of DNA·RNA substrates. This work was supported by National Institutes of Health Grant CA35035 and Department of Energy Grant DE-FGO2-88ER60621.

1. Sancar, A. & Rupp, W. D. (1983) *Cell* **33**, 249–260.
2. Yeung, A. T., Mattes, W. B., Oh, E. Y. & Grossman, L. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6157–6161.
3. Prakash, S., Sung, P. & Prakash, L. (1990) in *The Eukaryotic Nucleus*, eds. Strauss, P. R. & Wilson, S. H. (Telford, Caldwell, NJ), Vol. 1, pp. 275–292.
4. Bootsma, D., Keijzer, W., Jund, E. G. & Bohnert, E. (1989) *Mutat. Res.* **218**, 149–151.
5. Johnson, R. T., Elliott, G. C., Squires, S. & Joysey, V. C. (1989) *Hum. Genet.* **81**, 203–210.
6. Cleaver, J. E. & Kraemer, K. (1989) in *The Metabolic Basis of Inherited Disease*, eds. Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D. (McGraw-Hill, New York), Vol. 2, pp. 2949–2971.
7. Thompson, L. H., Shiomi, T., Salazar, E. P. & Stewart, S. A. (1988) *Somat. Cell Mol. Genet.* **14**, 605–612.
8. Thompson, L. H., Brookman, K. W., Dillehay, L. E., Mooney, C. L. & Carrano, A. A. (1982) *Somat. Cell Genet.* **8**, 759–773.
9. van Duin, M., de Wit, J., Odijk, H., Westerveld, A., Yasui, A., Koken, M. H. M., Hoeijmakers, J. H. J. & Bootsma, D. (1986) *Cell* **44**, 913–923.
10. Weber, C. A., Salazar, E. P., Stewart, S. A. & Thompson, L. H. (1990) *EMBO J.* **9**, 1437–1447.
11. Weeda, G., van Ham, R. C. A., Vermeulen, W., Bootsma, D., van der Eb, A. J. & Hoeijmakers, J. H. J. (1990) *Cell* **62**, 777–791.
12. Miller, R., Prakash, L. & Prakash, S. (1982) *Mol. Cell. Biol.* **2**, 939–948.
13. Wilcox, D. R. & Prakash, L. (1981) *J. Bacteriol.* **148**, 618–623.
14. Higgins, D. R., Prakash, S., Reynolds, P., Polakowska, R., Weber, S. & Prakash, L. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5680–5684.
15. Naumovski, L. & Friedberg, E. C. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4818–4821.
16. Sung, P., Prakash, L., Weber, S. & Prakash, S. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6045–6049.
17. Sung, P., Prakash, L., Matson, S. W. & Prakash, S. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8951–8955.
18. Sung, P., Higgins, D., Prakash, L. & Prakash, S. (1988) *EMBO J.* **7**, 3263–3269.
19. Koo, H.-S., Claassen, L., Grossman, L. & Liu, L. F. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1212–1216.
20. Oh, E. Y. & Grossman, L. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3638–3642.
21. Oh, E. Y. & Grossman, L. (1989) *J. Biol. Chem.* **264**, 1336–1343.
22. Matson, S. W. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4430–4434.
23. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
24. Kuhn, B., Abdel-Monem, M., Krell, H. & Hoffmann-Berling, H. (1979) *J. Biol. Chem.* **254**, 11343–11350.
25. Matson, S. W. & Kaiser-Rogers, K. A. (1990) *Annu. Rev. Biochem.* **59**, 289–329.
26. Stahl, H., Dröge, P. & Knippers, R. (1986) *EMBO J.* **5**, 1939–1944.
27. Scheffner, M., Knippers, R. & Stahl, H. (1989) *Cell* **57**, 955–963.
28. Bohr, V. A., Smith, C. A., Okumoto, D. S. & Hanawalt, P. C. (1985) *Cell* **40**, 359–369.
29. Madhani, H. D., Bohr, V. A. & Hanawalt, P. C. (1986) *Cell* **45**, 417–423.
30. Terleth, C., van Sluis, C. A. & van de Putte, P. (1989) *Nucleic Acids Res.* **17**, 4433–4439.
31. Sauerbier, W. & Hercules, K. (1978) *Annu. Rev. Genet.* **12**, 329–363.
32. Selby, C. P. & Sancar, A. (1990) *J. Biol. Chem.* **265**, 21330–21336.
33. Bedinger, P., Hochstrasser, M., Jongeneel, C. V. & Alberts, B. M. (1983) *Cell* **34**, 115–123.
34. Mellon, I., Spivak, G. & Hanawalt, P. C. (1987) *Cell* **51**, 241–249.
35. Mellon, I. & Hanawalt, P. C. (1989) *Nature (London)* **342**, 95–98.
36. Caron, P. R., Kushner, S. R. & Grossman, L. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4925–4929.
37. Husain, I., van Houten, B., Thomas, D. C., Abdel-Monem, M. & Sancar, A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6774–6778.
38. Reynolds, P., Higgins, D. R., Prakash, L. & Prakash, S. (1985) *Nucleic Acids Res.* **13**, 2357–2372.