Communication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 271, No. 31, Issue of August 2, pp. 18314–18317, 1996

RAD26, the Yeast Homolog of Human Cockayne's Syndrome Group B Gene, Encodes a DNA-dependent ATPase*

(Received for publication, March 6, 1996, and in revised form, May 16, 1996)

Sami N. Guzder, Yvette Habraken, Patrick Sung, Louise Prakash, and Satya Prakash‡

From the Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, Texas 77555-1061

Cells from Cockayne's syndrome (CS) patients are sensitive to ultraviolet light and defective in preferential repair of the transcribed DNA strand. CS patients suffer from complex clinical symptoms, including severe growth retardation, neurological degeneration, mental retardation, and cachexia. Two CS complementation groups, *CSA* and *CSB*, have been identified so far. *RAD26* encodes the yeast counterpart of the *CSB* gene. Here, we purify Rad26 protein to near homogeneity from yeast cells and show that it is a DNA-dependent ATPase. In contrast to the Mfd protein that functions in transcription-coupled repair in *Escherichia coli*, and which is a weak and DNA independent ATPase, Rad26 is a much more active ATPase, with a strict dependence on DNA. The possible role of Rad26 ATPase in the displacement of stalled RNA polymerase II from the site of the DNA lesion and in the subsequent recruitment of a DNA repair component is discussed.

Nucleotide excision repair (NER)[1] of ultraviolet (UV)-damaged DNA in eukaryotes is a complex process involving a large number of gene products. A defect in NER in humans results in xeroderma pigmentosum (XP). XP patients are extremely sensitive to sunlight due to an inability to remove UV-induced lesions from genomic DNA, and, consequently, they suffer from a high incidence of skin cancers. Seven XP complementation groups, A through G, have been identified so far.

Notably, UV-induced cyclobutane pyrimidine dimers in DNA are removed by the NER machinery at a faster rate from the transcribed strand of an active gene compared to the nontranscribed strand (1, 2). This preferential repair of the transcribed DNA strand, known as transcription-coupled repair (TCR), is restricted to genes transcribed by RNA polymerase II. In humans, mutations in the Cockayne's syndrome group A (*CSA*) and group B (*CSB*) genes result in defective TCR, such that in *CSA* and *CSB* mutant cells, repair of the transcribed strand falls to the level of the nontranscribed strand (3). Thus, CSA and CSB proteins are both required for coupling transcription to NER and, as such, can be considered as TCR factors. CS cells exhibit elevated UV sensitivity, but, unlike XP, no cancers have been reported in CS patients. CS symptoms include severe growth retardation that results in a characteristic physical appearance of cachectic dwarfism and progressive neurologic dysfunction with mental retardation and microcephaly. Other CS symptoms are sensorineural hearing loss, cataracts, pigmentary retinopathy, and dental caries. The mean age of death in CS is ≈12 years (4).

RAD26 is the *CSB* counterpart in *Saccharomyces cerevisiae*, and null mutations in *RAD26* severely reduce the proficiency of TCR (5). The proteins encoded by *CSB* and *RAD26* exhibit extensive homology to a large number of proteins that are members of the *SNF2* family. These proteins function in diverse cellular processes including transcription, recombination, and different DNA repair processes, and all of these proteins contain the seven conserved domains present in ATPases and DNA and RNA helicases (6, 7). Determination of the biochemical activities of CSB and Rad26 proteins and of other proteins required for TCR will be highly important for elucidating the molecular mechanism of TCR, but this has not yet been done. Here we purify Rad26 protein from yeast cells to near homogeneity and show that it is a DNA-dependent ATPase but has no apparent DNA helicase activity. Putative roles of the Rad26 ATPase in the displacement of RNA polymerase II stalled at the damage site and in the recruitment of NER proteins are discussed.

MATERIALS AND METHODS

Buffers—Buffers A, B, C, and D were as described in Sayre *et al.* (8), and buffer K was 20 mM KPO_4, pH 7.5, 0.5 mM EDTA, 10% glycerol, 1 mM dithiothreitol, 0.01% (w/v) Nonidet P-40.

Cloning of the RAD26 Gene—A DNA probe for the *RAD26* gene was generated by PCR using yeast genomic DNA and the following primers: 5′-CAC GCA ATG CAA CAG CAC AT-3′ and 5′-GAT CTG GGA GTT AAC GTG CT-3′. These primers anneal within the ORF of *RAD26* and generate a 1620-bp fragment. ^{32}P-labeled PCR product was used to probe the Carlson Botstein library. Nucleotide sequencing of clone pR26.1d thus obtained confirmed that it carries the *RAD26* gene.

Production of Antibodies—A portion of Rad26 protein encompassing amino acid residues 25 to 394 was fused to the N-terminal 116 amino acids of the *Escherichia coli* rho protein under the control of the λ-P_L promoter in plasmid pAS39 (9), yielding plasmid pR26.18. Induction of the rho-Rad26 fusion polypeptide in *E. coli* strain AR120.A6 harboring pR26.18 was accomplished by treatment with nalidixic acid (9). The insoluble fusion protein was purified from inclusion bodies by preparative SDS-polyacrylamide gel electrophoresis and used as antigen for raising polyclonal antisera in rabbits. Antibodies were purified by affinity chromatography on a cyanogen bromide Sepharose column (Pharmacia Biotech Inc.) to which the rho-Rad26 fusion protein had been covalently coupled.

Purification of Rad26 Protein—The entire open reading frame of *RAD26* from the ATG initiating codon to 714 nucleotides downstream of the TGA stop codon was placed under the control of the constitutively expressed *ADC1* promoter to yield plasmid pR26.23 (*ADC1-RAD26*; 2μ, *TRP1*). Yeast strain YPH/TFB1.6His (10) harboring pR26.23 was precultured in complete synthetic medium lacking tryptophan, diluted with 10 volumes of YPD, and grown to a cell density of 1×10^8 per ml. Extract was prepared from 360 g of yeast paste using a French press as described previously (11). The crude extract

* This work was supported by Grant CA35035 from NCI, National Institutes of Health and Grant DE-FGO3-93ER61706 from the Department of Energy. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Sealy Center for Molecular Science, University of Texas Medical Branch, 6.104 Medical Research Bldg., 11th and Mechanic St., Galveston, TX 77555-1061. Tel.: 409-747-8602 (office), 409-747-8600 (secretary); Fax: 409-747-8608; E-mail: sprakash@scms.utmb.edu.

[1] The abbreviations used are: NER, nucleotide excision repair; XP, xeroderma pigmentosum; TCR, transcription-coupled repair; PCR, polymerase chain reaction; bp, base pair(s); ss, single-stranded; ds, double-stranded.

(Fraction I) was clarified by centrifugation (100,000 × *g*, 60 min) and dialyzed against buffer A for 16 h to lower the ionic strength to ≈150 mM KOAc. The dialysate (fraction I, 450 ml) was clarified by centrifugation (20,000 × *g* for 30 min) and applied onto a column of Bio-Rex 70 (2.5 × 14 cm; 70-ml matrix) equilibrated in buffer A + 120 mM KOAc. After washing with 200 ml each of A + 120 KOAc and A + 200 KOAc, the Bio-Rex 70 column was eluted with A + 600 mM KOAc, collecting 4-ml fractions. The protein peak (Fraction II; 68 ml) was dialyzed against 2 liters of buffer B + 100 mM KOAc for 16 h before being applied onto a DEAE-Sephacel column (2.5 × 8 cm; 40-ml matrix) equilibrated in B + 100 KOAc. The DEAE-column was washed with 100 ml each of B + 100 and B + 200 and then eluted with B + 500 KOAc, collecting 2-ml fractions. The DEAE-pool (Fraction III; 20 ml) was dialyzed against 1 liter of B + 80 mM KOAc for 12 h, and the dialysate was loaded onto a column of hydroxyapatite (Bio-Gel HTP from Bio-Rad; 1.6 × 2.5 cm; 4.5-ml matrix) equilibrated in buffer C and developed with a 50-ml gradient from buffer C to buffer D, collecting 1-ml fractions. The hydroxyapatite fractions were screened by immunoblotting for Rad26 protein and for the TFIIH subunits, Rad3, Rad25, SSL1, and TFB1, as described previously (11, 12). For further purification of Rad26 protein, hydroxyapatite fractions 30–36 were pooled (Fraction IV; 4.5 ml), dialyzed against 1 liter of buffer K containing 50 mM KCl for 6 h, and fractionated in Mono S (HR 5/5) with a 30-ml KCl gradient from 50 to 500 mM in buffer K. Rad26 protein eluted from Mono S at ≈220 mM KCl, and the pool of which (Fraction V; 2 ml) was concentrated to ~0.3 ml using a Centricon-30 concentrator (Amicon) and subjected to molecular sizing in a Sephacryl S300 column (1 × 42 cm; 33-ml matrix) in buffer K + 75 mM KCl. The Rad26 peak fractions were pooled (Fraction VI; 3.0 ml) and further chromatographed in Source Q (Pharmacia; 0.5-ml matrix packed in an HR5/2 column) equilibrated in buffer K + 75 mM KCl. The column was developed with a 12-ml KCl gradient of 50–350 mM in buffer K, collecting 0.4-ml fractions. Rad26 protein eluted from Source Q at ≈200 mM KCl, and the pool of which (Fraction VII; 1.5 ml) was concentrated to 400 μg/ml. Fraction VII Rad26 protein was nearly homogeneous as determined by analyzing 1 μg of the protein in a 9% denaturing polyacrylamide gel and staining with Coomassie Blue.

ATPase Assay—The standard assay (10 μl final volume) was carried out at 36 °C in Buffer R (30 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM dithiothreitol, 100 μg/ml bovine serum albumin, 0.5 mM [γ-^{32}P]ATP), 150 ng of DNA cofactor, and the indicated amount of Rad26 protein. The reaction was stopped by addition of EDTA to 20 mM, and reaction products were analyzed by thin layer chromatography on polyethyleneimine cellulose sheets developed in 0.3 M LiCl, 1 M formic acid. The polyethyleneimine plates were subjected to autoradiography. The extent of ATP hydrolysis was determined by image analysis of autoradiograms in a Bio-Rad GS-670 Imaging Densitometer. UV irradiation of DNA (1000 J/m^2) was carried out using a 254 nm light source at a fluence rate of 10 J/m^2/s.

DNA Helicase Assay—The partial duplex used as DNA helicase substrate was constructed by hybridizing a 5′-^{32}P-labeled 17-base complementary fragment to the viral (+)-strand of M13mp18 as described (13). 5′-^{32}P-labeled 50-mer oligonucleotide A was hybridized to partially homologous 50-mer oligonucleotide E to give the forked helicase substrate containing a 20-nucleotide 5′-overhanging tail and a 20-nucleotide 3′-overhanging tail adjacent to a 30-bp duplex region, as described (14). The DNA helicase substrates, 2 ng each, were incubated with the indicated amount of Rad26 protein in 10 μl of buffer R containing 2 mM ATP. After incubation at 36 °C, the reaction was stopped by adding 5 μl of 1% SDS, 50 mM EDTA, 20% glycerol, and 0.02% bromphenol blue. Reaction mixtures were run in polyacrylamide gels which were dried and subjected to autoradiography.

RESULTS

Overproduction and Purification of Rad26 Protein—For purification of Rad26 protein, the *RAD26* gene was placed under the control of the alcohol dehydrogenase I (*ADCI*) promoter, yielding the multicopy plasmid pR26.23 (2μ, *ADCI-RAD26*), which was introduced into the yeast strain YPH/TFB1.6HIS (10). Probing of nitrocellulose blots of yeast cell extracts using affinity-purified antibodies raised against a rho-Rad26 fusion polypeptide produced in *E. coli* revealed that extract harboring pR26.23 contained an immunoreactive species of 125 kDa, which is in good agreement with the predicted size of 126.5 kDa for Rad26 protein (Fig. 1*B*, *lane 3*). The level of the Rad26 protein in wild type extract is too low to detect under the conditions employed. By immunoprecipitation, we have verified that Rad26 protein in wild type extract has the same size as the overproduced Rad26 protein shown in Fig. 1*B*, and that it was absent from otherwise isogenic yeast cells that contain a

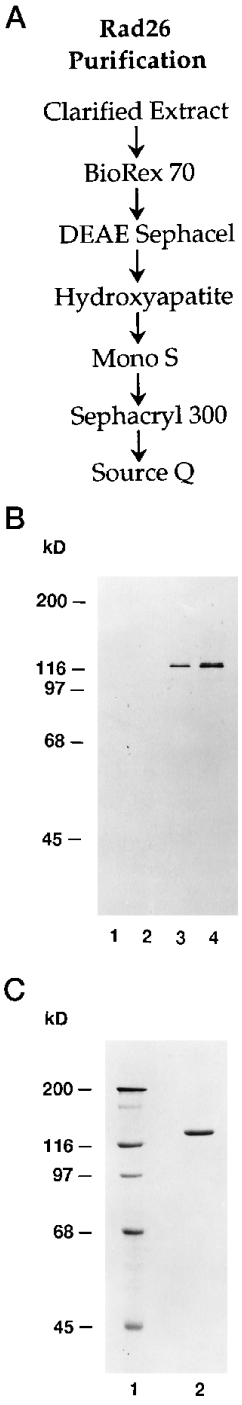

FIG. 1. **Overproduction and purification of Rad26 protein.** *A*, Rad26 purification scheme. *B*, overproduction of Rad26 protein. The nitrocellulose blot of a 7.5% denaturing polyacrylamide gel was probed with affinity-purified anti-Rad26 antibodies. *Lane 1*, extract from the *rad26*Δ mutant strain YHY1; *lane 2*, extract from yeast strain DBY747, which is the wild type parent of YHY1; *lane 3*, extract from yeast strain YPH/TFB1.6HIS harboring the overproducing plasmid pR26.23 (2μ, *ADC1-RAD26*); *lane 4*, 30 ng of purified Rad26 protein. *C*, a 7.5% denaturing polyacrylamide gel containing 1 μg of purified Rad26 protein (*lane 2*) and molecular size standards (*lane 1*) was stained with Coomassie Blue.

TABLE I
Rad26 is a DNA-dependent ATPase

Rad26 protein, 75 ng, was incubated with various DNA cofactors for 60 min, as described under "Materials and Methods"; 100% activity corresponded to the hydrolysis of 55% of the input ATP.

	ATPase activity
	%
No DNA	0
Single-stranded DNA	
M13mp18	100
ϕX174	98
ϕX174 ($-Mg^{2+}$)	0
UV irradiated (ϕX174)	99
Duplex DNA	
pBR322	31
M13mp18	33
ϕX174	30
ϕX174 ($-Mg^{2+}$)	0
UV irradiated (ϕX174)	33

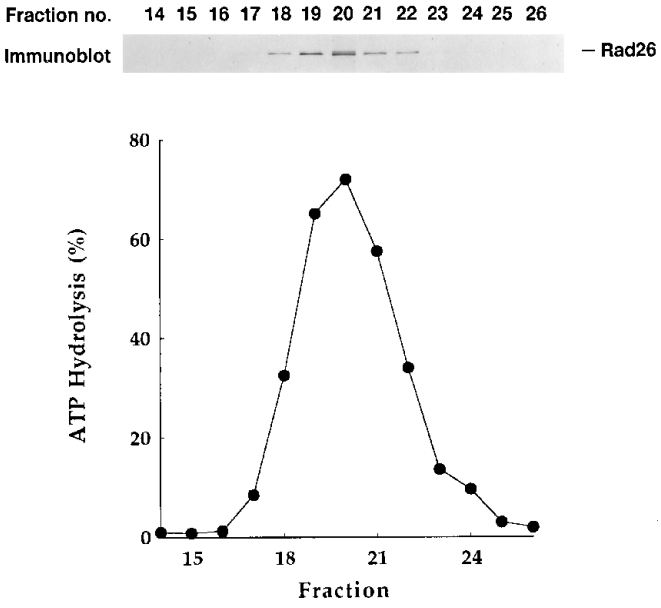

FIG. 2. **Co-elution of DNA-dependent ATPase activity with Rad26 protein.** Source Q column fractions 14 to 26 were examined for their content of Rad26 protein by immunoblotting and for ssDNA-dependent ATPase activity. No ATPase activity was observed in these Source Q fractions when the DNA cofactor was omitted (not shown).

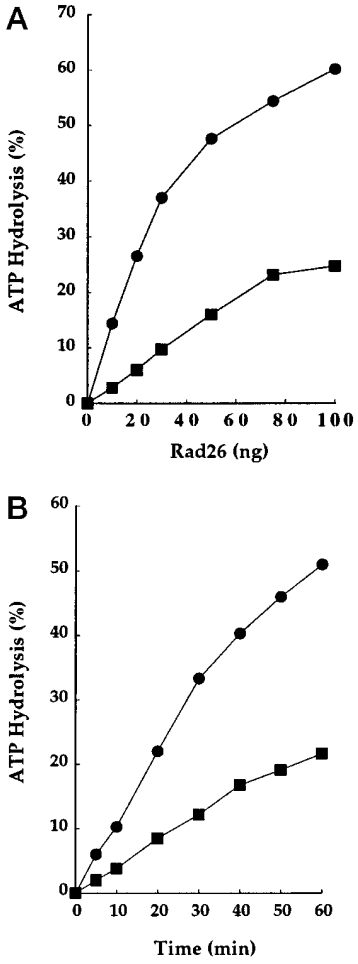

FIG. 3. **Kinetics of ATP hydrolysis.** *A*, ATP hydrolysis as a function of Rad26 protein concentration. The indicated amount of Rad26 protein was incubated with ϕX ssDNA (●) or ϕX linear dsDNA (■) for 60 min. *B*, ATP hydrolysis as a function of reaction time. Rad26 protein, 75 ng, was incubated with ϕX ssDNA (●) or ϕX dsDNA (■) for the indicated times.

genomic deletion mutation of the *RAD26* gene (data not shown).

Rad26 protein was purified from yeast strain YPH/TFB1.6HIS harboring pR26.23, by subjecting clarified cell extract to chromatographic fractionation in six columns, as outlined in Fig. 1*A* and described in details under "Materials and Methods." During purification, up until the molecular sizing step, the elution of Rad26 protein from the chromatographic columns was monitored by immunoblotting of column fractions. During molecular sizing in Sephacryl S300, Rad26 protein behaved as a monomer under the conditions employed (data not shown). When the final Source Q pool of Rad26 protein (Fraction VII) was analyzed by SDS-PAGE and Coomassie Blue staining (Fig. 1*A*), no protein species other than the Rad26 band was detected (Fig. 1*C*), indicating that the Rad26 protein preparation was nearly homogeneous. Fraction VII Rad26 protein was subjected to immunoblot analysis to verify its identity (Fig. 1*B*, *lane 4*) and was used in all the biochemical studies described below.

Rad26 Protein Is a DNA-dependent ATPase—Since Rad26 protein possesses sequence motifs found in proteins that bind and hydrolyze ATP, we examined whether purified Rad26 protein has ATPase activity. To do this, Rad26 protein was incubated with [γ-^{32}P]ATP in the absence of DNA and also in the presence of single-stranded or double-stranded DNA, followed by analyzing the reaction mixtures by thin layer chromatography in PEI-cellulose and autoradiography to determine the level of ATP hydrolysis in each case. As summarized in Table I, Rad26 protein hydrolyzed ATP in the presence of both circular single-stranded DNA and linear double-stranded DNA (Table I), but there was no hydrolysis when the DNA co-factor was omitted. Consistently, single-stranded DNA was more effective than double-stranded DNA by a factor of about 3 in promoting ATP hydrolysis by Rad26 protein (Table I and Fig. 3). Using α-^{32}P-labeled ATP, we determined that ADP and P_i are generated by Rad26. We have also determined that polyribonucleotides including poly(A), poly(U), and yeast total RNA do not promote ATP hydrolysis by Rad26 protein (data not shown). The Rad26 DNA-dependent ATPase activity is expressed over a broad range of pH from 6 to 8.5 and is optimal at pH 7.5 (data not shown), which was used in all the experiments described herein. The DNA-activated ATPase activity observed with the purified Rad26 protein is an intrinsic property of Rad26 because (i) Fraction VII Rad26 protein used in these studies was apparently homogeneous (Fig. 1*C*) and (ii) both of the ssDNA and dsDNA activated ATP hydrolytic activities co-eluted precisely with the Rad26 protein during the final chromatographic

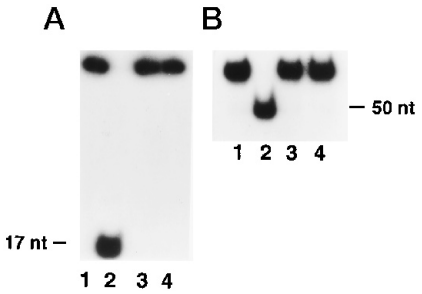

FIG. 4. **Rad26 protein does not unwind a partial DNA duplex or a forked DNA substrate.** The 17-bp partial duplex (*A*) and the forked DNA substrate containing 20-nucleotide-long 3′- and 5′-overhanging tails adjoining a 30-bp duplex region (*B*) were incubated at 36 °C without (*lane 1*) or with 200 ng and 500 ng of Rad26 protein (*lanes 3* and *4*) for 60 min. In *lane 2*, the DNA substrate was boiled for 2 min to release the annealed ^{32}P-labeled DNA fragment.

step in Source Q (Fig. 2; data not shown).

DNA-dependent ATP hydrolysis is proportional to the Rad26 protein amount and increases with the reaction time for at least 60 min (Fig. 3). Under the conditions employed and using 50 ng of Rad26, the k_{cat} for ssDNA-activated ATP hydrolysis is ~100 min^{-1} and that for dsDNA-activated ATP hydrolysis is 33 min^{-1}. In addition to a DNA cofactor, ATP hydrolysis by Rad26 protein also requires Mg^{2+}, which could not be substituted by the same molar concentration of Ca^{2+}, Co^{2+}, Mn^{2+}, or Zn^{2+}.

Since Rad26 protein has a DNA-dependent ATPase activity, we examined whether ATP hydrolysis by Rad26 can be used to unwind duplex DNA. For this purpose, Rad26 protein was incubated in the presence of ATP with M13 ssDNA to which a ^{32}P-labeled 17-base complementary fragment had been annealed (13), or with a ^{32}P-labeled forked helicase substrate which contained 20-nucleotide-long 3′- and 5′-overhanging tails adjacent to a 30-bp duplex region (14). Reaction mixtures were run in a polyacrylamide gel followed by autoradiography to determine whether there was any displacement of the annealed ^{32}P-labeled fragment from the partial duplex or dissociation of the forked substrate, which would be indicative of a DNA unwinding or DNA helicase activity in Rad26 protein. However, we observed no unwinding of either DNA helicase substrate by Rad26 protein (Fig. 4).

Rad26 Protein Does Not Associate with TFIIH—During chromatography on hydroxyapatite (see "Materials and Methods"), by immunoblotting with antibodies against the Rad3, Rad25, TFB1, and SSL1 subunits of TFIIH, we determined that the peak of TFIIH partially overlapped that of Rad26 protein, such that the pool of TFIIH contained ≈10% of the total Rad26 protein. Since the TFB1 protein in the yeast strain used contains an added 6-histidine sequence (10), we could obtain a 50-fold purification of TFIIH by immobilizing it on nickel-nitrilotriacetic acid agarose and eluting it with 100 mM imidazole (11, 12). The molar amount of Rad26 protein in the 100 mM imidazole eluate, representing ≈15% of the total Rad26 protein in the onput (hydroxyapatite pool), was substoichiometric to that of TFIIH. This fraction of Rad26 protein was not physically associated with TFIIH in the 100 mM imidazole eluate as indicated by (i) the lack of co-immunoprecipitation of Rad26 protein with TFIIH carried out under mild conditions, and (ii) a separation of Rad26 protein from TFIIH occurred upon subjecting the 100 mM imidazole eluate to molecular sizing in Sephacryl S300 (data not shown). Thus, our results suggest that Rad26 protein by itself does not have a high affinity for TFIIH.

DISCUSSION

In *Escherichia coli*, the *mfd* gene encodes the transcription-repair coupling factor (15). Like Rad26, Mfd protein is an ATPase, but neither protein appears to have a DNA helicase activity. In contrast to Rad26 (k_{cat} ≈100 min^{-1} with ssDNA as co-factor), Mfd is a much weaker ATPase (k_{cat} ≈3 min^{-1}), and while Rad26 ATPase activity is dependent on DNA, the Mfd ATPase is not affected by DNA (15). Mfd acts in TCR by binding RNA polymerase stalled at the DNA lesion and dissociating the polymerase from DNA in an ATP-dependent reaction. The DNA-bound Mfd then recruits the $UvrA_2B_1$ complex to the damage site *via* its affinity for the UvrA subunit of the excision nuclease (15).

Rad26 protein may function in TCR in a manner reminiscent of Mfd. Rad26 could have a role in the recognition and displacement of RNA polymerase II from the damage site and could also function in the subsequent recruitment of one or more of the integral components of the NER machinery. During TCR, TFIIH, consisting of six different polypeptides (16, 17), may be the NER component that is brought to the damage site by Rad26. In both yeast and humans, TFIIH is essential for the dual incision of UV-damaged DNA (11, 12, 17–19), and our recent studies involving reconstitution of TFIIH from its component subunits suggest that the entire TFIIH may be required in this process (11). The possibility that Rad26 (CSB) interacts with TFIIH is suggested from the observation that mutations in the XPB and XPD subunits of human TFIIH can cause XP as well as CS (reviewed in Ref. 20). The CS mutations in XPB and XPD proteins may reflect a defect in interaction of these mutant TFIIH subunits with CSB, and the CS phenotype associated with these XP mutations may be the result of such an interaction defect. Our observation that Rad26 by itself does not stably associate with TFIIH is not necessarily incompatible with the foregoing suggestions, since Rad26 (CSB) protein may require the yeast CSA counterpart for a stable association with TFIIH, or an interaction of these molecular entities occurs at the damage site.

REFERENCES

1. Mellon, I., Spivak, G., and Hanawalt, P. C. (1987) *Cell* **51,** 241–249
2. Mellon, I., and Hanawalt, P. C. (1989) *Nature* **342,** 95–98
3. van Hoffen, A., Natarajan, A. T., Mayne, L. V., van Zeeland, A. A., Mullenders, L. H. F., and Venema, J. (1993) *Nucleic Acids Res.* **21,** 5890–5895
4. Nance, M. A., and Berry, S. A. (1992) *Am. J. Med. Genet.* **42,** 68–84
5. van Gool, A. J., Verhage, R., Swagemakers, S. M. A., van de Putte, P., Brouwer, J., Troelstra, C., Bootsma, D., and Hoeijmakers, J. H. J. (1994) *EMBO J.* **13,** 5361–5369
6. Johnson, R. E., Henderson, S. T., Petes, T. D., Prakash, S., Bankmann, M., and Prakash, L. (1992) *Mol. Cell. Biol.* **12,** 3807–3818
7. Eisen, J. A., Sweder, K. S., and Hanawalt, P. C. (1995) *Nucleic Acids Res.* **23,** 2715–2723
8. Sayer, M. H., Tschochner, H., and Kornberg, R. D. (1992) *J. Biol. Chem.* **267,** 23376–23382
9. Mott, J. E., Grant, R. A., Ho, Y. S., and Platt, T. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 4350–4354
10. Svejstrup, J. Q., Wang, Z., Feaver, W. J., Wu, X., Bushnell, D. A., Donahue, T. F., Friedberg, E. C., and Kornberg, R. D. (1995) *Cell* **80,** 21–28
11. Sung, P., Guzder, S. N., Prakash, L., and Prakash, S. (1996) *J. Biol. Chem.* **271,** 10821–10826
12. Guzder, S. N., Sung, P., Prakash, L., and Prakash, S. (1996) *J. Biol. Chem.* **271,** 8903–8910
13. Guzder, S. N., Sung, P., Bailly, V., Prakash, L., and Prakash, S. (1994) *Nature* **369,** 578–581
14. Habraken, Y., Sung, P., Prakash, L., and Prakash, S. (1995) *J. Biol. Chem.* **270,** 30194–30198
15. Selby, C. P., and Sancar, A. (1993) *Science* **260,** 53–58
16. Feaver, W. J., Svejstrup, J. Q., Bardwell, L., Bardwell, A. J., Buratowski, S., Gulyas, K. D., Donahue, T. F., Friedberg, E. C., and Kornberg, R. D. (1993) *Cell* **75,** 1379–1387
17. Guzder, S. N., Habraken, Y., Sung, P., Prakash, L., and Prakash, S. (1995) *J. Biol. Chem.* **270,** 12973–12976
18. Mu, D., Park, C.-H., Matsunaga, T., Hsu, D. S., Reardon, J. T., and Sancar, A. (1995) *J. Biol. Chem.* **270,** 2415–2418
19. Mu, D., Hsu, D. S., and Sancar, A. (1996) *J. Biol. Chem.* **271,** 8285–8294
20. Friedberg, E. C., Walker, G. C., and Siede, W. (1995) *DNA Repair and Mutagenesis*, ASM Press, Washington, D. C.