Reconstitution of TFIIH and Requirement of Its DNA Helicase Subunits, Rad3 and Rad25, in the Incision Step of Nucleotide Excision Repair*

(Received for publication, January 3, 1996, and in revised form, March 10, 1996)

Patrick Sung, Sami N. Guzder, Louise Prakash, and Satya Prakash†
From the Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, Texas 77555-1061

Yeast TFIIH is composed of six subunits: Rad3, Rad25, TFB1, SSL1, p55, and p38. In addition to TFIIH, we have purified a subassembly of the factor that lacks Rad3 and Rad25 and which we refer to as TFIIHi. In the in vitro nucleotide excision repair (NER) system that consists entirely of purified proteins, we show that neither TFIIHi nor a mixture of purified Rad3 and Rad25 proteins is active in NER but that the combination of TFIIHi with Rad3 and Rad25 promotes the incision of UV-damaged DNA. These results provide the first evidence for a direct requirement of Rad3, Rad25, and of one or more of the TFIIHi subunits in the incision step of NER. The NER efficacy of TFIIH is greatly diminished or abolished upon substitution of Rad3 with the rad3 Arg-48 mutant protein or Rad25 with the rad25 Arg-392 mutant protein, respectively, thus indicating a role of the Rad3 and Rad25 DNA helicase functions in the incision of damaged DNA. Our results further indicate that the carboxyl-terminal domain kinase (CTD) TFIIK is dispensable for the incision of damaged DNA in vitro. These studies reveal the differential requirement of Rad3 DNA helicase and CTD kinase activities in damage-specific incision versus RNA polymerase II transcription.

†To whom correspondence should be addressed: Sealy Center for Molecular Science, University of Texas Medical Branch, 6104 Medical Research Bldg., 11th and Mechanic Streets, Galveston, TX 77555-1061. Tel.: 409-747-8602; Fax: 409-747-8608; E-mail: sprakash@cms.utmb.edu.

‡This work was supported by National Cancer Institute Grant CA35035 and Department of Energy Grant DEFG03-93 ER61706. 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.

*In NER, the direct requirement for Rad3 and Rad25 in an early step of the repair process is indicated by the extreme sensitivity of various rad3 and rad25 mutants to UV light and to other DNA-damaging agents by the existence of mutants that are only inactivated in the repair function and by the fact that these mutations confer a total defect in the incision of damaged DNA (1, 3, 4, 10). On the other hand, whether the other subunits of TFIIH, TFB1, SSL1, p55, and p38, are also directly required for the incision of UV-damaged DNA has not yet been established. Although UV-sensitive mutations of SSL1 and of TFB1 have been identified, these ssl1 and tfb1 mutations cause conditional lethality, indicating a defect in Pol II transcription, but confer only a low level of UV sensitivity, making it possible that the moderate UV sensitivity represents a side effect engendered by the primary defect in transcription (11). No UV-sensitive mutations of p55 and p38 have yet been reported. Thus, it is unclear whether only the Rad3 and Rad25 proteins participate in the incision of damaged DNA or whether the entire TFIIH is in fact required in this process.

In this work, we purify a subassembly of TFIIH that contains the TFB1, SSL1, p55, and p38 subunits but lacks the Rad3 and Rad25 proteins, which we refer to as TFIIH-incomplete or TFIIHi. The availability of a defined in vitro NER system and of purified Rad3 protein (12), Rad25 protein (4), and TFIIHi (this work) has now enabled us to address whether Rad3 and Rad25 together are sufficient for damage-specific incision to occur or whether the incision reaction in fact also requires the other TFIIHi subunits. Here, we show that in addition to Rad3 and Rad25, the incision of UV-damaged DNA is absolutely dependent upon the presence of TFIIH. These reconstitution studies provide the first direct evidence for the requirement of Rad3, Rad25, and of one or more of the TFIIHi subunits in the incision of damaged DNA.

In addition, we determine whether the DNA helicase activities of Rad3 and Rad25 are essential for the incision step of NER and examine the role of TFIIK in this process. Interestingly, our studies indicate that in contrast to Pol II transcription, where only the Rad25 DNA helicase activity is required (4), the DNA helicase activities of both Rad3 and Rad25 are essential for the incision of damaged DNA. TFIIK is dispensa-
ble for the incision of UV-damaged DNA in vitro, suggesting that association of TFIIK with TFIIH has functional relevance only for Pol II transcription.

**MATERIALS AND METHODS**

Buffers—Buffers A, B, C, and D were as described (13). Buffer E was 20 mM Tris acetate, pH 7.2, containing 20% glycerol, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 0.01% Nonidet P-40 (w/v), and 250 mM potassium acetate. Buffer F was 20 mM Tris acetate, pH 7.7, 20% glycerol, 1 mM DTT, 0.25 mM EDTA, and 0.01% Nonidet P-40. The concentration of potassium acetate (KOAc) added to the various buffers is indicated as a numerical value following the buffer designation; thus A + 100 is buffer A with 100 mM KOAc.

Purification of Different Forms of TFIIH—Extract was prepared from 1.3 kg of yeast strain YPH/TFB1-6HIS with the use of a French press in the presence of protease inhibitors (12) and was clarified by centrifugation (100,000 × g for 90 min). The supernatant obtained (fraction I, 900 ml) was dialyzed overnight against 10 liters of A, diluted with A to give ionic strength equivalent to 100 mM KOAc, and applied onto Bio-Rex 70 (5 × 15 cm; 300 ml total) equilibrated with A + 200. The column was washed with 500 ml of A + 200 and treated with A + 600 mM KOAc to elute bound proteins (fraction II, 150 ml). After being dialyzed against 2 liters of B + 100 overnight, the Bio-Rex 70 protein pool was applied onto DEAE-Sephaloc (2.6 × 14 cm; 75 ml total) equilibrated in B + 100. After washing with 150 ml of B + 200, proteins were eluted with B + 500 to give fraction III (40 ml). The DEAE pool was dialyzed against 1 liter of B + 70 for 12 h and applied onto Bio-Rad HTP hydroxyapatite (1.4 × 6 cm; 12 ml total) equilibrated in C without EDTA and developed with a 160-mI gradient from C to D, collecting 3.2-mI fractions. The HTP column fractions that contained TFIIH were identified by immunoblotting with affinity-purified antibodies specific for the Rad3, Rad25, TFB1, and SSL1 subunits of TFIIH, pooled (fraction IV, 22.4 ml), and dialyzed against 1 liter of E overnight. The dialysate was mixed with 1.2 ml of nickel-nitrilotriacetate-agarose (Qiagen) on a rocking platform for 3 h and then centrifuged to collect the matrix. The nickel matrix was resuspended in 6 ml of E + 10 mM imidazole and transferred into a glass chromatography column with an internal diameter of 0.6 cm. The nickel matrix was washed sequentially with 5 ml each of E + 20 mM imidazole, E + 30 mM imidazole, E + 40 mM imidazole, and E + 100 mM imidazole. The majority of the TFIIH (~75%) was present in the E + 100 mM imidazole wash (fraction V), which was dialyzed against F for 2 h to lower the ionic strength to 100 mM KOAc and chromatographed on Mono S (HR5/5) with a 20-mI gradient from F to F + 550, collecting 0.5-mI fractions. The Mono S fraction that contained the various TFIIH subunits, eluting at ~300 mM KOAc, were identified by immunoblotting and pooled (fraction VI, 2.5 ml). The Mono S pool was fractionated on Mono Q (HR5/5) with a 12-mI gradient of 300-1500 mM KOAc in F, collecting 0.3-mI fractions; TFIIH eluted at ~600 mM KOAc, while TFIIHI eluted at ~1000 mM KOAc.

**RNA Polymerase II Purification**—For the purification of RNA polymerase II, hydroxyapatite fractions 9–12 from TFIIH purification (see above) containing core RNA polymerase II were applied directly onto a Mono Q column (HR5/5), which was developed with a 40-mI gradient of 100-1500 mM KOAc in buffer F. Fractions containing RNA polymerase II, which elutes at ~1,100 mM KOAc, were pooled and concentrated to 2 mg/ml.

**RPB1 Phosphorylation**—Purified RNA polymerase II, 130 ng, was incubated with 125 fmol of TFIIH, TFIIH-TFIIK, or TFIIHi-TFIIK in a final volume of 10 μl of buffer CK (20 mM Na-HEPES, pH 7.3, 8 mM MgSO₄, and 2 mM DTT) containing 2 μCi of [γ-32P]ATP (3,000 Ci/mmol, Amersham Corp.) for 1 h at 25°C. The samples were mixed with an equal volume of SDS sample buffer and run in an 8% polyacrylamide gel, which was dried and subjected to autoradiography.

**NER in Vitro**—The NER reaction was carried out in buffer R (40 mM potassium HEPES, pH 7.9, 120 μg/ml bovine serum albumin, 1.5 mM DTT, 2 mM ATP, and an ATP-regenerating system consisting of 30 mM creatine phosphate, 200 ng of creatine kinase, and 60 mM KOAc and 20 mM KCl due to the addition of the various NER factors) in a final volume of 10 μl, and it contained 100 ng of M13 mp18 DNA (*~90% supercoiled form) that had or had not been irradiated with 400 J/m² UV light, 80 ng of RPA, 20 ng of the Rad3-Rad10 complex, 20 ng of Rad2 protein, 30 ng of Rad4-Rad23 complex, 15 ng of Rad14 protein, and 100 ng of TFIIH. In some cases, instead of TFIIH, 60 ng of TFIIHi, 20 ng of
Reconstitution of TFIIH

RESULTS

Purification of TFIIH and TFIIHi—In the last step of purification in a Mono Q column, TFIIH containing the six core subunits was found in fractions 21–26. Fractions 21–23 also contained three additional polypeptides with molecular sizes of 47, 45, and 33 kDa (Fig. 1A, lane 2); the level of these three proteins in fractions 24–26 was much lower (Fig. 1A, lane 3). These three additional protein species are constituents of the RNA polymerase II CTD kinase, TFIIK, because (i) they had the same molecular sizes described for the three subunits of TFIIK (B), (ii) they eluted in the same position in the Mono Q gradient relative to TFIIH as reported for TFIIK (8), and (iii) they eluted in the same position in the Mono Q gradient relative to TFIIH as reported for TFIIK (8), and (iii) they eluted in the same position in the Mono Q gradient relative to TFIIH as reported for TFIIK (8), and (iii) they eluted in the same position in the Mono Q gradient relative to TFIIH as reported for TFIIK (8), and (iii) they eluted in the same position in the Mono Q gradient relative to TFIIH as reported for TFIIK (8), and (iii) they eluted in the same position in the Mono Q gradient relative to TFIIH as reported for TFIIK (8), and (iii) they eluted in the same position in the Mono Q gradient relative to TFIIH as reported for TFIIK (8), and (iii) they eluted in the same position in the Mono Q gradient relative to TFIIH as reported for TFIIK (8), and (iii) they eluted in the same position in the Mono Q gradient relative to TFIIH as reported for TFIIK (8), and (iii) they eluted in the same position in the Mono Q gradient relative to TFIIH as reported for TFIIK (8), and (iii) they eluted in the same position in the Mono Q gradient relative to TFIIH as reported for TFIIK (8), and (iii) they eluted in the same position in the Mono Q gradient relative to TFIIH as reported for TFIIK (8), and (iii) they eluted in the same position in the Mono Q gradient relative to TFIIH as reported for TFIIK (8), and (iii) they eluted in the same position in the Mono Q gradient relative to TFIIH as reported for TFIIK (8), and (iii) they eluted in the same position in the Mono Q gradient relative to TFIIH as reported for TFIIK (8), and (iii) they eluted in the same position in the Mono Q
Rad25 was evident in these fractions (Fig. 1A, lane 1). By immunoblotting, we established that TFIIH and SSL1 were indeed present in these fractions but that there was neither Rad3 nor Rad25 (Fig. 1B, lane 2). We refer to this TFIIH subassembly as TFIIHi. It should be emphasized that TFIIHi was not an anomaly of this particular protein preparation, as we obtained the same results in three other independent preparations (data not shown). Interestingly, TFIIK also associates with TFIIHi (Fig. 1A, lane 1), and TFIIHi-TFIIK is as active in RBP1 phosphorylation as TFIIH-TFIIK (Fig. 1D, compare lanes 2 and 6).

Incision of UV-damaged DNA Requires the Combination of TFIIHi, Rad3, and Rad25—We have previously purified Rad3 and Rad25 proteins to near homogeneity from yeast strains genetically tailored to overproduce these proteins (4, 12). The availability of TFIIHi has made it possible to define the role of Rad3, Rad25, and TFIIHi in NER. Whereas the incision of DNA damaged by ultraviolet light occurred efficiently when TFIIHi was combined with the remainder of the NER factors (viz. Rad1-Rad10, Rad2, Rad4-Rad23, Rad14, and RPA; Fig. 2A, lane 5), neither TFIIHi nor the mixture of Rad3 and Rad25 proteins, when combined with the same set of NER factors, was active in the NER reaction (Fig. 2A, lanes 7 and 8). Likewise, Rad3 alone, Rad25 alone, the combination of Rad3-TFIIHi, or the combination of Rad25-TFIIHi, when used together with the remainder of the NER factors, also did not promote incision of the UV-damaged DNA (data not shown). Strikingly, when Rad3, Rad25, and TFIIHi were preincubated and then added to the NER reaction, incision of the UV-damaged DNA substrate occurred just as efficiently as when TFIIHi was used (Fig. 2A, lane 9). There was a strict dependence of the incision reaction on ATP, regardless of whether TFIIHi (Fig. 2A, lanes 5 and 6) or the combination of TFIIHi, Rad3, and Rad25 was used (Fig. 2A, lanes 9 and 10). We also purified the excision DNA fragments from the NER reactions and then subjected them to labeling with [α-32P]dideoxy-ATP and calf thymus terminal transferase. Excision DNA fragments were seen when the UV-damaged DNA was incubated with the combination of TFIIH and the remainder of the NER factors (Fig. 2B, lane 4), and generation of these excision DNA fragments was absolutely dependent on ATP (Fig. 2B, lanes 3 and 4). In agreement with the results obtained using the agarose gel assay (Fig. 2A), ATP-dependent dual incision of the UV-damaged DNA was observed when TFIIHi was combined with Rad3-Rad25 (Fig. 2B, lanes 7 and 8) but not when TFIIHi or when the mixture of Rad3-Rad25 was used alone (Fig. 2B, lanes 5 and 6). Taken together, our results indicate that in addition to Rad3 and Rad25, TFIIHi is absolutely required during the ATP-dependent incision phase of NER.

Requirement for Rad3 and Rad25 Helicase Functions—Both Rad3 and Rad25 proteins possess a DNA helicase activity that is fueled by ATP hydrolysis (4, 5). We have previously generated mutations in the highly conserved Walker type A motif (+ UV) were incubated without any NER factor (lanes 1 and 5) and with various combinations of NER factors at 30 °C for 12 min. The reaction mixtures in lanes 2–4 and in lanes 6–9 all contained Rad1-Rad10 complex, Rad2, Rad4-Rad23 complex, Rad14, and RPA. TFIIHi was added to the reaction mixtures in lanes 2–4 and in lanes 6–9, as indicated above the gel. The combinations of Rad3-Rad25 (lanes 2 and 7), Rad3-rad25 Arg-392 (lanes 4 and 9), and rad3 Arg-48-Rad25 (lanes 3 and 8) were used in various reaction mixtures, as indicated. C, UV-irradiated DNA (+ UV) was incubated at 30 °C for 30 min with or without NER factors. The reaction mixtures in lanes 2–4 all contained Rad1-Rad10 complex, Rad2, Rad4-Rad23 complex, Rad14, RPA, and TFIIHi and either the combination of Rad3 and Rad25 (lane 2), rad3 Arg-48 and Rad25 (lane 3), or Rad3 and rad25 Arg-392 (lane 4), as indicated. TFIIHi is abbreviated as IHI in C, rad3 Arg-48 as rad3, and rad25 Arg-392 as rad25 in B and C.
required in NER but is dispensable for RNA polymerase II transcription (14), whereas the Rad25 helicase activity is required in both NER and transcription (2, 4). However, in these genetic studies, it was not possible to address whether Rad3 and Rad25 helicase activities are required for the incision of damaged DNA or for a later stage of repair, such as for the postreplication turnover of the NER protein complex and repair synthesis. Also, because both the Rad3 and Rad25 proteins are essential for Pol II transcription, the possibility existed that the helicase function of these proteins affects the expression of a factor indispensable for incision of UV-damaged DNA or for another phase of the repair process in vivo.

The rad3 Arg-48 mutant allele exhibits negative dominance over the wild type RAD3 gene, as its overexpression renders wild type yeast cells sensitive to UV light (Fig. 3A). Since the rad3 Arg-48 mutation does not affect viability, we could purify the rad3 Arg-48 protein from a rad3a yeast strain (14). To facilitate the purification of rad25 Arg-392 mutant protein, we attached a 6-histidine tag to the amino terminus of the rad25 Arg-392-encoded protein, whose expression in yeast is driven by the PGK promoter in plasmid pR25.21. When we attempted to introduce plasmid pR25.21 into wild type Rad+ yeast cells, we failed to recover any transformant that overexpresses the rad25 Arg-392 mutant protein. However, transformants that overexpress the rad25 Arg-392 mutant protein were obtained at the expected frequency when plasmid pR25.21 was introduced into yeast cells harboring plasmid pR25.22 that overexpresses the rad25Arg392 mutant protein which lacks the 45 carboxyl-terminal amino acids of Rad25 protein is defective in NER (1, 10) but is competent in RNA polymerase II transcription (4). The failure to overexpress the 6-histidine-tagged rad25 Arg-392 mutant protein in Rad+ cells strongly suggests that the rad25 Arg-392 mutant allele also exerts negative dominance over the Rad25 gene. The rad25 Arg-392 mutant protein was purified using a five-step procedure that includes affinity chromatography on nickel-agarose.

We determined whether rad3 Arg-48 and rad25 Arg-392 mutant proteins can function in the NER reaction. As shown in Fig. 3B, when Rad3, Rad25, and TFIIH were mixed with the remainder of the NER factors, >85% of the supercoiled UV-damaged plasmid DNA was converted to the open circular form (lane 7). By contrast, when purified rad25 Arg-392 mutant protein was used instead of wild type Rad25, no incision of the UV-damaged DNA occurred (lane 9). Consistent with the results from the agarose gel assay, there was no excision DNA fragment formed by the rad25 Arg-392 protein as assayed by the 32P-labeling protocol (Fig. 3C, lane 4). We have also tagged the wild type Rad25 protein with the same 6-histidine sequence and overproduced the tagged protein by use of the PGK promoter. The 6-histidine-tagged Rad25 protein, unlike the 6-histidine-tagged rad25 Arg-392 mutant protein, could be overproduced in Rad+ yeast cells. The purified 6-histidine-tagged Rad25 protein has a level of activity comparable with the untagged form in the in vitro NER system (data not shown).

When we substituted wild type Rad3 protein with the rad3 Arg-48 mutant protein in the incision assay, reproducibly a low level of incision activity was observed, as evidenced by the conversion of ~3% of the supercoiled plasmid to the open circular form (Fig. 3B, lane 8). This incision activity is specific for UV damage, because the undamaged plasmid DNA was not acted on by the rad3 Arg-48 mutant protein (Fig. 3B, lane 3). However, we could not detect any excision DNA fragment using the 32P-labeling protocol (Fig. 3C, lane 3) when rad3 Arg-48 protein was used. Since the excision fragments are formed by a dual incision event (15), the failure to detect any excision DNA
fragment with rad3 Arg-48 protein raises the possibility that the small amount of open circular form (Fig. 3B, lane 8) arose by the introduction of one of the two incision nicks that are normally made.

TFIIK Is Dispensable for Incision in Vitro—TFIIH can be purified alone or as a complex with the CTD kinase TFIIK (8) (see also Fig. 1). To investigate whether TFIIK might be important for promoting damage-specific incision, we used the same molar amount of TFIIH that contains either a stoichiometric amount of TFIIK or only a trace of TFIIK (Fig. 1A, lanes 2 and 3) in theNER reaction and assessed the incision rate afforded by the two different forms of TFIIH. As shown in Fig. 4, A and B, there was no detectable difference in the rate of incision of the UV-damaged plasmid with the two different forms of TFIIH. The amount of excision products generated by the TFIIH-TFIIK complex was essentially the same as that obtained with TFIIK containing little TFIIK (Fig. 4C, lanes 2 and 3).

DISCUSSION

For both RAD3 and RAD25, mutations conferring extreme UV sensitivity and a total defect in incision as well as mutations that affect only the DNA repair function or the transcription function have been identified (1, 3, 4, 10). While these and other observations (16) have suggested a direct involvement of Rad3 and Rad25 in incision, it has remained unclear whether the other TFIIH subunits also play a direct role in this process. All the existing tfb1 and ssl1 mutants exhibit a much lower level of UV sensitivity than rad3 and rad25 mutants, and all the UV-sensitive tfb1 and ssl1 mutants also exhibit temperature-sensitive lethality (11), indicative of a transcriptional defect. A reduction in repair synthesis is seen in nuclear extracts prepared from tfb1 and ssl1 mutants, and this could be augmented by the addition of a chromatographic fraction that contained partially purified TFIIH (11). Since the repair synthesis method does not directly measure the incision of damaged DNA but rather measures the level of DNA synthesis presumably tied to incision, it is not clear whether reduced repair synthesis in the tfb1 and ssl1 mutant extracts was due to reduced incision activity or reduced DNA synthesis activity. In addition, since the chromatographic fraction used for complementing the repair synthesis deficiency in the mutant extracts contained, besides TFIIH, other proteins (11), it is possible that the repair synthesis-stimulating activity observed was due to factors other than TFIIH present in the partially purified fraction. It is also important to consider that because nuclear extracts rather than a purified system were used (11), there might conceivably be inhibitory factors present in the extracts that limit the extent of incision and repair synthesis, and stimulation of repair synthesis by an added protein fraction could be due to the removal of the inhibitory factors and may not necessarily indicate a direct involvement of the added protein fraction in incision or repair synthesis. Finally, it remains possible that the transcriptional defect caused by tfb1 and ssl1 mutations results in a lowering of the levels of protein factors involved in some stage of NER and of protein factors that function in other cellular processes. In fact, it has been reported that ssl1 mutants exhibit various ribosomal abnormalities including a reduction in polysemes and an in vitro deficiency in translation (17). Thus, previous results concerning the NER deficiency in tfb1 and ssl1 mutants could reflect either a side effect engendered by the primary defect in transcription or a direct role of TFB1 and SSL1 protein in incision or repair synthesis or an indirect role of TFB1 and SSL1 in helping overcome the effect of inhibitors that might be present in the nuclear extracts used, but for reasons outlined above, they do not distinguish among these possibilities.

Here, using a purified NER system, we show that the combination of RAD3, RAD25, and TFIIH promotes the dual incision of UV-damaged DNA just as efficiently as does core TFIIH. Importantly, omission of any of RAD3, RAD25, and TFIIH results in abolishment of damage-specific incision, providing direct biochemical evidence that NER requires all three purified entities, probably as a reflection that TFIIH in its entirety functions in the incision of UV-damaged DNA. While our results clearly indicate a direct involvement of RAD3, RAD25, and TFIIH in the incision reaction, it remains possible that they also function in postincision reactions such as in the turnover of the incision protein complex and in repair synthesis.

Previous work has indicated a requirement of RAD25 helicase activity in RNA polymerase II transcription initiation (4), but unexpectedly, RAD3 helicase activity is dispensable for transcription (14). By contrast, here we show that both the RAD3 and RAD25 helicase activities are required in the incision phase of NER. Our results are consistent with a model in which DNA unwinding by the combined action of the RAD3 and RAD25 helicase activities creates an unwound “bubble” DNA structure appropriate for dual incision to occur. The observed specificities of the Rad1-Rad10 endonuclease (18) and of the Rad2 endonuclease (19) on model DNA substrates are congruent with nicking of the damaged DNA strand by these nucleases at the 5’- and 3'-side of the damage, respectively. Our data, however, do not exclude the possibility that RAD3 and RAD25 helicase functions are also required in postincision reactions.

While stoichiometric amounts of TFIIK are essential for Pol II transcription (9), our results indicate that TFIIK does not affect the rate of incision of UV-damaged DNA in vitro. From results based on complementation of a rad3 mutant extract using the repair synthesis assay, it has been suggested that TFIIK is dispensable for repair synthesis (20). It remains to be determined whether TFIIK, as a transcription factor, influences the in vivo efficiency of NER by affecting the levels of DNA repair factors.

REFERENCES

1. Prakash, S., Sung, P., and Prakash, L. (1993) Annu. Rev. Genet. 27, 33–70
2. Qi, H., Park, E., Prakash, L., and Prakash, S. (1993) Genes & Dev. 7, 2161–2172
3. Guzder, S. N., Qiu, H., Sommers, C. H., Sung, P., Prakash, L., and Prakash, S. (1994) Nature 367, 91–94
4. Guzder, S. N., Sung, P., Bailly, V., Prakash, L., and Prakash, S. (1994) Nature 369, 578–581
5. Sung, P., Prakash, L., Matson, S. W., and Prakash, S. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 8951–8955
6. Feaver, W. J., Svejstrup, J. Q., Bardwell, L., Bardwell, A. J., Buratowski, S., Gulyas, K. D., Donahue, T. F., and Kornberg, R. D. (1993) Cell 75, 1379–1387
7. Guzder, S. N., Habraken, Y., Sung, P., Prakash, L., and Prakash, S. (1995) J. Biol. Chem. 270, 12973–12976
8. Feaver, W. J., Svejstrup, J. Q., Henry, N. L., and Kornberg, R. D. (1994) Cell 79, 1103–1109
9. Svejstrup, J. Q., Feaver, W. J., LaPointe, J., and Kornberg, R. D. (1994) J. Biol. Chem. 269, 28044–28048
10. Sung, P., Prakash, L., and Hanawalt, P. C. (1994) J. Biol. Chem. 269, 1852–1857
11. Wang, Z., Buratowski, S., Svejstrup, J. Q., Feaver, W. J., Xu, X., Kornberg, R. D., Donahue, T. F., and Kornberg, E. C. (1995) Mol. Cell. Biol. 15, 2289–2293
12. Sun, P., Prakash, L., Weber, S., and Prakash, S. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 6045–6049
13. Sayre, M. H., Tischchner, H., and Kornberg, R. D. (2002) J. Biol. Chem. 267, 23376–23382
14. Sun, P., Higgins, D., Prakash, L., and Prakash, S. (1988) EMBO J. 7, 3263–3269
15. Huang, J. S., Svoboda, D. L., Reardon, J. T., and Sancar, A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 3664–3668
16. Sung, P., Watkins, J. F., Prakash, L., and Prakash, S. (1994) J. Biol. Chem. 269, 8033–8038
17. Yoon, H., Miller, S. P., Patrich, E. K., and Donahue, T. F. (1992) Genes & Dev. 6, 2463–2477
18. Bardwell, A. J., Bardwell, L., Tomkinson, A. E., and Friedberg, E. C. (1994) Science 265, 2082–2085
19. Habraken, Y., Sung, P., Prakash, L., and Prakash, S. (1995) J. Biol. Chem. 270, 30194–30198
20. Svejstrup, J. Q., Wang, Z., Feaver, W. J., Xu, X., Bushnell, D. A., Donahue, T. F., and Kornberg, E. C. (1995) Cell 80, 21–28
Reconstitution of TFIIH and Requirement of Its DNA Helicase Subunits, Rad3 and Rad25, in the Incision Step of Nucleotide Excision Repair
Patrick Sung, Sami N. Guzder, Louise Prakash and Satya Prakash

J. Biol. Chem. 1996, 271:10821-10826.
doi: 10.1074/jbc.271.18.10821

Access the most updated version of this article at http://www.jbc.org/content/271/18/10821

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 20 references, 13 of which can be accessed free at http://www.jbc.org/content/271/18/10821.full.html#ref-list-1