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# Human Transcription-Repair Coupling Factor CSB/ERCC6 Is a DNA-stimulated ATPase but Is Not a Helicase and Does Not Disrupt the Ternary Transcription Complex of Stalled RNA Polymerase II*

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**Transcription is coupled to repair in *Escherichia coli* and in humans. Proteins encoded by the *mfd* gene in *E. coli* and by the *ERCC6/CSB* gene in humans, both of which possess the so-called helicase motifs, are required for the coupling reaction. It has been shown that the Mfd protein is an ATPase but not a helicase and accomplishes coupling, in part, by disrupting the ternary complex of *E. coli* RNA polymerase stalled at the site of DNA damage. In this study we overproduced the human CSB protein using the baculovirus vector and purified and characterized the recombinant protein. CSB has an ATPase activity that is stimulated strongly by DNA; however, it neither acts as a helicase nor does it dissociate stalled RNA polymerase II, suggesting a coupling mechanism in humans different from that in prokaryotes. CSB is a DNA-binding protein, and it also binds to XPA, TFIIH, and the p34 subunit of TFIIE. These interactions are likely to play a role in recruiting repair proteins to ternary complexes formed at damage sites.**

Transcribed DNA is repaired preferentially in both mammalian cells (1, 2) and *E. coli* (3). The preferential repair, to a large extent, is due to the high rate of repair of the template (transcribed) strand relative to the coding (nontranscribed) strand and transcriptionally inactive DNA (2). Mutations in the *mfd* gene in *E. coli* (4) and in the *CSA*[1] and *CSB* genes in humans (5) abolish preferential repair. The mechanism of action of the Mfd protein and of transcription-repair coupling in *E. coli* has been elucidated in considerable detail (6–8). However, attempts at understanding transcription-coupled repair in humans have been frustrated by the lack of an efficient system for transcription by RNA polymerase II *in vitro* and by the lack of purified CSA and CSB proteins, which are known to be essential for coupling[2] (9, 10).

The *CSA* (11) and *CSB* (12) genes have been cloned and sequenced. CSA is a protein of 44 kDa and belongs in the "WD-repeat" family of proteins (11, 13). The CSB protein has a predicted size of 168 kDa, contains helicase motifs, and belongs in the SWI/SNF family of proteins (12, 14, 15). Because of size, sequence, and apparent functional similarities between CSB and Mfd, it has been generally assumed that CSB may play a role similar to that of Mfd (4, 16, 17). Hence the purification and characterization of CSB were considered essential for understanding the phenomenon of transcription-coupled repair. In this paper we describe the purification and characterization of recombinant human CSB protein. ATPase activity was detected with CSB protein, and this activity was characterized with regard to allosteric effectors. Although our CSB was active as indicated by its DNA binding and ATPase activities, it did not remove a stalled RNA Pol II. While this work was in progress Guzder *et al.* (18) reported that the yeast CSB homolog Rad26 protein is a DNA-dependent ATPase with no detectable helicase activity.

## EXPERIMENTAL PROCEDURES

*Plasmid Constructs*—Plasmids for expression of TFIIE (19–21) and TFIIF (22, 23) have been described previously. The *ERCC6* gene cloned into the vector Bluescript and designated pcBLsSE6 (12) was provided by Dr. J. H. J. Hoeijmakers (Erasmus University). A *Hpa*I site was placed immediately upstream of the ATG initiation codon (5′-AGAATG to 5′-AGAGTTAACATG) by site-directed mutagenesis (24) to facilitate subcloning; this construct was designated pE6Hpa. To generate a template for *in vitro* transcription-translation, the *CSB* gene was subcloned as a *Hpa*I-*Xba*I fragment from pE6Hpa into the *Sma*I-*Xba*I site of pIBI24 (VWR Scientific, Inc.), which places the gene downstream from the T7 promoter; this construct was designated pIE6. A construct intended to produce a full-length CSB-MBP fusion protein was made by inserting the *Hpa*I-*Xba*I fragment from pE6Hpa into the *Xmn*I-*Xba*I site of pMal-c2 (New England Biolabs) and was designated pMALE6. A construct for producing a fusion of GST protein with amino acids 528-1222 of CSB was made by subcloning the internal *Eco*RI fragment of the *CSB* gene into the *Eco*RI site of pGEX3x (Pharmacia Biotech Inc.) and was designated pGSTE65. The same fragment of *CSB* was subcloned into the *Eco*RI site of pMal-c2 to obtain pMALE65.

The *CSA* gene was subcloned from a human cDNA library by PCR using primers 5′-AGAATTCCCGGGATGCTGGGGTTTTTGTCCGCACGCCAAACGGGTTT and 5′-GTGAGATCTAGATATTCATCCTTCTTCATCACTGCTGCTCCAGGCATC. These primers were made based upon the published sequence of the *CSA* gene (11) and place *Eco*RI and *Sma*I sites at the 5′ border and *Bgl*II and *Xba*I sites at the 3′ border of *CSA*. The PCR product was subcloned into the pCRII vector (Invitrogen) to give pCRCSA2. The *Sma*I-*Spe*I fragment of pCRCSA2 containing the entire *CSA* gene was subcloned into the *Xmn*I-*Xba*I site of pMal-c2 to give pMALCSA.

The p2Bac vector (Invitrogen) was used to generate constructs for expression of full-length CS proteins in Sf21 cells. The *Sma*I-*Hin*dIII fragment of pCRCSA2 was inserted into the *Pvu*II-*Hin*dIII site of p2Bac to give pBacCSA, in which the *CSA* gene is downstream from the

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[1] The abbreviations used are: CSA, protein complementing deficiencies in Cockayne's syndrome group A; CSB, protein complementing deficiencies in Cockayne's syndrome group B; CFE, cell-free extract; GST, glutathione *S*-transferase; MBP, maltose-binding protein; RNA Pol, RNA polymerase; TF, transcription factor; TRCF, transcription-repair coupling factor; bp, base pair(s); ATPγS, adenosine 5′-*O*-(thiotriphosphate).

[2] C. P. Selby, R. Drapkin, D. Reinberg, and A. Sancar, unpublished results.

polyhedrin promoter. To construct a plasmid expressing both *CSA* and *CSB* genes, the *Hpa*I-*Xba*I fragment of pE6Hpa was inserted into the *Sma*I-*Xba*I site of pBacCSA to obtain pBacCSAB, in which the *CSB* gene is downstream from the p10 promoter. The Baculo Gold transfection kit (Pharmingen) was used to harvest recombinant viral stocks expressing the *CS* genes.

*Antibody Preparation*—GST and MBP fusion proteins of CSA and CSB were made in *E. coli* DR153 (*recA3* Δ*uvrB*) and purified with glutathione-Sepharose or amylose-agarose affinity resins (7). The CSA-MBP protein was further purified using columns of DEAE-Sepharose, Affi-Gel blue, and SP-Sepharose. To make anti-CSB antibodies, the GST-CSB(528–1222) protein was first purified by glutathione-Sepharose affinity chromatography and then by SDS-polyacrylamide gel electrophoresis. The band containing the fusion protein was excised from the gel, and the gel slice was ground into small pieces and injected into a rabbit. To make an antibody affinity column, MBP-CSB(528–1222) was covalently linked to SulfoLink coupling gel using the Pierce antibody immobilization kit. The immune serum was passed through the column, and the anti-CSB antibodies were eluted from the column with low pH into tubes containing 1 M Tris·HCl, pH 8.0, and then dialyzed against and stored in Manley buffer (25 mM HEPES pH 7.9, 12 mM $MgCl_2$, 0.5 mM EDTA, 2 mM dithiothreitol, and 17% glycerol) with 100 mM KCl.

During the purification of the MBP-CSA protein, approximately the first half of the fusion protein eluted from the amylose resin as precipitate. This insoluble material was injected into a rabbit. An affinity resin for purifying anti-CSA antibodies from the immune serum was made by linking MBP-CSA to Affi-Gel-10 (Bio-Rad) as described by the manufacturer.

*Purification of CSB*—Full-length CSB protein was purified from Sf21 cells using both Coomassie Blue staining and immunoblotting to locate the fractions containing the protein. CSA was detected by immunoblotting with anti-CSA antibodies. Approximately 2 liters of Sf21 cells at approximately $10^6$ cells/ml were infected with our recombinant baculovirus, which expresses CSA and CSB, and incubated at 26 °C for 42–48 h. The cells were collected by centrifugation, and CFE was prepared by the procedure of Manley *et al.* (25). Most of the CSB and only a small fraction of CSA were soluble. The CFE was passed through a DE52 column equilibrated with Manley buffer plus 100 mM KCl. The flow-through that contained both proteins was applied to a SP-Sepharose column equilibrated with Manley buffer plus 100 mM KCl. CSA was in the flow-through of this column, but CSB was bound quantitatively. The column was washed with 0.2 M KCl, and then a linear gradient of 0.2–1.0 M KCl in Manley buffer was applied. CSB eluted at about 0.4 M KCl. Fractions containing CSB were pooled and diluted to a final concentration of 0.1 M KCl in Manley buffer plus 0.01% Nonidet P-40. The sample was applied to a single-stranded DNA cellulose column equilibrated in the same buffer. The bound proteins were eluted with a linear gradient of 0.1–1.0 M KCl in Manley buffer plus 0.01% Nonidet P-40. CSB protein eluted at about 0.25 M KCl. Fractions containing CSB were pooled and dialyzed against Buffer C (20 mM Tris·HCl, pH 7.9, 0.1 mM EDTA, 19% glycerol, and 10 mM 2-mercaptoethanol) plus 0.3 M KCl. Then, ammonium sulfate was added to 1.2 M, and the precipitate was removed by centrifugation. The supernatant was applied to a phenyl Superose 5/5 fast protein liquid chromatography column. The column was washed with loading buffer, and bound proteins were eluted with a linear gradient of 1.2 to 0.0 M ammonium sulfate in Buffer C. The CSB eluted at about 0.1 M ammonium sulfate. The combined fractions were dialyzed against Buffer C with 0.1 M KCl plus 0.01% Nonidet P-40 and then applied to a Mono Q 5/5 fast protein liquid chromatography column. The column was washed, and then a gradient of 0.1–1.0 M KCl in Buffer C plus 0.01% Nonidet P-40 was applied. CSB eluted at about 0.35 M KCl. Fractions were pooled, diluted to 0.12 M KCl in Buffer C plus 0.01% Nonidet P-40, and applied to a Mono S 5/5 column. A linear gradient of 0.1–0.8 M KCl in Buffer C plus 0.01% Nonidet P-40 was applied, and CSB eluted at about 0.45 M KCl. The pooled fractions were diluted to 0.1 M KCl in Buffer C plus 0.01% Nonidet P-40 and applied to a 0.4-ml column of Q-Sepharose. The protein bound quantitatively and was eluted with 0.55 M KCl in Buffer C plus 0.01% Nonidet P-40. Extract preparation and all chromatographic steps except the last were performed in the presence of 1 μg/ml aprotinin and 1 μg/ml pepstatin A (Boehringer Mannheim).

*Transcription Proteins*—RNA polymerase II was purified using DE52, Mono Q, hydroxyapatite, and S300 columns. yTBP and recombinant TFIIB were gifts from Dr. D. Reinberg (Robert Wood Johnson Medical School, Piscataway, NJ). TFIIE, in which the p56 subunit was fused to MBP, was purified by amylose affinity chromatography. Recombinant TFIIF subunits were purified from *E. coli* and renatured as described (22, 23). TFIIH was purified from HeLa CFE using DE52, Affi-Gel blue, SP-Sepharose, S300, and Mono S columns. The *E. coli* Mfd protein was purified as described previously (6); *E. coli* DNA helicase II was the gift of Dr. S. Matson (University of North Carolina).

*Methods—In vitro* transcription-translation was performed with the TNT-coupled reticulocyte lysate system from Promega.

ATPase, helicase, and DNA-binding assays were conducted in reaction buffer that contained 40 mM HEPES, pH 7.9, 60 mM KCl, 8 mM $MgCl_2$, 2.4 mM dithiothreitol, 1.1 mM EDTA, and 6.8% glycerol. ATPase assays were performed as described (26) except that the reaction conditions were not restricted to hydrolysis of 15% of the substrate. For antibody inhibition experiments, antibodies and the ATPases were mixed in the reaction buffer and incubated on ice for 1 h before mixing with ATP substrate. For gel shift analysis, DNA and protein were incubated in 10 μl of reaction buffer at 30 °C for 30 min and then separated on a 5% native polyacrylamide gel run at room temperature at 60 V in 45 mM Tris borate and 0.5 mM EDTA, pH 8.3. Helicase assay was conducted as described by Matson *et al.* (27), and the protein pull-down assay was as described previously (7).

To examine ternary complex stability, we used template pMLU112, which contains a "U-less cassette" as the first 112 bp of the transcription unit (28). The plasmid was transcribed with a reconstituted human RNA Pol II transcription system in the absence of UTP, producing a stable, stalled RNA Pol II at the end of the cassette. The RNA was labeled using α-$^{32}$P-CTP. *Pvu*II restriction endonuclease was then added, and the mixture was incubated for 20 min at 32 °C to cleave the DNA downstream from the stalled polymerase. Then, CS proteins were added to the stalled polymerase complex in 31 mM HEPES, 6 mM Tris·HCl, pH 7.9, 120 mM KCl, 3.8 mM $MgCl_2$, 2.1 mM EDTA, 3% polyethylene glycol, 6.8% glycerol, and 208 μM each of ATP and GTP, and 2 μM CTP. After 45 min at 32 °C the transcript was chased by adding 400 μM UTP and 800 μM of cold CTP and incubating for 12 min. RNA products were resolved on a 5% denaturing polyacrylamide gel.

## RESULTS

*Preparation of Anti-CSB and Anti-CSA Antibodies*—To assist in characterizing the CSB protein we prepared antibodies against the putative catalytic region of the protein. This was done by using as antigen a peptide containing the helicase motifs region of CSB, amino acids 528-1222, fused to GST. Immune serum was purified using an affinity column ligand consisting of MBP fused to amino acids 528-1222 of CSB. Our antibody was capable of detecting native CSB among the proteins in CFE by Western analysis, although there was considerable cross-reactivity (see below). The band identified as CSB was absent when CS-B CFE was examined and when normal human CFE was examined using preimmune serum. CSB made by *in vitro* transcription-translation and by expression in Sf21 cells was also detected with our antibody. Most importantly, the CSB protein expressed in Sf21 cells co-migrated with native protein. In contrast, our anti-CSA antibody was of poor quality in that Western analysis of CFE did not permit identification of CSA protein among overwhelming cross-reactivity. However, the anti-CSA antibodies did allow detection of CSA overproduction in bacteria and Sf21 cells by immunoblotting (data not shown).

*Purification of CSB*—Several approaches were taken to generate purified CSB protein for our investigations. The full-length protein was not appreciably expressed in *E. coli* even when fused to GST or MBP. Therefore we attempted to purify CSB from HeLa CFE. However, after three or four chromatographic steps, the protein consistently underwent extensive degradation. Next, we attempted to overproduce the protein using the baculovirus/Sf21 insect cell system. CSB protein was greatly overproduced 24–48 h after infection of Sf21 cells with our recombinant baculovirus. We were able to obtain about 30 μg of CSB of high purity from $2 \times 10^9$ cells after a seven-column purification procedure. As seen in Fig. 1 the final step contained a single band that was reactive with anti-CSB antibodies. Even though the expression vector contained both *CSA* and *CSB* genes, the majority of CSA protein was insoluble, and the soluble fraction separated from the CSB protein completely

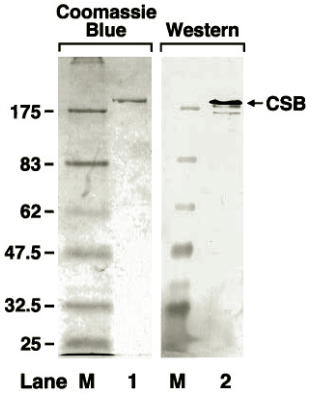


FIG. 1. **CSB protein purified from Sf21 cells.** A sample from the last chromatographic step was resolved by SDS-polyacrylamide gel electrophoresis, and proteins were analyzed by Coomassie Blue staining in *lane 1* and immunoblotting in *lane 2*. CSB migrates slightly slower than the 175-kDa prestained marker (MBP-β-galactosidase) even though its size, based upon its sequence, is 168 kDa (12). The native human protein and the CSB from Sf21 cells had the same mobility.

TABLE I
*DNA-stimulated ATPase activity of CSB*[a]

CSB was assayed for ATPase in the presence and absence of 11 nM DNA cofactor.

| Addition | $k_{cat}$ ($min^{-1}$) |
|---|---|
| None | 3.4 |
| ds DNA | 45 |
| ss DNA | 53 |

[a] ds, double-stranded; ss, single-stranded.

in the second chromatographic step, suggesting that CSA and CSB do not make a stable complex (data not shown). van Gool (10) also found that CSA and CSB proteins readily separate upon fractionation of cell extract, although it has been reported that CSA and CSB can interact as revealed by immunoprecipitation assays (11).

*Functional Analysis of CSB*—The prototype transcription-repair coupling factor (TRCF) encoded by the *E. coli mfd* gene possesses the so-called helicase motifs and is a DNA-independent ATPase and a DNA-binding protein with no detectable helicase activity (6, 8). Because the putative human coupling factor also possesses the helicase motifs, we decided to test the purified CSB for ATPase, DNA-binding, and helicase activities.

CSB exhibited ATPase activity with a $k_{cat}$ ~3 $min^{-1}$, which increased to 45–53 $min^{-1}$ in the presence of single- or double-stranded DNA (Table I). The ATPase activity is intrinsic to CSB and is not due to a contaminating ATPase as evidenced by the fact that anti-CSB antibodies specifically inhibited the ATPase activity of CSB without affecting ATP hydrolysis by Mfd or UvrD (helicase II), both of which also have helicase motifs (Fig. 2). CSA-MBP did not exhibit ATPase activity and did not affect the ATPase activity of CSB.

DNA binding was examined by gel shift assay using a 90-bp DNA fragment. The results in Fig. 3 show that unlike Mfd protein, CSB readily bound to DNA in the absence of nucleotide. At low concentrations of CSB, a single retarded band was detected, indicated as *B1* in Fig. 3. At higher CSB concentrations, there were apparently multiple CSB proteins bound per duplex as indicated by the appearance of discreet lower mobility bands, labeled *B2– B4*. The presence of ATPγS increased the amount of DNA bound by CSB. ATP and, to a lesser extent, ATPγS decreased the number of CSB proteins/duplex. An alternative explanation for *B2–B4* is the formation of intermolecular aggregates of *B1*.

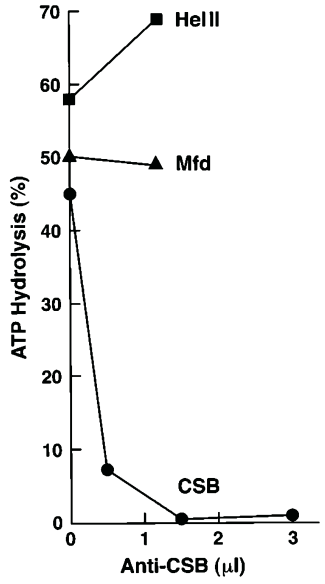


FIG. 2. **ATPase activity of CSB is inhibited by anti-CSB antibody.** CSB (100 ng), Mfd (1.2 μg), and DNA helicase II (87 ng) were incubated with and without anti-CSB antibody, and then the amount of ATP hydrolyzed by the proteins was measured after a 2-h incubation. Single-stranded M13 DNA (150 ng) was included in the hydrolysis reactions of CSB and DNA helicase II. The background level of hydrolysis observed in the absence of protein (8–11%) has been subtracted from the values shown. The antibody preparation did not hydrolyze ATP. The DNA-independent ATPase activity of CSB was also inhibited by anti-CSB antibodies (data not shown).

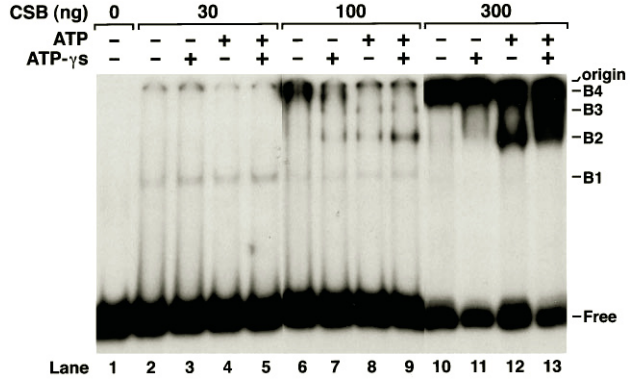


FIG. 3. **CSB DNA binding detected by gel shift assay.** Various amounts of CSB were incubated with a 90-bp DNA duplex in the presence of combinations of ATP and ATPγS, as indicated in the figure. Free and protein-bound DNA were separated on a 5% gel. CSB bound even in the absence of nucleotide, and binding was stimulated by ATPγS. Free DNA (*F*) is indicated as are four discrete bands of protein-bound DNA (*B1–B4*).

Helicase activity was tested using as substrate a labeled 24-mer annealed to a circular, single-stranded DNA molecule. Even though CSB binds to DNA and exhibits DNA-stimulated ATPase activity, Fig. 4 shows that CSB cannot displace the 24-mer, whereas helicase II releases it quantitatively. CSA-MBP did not exhibit helicase activity and did not confer helicase activity to CSB. Thus we conclude that CSB, like its *E. coli* and *Saccharomyces cerevisiae* counterparts Mfd (6) and Rad 26 (18), respectively, is a protein with helicase motifs but no helicase activity.

*Interactions of CSB with Transcription and Repair Proteins*—The Mfd protein brings about transcription-coupled repair by interacting with RNA Pol stalled at a lesion and then removing the polymerase and directing the damage recognition subunit of the bacterial excision nuclease to the lesion (6–8). Similarly, it has been found that CSB interacts with CSA and

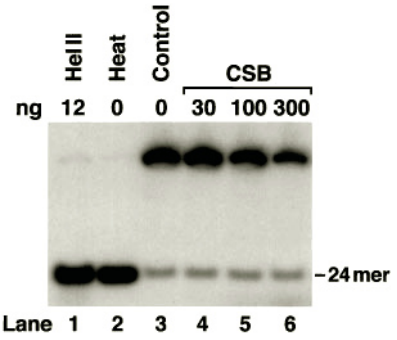


FIG. 4. **CSB lacks helicase activity.** Various amounts of CSB were incubated with single-stranded pPU192 circular DNA annealed to a $^{32}$P-labeled 24-mer. Substrate and product were separated with a 10% polyacrylamide gel. The mobility of the 24-mer released by either heat treatment (*lane 2*, 95° C, 5 min) or DNA helicase II (*Hel II*) is indicated.

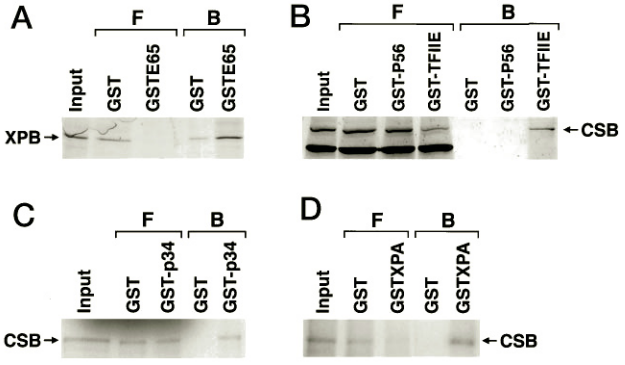


FIG. 5. **CSB can interact with TFIIH, TFIIE, and XPA.** Pull-down assays were performed to examine the possible protein-protein interactions of CSB. For *panel A*, we used GSTE65 in which GST was fused to amino acids 528-1222 of CSB. GST and GSTE65 were coupled to glutathione-Sepharose resin, and then the resins were incubated with HeLa CFE. After incubation and pelleting, the free proteins (*F*) in the supernatant were recovered, and the resin-bound proteins (*B*) were recovered after washing the resin. Fractions were resolved by SDS-polyacrylamide gel electrophoresis, and a Western blot of the gel hybridized with anti-XPB antibody is shown. It is possible that the TFIIH-CSB interaction shown in *panel A* is mediated by CSA because CSA has been reported to interact with both CSB and TFIIH (11). *Panels B* and *C* show experiments with the p34 and p56 subunits of TFIIE. In *panel B*, three resins were used: GST, GST-p56, and GST-p56 bound to p34 (TFIIE). These resins were incubated with HeLa CFE, and free and bound fractions were examined by Western blot using anti-CSB antibodies. There is a strongly cross-reacting, unidentified protein of about 145 kDa in size that did not bind to any of the resins. CSB bound to the resin only when p34 was present. In *panel C*, we used glutathione-Sepharose bound to either GST or GST-p34. $^{35}$S-labeled CSB protein made with an *in vitro* transcription-translation system was applied to the resins, and the presence of CSB in free and bound fractions was detected with the autoradiograph shown. In *panel D*, $^{35}$S-labeled CSB was applied to either GST or GST-XPA resins, and the autoradiograph shows the presence of CSB in the free and bound fractions.

with the XPG protein (29) and that CSA interacts with the p44 subunit of TFIIH (11), suggesting that a related mechanism may be operative in humans. We wished to know if CSB exhibited binding and functional properties similar to those of Mfd, in particular binding to the damage recognition protein and disruption of the stalled RNA Pol complex.

First, we have found that CSB interacts with TFIIH as shown in Fig. 5*A*. Furthermore, because the fusion protein used in this assay comprised only amino acids 528-1222 of CSB, our result narrows down the interaction site to this domain. TFIIH is both a transcription and an excision repair factor; in transcription, it facilitates initiation of RNA synthesis in conjunction with TFIIE (30, 31). However, in transcription-independent excision repair TFIIH functions independently of TFIIE,

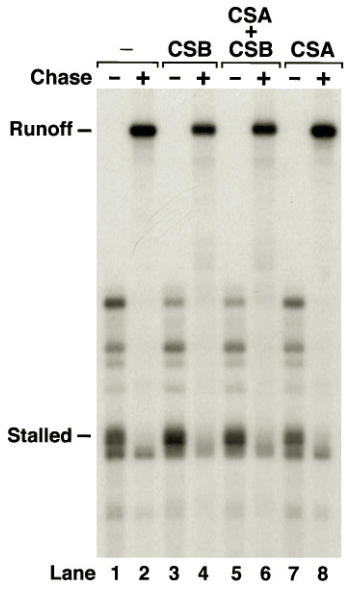


FIG. 6. **CSB does not remove stalled RNA Pol II from the template.** Radiolabeled transcripts were made in the absence of UTP using pMLU112 as template with a reconstituted RNA Pol II transcription system. The principal product (labeled *Stalled*) is a 112-nucleotide transcript formed when RNA Pol II stalls at the end of the U-less cassette of pMLU112. These complexes were then digested with *Pvu*II, which cleaves downstream of the stall site, and then incubated with combinations of CSB and CSA-MBP proteins. Then, in the *even-numbered lanes*, reactions were chased by incubation with UTP to allow transcription to the end of the template plus cold CTP to dilute the radiolabel, and the products were separated on the 5% sequencing gel shown. *Runoff* indicates the products of transcription to the end of the templates. The bands longer than stalled transcript in the absence of chase are presumably due to minor contamination of one or more of the three rNTPs with UTP.

and no effect of TFIIE on this mode of repair could be detected (32). However, because CSB is presumed to act at the transcription/repair interface, we considered the possibility that it may interact with TFIIE. Interestingly, we found that CSB binds to TFIIE, and the binding is mediated by the p34 subunit (Fig. 5, *B* and *C*). Finally, because a working model for coupling presupposes the recruitment of damage recognition proteins by CSB, we tested for binding to XPA. Fig. 5*D* shows that XPA specifically binds to CSB, consistent with CSB being a bridge between transcription and repair proteins.

*Effect of CSB on Stalled RNA Pol II*—A striking feature of transcription-repair coupling in *E. coli* is the removal of stalled RNA Pol by the Mfd protein (6). Mfd removes RNA Pol stalled at a lesion, stalled by nucleotide starvation, or blocked by a DNA-bound protein (8). We tested CSB for an analogous activity. RNA Pol II was stalled 112 nucleotides into a U-less cassette by omission of UTP, and then the ternary complex was incubated with CS proteins. With this experimental setup, the subsequent addition of UTP leads to elongation of RNA to the end of the template (Fig. 6, *lanes 1* and *2*) in the absence of other factors. We found that incubation with CSB and CSA by themselves or in combination does not interfere with the subsequent formation of runoff transcripts (Fig. 6, *lanes 3, 5,* and *7 versus lanes 4, 6,* and *8*). Thus, CSB clearly does not release stalled RNA Pol II.

## DISCUSSION

Transcription-repair coupling occurs in many organisms, including prokaryotes and eukaryotes. Initial biochemical investigations of this phenomenon revealed that in *E. coli*, a TRCF encoded by the *mfd* gene was required and sufficient to couple the transcription and repair processes. It did so by removing

TABLE II
*Biochemical properties of TRCFs*

| | Mfd | | Rad26[c] (*S. cerevisiae*) | CSB[d] (*Homo sapiens*) |
|---|---|---|---|---|
| | *E. coli*[a] | *B. subtilis*[b] | | |
| Size (kDa) | 130 | 133 | 127[e] | 168[f] |
| Helicase motifs | + | + | +[e] | +[f] |
| Helicase activity | − | − | − | − |
| DNA binding | | | | |
| − ATPγS | − | − | ? | + |
| + ATPγS | + | + | + | + |
| ATPase, $k_{cat}$ ($min^{-1}$) | | | | |
| −DNA | 3 | 2 | 0 | 3 |
| +DNA | 3 | 2 | 33–100 | 45–53 |
| Dissociate stalled RNA Pol | + | + | ? | − |
| Protein-protein interactions | | | | |
| Transcription proteins | RNA Pol | RNA Pol | ? | TFIIH, TFIIE |
| Repair proteins | UvrA | ? | ? | TFIIH, XPG,[g] XPA |

[a] Selby and Sancar (6–8).
[b] Ayora *et al.* (33). A RNA Pol-Mfd interaction is assumed because Mfd removes RNA Pol stalled at a lesion.
[c] Guzder *et al.* (18). DNA binding is assumed because DNA is required for ATPase activity.
[d] This study.
[e] van Gool *et al.* (34).
[f] Troelstra *et al.* (12).
[g] Iyer *et al.* (29).

the stalled RNA Pol and delivering the repair proteins to the lesion at an accelerated rate compared to lesions in which polymerase was not stalled (6). A protein from *Bacillus subtilis* was found to have similar structure and function, notably, a region containing the seven so-called helicase motifs, and the ability to remove a stalled RNA Pol (33). Based on genetic data and *in vivo* studies, humans require two proteins for transcription-repair coupling, the CSA and CSB proteins (5). CSA is a member of the "WD-repeat" family of proteins, which so far have been found to have structural and regulatory roles but no enzymatic activity (11, 13). CSB (12) and its yeast homolog, Rad26 (34), are clearly more similar to the prokaryotic coupling factors than CSA. The properties of these four TRCFs are summarized in Table II.

Aside from having identical cellular functions, the TRCFs in Table II are similar in being relatively large and possessing the helicase motifs. Although all four proteins can bind to DNA and hydrolyze ATP, none exhibit detectable helicase activity. This property may not be unique to this class of enzymes because two other proteins with helicase motifs, SNF2 (35) and Rad5 (36), have also been reported to lack helicase activity.

In those cases in which protein-protein interactions have been investigated, TRCFs have been found to associate with various transcription and nucleotide excision repair proteins (Table II). Although these interactions are valuable toward devising models and working hypotheses, with the human proteins they have been largely identified by assays designed to detect weak protein-protein interactions. The prokaryotic interactions listed are more likely to reflect functional interactions because they have been corroborated by independent approaches. However, in no case have transcription or repair proteins been found to form a tight complex with a TRCF.

Previous studies have indicated that DNA binding and ATP hydrolysis by *E. coli* Mfd protein are involved in dissociation of stalled RNA Pol. It was inferred that the Mfd protein must bind nucleotide to bind to DNA and that hydrolysis of nucleotide is associated with dissociation of Mfd from DNA. It was proposed that, for dissociation of stalled RNA Pol, first Mfd-ATP binds to the ternary complex, both to the DNA and to RNA Pol. Then, hydrolysis of ATP brings about the release of Mfd from DNA together with the associated RNA Pol (7). CSB is strikingly different from Mfd in both DNA binding (ATPγS-independent) and ATP hydrolysis (strongly stimulated by DNA). Thus it is not surprising that CSB, in contrast to Mfd, does not function to remove its cognate stalled RNA Pol from DNA.

A most interesting aspect of transcription-repair coupling is the reaction mechanisms and in particular the disposition of the RNA Pol stalled at the lesion. In *E. coli*, RNA Pol stalled at the lesion inhibits repair, and Mfd removes it. Mfd also binds to the repair enzyme, which is delivered to the transcription-blocking lesion at an accelerated rate (6, 37). In humans, RNA Pol stalled at a lesion does not inhibit repair,[2] and CSB does not remove the stalled polymerase. CSB does, however, bind to repair proteins and may, like Mfd, function to deliver them to the transcription-blocking lesion at an accelerated rate. Such a mechanism, in which RNA Pol is not removed from the template during repair, could permit subsequent transcription by the polymerase without reinitiation at the promoter, as described by Hanawalt (16).

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