Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 11574–11578, December 1991
Biochemistry

Escherichia coli mfd mutant deficient in "mutation frequency decline" lacks strand-specific repair: *In vitro* complementation with purified coupling factor

(transcription-repair coupling/UV mutagenesis/SOS response/nonsense suppressors)

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ABSTRACT **Mutation frequency decline (MFD) is the rapid decrease in the frequency of certain induced nonsense suppressor mutations occurring when protein synthesis is transiently inhibited immediately after irradiation. MFD is abolished by mutations in the *uvrA*, *-B*, or *-C* genes, which prevent excision repair, or by a *mfd* mutation, which reduces the rate of excision but does not affect survival. Using an *in vitro* repair synthesis assay we found that although wild-type cells repair the transcribed (template) strand preferentially, *mfd*$^-$ cells are incapable of strand-specific repair. The deficiency in strand-selective repair of *mfd*$^-$ cell extract was corrected by adding highly purified "transcription-repair coupling factor" to the reaction mixture. We conclude that *mfd* is, most likely, the gene encoding the transcription-repair coupling factor.**

In recent years *in vivo* studies have shown that, in general, actively transcribing genes are repaired at a faster rate than the rest of the genome (1–3). In the majority of the cases gene-specific repair appears to be due to strand-specific repair—that is, in an actively transcribing gene the template (transcribed) strand is repaired at such a high efficiency as to account for all of the gene-specific repair, whereas the coding (nontranscribed) strand is repaired at essentially the same rate as the rest of the genome (4, 5). Recently, we have developed an *in vitro* system (6, 7) capable of gene- and strand-specific repair and we have partially purified an *Escherichia coli* protein that confers strand specificity onto the *E. coli* nucleotide excision repair enzyme, (A)BC excinuclease. In this communication we describe the purification of the "transcription-repair coupling factor" (TRCF) to near-homogeneity and the preliminary identification of the coupling factor as the *mfd* gene product.

MFD (mutation frequency decline) is operationally defined as the rapid and irreversible decrease in the frequency of certain damage-induced suppressor mutations that occurs when protein synthesis is transiently inhibited immediately after irradiation (8–12). MFD-promoting posttreatments do not alter survival, nor do they reduce the yields of other induced mutations, yet the absence of MFD in *uvr*$^-$ strains indicates that it represents a specialized type of excision repair (9). In addition to *uvr* genes, mutation in another gene, called *mfd*, abolishes MFD; the *mfd* mutation, in contrast to *uvr* mutations, does not reduce survival (9). Taken together these results would imply that the *mfd* gene product directs (A)BC excinuclease to carry out the specialized repair resulting in MFD.

MFD experiments have usually utilized amino acid-requiring strains owing their auxotrophy to ochre or amber mutations, and the induced mutation frequency is measured as reversion to prototrophy. Nonsense suppressor mutations account for nearly all of the UV-induced reversions, and only the suppressors exhibit MFD (12–14). Bockrath and colleagues (15–18) conducted a series of elegant genetic experiments on the MFD effect and based on the results of these experiments concluded that "MFD is a unique process involving excision repair of premutational lesions located only in the transcribed strand of DNA" (16). The apparent strand specificity of MFD led us to consider whether the *mfd* gene might encode or control the synthesis of the TRCF we detected in our *in vitro* assay. Therefore, cell-free extracts from *E. coli* B/r and its *mfd*$^-$ derivative were tested for strand-specific repair *in vitro*. We found that *E. coli* B/r, like *E. coli* K-12, was capable of strand-specific repair. In contrast, *E. coli* B/r *mfd*$^-$ extract was totally deficient in strand-specific repair. When we added the purified TRCF to the mutant cell extract it restored the strand-specific repair to the wild-type level. The most likely explanation of our data is that *mfd* encodes the TRCF.

MATERIALS AND METHODS

Cells and Plasmids. *E. coli* K-12 derivatives AB1157 (wild type) and AB1886 (*uvrA*$^-$) were used for making extracts for routine repair synthesis assay and for purification of the TRCF, respectively. *E. coli* B/r derivative WU3610 (which is Leu$^-$ and Tyr$^-$ because of UAG and UAA mutations) and its derivative WU3610-45 (*mfd-1*) are the strains that have frequently been used in studies on MFD (11). The plasmid pDR3274 (19) contains the *uvrC* gene under the strong *tac* promoter. Transcription from this promoter can be inhibited by rifampicin (Rif) or specifically by the *lac* repressor (7). To prepare repair substrate, the plasmid DNA (30 μg/ml in 10 mM Tris·HCl, pH 7.4/10 mM NaCl/1 mM EDTA) was irradiated with 225 J·m^{-2} of 254-nm light from a Sylvania germicidal lamp.

Materials. The chromatographic resins DEAE agarose (Bio-Rad), AcA 34 (LKB), blue Sepharose (Sigma), and heparin agarose (Sigma) were from the indicated sources. [α-^{32}P]dCTP (6000 Ci/mmol; 1 Ci = 37 GBq) was from New England Nuclear, Rif was from Sigma, and restriction enzymes, DNA polymerase I, and T4 DNA ligase were from GIBCO/BRL. RNA polymerase (RNA Pol) was from Promega. *E. coli* UvrA, -B, -C, and -D proteins were purified as described (20, 21).

Purification of the TRCF. Twelve liters of *E. coli* AB1886 was grown in Luria broth to A_{600} = 0.95. The cells were collected by centrifugation and washed, and cell-free extract was prepared as described (7, 22). The yield was 19 ml of

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Abbreviations: MFD, mutation frequency decline; TRCF, transcription-repair coupling factor; RNA Pol, RNA polymerase; Rif, rifampicin.

protein at 63 mg/ml (F1). This fraction was loaded onto a 55-ml DEAE agarose column equilibrated with 0.1 M KCl in buffer A (50 mM Tris·HCl, pH 7.5/1 mM EDTA/10 mM 2-mercaptoethanol/20% glycerol). The column was washed with 100 ml of buffer A plus 0.1 M KCl and then washed with 50 ml of buffer A plus 1.0 M KCl. The TRCF was found in the low-salt fraction (F2). The active fractions were combined and loaded onto a 40-ml blue Sepharose column equilibrated with 0.1 M KCl in buffer A. The column was washed with 70 ml of buffer A plus 0.1 M KCl and then developed with a 160-ml gradient of 0.1–1.0 M KCl in buffer A. The TRCF eluted at about 0.2 M KCl (F3). The F3 was concentrated to 2 ml and applied to an AcA 34 column (1.6 cm × 64 cm) equilibrated with buffer A plus 0.3 M KCl. Fractions corresponding to a molecular mass of ≈100 kDa contained transcription-repair coupling activity (F4). The active fractions were combined and dialyzed against buffer A plus 0.05 M KCl and then loaded onto a 13-ml heparin agarose column that was washed with 26 ml of the same buffer and then developed with a 65-ml gradient of 0.05–0.3 M KCl in buffer A. The activity eluted at about 0.2 M KCl. The active fractions were combined (F5), aliquoted, frozen in dry ice/ethanol, and stored at −80°C. The activity was stable for at least 3 months under these conditions.

Repair Assays. The transcription-repair buffer contained 40 mM Hepes (pH 7.8), 50 mM KCl, 8 mM $MgCl_2$, 5 mM dithiothreitol, 4% glycerol, 100 μg of bovine serum albumin per ml, 6% (wt/vol) polyethylene glycol 6000, 500 μM NAD, 2 mM ATP, 200 μM (each) CTP, GTP, and UTP, 40 μM (each) dATP, dGTP, and dTTP, 4 μM unlabeled dCTP, and 5 μCi of [α-^{32}P]dCTP plus pDR3274 substrate at 1.3 nM (plasmid). When transcription/repair was carried out with cell-free extract, F1 was added to 1.2 mg/ml. When the reaction was conducted with the defined system, F1 was substituted by 1.2 units of RNA Pol per ml, 4 nM UvrA, 100 nM UvrB, 70 nM UvrC, 5 nM UvrD (helicase II), 80 units of Pol I per ml, and 48 units of T4 DNA ligase per ml. Column fractions or purified TRCF was added to the reaction mixture (25 μl) in 1–3.5 μl when indicated. The reactions were carried out at 37°C for 25 min.

The DNA was then extracted with phenol and ether, precipitated with ethanol, dissolved, digested with restriction enzymes, and separated on either an agarose gel (overall repair) or a sequencing gel (strand-specific repair). Repair was measured by autoradiography followed by densitometry as described (7). Overall repair is a measure of radiolabel incorporated into the whole pDR3274 plasmid, in which the strongly transcribed *uvrC* gene constitutes about one-third of the total length. Strand-specific repair is a measure of relative repair synthesis in the coding and template strands of a 299-base-pair (bp) promoter-proximal fragment of *uvrC* gene of the same plasmid (7). To measure repair under nontranscription conditions, Rif (22 μg/ml) was added to the reaction mixtures. We have found that under our reaction conditions Rif inhibits transcription >90% (7). For quantitative analysis of the repair synthesis, the autoradiographs of the gels were scanned using a Biomed Softlaser scanning densitometer.

RESULTS

Purification of TRCF. Previously we demonstrated transcription-directed preferential nucleotide excision repair synthesis of the template DNA strand using extracts from the *E. coli* K-12 derivative AB1157 and the UV-irradiated substrate pDR3274 (which carries the *uvrC* gene linked to a *tac* promoter). In our assay, following repair synthesis reactions, pDR3274 was digested with restriction enzymes to generate a 299-bp *Bgl* I/*Nsi* I fragment originating near the 5′ end of the strongly transcribed *uvrC* gene. When the 299-base template and 300-base coding strands were separated on a sequencing gel it was found that transcription enhanced repair synthesis (incorporation of radiolabeled dCMP) of the template strand by 4- to 5-fold but had negligible effect on repair synthesis in the nontranscribed strand or DNA from the weakly transcribed *tet* gene (7).

To purify the putative protein that couples transcription to repair, we have used a defined transcription-repair system consisting of purified transcription (RNA Pol) and repair (UvrA, UvrB, UvrC, UvrD, Pol I, ligase) proteins and pDR3274 as substrate. Repair synthesis reactions were conducted using the defined system, which does not perform strand-directed repair, mixed with chromatographic fractions of cell extract (which were incapable of excision repair). Active fractions were identified based upon their ability to enhance repair synthesis of the template strand of the *Bgl* I–*Nsi* I fragment relative to the coding strand (7), and in this way we have purified a transcription-repair coupling activity through four chromatographic steps. The final fraction (F5) contained only three bands when analyzed on SDS/PAGE followed by silver staining (Fig. 1). Of the three proteins in this fraction the elution profile of the one with a molecular mass of 121 kDa most closely correlates with the transcription-repair coupling activity over the last two columns and therefore we ascribe the transcription-repair coupling activity to this protein. The sequence of the amino-terminal 15 amino acids of this protein (not shown) did not match any protein in GenBank, indicating that the TRCF is not a previously described protein.

Lack of TRCF in *E. coli mfd-1*. Cell-free extracts from wild-type *E. coli* but not from *uvrA*$^-$ cells are capable of strand-specific repair (7). Since genetic data suggest that, in addition to *uvr* genes, *mfd* may also be involved in strand-specific repair (16–18), we wished to examine the effect of the *mfd-1* mutation on repair in our *in vitro* assay. Cell-free extracts were prepared from the *E. coli* B/r strains WU3610 (*mfd*$^+$) and WU3610-45 (*mfd-1*) and tested. The results of the overall and strand-specific repair synthesis assays (7) for the two strains are summarized in Tables 1 and 2, respectively.

Under our assay conditions in the absence of transcription both strains carried out overall repair synthesis to about the same extent (for unknown reasons B/r strains have a higher

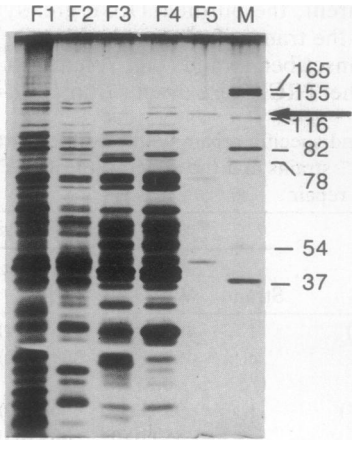

FIG. 1. Purification of TRCF. Samples from the indicated fractions were separated on a 10% SDS/polyacrylamide gel that was silver stained and photographed. Molecular mass markers (lane M) were *E. coli* RNA polymerase β and β′ (165 and 155 kDa) β-galactosidase (116 kDa), RNA Pol σ subunit (82 kDa), UvrB (78 kDa), photolyase (54 kDa), and RNA Pol α subunit (37 kDa). The TRCF activity profiles followed those of TRCF and not those of the low molecular mass species on the two last columns. The TRCF is indicated by an arrow. The purification lanes contained the following amounts of protein: F1, 1.3 μg; F2, 1.1 μg; F3, 1.4 μg; F4, 2.4 μg; F5, 0.1 μg.

Table 1. Overall repair synthesis in Mfd$^+$ and Mfd$^-$ strains

Strain	Repair synthesis		
	With Rif*	Without Rif	Without Rif, with TRCF
AB1157 (*mfd*$^+$)	227 (100)	305 (134)	364 (160)
WU3610 (*mfd*$^+$)	38 (100)	58 (152)	65 (171)
WU3610-45 (*mfd-1*)	66 (100)	61 (92)	89 (135)

Background synthesis observed in undamaged DNA (which was substantially lower in AB1157 than the other two strains) has been subtracted. Repair is in arbitrary densitometric units and is also expressed (in parentheses) relative to that obtained in the absence of transcription (With Rif), which was taken to be 100 for each strain. Values are the averages of two experiments.

*High background values (126 units for WU3610 and 104 units for WU3610-45) make it difficult to judge whether the difference in repair synthesis of the two strains relative to one another under this condition (no transcription) is significant.

background in the repair synthesis assay compared to K-12 strains); however, a striking difference was observed when transcription and repair synthesis occurred simultaneously. Under transcription conditions, the level of overall repair increased by a factor of 1.4–1.5 for *E. coli* K-12 and B/r strains but did not increase in the B/r *mfd-1* strain (Table 1). A more striking difference was observed when repair syntheses in the two strands were compared (Fig. 2 and Table 2). The wild-type strain, as was the case in *E. coli* K-12, repaired the transcribed strand about four times more efficiently than the coding strand; in contrast, the *mfd*$^-$ strain was totally defective in transcription-promoted strand-specific repair. Thus, it appears that WU3610-45 (*mfd-1*) is deficient in one or more factors necessary for transcription-directed strand-specific repair.

Complementation of the *mfd-1* Defect *in Vitro*. If MFD results from lack of preferential repair of the transcribed strand of tRNA genes because of a deficiency in the coupling factor, then the addition of the purified TRCF to the cell-free extract from WU3610-45 (*mfd-1*) should restore its strand-specific repair activity. The results of such an experiment are shown in Fig. 2, and quantitative analysis of two experiments carried out under identical conditions is summarized in Table 2. As is apparent, the purified TRCF greatly enhanced the repair rate of the transcribed strand with only a minor effect on the nontranscribed strand (Fig. 2, lane 10). Interestingly, addition of the TRCF to extracts from wild-type cells enhanced the strand-selective repair already present (Fig. 2, lane 3 vs. lane 4 and lane 7 vs. lane 8; Table 2), indicating that the TRCF is limiting in our *in vitro* system. However, the most important conclusion that emerged from these experiments is that *mfd* apparently codes for the TRCF and that MFD is due to the selective repair of the transcribed strand of a tRNA gene.

Table 2. Strand-specific repair synthesis in cell-free extracts of Mfd$^+$ and Mfd$^-$ strains and the effect of the TRCF on strand-specific repair

Strain	Strand	Repair synthesis		
		With Rif	Without Rif	Without Rif, with TRCF
AB1157 (*mfd*$^+$)	t	402 (87)	946 (204)	1237 (267)
	c	462 (100)	379 (82)	412 (89)
	t/c	**0.87**	**2.5**	**3.0**
WU3610 (*mfd*$^+$)	t	95 (111)	400 (470)	489 (575)
	c	85 (100)	88 (103)	125 (147)
	t/c	**1.1**	**5.3**	**3.9**
WU3610-45 (*mfd-1*)	t	255 (111)	276 (116)	836 (351)
	c	238 (100)	279 (117)	346 (145)
	t/c	**1.1**	**0.99**	**2.4**

Repair synthesis is expressed in densitometric units and has been normalized (parentheses) to the repair synthesis obtained under the nontranscription condition for the coding (nontranscribed) strand for each strain. The values for AB1157 are from one experiment; those for the other two strains are averages of two experiments. t, c, and t/c, transcribed, coding strands, and the ratio of the two (in bold type), respectively.

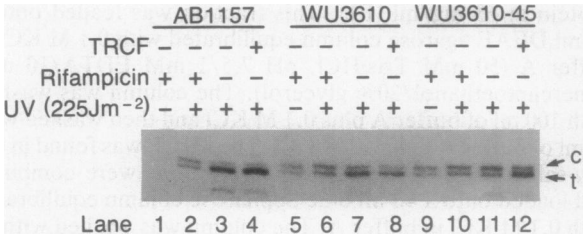

FIG. 2. Lack of strand-specific repair in the *E. coli mfd-1* strain. Repair synthesis reactions were conducted with cell-free extracts from the indicated strains. Where indicated, 12 ng of purified TRCF was added to the reaction mixture. Following repair synthesis, the plasmid (pDR3274) was digested with the appropriate restriction enzymes (7), and DNA was separated on a 3.6% sequencing gel. The region of the gel containing the 299-bp promoter-proximal *Bgl* I–*Nsi* I fragment is shown. Note the exceptionally high background synthesis in *E. coli* B/r strains compared to the K-12 strain. t, Template; c, coding strand.

DISCUSSION

We would like to comment on several issues relating to MFD and strand-specific repair in light of results reported in this communication.

Molecular Mechanism of Transcription-Coupled Repair. The strand specificity of MFD and the deficiency in MFD and TRCF activity in the *mfd* mutant, considered together, lead to the following simple model: the *mfd* gene encodes TRCF, which targets (A)BC excinuclease to the template strand of the tRNA gene, permitting rapid excision of UV lesions on that strand. More specifically, we propose that RNA Pol stalls at a UV lesion on the template strand and that the ternary complex of DNA template–RNA Pol–RNA is recognized by the Mfd (TRCF) protein, which binds to the complex and, either by itself or in conjunction with a subset of the components of the complex, constitutes a high-affinity binding site for the damage recognition subunit of (A)BC excinuclease, resulting in rapid excision repair on the template strand.

Although we believe that this model accounts for strand-specific repair in general, the properties of TRCF alone do not explain the medium dependence of MFD. In particular, although TRCF activity was enhanced on transcriptionally active templates (7), most (albeit not all) MFD-promoting conditions (amino acid starvation, nutritional shift-down) elicit the stringent response, causing the cessation of tRNA gene transcription. Moreover, there is considerable evidence that photoproducts at suppressor mutation sites are uniquely refractory to excision in rich medium (12), under the conditions most favorable for transcription of tRNA genes and therefore presumably for TRCF activity. These inconsistencies may arise from unusual conditions in the tRNA genes where MFD-susceptible suppressor mutations originate.

Site Specificity of MFD. MFD is a highly specialized repair response not known to affect any premutational lesions in DNA except those located at the site corresponding to the first letter of the anticodon on the template strand of tRNA genes *glnU* (ochre) and *glnV* (amber) (16, 17, 23), a region rich in potential secondary structure. Even if the effect of TRCF is to stimulate repair on the template strand as a whole, it may not do so at that specific location because of a unique

configuration assumed by the anticodon region during protein synthesis. Furthermore, the regulation of the seven-tRNA operon containing these genes (24) has not been investigated, and it may prove to include unexpected features that contribute to their anomalous repair properties. A full understanding of MFD, therefore, may not illuminate the broader process of strand-specific repair, although it could reveal how this process is influenced by a singularity at a particular site.

Mfd⁻ Phenotype. The *mfd-1* allele causes no detectable phenotypic effects in excision-deficient Uvr⁻ strains. Therefore, its manifestations in Uvr⁺ strains can be taken as results of the occurrence of excision repair without the participation of TRCF. The Mfd mutant phenotype has been extensively characterized (9, 25, 26). The mutant is no more sensitive to UV than its *mfd*⁺ parent, yet its initial rate of excision is one-third that of wild-type cells. Its UV resistance implies that repair of potentially lethal DNA damage, although accomplished more slowly, is ultimately as complete as in the wild type. Resumption of UV-inhibited DNA replication is delayed, very likely contributing to UV resistance by minimizing the number of unexcised lesions that encounter a replication fork.

SOS functions in the *mfd* mutant are expressed at lower UV fluences than in the wild type, probably because more single-stranded DNA (a cofactor required for SOS induction) is exposed after UV irradiation, owing to the persistence of excision gaps and more active DNA degradation in the mutant (26). UV mutability is 5-fold higher than wild type in all genes tested and at all UV fluences. In wild-type strains, therefore, transcription-coupled excision not only protects the integrity of protein synthesis but it is also evidently a powerful antimutagenic influence in the DNA-damaged cell.

Strand Specificity of Induced Mutations. Bockrath and Palmer (16) found that, when the photoproduct in the tRNA gene causing suppressor mutation is located on the coding strand, rather than on the template strand (as it is when an amber suppressor mutation in *glnV* is converted to an ochre suppressor), not only does very little MFD occur, but the mutation frequency is extraordinarily high. Slow excision apparently increases mutagenesis on the wild-type coding strand as well as in the *mfd* mutant. In the *lacI* gene, almost all UV-induced point mutations map at the sites of UV photoproducts on the coding strand, although the distribution on the two strands is nearly equal in a *uvrB* strain (27, 28). We predict that the distribution of induced mutations in the *mfd* mutant will not exhibit a strand bias favoring the coding strand.

Mutagenic Effect of Slow Excision. Two distinct pathways of SOS mutagenesis have been delineated in excision-proficient wild-type strains. MFD-susceptible suppressor mutations comprise the only class of UV-induced mutations known to require semiconservative DNA replication for their fixation as stable genetic changes, >90% of them remaining susceptible to elimination either by MFD-inducing posttreatments (29) or by photoreactivating light (30) until DNA replicates, indicating that they are caused by unexcised photoproducts. Other types of induced mutations in wild-type strains (true back-mutations to prototrophy, mutations to streptomycin resistance), which are an order of magnitude less frequent, in any medium, than suppressor mutations in rich medium, lose their photoreversibility much more rapidly (30–32). These mutations are believed to originate before replication, in genes from which all premutational photoproducts are excised before replication resumes, by error-prone SOS processing of a small number of excision gaps.

Slow excision leads to increased UV mutability at many loci, possibly throughout the genome, in the *mfd* mutant, where excision is slow on both strands, and also on the coding strand of the wild type. For the suppressor mutations in *glnV* and *glnU*, a likely explanation is that slow excision converts the fate of some premutational lesions from excision before replication to replication before excision—that is, from error-free repair to error-prone translesion replication. However, it would be difficult to reconcile the wild-type UV resistance of the *mfd* mutant with a 5-fold genome-wide increase in the number of unexcised lesions present during DNA replication. We suggest, instead, that in nonsuppressor genes, all premutational lesions are excised, albeit more slowly than in the wild type, before DNA replication resumes. (Replication resumes after an extended lag in the *mfd* mutant, perhaps indicative of coordination between the end of excision and the resumption of DNA replication.)

The elevated UV mutability in nonsuppressor genes could be due to the longer persistence and larger size of excision gaps (enlarged by DNA degradation) on both DNA strands of the *mfd* mutant as well as on both strands of repressed genes and on the coding strand of active genes in wild-type strains. Many of these larger, longer-lasting gaps could persist until well after full induction of the SOS response. Some of them could come to be located opposite an unexcised replication-blocking UV photoproduct on the opposite strand, requiring mutagenic SOS processing rather than simple repair synthesis for gap filling (33).

Mutagenesis and Strand-Specific Repair in Eukaryotes. Considering that transcription-coupled strand-specific repair occurs in nearly all organisms tested, one might expect its consequences for mutagenesis in eukaryotes to be similar to those we have discussed in *E. coli*. In reality, however, the situation in eukaryotes is not strictly analogous to *E. coli*. Although several studies (34–36) have shown that ≈90% of the mutations in dihydrofolate reductase or hypoxanthine phosphoribosyltransferase genes of hamster or human cell lines arose from lesions in the nontranscribed strand, ≈90% of UV-induced mutations were found at sites of dipyrimidines in the template strand of the sup4-0 tRNA gene of yeast (37). We do not believe that these seemingly paradoxical results indicate a fundamental difference between the transcription-repair coupling mechanisms between prokaryotes and eukaryotes.

Rather, these differences reflect differences in the transcriptional complexes of the two systems. In *E. coli* all three types of RNA are transcribed by a single RNA Pol, whereas in eukaryotes RNA Pol I transcribes rRNA, RNA Pol II transcribes heterogeneous nuclear RNA (mRNA), and RNA Pol III transcribes 5S RNA and tRNAs. The TRCF couples repair to RNA Pol stalled at any gene in *E. coli* by protein–protein interactions. In contrast, in eukaryotes the TRCF can interact only with RNA Pol II (38) and, as a result, only in genes transcribed by this RNA Pol is there transcription-enhanced repair and strand-specific mutagenesis. This model explains the lack of gene-specific repair of psoralen damage in an rRNA gene in Chinese hamster ovary cells (39) as well as the apparent lack of repair of a tRNA gene in yeast (37). In fact, the preferential induction of mutation in the template strand of the yeast tRNA gene (37) is consistent with the idea that an RNA Pol stalled at a lesion might preferentially interfere with the repair of that strand. This was shown to be the case in an *in vitro* system with *E. coli* RNA polymerase in the absence of the TRCF (6). The *in vitro* results in fact predict that in the *E. coli mfd*⁻ strain and in cell lines from the Cockayne syndrome, which appears to be the human analogue of *mfd*⁻ (40) in that it lacks strand-specific repair, the absence of the coupling factor will reverse the strand preference for mutations in active target genes.

We are grateful to Richard Bockrath and Douglas Brash for their insight and for stimulating discussions. This work was supported by National Institutes of Health Grant GM32833.

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