JOURNAL OF BACTERIOLOGY, Dec. 1993, p. 7509–7514
0021-9193/93/237509-06$02.00/0

Vol. 175, No. 23

MINIREVIEW

Transcription-Repair Coupling and Mutation Frequency Decline

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INTRODUCTION

Mutation frequency decline (MFD) is the rapid and irreversible decrease in the frequency of certain damage-induced mutations that occurs in *Escherichia coli* when protein synthesis is transiently inhibited immediately after the damaging treatment (49). Transcription-repair coupling (TRC) is the preferential repair of transcribed regions at a faster rate compared to the rest of the genome (5). These two phenomena are intimately connected in *E. coli*. It is our aim to review the current state of knowledge on MFD, TRC, and the transcription-repair coupling factor (TRCF) which is the key protein in both MFD and TRC.

MUTATION FREQUENCY DECLINE

For convenience, the initial studies on UV mutagenesis were conducted on auxotrophic *E. coli* strains, and mutants were scored as revertants to prototrophy (50). It was found that if irradiated cells, prior to being plated on a rich medium, were held in a medium that contained an energy source (glucose) but that was not conducive to protein synthesis, then the frequency of mutations declined even though no significant change in cell survival occurred during this period (49–52). This was called mutation frequency decline (MFD). Later work revealed that the vast majority of revertants detected in these experiments were due to suppressor mutations (7).

A study by Bockrath and Palmer (4) revealed that "de novo suppressor mutations" (mutations in the anticodon of a tRNA which make it a suppressor) were subject to MFD but that neither "conversion-type suppressor mutations" (mutations which convert a suppressor tRNA from recognizing one type of a nonsense codon to a form that recognizes another nonsense triplet) nor true back revertants were susceptible to MFD. It was concluded that MFD must be the consequence of a specific repair event operating on the tRNA genes. Furthermore, upon analysis of the potential lesion sites in the particular tRNA genes which would give rise to de novo and conversion-type mutations, it was realized that both types of mutations would arise from UV-induced lesions at a TC sequence, converting it to TT (Fig. 1). However, in the case of the de novo suppressor, the target TC sequence is in the transcribed (template) strand, and in the case of the conversion type suppressor, the target TC is in the coding (nontranscribed) strand. This led to the conclusion that "MFD ... is a unique process involving excision repair of premutational lesions located only in the transcribed strand of DNA" (4). A final piece of evidence for the special relationship between the lesion in the transcribed strand and MFD came from experiments with ethyl methanesulfonate mutagenesis. In contrast to UV mutagenesis, conversion-type mutations which arose from an O^6-ethylguanine in the transcribed strand were susceptible to MFD, whereas de novo-type suppressors caused by an ethylated G in the nontranscribed strand were not (Fig. 1) (3).

Furthermore, since MFD occurred when cells were starved for amino acids, a condition which severely represses tRNA synthesis (stringent response), it was proposed that the preferential repair of the transcribed strand of the tRNA gene was actually inhibited by transcription and that under MFD conditions (repressed transcription) the transcribed strand containing the photoproduct would hybridize with its cognate tRNA and "place the photoproduct in a double-stranded configuration necessary for incision" by the repair enzyme (4). This model raised the possibility of strand-specific repair for the first time, but it also implied that the strand-specific repair was due to the peculiarity of tRNA genes and that it occurred in the transcribed strand only when transcription was not occurring.

However, when the same experiments were repeated in a *rel* mutant in which tRNA synthesis is not repressed by amino acid starvation (44), MFD did occur as it did in a rel^+ strain, leading the authors to conclude that "transcription activity of tRNA genes does not influence MFD, and therefore MFD should not be cited as an example where the state of transcription affects repair" (10). Since strand-specific repair appeared to be independent of transcription, another potential source of strand asymmetry was considered, i.e., replication. Lesions may be mutagenic to different degrees depending on whether they are in the template strand for leading or lagging-strand synthesis (47). However, when isogenic strains carrying the $tRNA^{gln}_{CAG}$ gene in opposite orientations were tested for MFD, no difference was found (9), suggesting that the direction of replication was not a determinant for MFD.

In conclusion, studies of MFD revealed that the transcribed and nontranscribed strands of tRNA genes were unequal with regard to susceptibility to repair, but it also appeared that this asymmetric behavior of the two strands with regard to repair was independent of the transcriptional status of the gene. Therefore, when the transcription-dependent gene- and strand-specific preferential repair phenomena were discovered (5, 25), the connection of MFD with these phenomena was not immediately evident to many researchers in the field.

PREFERENTIAL NUCLEOTIDE EXCISION REPAIR

Both MFD and preferential repair are special manifestations of nucleotide excision repair (11, 12, 25, 51), which acts on all unnatural base modifications, is the only mechanism for bulky adducts, and involves the excision of an oligomer containing the damaged base(s) by an ATP-dependent nuclease (see references 32 and 35). In *E. coli*, the excision nuclease,

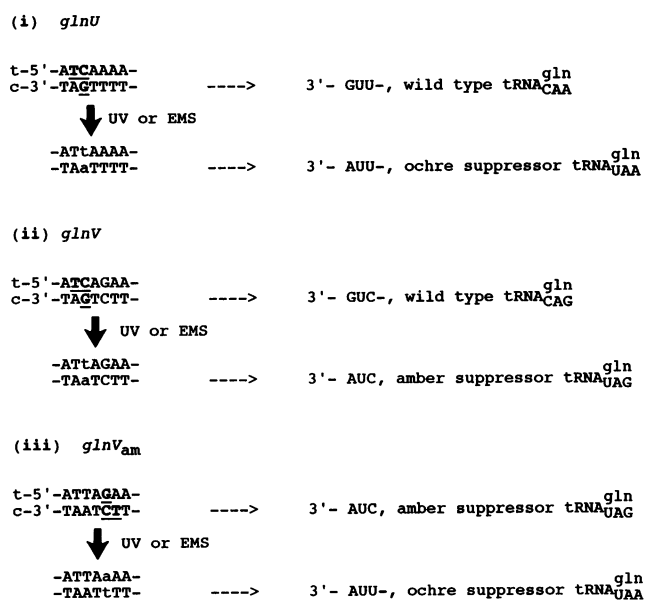


FIG. 1. Formation of suppressor mutations in the *glnU* and *glnV* tRNA genes. Pathways i and ii show formation of suppressors de novo, by mutation of the wild-type genes, and pathway iii shows how mutation can produce conversion from an amber to an ochre suppressor. Mutation is by GC→AT transition (lowercase) following either the formation of a UV photoproduct at the TC sequence (shown in boldface type and underlined) or the formation of an ethylated guanine residue (boldface type and underlined) by treatment with ethyl methanesulfonate (EMS). t, template strand; c, coding strand.

(A)BC excinuclease (35), results from the sequential and partially overlapping actions of the UvrA, UvrB, and UvrC proteins. UvrA is a molecular matchmaker (34) and a damage-specific DNA-binding protein. It makes an A_2B_1 complex with UvrB (which on its own has no affinity to DNA), delivers UvrB to the damage site, and dissociates from the UvrB-DNA complex (30). UvrC recognizes the UvrB-DNA complex and binds to it, causing a conformational change in UvrB which hydrolyzes the fifth phosphodiester bond 3′ to the lesion which, in turn, triggers the hydrolysis of the eighth phosphodiester bond 5′ to the lesion by UvrC. Helicase II (UvrD) displaces UvrC and the excised 12- to 13-mer. DNA polymerase I fills in the gap and displaces UvrB, and finally the patch is sealed by ligase (14, 21). The rate-limiting step in the overall reaction is the loading of UvrB to the damage site because it involves probing of DNA for subtle structural abnormalities by the A_2B_1 complex using helicase-like action (18, 28, 31). The details of the action mechanism of human excision nuclease are not known. However, the basic excision mechanism is similar but not identical to that of *E. coli* (15).

The first evidence for preferential repair of a gene undergoing active transcription was obtained by Bohr et al. (5; cf. reference 27), who analyzed the formation and repair of pyrimidine dimers (Pyr<>Pyr) in the dihydrofolate reductase (DHFR) gene of UV-irradiated Chinese Hamster ovary cells. The repair rate of the DHFR gene was found to be fivefold higher than the average repair rate for the entire genome. This study had a major impact in the field and led to similar studies and similar findings in other genes, other lesions, and other organisms, including humans.

Initially, the molecular explanation of "gene-specific repair" seemed simple enough; transcription causes, or is associated with, a loose chromatin structure that makes DNA accessible to repair enzymes, as it does for probes of chromatin structure (see reference 8). This view was soon challenged by the results of Mellon et al. (25), who found that the transcribed (template) strand of the DHFR gene in CHO cells was repaired 10 times faster than the nontranscribed strand, which had a repair rate equal to that of the bulk DNA. Still, the strand-specific repair could be explained within the general framework of open chromatin conformation (43): a lesion in the template strand (but not in the coding strand) blocks RNA polymerase (RNAP) and thus retains the "open chromatin" conformation long enough for the repair enzyme to remove the lesion. However, this was inconsistent with three other observations. First, Mellon and Hanawalt (24) discovered strand-specific repair in the *E. coli lac* operon, and even though *E. coli* does have histone-like proteins it does not have a stable nucleosome structure which would inhibit repair. Second, Selby and Sancar (37) found in vitro that an RNAP stalled at a Pyr<>Pyr dimer inhibited repair by steric interference with (A)BC excinuclease. Third, in eukaryotes, genes transcribed by RNAP I (48) and, apparently, those transcribed by RNAP III (1) are not subject to gene-specific repair, yet these polymerases are also blocked by lesions, and genes transcribed by these RNAPs are also associated with an open chromatin conformation. It became apparent that the act of transcription and the particular RNAP involved, rather than an open chromatin structure, played pivotal roles in the increased repair rate. To understand transcription-stimulated repair it was essential to understand the interaction of the transcription apparatus with lesions in the template and coding strands and with the nucleotide excision repair enzyme.

EFFECT OF DNA DAMAGE ON TRANSCRIPTION AND OF TRANSCRIPTION ON REPAIR

The seminal work of Michalke and Bremer (26) showed that the length of nascent RNA decreased with increasing UV dose to *E. coli*, suggesting that UV lesions block RNAP. Similar results were obtained by Sauerbier et al. (36), who subsequently developed an elegant method of gene sizing and operon mapping based on inactivation by single-hit kinetics. Knowing whether transcription was blocked by a lesion in either DNA strand or whether it was blocked only by a lesion in the template strand was not essential for this analysis, but it was critical for developing an in vitro system for studying TRC.

Toward this goal, Selby and Sancar (37) adapted the transcription-repair template/substrate system of Shi et al. (42) to the study of thymine dimer (T<>T) repair in a defined system. It was found that a T<>T in the coding (nontranscribed) strand had no discernible effect on transcription. However, a T<>T in the template (transcribed) strand constituted an absolute block for RNAP, with <1% translesion synthesis, and gave rise to a stable elongation complex. Thus, it appears that in *E. coli* the so-called bulky adducts block RNAP only when present in the template strand, giving rise to a stable elongation complex.

The effect of transcription on repair was investigated by using a defined system consisting of two DNA duplexes containing a T<>T in the template or coding strand, purified *E. coli* RNAP, and the UvrA, UvrB, and UvrC subunits of (A)BC excinuclease (37). When repair was measured in the absence of ribonucleoside triphosphates (rNTPs), it was found that the promoter-bound RNAP had no effect on repair of a T<>T downstream from the transcriptional initiation site regardless of whether the T<>T was in the coding or template strand. However, when repair was carried out in the presence

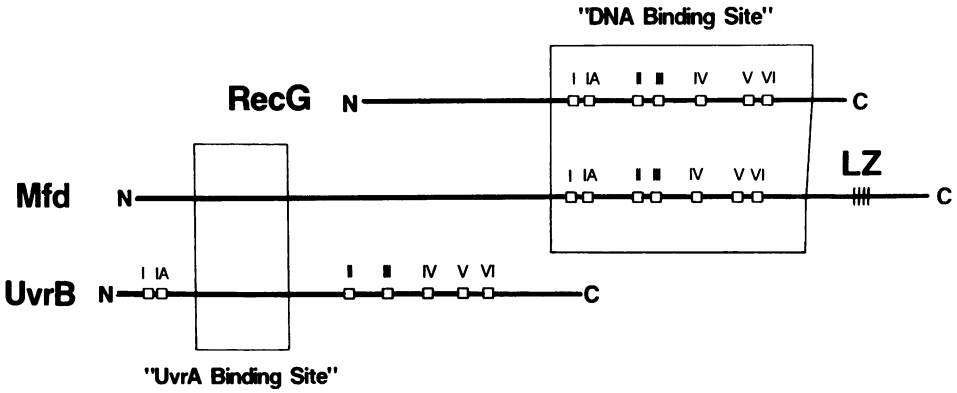


FIG. 2. Sequence homologies between Mfd (TRCF), UvrB, and RecG proteins. I through VI indicate the so-called helicase motifs. Neither Mfd nor RecG has helicase activity. LZ, potential leucine zipper.

of rNTPs, a paradoxical result was obtained. In contrast to the in vivo data, transcription specifically inhibited the repair of the transcribed strand, with no effect on the repair of the coding strand. The net effect was that in this defined system, the coding strand was repaired preferentially in the actively transcribed DNA. This unexpected finding forced the conclusion that cells must possess a transcription-repair coupling factor (TRCF) which performs two tasks: overcoming the repair-inhibitory effect of a stalled RNAP and accelerating the rate-limiting step of excision repair, i.e., the delivery of UvrB to the damage site.

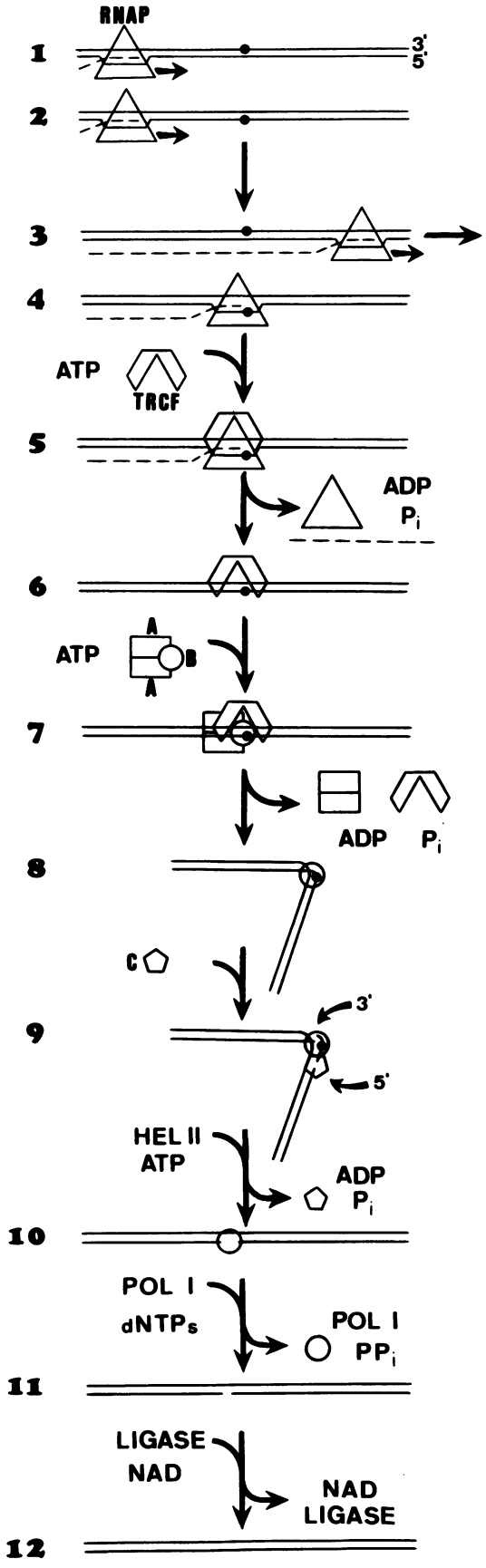
PURIFICATION AND PROPERTIES OF TRCF

When a defined system consisting of template/substrate, *E. coli* RNAP, and the UvrA, -B, and -C proteins failed to achieve strand-specific repair, it was concluded that the system lacked a TRC protein present in cells (37). Therefore, *E. coli* cell extract (CE) supplemented with 6% polyethylene glycol for macromolecular crowding was tested for transcription-coupled repair (38). Repair was measured by incorporation of label into the DNA of a plasmid. When the distribution of the repair synthesis within the plasmid was analyzed, it was found that there was about twofold more repair synthesis in the *tac* transcriptional unit compared with the other regions of the plasmid. This preferential repair was eliminated by inhibiting transcription with either rifampin or the *lac* repressor. When repair synthesis within the two strands of the *tac* transcriptional unit was analyzed, it was found that the template strand was repaired fivefold faster than the nontranscribed strand. There was no such difference between the two strands of transcriptionally quiescent parts of the plasmid. In this system, gene- and strand-specific repair also occurred when the DNA was damaged with psoralen or cisplatin, two other agents known to be substrates for (A)BC excinuclease. Thus, in the in vitro system preferential repair was strand specific and transcription dependent and was elicited by any lesion which blocked RNAP.

It became apparent from biochemical studies that a single protein in *E. coli* was responsible for TRC (38, 41). The *mfd* gene product was considered as a possible candidate because of the genetic evidence linking *mfd* to preferential repair of the transcribed strand of tRNA genes (3, 4), even though it had been reported that this preferential repair was transcription independent (10). CFE was prepared from *mfd* mutant cells and tested for strand-specific repair. The results were unambiguous: there was no strand-specific repair in this strain (41). This defect was complemented with partially purified TRCF, and it was concluded that *mfd* encodes the TRCF and that the Mfd^- phenotype was due to a lack of TRC.

The *E. coli* TRCF gene *mfd* (and its human counterpart *ERCC6*) have been cloned and sequenced (39, 46). The *mfd* gene is located at 25.3 min of the genetic map (39). The sequence of the 1,148-amino-acid protein encoded by the *mfd* gene (Fig. 2) reveals three features of interest. First, the sequence of a 140-amino-acid region near the NH_2-terminus of TRCF is homologous to the corresponding region of UvrB. This region may be a UvrA-binding domain (see below). Second, near the middle of TRCF there are the seven motifs that are found in known or putative helicases (13). Over a stretch of 400 amino acids covering this so-called helicase motif, the TRCF shares 38% sequence identity with the *E. coli* RecG protein, which is involved in branch migration of Holliday junctions (22). Finally, in the COOH-terminal region there are four leucines at seven-amino-acid intervals (potential leucine zipper). This region might be involved in binding other proteins, such as RNAP.

The Mfd protein (TRCF) is a relatively abundant protein with about 500 copies per cell (40). Cells expressing TRCF to an extent that it constitutes about 5% of total cellular proteins appear normal and have normal viability (39). The Mfd protein is a monomer, binds to DNA nonspecifically, and has a weak ATPase activity ($k_{cat} = \sim 3\ min^{-1}$). The ATPase activity is not affected by DNA; however, γS ATP stimulates the nonspecific DNA binding. A most interesting finding is the lack of helicase activity. No helicase activity could be detected even when the DNA oligomer to be displaced was only 17 nt long. Similarly, it was unable to displace a 71-nt RNA annealed to DNA. Since TRCF has a Rho protein-like function in that it dissociates a stalled ternary complex (see below) and since Rho requires a 5′ single-stranded RNA tail of at least 60 nt to perform its RNA-DNA helicase activity, the TRCF was tested with such a substrate and found unable to dissociate a 49-bp RNA-DNA hybrid with 222-nt 5′ RNA tail. RecG, the protein with the highest sequence homology to Mfd, also lacks helicase activity, even though RecG in an ATP-dependent reaction promotes branch migration of a synthetic Holliday junction (22). In contrast to these functionally unrevealing characteristics of TRCF, when the Mfd protein was tested for the two properties that it was predicted to have, that is, interaction with a stalled ternary complex and with the damage recognition subunit of



the excision nuclease, the results were quite informative regarding the molecular mechanism of TRC.

MOLECULAR MECHANISM OF TRC

The two functions that the TRCF must perform are the displacement of the stalled RNAP from the lesion and the recruitment of the repair enzyme to the damage site. Regarding the displacement of the stalled complex, in both prokaryotes and eukaryotes there are a number of proteins which interact with stalled RNAP. In *E. coli*, Rho binds to nascent RNA and dissociates the ternary complex stalled at Rho-dependent termination sites. NusG binds to the RNAP core and enhances the efficiency of Rho-dependent termination. NusA also binds to RNAP and increases the efficiency of intrinsic terminators. In contrast, NusB and S10 proteins bind to a specific sequence in the *rrn* transcript and act as antiterminators. A most interesting class of antiterminators that have been discovered in both *E. coli* and humans are the transcript cleavage factors (17). In *E. coli*, GreA and GreB proteins bind to a stalled RNAP and activate its intrinsic RNase activity. This enables RNAP to hydrolyze from 2 to 10 nucleotides from the 3′ end, retract from its trapped conformation, and resume its effort to bypass a transcription-blocking structure (6, 45). In humans, TFIIS performs the same function for RNAPII (16). Surprisingly, there is no evidence for involvement of any of these factors in TRC. Instead, it appears that, at least in *E. coli*, TRCF performs its function independently of these other factors and by an entirely different mechanism.

In a completely defined system (39), *E. coli* TRCF specifically interacts with RNAP stalled at a lesion and dissociates the ternary complex. No other protein is required for this function, nor is the RNA cleaved before or during dissociation from the RNAP. The coupling factor does not dissociate an initiation complex, and TRCF-stimulated repair begins at the 15th base and continues beyond in the transcriptional unit. At approximately this point in the template (i.e., the 15th base), RNAP is known to undergo several structural and functional changes (19): it forms a stable elongation complex which no longer undergoes repetitive, abortive RNA synthesis; it has a smaller DNase I footprint compared with earlier transcribing complexes; and sigma factor dissociates from the core polymerase. It is not yet clear what feature or subunit of the fully committed core polymerase is recognized by the coupling factor, although the beta subunit, like the TRCF, possesses a potential leucine zipper structural motif at residues 946 to 967. It is likely that the coupling factor also interacts with the transcription bubble. RecG and TRCF are structurally similar in their putative DNA-binding domains, and RecG interacts with a synthetic Holliday junction, which bears structural similarities to a transcription bubble. DNA damage is not required for dissociation of a stalled RNAP, since the TRCF dissociates RNAP stalled at a protein-DNA "roadblock" or at a "missing-base" site (40). Another component of the substrate that is not required is the free RNA "tail", since TRCF does not bind to free RNA and stimulation of repair occurs at about +15 nt, when essentially all of the transcript is in the form of an RNA-DNA hybrid. This is an important difference between the TRCF-dependent and Rho-dependent terminations. In Rho-dependent termination, an RNA tail of >50 nt is required

FIG. 3. Molecular model for TRC in *E. coli*. RNAP, RNA polymerase; A, B, and C, UvrA, -B, and -C, respectively; HELII, helicase II; PolI, DNA polymerase I.

for binding of Rho, entry into the ternary complex, and eventual dissociation by Rho's RNA-DNA helicase activity (see reference 33). However, the TRCF- and Rho-mediated terminations have some similarities: (i) both proteins are ATPases requiring ATP hydrolysis for dissociation of the complex, (ii) both proteins dissociate ternary complexes formed at protein blocks such as *trp* repressor or *Eco*RI endonuclease, and (iii) neither Rho nor TRCF forms a stable complex with free RNAP.

The second part of TRC is the increased rate of repair by (A)BC exinuclease. The rate-limiting step of this enzyme is the delivery of UvrB to the damage site by UvrA (30, 31). Therefore, the only way to increase the repair rate is to facilitate this process. Although no interaction could be detected between the coupling factor and UvrA by conventional hydrodynamic methods, UvrA does specifically bind to a TRCF affinity column, which indicates that the interaction is relatively weak. The functional form of UvrA is the A_2B_1 complex (30), and under physiological conditions all of UvrA is in this form. Thus, the true damage recognition entity of (A)BC excinuclease is the A_2B_1 complex. Surprisingly, when this complex was applied to a TRCF affinity column, only UvrA was retained. As noted previously, TRCF and UvrB share a 140-amino-acid region of homology in their NH_2-terminal regions. These regions are potential UvrA-binding domains in both proteins, and UvrA may possess a UvrB/TRCF dual binding site where the binding sites for UvrB and TRCF overlap (39, 40). It is possible that TRCF binds weakly to the A_2B_1 complex initially by interacting with UvrA outside the area of the UvrB/TRCF dual binding site. The TRCF then replaces UvrB at the UvrB/TRCF-binding site, which aids in dissociating UvrA from the UvrB-DNA complex.

These properties of TRCF are consistent with the following model for TRC (Fig. 3). An RNAP stalled at a lesion is recognized by the TRCF, which binds to the ternary complex and releases the RNAP and the truncated RNA. In a concerted but nonsynchronous reaction, TRCF binds to UvrA in the A_2B_1 complex and recruits the complex to the damage site. As RNAP leaves, the A_2B_1 complex replaces it at the lesion site. The TRCF binds to the UvrB/TRCF-binding domain on UvrA and thus facilitates the dissociation of UvrA from the A_2B_1-DNA complex and the formation of the preincision UvrB-DNA complex. These series of reactions leading up to the formation of the preincision complex are examples of molecular matchmaking to achieve high-specificity DNA binding that does not rely on DNA sequence (34). Thus, stalled RNAP acts as a molecular matchmaker for TRCF, which acts as a molecular matchmaker for UvrA, which in turn is the molecular matchmaker for UvrB and damaged DNA. Following the formation of the UvrB-damaged DNA complex, UvrC binds to it with high affinity and excision progresses via the UvrB-UvrC-DNA complex (above).

In agreement with this model for strand-specific repair are the in vivo mutagenesis results. Most mutations in an active gene arise from lesions in the nontranscribed strand in wild-type cells and from lesions in the transcribed strand in *mfd* mutant cells (29), presumably because lesions in the template strand are repaired relatively rapidly in wild-type cells and lesions in the coding strand are repaired more efficiently in *mfd* mutant cells (20).

STRAND-SPECIFIC REPAIR AND MUTATION FREQUENCY DECLINE

MFD occurs under conditions causing the stringent response which turns off tRNA transcription. However, it also depends on TRCF, which couples repair to transcription. This poses a paradox. A possible explanation is as follows. tRNA genes are transcribed very efficiently, and it is possible that even though during the stringent response the transcription rate goes down, there is sufficient transcription to target the transcribed strand of tRNA genes for rapid repair. In fact, it has been shown that the level of strand-specific repair in *Saccharomyces cerevisiae* is not a simple function of the level of transcription (2). In *E. coli*, it is possible that the high rate of transcription of tRNA genes in rich medium would interfere with repair because even if the first and second stages of TRC occurred, resulting in rapid loading of UvrB to the damage site, a second RNAP molecule may reach the UvrB-DNA complex before UvrC and displace UvrB or make it inaccessible to UvrC. A preliminary report indicating the inhibition of repair of *E. coli* rRNA genes by a high transcription rate (23) is consistent with this model. We expect that similar studies employing controlled expression of tRNA genes as well as mRNA-encoding genes will aid in solving this long-standing mystery called MFD.

ACKNOWLEDGMENTS

We thank Promila Aghi and Jan Sumerel for comments.

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