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Mechanisms of Transcription-Repair Coupling and Mutation Frequency Decline

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INTRODUCTION

"The yield of ultraviolet-induced prototrophs obtained from most auxotrophic strains of *Escherichia coli* and *Salmonella typhimurium* is drastically reduced when the irradiated cells are incubated with chloramphenicol, or in a medium lacking amino acids." This is how Witkin and Theil (102) described the phenomenon known as "mutation frequency decline" (MFD) (20) (Fig. 1), which was originally discovered in 1956 (99). After nearly 4 decades of work, we still cannot explain MFD in molecular mechanistic terms, although there has been significant progress in this direction.

"We find a dramatic difference in the repair of the two strands (of the *lac* operon) only when transcription is induced. Most (pyrimidine) dimers are removed from the transcribed strand of the induced operon within five minutes of irradiation. In the nontranscribed strand repair is significantly slower." This is how Mellon and Hanawalt (51) described the phenomenon of strand-specific repair which was discovered by Mellon et al. (52) 30 years after the discovery of MFD and which is intimately related to MFD. The discovery of strand-specific repair, in contrast to that of MFD, was timely because it occurred when both transcription and excision repair could be carried out in vitro in defined systems to directly test the various models proposed to explain the so-called "preferential repair of transcribed genes" (7). Nomenclature for the strands which compose a transcribed gene is given in Fig. 2.

Whereas attempts to reconstitute preferential repair in vitro were greatly stimulated by the discovery of strand-specific repair first in mammalian cells (52) and then in *Escherichia coli* (51), the phenomenon of MFD had, initially, no effect on this line of research. In fact, gene- and strand-specific repair were achieved in vitro and the transcription-repair coupling factor (TRCF) was extensively purified (75) before a connection between MFD and preferential repair was established (77). This was despite the fact that Bockrath and Palmer (5) had stated that "MFD . . . is a unique process involving excision repair of premutational lesions located only in the transcribed strand of DNA" a full decade before the direct demonstration of such a phenomenon in vivo (52). Our intention is to review the present state of knowledge on MFD (which we still cannot entirely explain in mechanistic terms), transcription-repair coupling (TRC) (about which we have a reasonable understanding), and the TRCF (Mfd protein in *E. coli* and *ERCC6/CSBC* in humans), which is the crucial protein in both phenomena.

MFD

For convenience, auxotrophic *E. coli* strains were used for initial studies on UV mutagenesis, and mutants were scored as revertants to prototrophy (19). If, before being plated on a rich medium, irradiated cells were maintained in a medium having an energy source (glucose) but not favoring protein synthesis, then the frequency of mutations declined (MFD [Fig. 1]) even though cell survival showed no substantive change during this time (99). Subsequent studies demonstrated that almost all of the revertants found in these experiments were due to suppressor mutations and that a subclass of the revertants which arose from true back mutation was not subject to MFD (9).

An important step towards understanding the mechanism of

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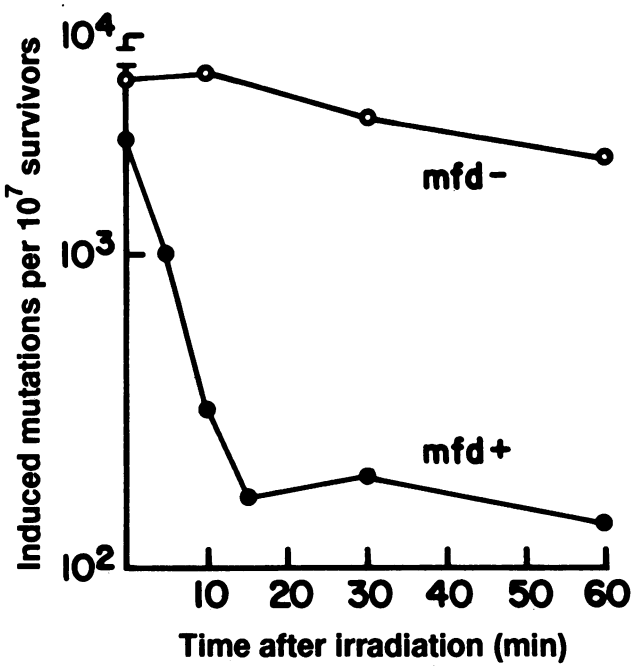

FIG. 1. MFD and its absence in the Mfd$^-$ mutant. Wild-type (WU3610) and Mfd$^-$ (WU3610-45) cells in saline were irradiated with 60 J of UV light per m^2 and then diluted into and held in minimal media. At the times indicated, cells were plated on semienriched medium to select revertants to tyrosine-independent growth. Redrawn from reference 100.

MFD was the identification of two classes of mutant strains which were unable to undergo MFD (101, 102). One class, exemplified by *uvr* mutants, was deficient in excision repair and was UV sensitive; the second class was defined by a single gene, *mfd*. Further studies of the *mfd* mutant provided some clues for how this gene might function (26–28): (i) the *mfd* mutant was capable of excision repair; (ii) Mfd$^-$ cells were only slightly more sensitive to UV than Mfd$^+$ cells; (iii) Mfd$^-$ cells had a normal spontaneous mutation rate; (iv) Mfd$^-$ cells had a fivefold higher UV-induced mutation rate than wild-type cells at all loci tested; (v) Mfd$^-$ cells removed pyrimidine

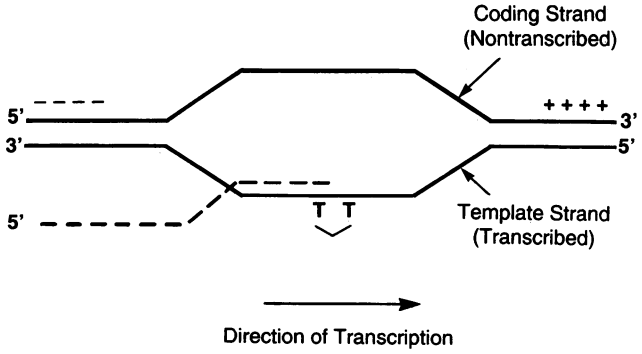

FIG. 2. Representation of a transcription bubble. ++++, transient positive supercoiling preceding, and ----, transient negative supercoiling following, the transcribing RNAP. The dashed line denotes RNA. In this case, the transcribing RNAP (not shown) has been blocked by a thymine dimer (T<>T) in the template and has inserted a base across from the 3′ T of the dimer.

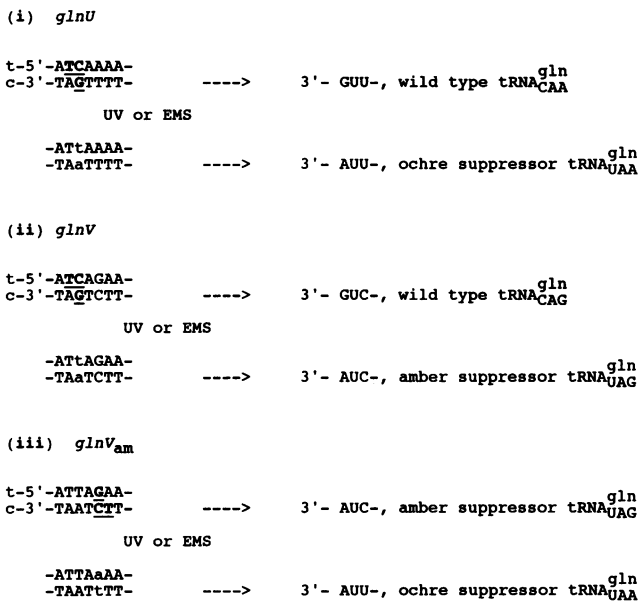

FIG. 3. Formation of suppressor mutations in the *glnU* and *glnV* tRNA genes. These two genes are tandem duplicates located in an operon comprising seven tRNA genes (55). Pathways i and ii show formation of suppressors de novo, by mutation of the wild-type genes, and pathway iii shows how mutation can produce conversion from an amber to an ochre suppressor. Mutation is via GC→AT transition (lowercase) after either the formation of a UV photoproduct at the TC sequence shown in boldface and underlined or the formation of an ethylated guanine residue (boldface and underlined) by treatment with ethyl methanesulfonate (EMS). t, template strand; c, coding strand. In UV mutagenesis studies (5), de novo suppressor mutations, but not conversion-type suppressors, were subject to MFD. In ethyl methanesulfonate mutagenesis studies (4), the reverse was the case: de novo suppressor mutations were not subject to MFD but conversion-type suppressors were. Reprinted from reference 76a with permission of the publisher.

dimers (Pyr<>Pyr) from DNA at one-third the rate of wild-type cells; and (vi) in the *mfd* mutant, the MFD phenomenon was not totally abolished; rather it became exceedingly slow. MFD appeared to result from the removal of a subclass of premutational lesions by the excision nuclease targeted to these lesions by the *mfd* gene product before the mutations were fixed by replication.

The occurrence of MFD for certain types of mutations but not others has been the source of considerable speculation and confusion and at the same time has led to a surprisingly accurate insight into the function of the *mfd* gene. Bridges et al. (9) reported that MFD affects suppressor mutations but not true back mutations. Bockrath and Palmer (5) revealed that UV-induced "de novo suppressor mutations" (mutations in the anticodon of a glutamine tRNA gene making it a suppressor [Fig. 3]) were but "conversion type suppressor mutations" (mutations which convert a suppressor tRNA from recognizing an amber codon to recognizing an ochre codon) and true back revertants were not susceptible to MFD. They concluded that MFD must be the result of a specific repair event operating on the glutamine tRNA genes (5, 26a, 29b).

Analysis of the potential lesion sites in the particular tRNA genes, illustrated in Fig. 3, suggested that both types of suppressor mutations would arise from UV-induced lesions at a TC sequence and change it to TT (24a, 24b). However, the target TC sequence is in the transcribed (template) strand for

the de novo suppressor (susceptible to MFD) and in the coding (nontranscribed) strand for the conversion-type suppressor (not susceptible to MFD). This led to the logical conclusion that "MFD . . . is a unique process involving excision repair of premutational lesions located only in the transcribed strand of DNA" (5). Experiments with ethyl methanesulfonate mutagenesis provided strong support for this conclusion. In contrast to UV mutagenesis, conversion-type mutations arising 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. 3) (4).

Since MFD occurred at times of amino acid starvation of cells, 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. "The tRNA:DNA hybrid would place the photoproduct in a double-stranded configuration necessary for incision" by the repair enzyme (5). While this model raised the possibility of strand-specific repair for the first time, it attributed the strand-specific repair to the peculiarity of tRNA genes and stated that it happened 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 (85), MFD occurred at the same rate and to the same extent as in a rel^+ strain (24, 26), leading to the conclusion "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" and that the results "do not lend support to the tRNA:DNA hybridization mechanism as an explanation for MFD" (24).

Since strand-specific repair seemed to be unconnected to transcription, another possible source of strand asymmetry was considered. Lesions may be mutagenic to different degrees depending on whether they are in the template strand for leading or for lagging-strand synthesis (48, 92). However, no difference was found when isogenic strains carrying the $tRNA^{gln}_{CAG}$ gene in opposite orientations were tested for MFD, suggesting that the direction of replication was not a determinant for MFD. These experiments demonstrated that MFD was not unique to UV mutagenesis or to de novo suppressors and that it was associated with mutations arising from damage in the transcribed strand of tRNA genes regardless of the direction of replication, the type of lesion, or the particular mutation site.

The scenario that emerged from these studies was as follows. Incubation of cells with damaged DNA in a medium with an energy source (e.g., glucose) but lacking an essential nutrient for growth resulted in a rapid decline of mutations in suppressor tRNA genes when these mutations were caused by a lesion in the transcribed strand. However, this rapid decline in mutation frequency was not dependent upon or inhibited by transcription. MFD in the original sense, that is, a rapid decline in mutation frequency with a half-life of <10 min (which is faster than the rate for bulk dimer excision [29b]), does not occur for mutations caused by lesions in the nontranscribed strand of tRNA genes or for mutations by lesions in structural genes encoding proteins. It must be stated, however, that an MFD with much slower kinetics occurs in mutations caused by all lesions which are substrate for excision repair regardless of their location. This is because of a phenomenon called liquid holding recovery. Incubation of UV-irradiated cells in a buffer with glucose but no nitrogen source results in a slow but steady excision of DNA lesions up to 8 h (87, 88), and this excision, naturally, results in an overall reduction of mutation frequency. However, this decline in the fraction of lesions and fraction of mutations never exceeds 50% of the initial values, and it is apparently unaffected by the *mfd* gene (4, 100, 102).

In conclusion, studies of MFD revealed that the transcribed and nontranscribed strands of tRNA genes were not equally susceptible to repair, but it also seemed that this asymmetric behavior of the two strands with regard to repair was not connected to the transcriptional status of the gene. Moreover, even though the overall induced mutation frequency is increased fivefold in *mfd* cells, there was no indication that this had anything to do with transcription. Therefore, when the transcription-dependent gene- and strand-specific preferential repair phenomena were discovered (7, 52), the link between MFD and these phenomena was not immediately evident.

NUCLEOTIDE EXCISION REPAIR

Both MFD and preferential repair are special manifestations of nucleotide excision repair (27, 28, 52, 101), 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 30 and 63). In *E. coli*, the excision nuclease, (A)BC excinuclease (70), results from the sequential and partially overlapping actions of UvrA, UvrB, and UvrC proteins. UvrA is a molecular matchmaker (69) 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 (59). 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, in turn triggering 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 (31, 45, 61). The loading of UvrB to the damage site is the rate-determining step in the overall reaction because it involves probing of DNA for subtle structural abnormalities by the A_2B_1 complex through a helicase-like action (41, 57, 60). 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* (81). The human excinuclease, resulting from the coordinate actions of at least eight subunits, hydrolyzes the 22nd to 24th phosphodiester bond 5′ and the 5th phosphodiester bond 3′ to the lesion and thus excises the lesion in a 27- to 29-mer, in contrast to the 12- to 13-mer of *E. coli* (32). Humans deficient in this enzyme are afflicted with the xeroderma pigmentosum syndrome, which is characterized by actinic keratoses and cancers and in many cases neurological abnormalities.

PREFERENTIAL REPAIR

Repair of certain regions, structures, sequences of the genome, or one of the two strands of the duplex at a faster rate compared with the rest of the genome is called preferential repair (7). Curiously enough, a forerunner to this topic reported inhibition of photoreactivation of the *E. coli* galactokinase gene upon induction of transcription (40). Wide interest and investigation began only after the nucleosome-chromatin structures were elucidated in the 1970s and the effects of these

structures on DNA functions (replication, transcription, recombination, and repair) were appreciated. Studies of the effects of chromatin structure on transcription and on repair, in general, paralleled one another. The "nuclease sensitivity assay," which became one of the most important tools in investigating "active chromatin" (see reference 22), was immediately adapted to DNA repair. Studies with this assay yielded results similar to those obtained with "transcription-active chromatin" in terms of chromatin structure and function. Thus, "repair-active chromatin" was also found to have increased susceptibility to micrococcal nuclease (6) and DNase I (2). Furthermore, it was found that the recovery of RNA synthesis following UV irradiation preceded the removal of Pyr<>Pyr from the genome, and it was concluded that this was because the transcribed regions of the genome were repaired faster than the bulk DNA (49, 50). Finally, a high-resolution assay was developed by Nose and Nikaido (56) to investigate repair at the gene level. In this assay, following DNA damage by an alkylating agent and repair, DNA was isolated, digested with a restriction enzyme, treated with alkali which cleaves at AP (apurinic-apyrimidinic) sites caused by alkylation damage, resolved on a denaturing gel, and analyzed by Southern hybridization. From the fraction of full-length fragment present at various time points following DNA damage, the rate of adduct formation and repair could be measured. Nose and Nikaido (56) found that the alkali-labile sites caused by MNNG in the actively expressed procollagen gene and the inactive β-globin gene in human fibroblasts were repaired at the same rate. As it turns out, this lack of transcription-stimulated repair may result from a failure of this lesion to block transcription and from the action of alternative repair pathways that are not stimulated by transcription.

The first support for preferential repair of an actively transcribing gene was obtained by Bohr et al. (7), who analyzed the formation and repair of pyrimidine dimers (Pyr<>Pyr) in the dihydrofolate reductase gene of UV-irradiated Chinese hamster ovary cells by treating the restriction enzyme-digested DNA with T4 endonuclease V (which incises DNA at Pyr<>Pyr) before separation on a denaturing gel and Southern hybridization. They found that the repair rate of the dihydrofolate reductase gene was fivefold higher than the average rate for the entire genome. This study had a significant effect in the field and led to similar studies and similar findings for other genes, other lesions, and other organisms including humans.

At first, 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 22). This idea was challenged by the results of Mellon et al. (52), who discovered that the transcribed (template) strand of the dihydrofolate reductase gene in CHO cells was repaired at a rate 10 times higher than that of the nontranscribed strand, which was repaired at a rate equal to that of the bulk DNA. In other words, the entire gene-specific repair phenomenon (fivefold increase in repair rate) could be explained by strand-specific repair (10-fold increase in repair rate). The strand-specific repair could still be explained within the general framework of open chromatin conformation (83): a lesion in the template strand (but not in the coding strand) blocks RNAP and thus retains the open chromatin conformation long enough for the repair enzyme to excise the lesion. However, this explanation was inconsistent with three other observations. First, Mellon and Hanawalt (51) 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 (74) found that in vitro an RNAP stalled at a Pyr<>Pyr inhibited repair by steric interference with (A)BC excinuclease. Third, in eukaryotes, genes transcribed by RNAPI (95) and apparently by RNAPIII (1) are not subject to gene-specific repair, yet these polymerases are also blocked by lesions, and the transcribed genes are associated with an open chromatin conformation. It was seen that the act of transcription and the particular polymerase involved, rather than an open chromatin structure, had crucial roles in the increased repair rate. To understand transcription-stimulated repair, it was necessary to understand the interaction of the transcription apparatus with lesions in the template and coding strands and with the nucleotide excision repair enzyme.

EFFECTS OF DNA DAMAGE ON TRANSCRIPTION AND OF TRANSCRIPTION ON REPAIR

Effect of DNA Lesions on Transcription

UV irradiation of *E. coli* inhibits all macromolecular syntheses to varying degrees (78). Evidence that UV damage inhibits transcription directly was first obtained by Starlinger and Kolsch (84), who found a polar effect of UV irradiation on the *gal* operon of *E. coli*. The seminal work of Michalke and Bremer (53) provided a molecular explanation of the phenomenon; they showed that the length of nascent RNA chains became progressively shorter with increasing UV dose to *E. coli*, suggesting that UV lesions block RNAP. Using an in vitro system with irradiated T4 phage DNA as a template and developing an elegant method of gene sizing and operon mapping based on inactivation by single-hit kinetics, Sauerbier et al. (73) obtained results similar to those of Michalke and Bremer. Thus, the larger the gene the more sensitive it is to UV inactivation. Similarly, in an operon of several genes the promoter-distal genes are more sensitive to inactivation. Thus, the size of a given gene and the order of several genes in an operon can be determined by UV inactivation kinetics (72). Knowing whether transcription was blocked by a lesion in either DNA strand or only by a lesion in the template strand was not essential for this analysis, but this knowledge became important in developing an in vitro system for studying TRC.

The first progress towards such a system was made by Shi et al. (79, 80), who constructed two 137-bp templates, bearing promoters for *E. coli* and T7 phage RNAPs and a psoralen-thymine monoadduct located downstream in either the template or the coding strand. With this system, it was found that a psoralen adduct in the coding strand had no effect on transcription whereas an adduct in the template strand caused premature termination 1 base before the psoralen-adducted thymine. The RNAPs made stable elongation complexes at the lesion site as revealed by DNase I footprinting. Selby and Sancar (74) adopted this system to the thymine dimer (T<>T). 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 strand constituted an absolute block for RNAP with <1% translesion synthesis and gave rise to a stable elongation complex. In the case of T<>T, in contrast to the psoralen-thymine adduct, the truncated transcript terminated with a base (possibly A) across from the 3′ base of the T<>T (Fig. 2). Chen and Bogenhagen (12) conducted similar studies with phage T7 RNAP and DNA containing an AP site, 8-oxodG, and AAF- or AF-modified guanine. They found that none of these lesions blocked transcription when in the coding strand, but with the exception of 8-oxodG, they did inhibit it to varying degrees when present in the template strand. In contrast, studies by Zhou and

Doetsch (103) with an abasic (AP) site lesion and RNAPs from *E. coli* or SP6 revealed that both RNAPs bypassed the lesion whether it was in the coding or the template strand. Corda et al. (15a, 15b) used dinucleotides as primers for elongation by *E. coli* RNAP and wheat germ RNAPII and examined the effects of various platinum adducts located downstream on elongation. Elongation by the RNAPs was only slightly inhibited by *cis*-platin intrastrand cross-links located on the nontranscribed strand but was strongly blocked by the intrastrand cross-links on the template strand and by an interstrand cross-link. A platinum monoadduct and a *trans*-platinum intrastrand cross-link also blocked elongation by the RNAPs, although with these lesions some bypass was observed. Finally, Chen et al. (13) found that both *N*-acetyl-2-aminofluorene-guanine and 2-aminofluorene-guanine adducts in the coding strand had no effect on transcription but blocked *Xenopus laevis* RNAPIII when present in the template strand, causing termination 1 nucleotide (nt) before the modified guanine. It was not determined whether a stable elongation complex was formed at the site of the lesion in the eukaryotic systems. Thus, it appears that most of the so-called bulky adducts block RNAP but only when present in the template strand. In the case of the phage and the *E. coli* RNAPs, blockage has been shown to give rise to a stable elongation complex.

Effect of Stalled RNAP on Repair

Selby and Sancar (74) investigated the effect of transcription on repair 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. When repair was measured in the absence of ribonucleoside triphosphates, it was found that the promoter-bound RNAP had no effect on repair of a T<>T downstream from the transcriptional initiation site whether the T<>T was in the coding or in the template strand. However, when repair was performed in the presence of ribonucleoside triphosphates, 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. This unanticipated finding compelled the conclusion that cells must possess a TRC mechanism which performs two tasks: overcoming the repair-inhibitory effect of a stalled RNAP and accelerating the rate-limiting step of excision repair.

TRC MECHANISMS

Models

Models that attempt to describe the mechanism of preferential repair of transcribed genes are divided below into four general areas that represent different aspects of the same phenomenon. They are the overall conformation of transcribed DNA, the unique topology of the transcription bubble, the structure of a stalled complex and its protein components, and the existence of a protein distinct from the known transcription and repair proteins which function to couple the two processes.

(i) Chromosome conformation. The most general model is based on the well-known inaccessibility of folded chromosomes to enzymes and the increased susceptibility of transcriptionally active chromatin to all kinds of probes including enzymes. Simply put, this model suggests that the open chromatin structure and perhaps even the nucleosome-free state of the transcribed region make it accessible to repair proteins. The in vivo study of Smerdon and Thoma (3, 83) with a *Saccharomyces cerevisiae* minichromosome that has precisely mapped nucleosome and transcriptional units found a correlation between high repair rate and nucleosome instability caused by transcription or by unknown factors. The higher rate of repair in the transcribed strand compared with the nontranscribed strand was explained by assuming that a lesion in the transcribed strand blocks RNAP and as a consequence maintains the nucleosome-free state for longer periods compared with a lesion in the nontranscribed strand. Undoubtedly, this model and other versions of it have some validity because a folded chromosome is generally a poor substrate for an enzyme, including most repair enzymes. However, this model assumes that a stalled RNAP does not interfere with the excision nuclease, which is unlikely, and also is of questionable value for *E. coli* which lacks the well-defined nucleosome-chromatin structure found in eukaryotes.

(ii) Configuration of the transcription bubble. The transcription bubble (Fig. 2) has a unique topology (25). In the *E. coli* elongation complex, the DNA is unwound by approximately 17 bp, and within the unwound region, approximately 12 nt of the template strand is in an RNA-DNA hybrid; in eukaryotes, the unwound region is at least 10 to 11 bp. Progression of the transcription bubble causes overwinding in front of and underwinding behind the transcription complex (25), which under appropriate circumstances can create positive superhelical turns preceding and negative superhelical turns following RNAP (46). It is conceivable that transient changes in superhelicity caused by a stalling RNAP make the transcription-blocking lesion a better substrate for the excision nuclease. For example, it is known that transcription-stimulated supercoiling can stimulate Tn*1000*-mediated recombination (21). Similarly, Munn and Rupp (54) found that a psoralen cross-link was >100-fold better as a substrate for (A)BC excinuclease when it was located in a supercoiled compared with a relaxed plasmid. However, no such difference was seen with other psoralen-cross-linked substrates (35, 91). More importantly, superhelicity has no or only an inhibitory effect on the removal of monoadducts and intrastrand diadducts by (A)BC excinuclease (97) or human excinuclease (82). Thus, the topological model, although likely to be a factor in fine-tuning of repair of certain lesions, is insufficient to explain the drastic differences between the rates of repair of transcribed and nontranscribed DNA.

(iii) Protein tagging of lesions. Excision nucleases recognize and excise bulky adducts. It is conceivable that the presence of RNAP stalled at a lesion would enhance the repair of the lesion by increasing its apparent bulkiness. A potential precedent for this is the photolyase-excinuclease interaction. Photolyase binds to Pyr<>Pyr with high affinity and splits the dimer upon exposure to light. Without light, the enzyme remains stably bound to Pyr<>Pyr, and this complex is apparently recognized with much higher efficiency by excinucleases in *E. coli* (68) and in *S. cerevisiae* (71), since the photolyase-bound Pyr<>Pyr is removed about threefold faster than the unbound Pyr<>Pyr. It is possible that other proteins, including RNAP, that bind to lesions facilitate recognition by the excision nuclease proper and promote rapid repair. However, the cooperative action between photolyase and (A)BC excinuclease is possible because photolyase contacts mostly the damaged strand (33) while (A)BC excinuclease binds mostly to the undamaged strand, enabling a coordinate action. In fact, the binding surfaces on DNA for the two enzymes are so tightly complementary that photolyases from different species inhibit rather than stimulate excinucleases of other species (37, 71, 82). Thus, no a priori prediction can be

TABLE 1. Properties of TRCF in *E. coli* and humans

Property	*E. coli*	Human
Gene	*mfd*	*ERCC6/CSBC*
Location	25.3 min	10q11-q21
No. of amino acids	1,148	1,493
Size (kDa)	130	168
Helicase motif	Yes	Yes
Sequence similarity	RecG, UvrB	SNF2 family
Cognate RNAP	*E. coli* RNAP	RNAP II
Interacting repair protein	UvrA	ERCC2, ERCC3
ATPase	Yes	ND[a]
DNA binding	Yes	ND
Helicase activity	No	ND
No. of copies/cell	~500	ND
Mutant phenotype	Mfd$^-$	Cockayne's
UV sensitivity of mutant	Moderate	Moderate

[a] ND, not determined.

made whether a protein bound to (stalled at) a lesion will stimulate or inhibit repair. When the experiment was done with *E. coli* RNAP and (A)BC excinuclease, the stalled RNAP inhibited repair presumably because of steric hindrance (74). An analogous experiment has not been done in an in vitro eukaryotic system. However, in vivo data suggest that the same may be true for *S. cerevisiae*. Most UV-induced mutations in the yeast tRNA gene are caused by lesions in the template strand (1), suggesting that RNAPI stalls at photoproducts in the template strand and inhibits repair. Thus, clearly neither the topology of the transcription bubble nor the lesion-bound RNAP is a high-affinity site for the repair enzyme. However, they must be intrinsic components of transcription-coupled repair and thus could be high-affinity sites for a protein(s) specifically designed to recognize such structures and function as an intermediary between the stalled RNAP complex and the repair enzyme.

(iv) TRCF. The coupling factor could be a multiprotein complex ("couplingsome") or a single polypeptide capable of interacting with both a stalled elongation complex and the excision nuclease. The latter possibility would predict that a single gene is responsible for preferential repair. In fact, such a gene has been identified both in *E. coli* and in humans. In *E. coli*, mutation in the *mfd* gene (77) and, in humans, mutation in the *CSBC/ERCC6* gene (which causes Cockayne's syndrome) (93, 94) completely abolish gene- and strand-specific repair (Table 1). Thus, it appears that in both organisms a protein functions to displace the stalled RNAP and recruit the excision nuclease.

In Vitro Systems

When a defined system consisting of template-substrate; *E. coli* RNAP; and the UvrA, UvrB, and UvrC proteins failed to achieve strand-specific repair, it was concluded that the system lacked a TRC protein present in cells (74). Therefore, unfractionated *E. coli* cell extract supplemented with 6% polyethylene glycol for macromolecular crowding was tested for transcription-coupled repair (75). A plasmid with a *tac* promoter was irradiated with UV and mixed with the cell extract in the presence of four deoxynucleoside triphosphates (with [α-^{32}P] dCTP as a label), four ribonucleoside triphosphates, and NAD. Repair was measured by incorporation of label into the plasmid DNA. When the distribution of the repair synthesis within the plasmid was analyzed, it was found that the *tac* transcriptional unit contained about twofold more repair synthesis compared with the other regions of the plasmid. This preferential incorporation was inhibited by adding either rifampin or *lac* repressor to inhibit transcription in the reaction mixture. In analyzing repair synthesis within the two strands of the *tac* transcriptional unit, it was found that the template strand was repaired fivefold faster than the nontranscribed strand. In contrast, there was no difference between the rates of repair of complementary strands originating from transcriptionally quiescent parts of the plasmid. Strand-specific repair within the *tac* transcriptional unit was inhibited by the *lac* repressor, and this inhibition could be suppressed by the *lac* inducer IPTG (isopropyl-β-D-thiogalactopyranoside). With cell extract, 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, this in vitro system had all the hallmarks of gene- and strand-specific repair as defined by in vivo experiments: the preferential repair was strand specific and transcription dependent, it was elicited by any lesion which blocked RNAP, and it utilized the nucleotide excision repair pathway since preferential repair was absent from extracts made from *uvrA* mutant cells.

A similar defined system consisting of purified *E. coli* RNAP, UvrA, UvrB, UvrC, helicase II, polymerase I, and DNA ligase performed nucleotide excision repair synthesis but failed to carry out preferential repair when provided with the UV-irradiated plasmid with the *tac* promoter. However, strand-specific repair was observed when the defined system was supplemented with certain fractions of *E. coli* cell extract. This assay of transcription-dependent preferential repair was utilized to successively purify TRC activity from *E. coli* cell extract through four to five chromatographic steps. A large (130-kDa) protein was partially purified and identified as the TRCF (75, 77).

The TRCF Is Encoded by *mfd*

From biochemical studies, it became apparent that a single protein in *E. coli* was responsible for TRC. 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, even though it had been reported that this preferential repair was transcription independent (24). Cell extract was prepared from *mfd* mutant cells and tested for strand-specific repair. The results were clear: there was no strand-specific repair (77). This defect was complemented with purified TRCF, and it was concluded that *mfd* encodes the TRCF and that the Mfd$^-$ phenotype was due to a lack of TRC.

Confirmation that *mfd* encodes the TRCF was obtained through the cloning and functional analysis of the *mfd* gene and protein. Preliminary mapping linked *mfd* to *umuDC* (26 min [101a]). Higher-resolution mapping by P1 cotransduction utilized the available *mfd* mutant WU3610-45 and a series of K-12 derivatives with defined, chromosomal Tn*10* insertions (82a) located in the region of *umuDC*. In these experiments, transduction of the *mfd*$^+$ gene from the K-12 derivatives into WU3610-45 was analyzed by testing for the Mfd phenotype, and *mfd* was mapped to 25.3 min. In addition, a *mfd* mutant transductant of WU3610-45 was used as a source of P1 lysate to move the gene into AB1157, and the *mfd* mutant strain created (termed UNC361045) was identified on the basis of failure of cell extract to perform strand-specific repair in vitro. A degenerate oligonucleotide probe was synthesized on the basis of the amino-terminal sequence of the partially purified TRCF protein. This probe hybridized weakly to phages 237 and 238 from the Kohara library (39) miniset (76), both of which possess chromosomal DNA from the 25.3-min region.

DNA from phage 238 was partially digested with *Sau*3A, fragments were inserted into the *Bam*HI site of pBR322, and transformants of WU3610-45 were screened for the Mfd$^+$ phenotype. Constructs that conferred Mfd$^+$ were transformed into UNC361045 and were found to confer strand-specific repair when extracts of the transformants were tested for biochemical activity. The constructs had chromosomal DNA inserts that were 6 to 8 kb in size. Finally, from the cloned gene the protein was overproduced and purified, and it was used to reconstitute strand-specific repair in a completely defined system (76, 76b). Consequently, TRCF and Mfd protein both refer to the *mfd* gene product and TRCF has been used generically to refer to an enzyme from any organism which functions as the Mfd protein functions.

Undoubtedly related to *mfd* is the finding that, in wild-type bacterial and eukaryotic cells, lesions in the nontranscribed strand are more mutagenic than lesions in the transcribed strand (10, 11, 38, 64, 65, 67, 96). Presumably, most mutations arise from lesions in the nontranscribed strand because, in wild-type cells, lesions in the transcribed strand are more rapidly repaired. In contrast, it was predicted that in cells that lack TRCF, lesions in the transcribed strand should be more mutagenic (77). This prediction was based on the finding that, in vitro, a lesion in the template but not the coding strand blocked RNAP and the stalled RNAP inhibited repair in the absence of TRCF (74). When the TRCF was shown to be the *mfd* gene product, *mfd* mutant cells were used to test this prediction. The results were striking: in the *lac*I target gene, mutations arose 3.2 times more frequently from lesions in the nontranscribed strand in wild-type cells, while in *mfd* mutant cells mutations arose 4.5 times more frequently from lesions in the template strand (58). These results indicated that in vivo, in the absence of TRCF, when RNAP encounters a lesion in the template, it forms a stable, stalled elongation complex that inhibits repair. The *mfd* gene product must overcome this inhibition and target the lesion for enhanced repair in vivo.

In analogy with *mfd*, the *ERCC6* gene encodes the human TRCF because it confers the wild-type phenotype on Cockayne's syndrome complementation group B cells (90), whose only biochemical defect is the lack of gene- and strand-specific repair (93, 94). It has been predicted that Cockayne's syndrome group B cells should exhibit the same strand bias for mutation induction as *mfd* mutants (77), but this prediction has not been tested experimentally.

STRUCTURE AND PROPERTIES OF TRCF

Both the *E. coli* and human TRCF genes have been cloned and sequenced (76, 89, 90). The sequence of the 1,148-amino-acid protein encoded by the *mfd* gene reveals three features of interest (76) (Fig. 4). First, the sequence of a 140-amino-acid region near the NH_2 terminus of TRCF is homologous to the corresponding region of UvrB. Since both TRCF and UvrB bind UvrA (see below), this region of 140 amino acids may form a UvrA binding domain. Second, near the center of TRCF there are the seven motifs that are found in many known or putative helicases (29). Over a stretch of 400 amino acids covering this so-called helicase motif region, the TRCF shares 38% sequence identity with the *E. coli* RecG protein, which is involved in branch migration of Holliday junctions (47). Finally, in the COOH-terminal region between positions 1039 and 1060 there are four leucines at 7-amino-acid intervals (potential leucine zipper). This region might be involved in binding other proteins, such as RNAP. The *ERCC6* gene also encodes a large (168-kDa) protein (89, 90) that possesses the seven helicase motifs; however, aside from these general features there are no striking similarities between the Mfd and ERCC6 sequences. Other comparisons of the human and *E. coli* TRCFs are given in Table 1.

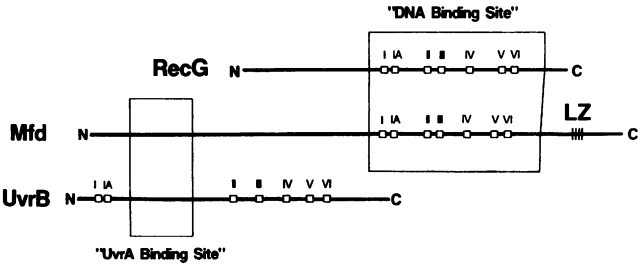

FIG. 4. Sequence homologies among Mfd (TRCF), UvrB, and RecG proteins. I to VI indicate the so-called helicase motifs. Neither Mfd nor RecG has helicase activity. LZ, potential leucine zipper structural motif. Reprinted from reference 76a with permission of the publisher.

The Mfd protein (TRCF) is a relatively abundant protein with about 500 copies per cell. Cells carrying the *mfd* gene on a multicopy plasmid and expressing the TRCF to about 5% of total cellular proteins appear normal and have normal viability. Overproducing constructs were also found to confer the Mfd$^+$ phenotype on WU3610-45 in a semiquantitative spot test. The Mfd protein is a monomer, binds to DNA nonspecifically, and has a weak ATPase activity (k_{cat} = ~3 min^{-1}). The ATPase activity is not affected by DNA, although ATPγS greatly stimulates the nonspecific DNA binding (76). A most interesting finding from a functional standpoint is the lack of helicase activity. No helicase activity could be detected when the DNA oligomer to be displaced was only 17 nt long (76b). Similarly, it was unable to displace a 48-nt-long 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 at least 60 nt long to perform its RNA-DNA helicase activity, the TRCF was tested with such a substrate. TRCF did not dissociate a 49-bp RNA-DNA hybrid with a 222-nt-long 5′ RNA tail (76). 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 (47). 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 revealing.

MOLECULAR MECHANISM OF TRC

The two functions that the TRCF must carry out are the displacement of the stalled RNAP from the lesion and the recruitment of repair enzyme to the damage site. In regard to the displacement of the stalled complex, in both prokaryotes and eukaryotes there are a number of proteins which interact with stalled RNAP (reviewed in reference 66). 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 improves the efficiency of Rho-dependent termination. NusA also binds to RNAP and enhances the efficiency of intrinsic terminators. In contrast, NusB and S10 proteins bind to a specific sequence in the *rrn* transcript and act

as antiterminators. In eukaryotes, TTF1 binds to 3′ DNA sequences to affect RNAPI termination and La protein binds to the 3′ end of the transcript to affect RNAPIII termination. No similar factors are known for RNAPII (66). A most fascinating class of antiterminators that have been discovered in both *E. coli* and humans are the transcript cleavage factors (36). In *E. coli*, GreA and GreB proteins bind to a stalled RNAP and activate its intrinsic RNase activity. This enables RNAP to hydrolyze 2 to 10 nt from the 3′ end, retract from its trapped conformation, and resume its effort to bypass a transcription-blocking structure (8, 86). In humans, TFIIS performs the same function for RNAPII (34). Surprisingly, there is no evidence for involvement of any of these factors in TRC. Instead, it appears that TRCFs both in *E. coli* and in humans perform their functions independently of these other factors and by a completely different mechanism. As yet, only the reaction mechanism of the *E. coli* TRCF has been elucidated.

In a fully defined system, *E. coli* TRCF specifically interacts with RNAP stalled at a lesion and dissociates the ternary complex. No other protein is needed for this function, and the RNA is not cleaved before or during dissociation from the RNAP. The coupling factor does not seem to dissociate an initiation complex, and TRCF-stimulated repair starts at the 15th base and continues beyond in the transcriptional unit (76). Transcription-enhanced repair is not seen at damage closer to the transcription start site (76b). At approximately this point in the template (15th base), RNAP is known to undergo several structural and functional changes (42): 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 apparent what feature or subunit of the entirely 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 (62). Since the coupling factor binds to DNA nonspecifically, it may interact with some nucleic acid component of the transcription bubble. It is known that DNA damage is not needed for dissociation of a stalled RNAP since the TRCF dissociates RNAP stalled either by a protein-DNA roadblock or by the absence of a nucleotide triphosphate precursor (42, 62b, 76b). Another component of the substrate that is probably not essential is the free RNA tail, since TRCF does not bind to free RNA and because stimulation of repair occurs at about nt +15 when virtually all of the transcript is in the form of an RNA-DNA hybrid (76). This is a significant difference between the TRCF-dependent and Rho-dependent terminations. In Rho-dependent termination, an RNA tail of >50 nt is required for binding of Rho, entry into the ternary complex, and eventual dissociation by Rho's RNA-DNA helicase activity. However, the TRCF- and Rho-mediated terminations have some similarities: both proteins are ATPases and ATP hydrolysis is required for dissociation of the complex, both proteins dissociate ternary complexes formed at protein blocks such as *trp* repressor or *Eco*RI endonuclease, and 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 excinuclease. The delivery of UvrB by UvrA to the damage site is the rate-limiting step of the repair reaction. Therefore, the only way to increase the repair rate is to facilitate this process. This is accomplished by specific binding between the coupling factor and UvrA. Although no interaction between these two subunits could be detected by standard hydrodynamic methods, UvrA does specifically bind to a

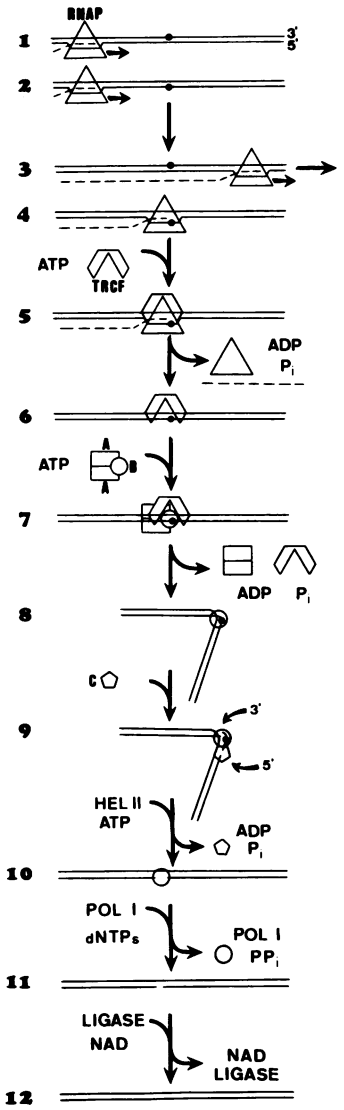

FIG. 5. Molecular model for TRC in *E. coli*. A, B, and C, UvrA, -B, and -C, respectively; HEL II, helicase II; POL I, DNA polymerase I. Reprinted from reference 76a with permission of the publisher.

TRCF affinity column, which indicates that the interaction is relatively weak. The functional form of UvrA is the A_2B_1 complex (59), 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-long region of homology in their NH_2-terminal regions. These regions are probable 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 (76, 76b). Possibly, the TRCF binds weakly to the A_2B_1 complex at first by interacting with UvrA outside the area of the UvrB-TRCF dual binding site. Then, the TRCF replaces UvrB at the UvrB-TRCF binding site, which aids in dissociating UvrA from the UvrB-DNA complex.

These characteristics of TRCF are consistent with the following model for TRC (Fig. 5). An RNAP stalled at a lesion is recognized by the TRCF, which binds to the ternary complex

and releases the RNAP and the nascent 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 departs, 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 (69). 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. After the formation of the UvrB-damaged DNA complex, UvrC binds to it with high affinity, and in the resulting UvrB-UvrC-DNA complex, UvrB makes the first incision at the fifth phosphodiester bond 3′, and then UvrC incises the eighth phosphodiester bond 5′ to the lesion. The postincision UvrB-UvrC-DNA complex is stable; UvrC and the excised oligomer are released by helicase II (UvrD), and the UvrB is displaced from the repair gap by polymerase I, which synthesizes a repair patch exactly matching the gap (without nick translation). Finally, the patch is ligated by DNA ligase. Although this model is consistent with all available data, some of the key intermediates have not been experimentally demonstrated as yet. In particular, intermediates involving DNA-RNA-RNAP-TRCF-A_2B_1, or DNA-TRCF and DNA-TRCF-A_2B_1, have not been captured by either hydrodynamic or footprinting techniques.

There has not been any biochemical study of TRC in humans. However, both in vitro transcription systems (15) and repair systems (63) are available and the human TRCF gene *ERCC6/CSBC* has been cloned and sequenced (90). It is possible that TRC in humans occurs by the same mechanism as in *E. coli*. Both Mfd and ERCC6 are relatively large proteins and possess "helicase motifs" (Table 1). Furthermore, in keeping with the specificity of the TRCF-RNAP interaction, coupling occurs only with a stalled RNAPII (14, 44, 95). Mutagenesis studies suggest (1) that repair of the template strand of genes transcribed by RNAPI or RNAPIII is inhibited by transcription. This is consistent with the data obtained with the *E. coli mfd* mutant. When *E. coli* RNAP does not interact with a TRCF, it actually inhibits repair of the transcribed (43, 74) strand. This inhibition results in preferential repair of the coding strand and preferential mutagenesis of the template strand (58).

The eukaryotic TRC reaction may have some novel features as well, as suggested by the reports that the ERCC3/XPB and ERCC2/XPD gene products are subunits of the RNAPII transcription factor TFIIH (20a, 73a). The yeast homolog of ERCC3 is SSL2 (Rad 25) (29a, 62a). A mutational analysis of the SSL2 gene product revealed that some mutants conferred UV sensitivity and some mutant constructs were not viable, suggesting two functions for the protein, one in DNA repair and one essential function related to transcription or translation. The SSL2 gene was isolated by using a genetic system to detect suppression of the inhibitory effect of a stem-loop structure in the 5′ end of a mRNA on translation, suggesting a role in translation initiation. It should be noted that an additional mutant isolated by this system, SSL1, also confers UV sensitivity (102a). However, the roles of these eukaryotic repair-related proteins (ERCC2, ERCC3, SSL2, SSL1) in transcription and TRC are far from clear.

STRAND-SPECIFIC REPAIR AND MFD

Although the phenomenon of MFD greatly aided in understanding the mechanism of TRC, the model for TRC presented here cannot explain MFD entirely. MFD occurs under stringent response incubation conditions in which the synthesis of mRNA is elevated and tRNA is depressed. It results from preferential repair of the transcribed strand of tRNA genes but apparently not that of mRNA genes. This is the exact opposite of what one would have predicted from the model. Yet it also is true that when the *mfd* gene which encodes TRCF is inactivated no MFD occurs (100). How can the model be reconciled with the MFD phenomenon? We will address the questions of repair of tRNA and mRNA genes as they relate to MFD separately.

tRNA genes are transcribed very efficiently, and it is quite possible that even though during the stringent response incubation the transcription rate goes down, there is sufficient transcription to target the transcribed strand of tRNA genes for rapid repair. In rich media, it is possible that the high rate of transcription of tRNA genes would actually 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 report indicating an apparent inhibition of strand-specific repair by a high transcription rate (33a) is consistent with this model. In this study, the strongly transcribed *rpsL* gene was introduced into *ada ogt* mutant cells on a plasmid. It was found that *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine-induced mutations in the *rpsL* gene were targeted more often by lesions in the transcribed strand than by those in the nontranscribed strand.

The second question deals with the lack of MFD in true back mutations. In rich media, genes encoding some biosynthetic and degradative enzymes are turned off, and in minimal media, they are induced. Thus, it would be expected that in minimal media (MFD condition) these genes would be repaired faster because of TRC whereas repair would be slower in rich media. In terms of an operational definition of MFD, this would mean that if the cells were plated on rich media immediately following UV irradiation, a high mutation rate would be observed because replication would "fix mutation" before repair. In contrast, if cells were held in a minimal medium before plating, active transcription would be expected to increase the rate of repair and thus lower the chance of translesion mutagenic replication. In fact, in cases in which this has been tested by scoring true back mutations in Leu^- or Tyr^- auxotrophs only marginal MFD was seen (24). It is possible that in these cases strand-specific repair proceeds at near-maximal efficiency in the rich medium condition, and the increased transcription rate produced in minimal media as a result of the stringent response can only marginally enhance the rate of strand-specific repair. Also, it is possible that the scoring of back mutations imposes certain restrictions on the types of mutations that can be observed and thus obscures any MFD that might be present. In any event, it is known that (i) the overall induced mutation frequency is increased fivefold in the *mfd* mutant strain (58, 101); that (ii) the majority of UV-induced mutations in *lac*I in wild-type cells are due to lesions in the coding strand (38) which (iii) are repaired at about 15% the rate of those in the template strand (43); and that (iv) in the *mfd* mutant strain the repair of the *lac*I template strand is inhibited (43), mutation frequency is increased fivefold, and most of the mutations are caused by lesions in the template strand (58). Thus, clearly MFD occurs in forward

mutations in genes encoding mRNA. We predict that a quantitative analysis of MFD which takes into account the absolute rates of transcription (see references 3 and 73b), repair, TRC, and replication for any gene will yield results that are consistent with the model that the Mfd protein reduces the mutation rate by targeting (A)BC excinuclease to the transcribed strand.

CONCLUSIONS AND PROSPECTS

The reconstitution of TRC in a completely defined system (75, 76, 76a) has eliminated many plausible scenarios for such coupling. Within this framework, we wish to make the following generalizations.

(i) TRC involves specific protein-protein interactions between the TRCF on the one hand and RNAP and the excision nuclease on the other. As a consequence, in *E. coli* only genes transcribed by *E. coli* RNAP and not by phage RNAP and in humans only genes transcribed by RNAPII and not those transcribed by RNAPI and -III are subject to preferential repair.

(ii) Similarly, since the TRCF interacts specifically with the excision nuclease, only nucleotide excision repair is subject to TRC. Other repair enzymes are most likely inhibited by an RNAP stalled at a lesion.

(iii) Lesions which block RNAP are subject to TRC. Therefore, it should be sufficient to find out whether a specific lesion blocks RNAP in *E. coli* and RNAPII in humans to decide whether that lesion is repaired preferentially.

(iv) Since TRC involves multiple protein-protein interactions, mutations in more than one protein should give rise to the coupling-defective phenotype, Mfd^- in *E. coli* and Cockayne's syndrome in humans. In *E. coli*, mutations in any of three genes should give rise to the Mfd^- phenotype: mutations in *mfd* itself, mutations in *uvrA* which interfere with its binding to TRCF without affecting its other functions, and finally mutations in *rpo* genes (most likely *rpoB*) which interfere with the interaction of RNAP with TRCF without disrupting its transcription activity. So far, only an *mfd* mutant is available. Work is under way to isolate *uvrA* and *rpo* mutants with the Mfd^- phenotype.

In contrast to *E. coli*, mutations in several genes give rise to Cockayne's syndrome in humans. In addition to mutations in the *CSBC/ERCC6* gene which encodes the TRCF, certain mutations in *XPBC/ERCC3*, *XPDC/ERCC2*, and perhaps *XPGC/ERCC5* cause a clinical syndrome with symptoms of xeroderma pigmentosum and Cockayne's syndrome (30). The latter three genes are basal subunits of human excinuclease (63), and thus it is quite conceivable that ERCC6 directs the human excision nuclease to a stalled RNAPII complex by interacting with the ERCC2, ERCC3 (98), and possibly ERCC5 proteins. Apparently, mutations in these XP genes severely impair their repair functions; furthermore, the residual repair is uncoupled from transcription because these mutations also interfere with the interaction of these proteins with ERCC6, thus resulting in a dual phenotype.

(v) Most organisms tested so far carry out TRC. However, it has been reported that *Drosophila melanogaster* lacks gene- and strand-specific repair (16–18). While this finding needs further confirmation, it is possible that certain species are truly defective in preferential repair. Certainly, *E. coli* with a deletion of the *mfd* gene has normal growth characteristics under physiological conditions. Similarly, humans completely defective in ERCC6 protein do grow to adult age although they have serious musculoskeletal and neurological abnormalities.

In conclusion, TRC is an important mechanism in preventing mutations, and it also reveals the intimate relationship between two essential cellular functions, transcription and repair. Further investigation on this subject is likely to facilitate our understanding of the molecular mechanics of both repair and transcription.

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