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Transcription-coupled nucleotide excision repair of a gene transcribed by bacteriophage T7 RNA polymerase in *Escherichia coli*

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ABSTRACT

Transcription-coupled nucleotide excision repair (TC-NER) removes certain kinds of lesions from the transcribed strand of expressed genes. The signal for TC-NER is thought to be RNA polymerase stalled at a lesion in the DNA template. In *Escherichia coli*, the stalled polymerase is dissociated from the lesion by the transcription repair coupling factor (Mfd protein), which also recruits excision repair proteins to the site resulting in efficient removal of the lesion. TC-NER has been documented in cells from a variety of organisms ranging from bacteria to humans. In each case, the RNA polymerase involved has been a multimeric protein complex. To ascertain whether a gene transcribed by the monomeric RNA polymerase of bacteriophage T7 could be repaired by TC-NER, we constructed strains of *E. coli* in which the chromosomal *lacZ* gene is controlled by a T7 promoter. In the absence of T7 RNA polymerase, little or no β-galactosidase is produced, indicating that the *E. coli* RNA polymerase does not transcribe *lacZ* efficiently, if at all, in these strains. By introducing a plasmid (pAR1219) carrying the T7 gene 1 under control of the *E. coli* lac UV5 promoter into these strains, we obtained derivatives in which the level of T7 RNA polymerase could be regulated. In cultures containing upregulated levels of the polymerase, β-galactosidase was actively produced indicating that the T7 RNA polymerase transcribes the *lacZ* gene efficiently. Under these conditions, we observed that UV-induced cyclobutane pyrimidine dimers were removed more rapidly from the transcribed strand of *lacZ* than from the nontranscribed strand, supporting the conclusion that TC-NER occurred in this gene. This response was absent in an *mfd-1* mutant, indicating that the underlying mechanism may be similar to that for the bacterial RNA polymerase.

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1. Introduction

Nucleotide excision repair (NER) removes a variety of lesions from DNA. It is generally accepted that NER comprises two subpathways: transcription-coupled nucleotide excision repair (TC-NER), and global genome nucleotide excision repair (GG-NER) (reviewed in [1]). The former repairs lesions in the transcribed strand of expressed genes, while the latter repairs lesions in the genome overall. When bacteriophage T7 damaged by UV light infects *Escherichia coli*, the phage DNA can be repaired by the bacterial NER system, resulting in "host cell reactivation" of the phage [2]. It is not known, however, whether TC-NER can contribute to this reactivation.

In *E. coli*, TC-NER of damaged bacterial DNA requires RNA polymerase and Mfd, the product of the *mfd* gene, in addition to the UvrA, UvrB, and UvrC proteins, which recognize a DNA lesion and incise the strand containing it [1,3,4]. Mutants deficient in Mfd do not show TC-NER [5–7]. According to current ideas, lesions that block the RNA polymerase (e.g. cyclobutane pyrimidine dimers (CPDs)), are substrates for TC-NER. The blocked polymerase, however, occludes the lesion and prevents access by the incision complex [8–10]. The Mfd protein, also known as the transcription repair coupling factor, is required to displace the polymerase, allowing repair to occur. In addition to displacing the RNA polymerase, Mfd is thought to recruit UvrA to the site to initiate NER, thus enhancing the rate of repair [3,4,11,12]. TC-NER is detected experimentally when the transcribed strand of an expressed gene is repaired more rapidly than the nontranscribed strand of the gene [13,14].

The RNA polymerase of *E. coli* is a complex of several different proteins. In contrast, the RNA polymerase of T7 is a monomeric protein. The elongation complex of the T7 enzyme, like that of *E. coli*, can be stalled by a CPD in the transcribed strand of a gene [10]. Results obtained from studies of defined systems in vitro, however, indicate that the T7 polymerase can bypass CPDs more readily than

Abbreviations: NER, nucleotide excision repair; TC-NER, transcription-coupled nucleotide excision repair; GG-NER, global genomic nucleotide excision repair; IPTG, isopropyl-β-D-thiogalactopyranoside; ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

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Table 1
E.coli K-12 derivatives used in these experiments. Strains with HL numbers were produced for this paper.

Designation	Genotype	Source
SR108	F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1	J. Cairns
NC397	F-, pglΔ8, gal490, ΔCI857, Δ (cro, bioA), (In NC397 the *cat/sacB* cassette replaces the *lacZ* promoter (the *lacI* promoter, 5′ end of lacI and RBS of *lacZ* remain), a kanamycin resistance gene and transcription terminator are inserted directly upstream of *cat/sacB*.)	D.L. Court
UNC3610-45	F- λ-, thr-1, ara-14, leuB6, Δ(gpt-proA)62, lacY1, tsx-33, supE44, galK2, rac-, hisG4(Oc), rfbD1, mgl-51, rpsL31, kdgK51, xyl-5, mtl-1, argE3, thi-1, zcf-117::Tn10, mfd-1	C. Selby
HL1132	F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ	
HL1134	F-, pglΔ8, gal490, λCI857, Δ(cro, bioA), kan-T7p-lacZ	
HL1159	F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ/pAR1219	
HL1160	F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ/pAR1219	
HL1166	F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ, zcf-117::Tn10	
HL1167	F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ, zcf-117::Tn10, mfd-1	
HL1172	F-, pglΔ8, gal490, λCI857, Δ (cro, bioA), kan-T7p-lacZ	
HL1173	F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ	
HL1176	F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ/pAR1219	
HL1177	F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ/pAR1219	
HL1179	F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ, zcf-117::Tn10, mfd-1/pAR1219	
HL1180	F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ, zcf-117::Tn10, mfd-1/pAR1219	

can the multimeric polymerases [15,16]. Furthermore, unlike the *E. coli* RNA polymerase, the T7 polymerase does not appear to interact with the Mfd protein in vitro [10]. Therefore, as a test of the generality of the proposed mechanism for TC-NER, it was of interest to evaluate the possibility that a gene transcribed by T7 RNA polymerase might show this type of repair.

We constructed *E. coli* derivatives in which a T7 promoter controls the *lac* operon, and the T7 RNA polymerase is supplied by a plasmid carrying the T7 gene 1. Our results show that TC-NER can occur in the *lac* operon when it is transcribed by the T7 RNA polymerase; furthermore, in *mfd-1* derivatives, we did not observe TC-NER, indicating that even when a gene is transcribed by the T7 RNA polymerase Mfd is required for TC-NER.

2. Materials and methods

2.1. Bacteria

Table 1 lists the derivatives of *E. coli* K-12 used.

2.2. Plasmid

The plasmid used, pAR1219 [17], contains the T7 gene 1, which encodes the T7 RNA polymerase. The gene is regulated by the lac UV5 promoter. The plasmid also carries the *E. coli lacI* gene, which codes for the lac repressor protein. When bacteria carrying this plasmid are grown in the presence of an inducer like isopropyl-β-D-thiogalactopyranoside (IPTG), the inducer binds to the repressor, preventing it from associating with the UV5 promoter. In the absence of an inducer, the repressor can bind to the promoter and prevent synthesis of the T7 RNA polymerase.

2.3. Media

The minimal medium (MM) was Difco Minimal Broth Davis containing 0.4% glucose and 0.05% vitamin-free casamino acids. For plates, 1.5% Difco Bacto agar was added. The complex medium was LB [18]. For LB plates, 1.2% Difco Bacto agar was added. L-broth [19] containing 1 g/l of glucose was used for preparing P1vir phage.

For thymine-requiring strains, 10 μg/ml of thymine was added to all media.

When needed, kanamycin was incorporated into plates at a concentration of 25 μg/ml, chloramphenicol at a concentration of 30 μg/ml, ampicillin at a concentration of 30 μg/ml, and tetracycline at a concentration of 15 μg/ml. To select against *sacB*, bacteria were plated on Difco Minimal Broth Davis containing 0.2% glycerol, 5% sucrose and 0.05% vitamin-free casamino acids.

The T7 gene 1 carried on the plasmid, pAR1219, was induced by growing cultures in MM containing 1 mM IPTG.

2.4. Recombineering

To construct *E. coli* derivatives with the *lacZ* gene under control of the T7 promoter, we used recombineering [20] with NC397 as the starting strain ([21], Supporting Information). NC397 contains the *cat-sacB* cassette for counterselection upstream from *lacZ*, and the gene conferring kanamycin resistance upstream from the *cat-sacB* cassette. Using a 100 base lagging strand oligonucleotide (5′GGGTGGCGGGCAGGACGCCCGCCATAAACTGC-CAGGAATTtaatacgactcactataggg ACAGGAAACAGCTATGACCATGA-TTACGGATTCACTGGCC) containing the T7 promoter sequence (lower case letters) flanked by 40 bases of kan on one side and 40 bases of *lacZ* on the other, we were able to replace the *cat-sacB* cassette with the T7 promoter (Fig. 1). The products of recombineering were sensitive to chloramphenicol, resistant to sucrose, and resistant to kanamycin. The resulting kan-T7p-lacZ sequence was transferred to SR108 by P1 transduction. Finally, the plasmid pAR1219 [17] was introduced by electroporation.

Oligonucleotides for recombineering and for PCR were purchased from Midland Certified Reagent Company, Inc. (www.mcrc.com).

2.5. Transduction

Lysates of P1vir were prepared, and transductions performed, essentially as described by Miller [22].

2.6. Electroporation

Late exponential phase cultures (25 ml) were pelleted by centrifugation, the pellets were washed with 25 ml of chilled, sterile water, and pelleted again. The pellets were resuspended in 1 ml chilled water and the suspensions were transferred to 1.5 ml

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