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Transcription coupled nucleotide excision repair in *Escherichia coli* can be affected by changing the arginine at position 529 of the β subunit of RNA polymerase

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ABSTRACT

The proposed mechanism for transcription coupled nucleotide excision repair (TCR) invokes RNA polymerase (RNAP) blocked at a DNA lesion as a signal to initiate repair. In *Escherichia coli*, TCR requires the interaction of RNAP with a transcription-repair coupling factor encoded by the *mfd* gene. The interaction between RNAP and Mfd depends upon amino acids 117, 118, and 119 of the β subunit of RNAP; changing any one of these to alanine diminishes the interaction [1]. Using direct assays for TCR, and the *lac* operon of *E. coli* containing UV induced cyclobutane pyrimidine dimers (CPDs) as substrate, we have found that a change from arginine to cysteine at amino acid 529 of the β subunit of the RNAP inactivates TCR, but does not prevent the interaction of RNAP with Mfd. Our results suggest that this interaction may be necessary but not sufficient to facilitate TCR.

1. Introduction

Nucleotide excision repair (NER) removes many different kinds of lesions from DNA, including those resulting from exposure to UV. Two subpathways of NER have been described: global genome repair (GGR), which removes lesions from the genome overall; and transcription coupled repair (TCR), which removes lesions from the transcribed strand of active genes [2–4]. In *Escherichia coli* the product of the *mfd* gene is required for TCR [5–8]; *mfd* mutants do not exhibit TCR [9]. It has been proposed that, in addition to the Mfd protein, RNA polymerase (RNAP) is intimately involved in TCR, facilitating recognition of lesions in actively transcribed DNA strands [10], and interacting with Mfd which then recruits UvrA, which, together with UvrB and UvrC, is required for the initiation of NER (both TCR and GGR) [11–15]. Studies using RNAP from various sources have shown that cyclobutane pyrimidine dimers (CPDs), the prototype substrate for TCR *in vivo*, block transcription *in vitro*. The blocked RNAP occludes the lesion and interferes with its repair [5,16–20]. Mfd can dissociate RNAP from the lesion site allowing access to repair proteins [6–8]. The *E. coli* RNAP elongation complex consists of five subunits, α_2, β, β′, and ω Interaction with Mfd involves the N-terminal portion of the β subunit [1,7,8,21].

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Table 1 – *E. coli* K-12 derivatives used in these experiments

Lab no.	Other no.	Genotype	Source
HL108	SR108	F-λ-, *thyA36*, *deoC2*, *IN*(*rrnD-rrnE*)1	J. Cairns
HL961	MG1655	F-λ-, *rph-1*	C. Gross
HL978	CAG3008	F-λ-, *rph-1*, *rpoB3401*, *btuB*::*Tn10*	C. Gross
HL979		F-λ-, *rph-1*, *rpoB3401*, *btuB*::*Tn10*	C. Gross
HL1080		F-λ-, *rph-1*, *btuB*::*Tn10*, *rpoB*(1552 AT → GC)	Ding Jin
HL1082	DJ1017	F-λ-, *rph-1*, *cpsF4*::*Tn10*, *rpoB111*	Ding Jin
HL1126		F-λ-, *thyA36*, *deoC2*, *IN*(*rrnD-rrnE*)1, *btuB*::*Tn10*	This work
HL1128		F-λ-, *thyA36*, *deoC2*, *IN*(*rrnD-rrnE*)1, *btuB*::*Tn10*, *rpoB3401*	This work

RNAP may stall on undamaged DNA templates when it encounters a pause sequence or a bound protein. Under appropriate conditions Mfd can interact with the β subunit of stalled RNAP and either terminate transcription or enable transcription to restart [7,21]. Results obtained with a yeast 2-hybrid system indicated that the binding site for Mfd lay within the first 142 amino acids of the N-terminal region of the β subunit [21]. More recently, Smith and Savery [1] identified amino acids 117 (isoleucine), 118 (lysine), and 119 (glutamic acid) within this region as those required for the interaction. Substitution of any one of these with alanine reduced the interaction of RNAP with Mfd.

The *rpoB* gene codes for the β subunit of RNAP. Because of the potential importance of the β subunit for TCR, we analyzed TCR in *rpoB* mutants, specifically those resistant to the antibiotic rifampicin (Rif). In mutants in which the arginine at position 529 in the β protein had been changed to cysteine we observed a loss in the capacity for TCR. This response was unexpected because this part of the RpoB protein has not previously been implicated in interactions with Mfd, and it suggests that the ability to interact with Mfd is not the only property of RNAP required for TCR *in vivo*.

2. Materials and methods

2.1. Bacteria

Table 1 lists the derivatives of *E. coli* K-12 used. Table 2 lists the nucleotide and predicted amino acid changes in each strain.

2.2. 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 [22]. For LB plates, 1.2% Difco Bacto agar was added. L-broth [23] 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. Rifampicin was used at a concentration of 100 μg/ml, ampicillin at 30 μg/ml, and tetracycline at 15 μg/ml.

2.3. Transduction

Lysates of P1vir were prepared and transductions performed essentially as described by Miller [24]. To avoid isolating new *rpoB* mutants instead of the desired *rpoB* transductants, selection for tetracycline resistance (*btuB*::Tn10), not rifampicin resistance, was used. About 70% of *btuB*::Tn10 transductants acquire the linked *rpoB* allele of the donor strain [25]. Rifampicin resistance was used only to distinguish between transductants that had acquired the *rpoB* mutation and those that had not.

2.4. Irradiation

The UV source was an unfiltered 15 w germicidal bulb emitting primarily 254 nm. The incident dose rate was 0.6 J/(m^2 s).

2.5. Assays for β-galactosidase

Enzyme activity was assayed with o-nitrophenyl-β-D-galactoside (ONPG) essentially as described by Miller [22].

2.6. Repair assays

The frequencies of CPDs in individual strands of the *lac* operon were measured as previously described [10,26]. Exponentially growing cultures of *E. coli* in defined medium were irradiated

Table 2 – Mutations and amino acid changes in the strains used

Strain	RpoB allele	Nucleotide change	Amino acid change
HL108 (SR108)	Wild type	None	None
HL961 (MG1655)	Wild type	None	None
HL978	3401	1585 CG → TA	R529C
HL979	3401	1585 CG → TA	R529C
HL1080		1552 AT → GC	N518D
HL1082	111	1691 GC → AT	P564L
HL1126	Wild type	None	None
HL1128	3401	1585 CG → TA	R529C