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Transcription promotes guanine to thymine mutations in the non-transcribed strand of an *Escherichia coli* gene

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Abstract

Transcription of DNA opens the chromatin, causes topological changes in DNA and transiently exposes the two strands to different biochemical environments. Consequently, it has long been argued that transcription may promote damage to DNA and there are data in *Escherichia coli* and yeast supporting a correlation between high transcription and mutations. We examined the transcription-dependence of the reversion of a nonsense codon (TGA) in *E. coli* and found that there was a strong dependence of mutations on transcription in strains defective in the repair of 8-oxoguanine in DNA. Under conditions of high transcription there was a three to five-fold increase in mutations that changed TGA in the non-transcribed strand to a sense codon. Furthermore, in both *mutY* and *mutM mutY* backgrounds the mutations were overwhelmingly G:C to T:A. In contrast, when the TGA was in the transcribed strand in relation with the inducible promoter, high transcription decreased the rate of reversion. Similar results were obtained in a strain defective in the transcription-repair coupling factor, Mfd, suggesting that transcription dependent increase in base substitutions does not require transcription-dependent DNA repair. However, Mfd does modulate the magnitude of the mutagenic effect of transcription. These data are consistent with a model in which the non-transcribed strand is more susceptible to oxidative damage during transcription than the transcribed strand. These results suggest that the magnitudes of individual base substitutions and their relative numbers in other studies of mutational spectra may also be affected by transcription.

Keywords: Reactive oxygen species; Singlet oxygen; Hydroxyl radical; G to T transversions

1. Introduction

Cellular metabolism creates chemicals that damage the genomic DNA and cause mutations. Endogenous DNA damaging chemicals include OH^-, various radicals and alkylating agents such as *S*-adenosylmethionine [1]. They cause deamination and loss of DNA bases, and create a wide variety of alkylation and oxidation products [2]. It should be noted that the inward orientation and Watson–Crick pairing of the bases in double-stranded (DS) DNA substantially protects them against chemical attack [1,3,4]. For example, cytosines in single-stranded (SS) DNA are 100–1000-fold more susceptible to hydrolytic deamination than those in DS DNA [5,6] and dATP is ∼67-fold more readily oxidized than dA in DS DNA [4]. When essential cellular processes such as transcription and replication cause transient disruption of base-pairing, the bases become more susceptible to damage. In the case of transcription, an RNA polymerase briefly separates the two DNA strands and copies one strand (transcribed strand, TS) as mRNA. The other strand (non-transcribed strand, NTS) becomes exposed as it passes through a channel on the roof of the polymerase [7]. Results from chemical and enzymatic footprinting, and X-ray crystallography have consistently demonstrated that several nucleotides in the NTS part of the bubble are exposed to the aqueous environment within the cell. Based on the structure and properties of the transcription bubble, we [8] and others have proposed that transcription should promote damage to DNA [9–12] and this should occur in a strand-biased manner [8,12].

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Consistent with these proposals, transcription has been linked to increased spontaneous mutagenesis in yeast [9], *E. coli* [8,10,12] and *Salmonella enterica* [13]. This phenomenon has since been referred to as transcription-induced mutations (TIM; [8]) or transcription-associated mutations [9]. We showed that in *E. coli* transcription promotes deamination of cytosines to uracil in the NTS, but not the TS [8]. This results in an excess of C to T mutations in the NTS compared to TS even in strains proficient in uracil removal from DNA. The increase in mutations was shown to occur in different *E. coli* genes and DNA sequence contexts [14]. Furthermore, it occurs regardless of whether transcription is performed by the T7 RNA polymerase [15] or the *E. coli* enzyme [8]. TIM also does not depend on protein synthesis [16], suggesting that it does not depend on the induction of synthesis of new mutator proteins. It appears to be the property of transcription itself.

Recently, we described a genetic reversion system that scores all base pair substitutions except for C to T (hereafter referred to as non-C-to-T mutations) in the bleomycin-resistance gene, *ble* [17]. Using this system, we showed that induction of high level transcription of *ble* promotes non-C-to-T mutations. These experiments were done in repair-proficient *E. coli* and the rate of non-C-to-T mutations was shown to increase by up to four-fold [17]. The most frequent class of base change observed in the high transcription state was G to T transversions.

G to T transversions are the hallmark of oxidative damage to DNA. Reactive oxygen species (ROS) damage nucleic acids and the attack of hydroxyl radicals at C-8 of guanine followed by oxidation creates 7,8-dihydro-8-oxo-guanine (8-oxoG; [4]). 8-OxoG in DNA can adopt *syn* conformation and mispair with an incoming dATP during replication promoting G:C to T:A transversions [18]. As 8-oxoG is a major product of oxidative damage to DNA [19], it is thought to be a major source for G to T transversions.

Here, we investigated the mechanism underlying G:C to T:A transversions promoted by high transcription in DNA repair-proficient *E. coli* [17]. We hypothesized that these increases in mutation rates were due to a preferential attack of ROS on the guanine base when it is present in the NTS, but not in the TS. We quantified the increases in the rates of mutations in a termination codon (TGA) promoted by transcription in cells defective in the removal of 8-oxoG and determined the spectrum of resulting mutations. Our results are presented below.

2. Materials and methods

2.1. Bacterial strains

The *E. coli* strains used in this work were derived from AB1157 and are listed in Table 1. The *mfd* strains used here were constructed from UNC361045 (=AB1157 *mfd-1 zcf-117*::Tn*10*) [20]. To allow the introduction of new markers into this strain, it was first made tetracycline-sensitive (Tet^S) using the method described by Bochner et al. [21]. Briefly, the selective plates used for the selection of tetracycline sensitive colonies plates contained 1.5% Bacto-Agar, 1% NaCl, 0.4% Bacto-Tryptone and 1% $NaH_2PO_4{\cdot}H_2O$. The pH was adjusted to 5.5 and chlortetracycline hydrochloride was added to a final concentration of 50 μg/ml. After sterilization, zinc chloride and fusaric acid were added to final concentrations of 0.1 mM and 12 μg/ml, respectively. Dilutions of overnight UNC361045 culture were spread on these plates and incubated at 37 °C for ∼48 h. Surviving colonies were replica plated on Luria broth (LB) and LB plates supplemented with 20 μg/ml tetracycline to test for the loss of the drug resistance. The Mfd^- phenotype of this strain, BH203 (=AB1157*mfd*), was kindly confirmed by Bockrath using the procedure described previously [22].

2.2. Construction of plasmids

The construction of pUP21-op75 was previously described [17]. Plasmid pUP27-op75 was constructed from pUP21 [14] as follows. Two unique restriction sites *Xba*I and *Nhe*I, were introduced to flank the *kan-ble* cassette and the endogenous P_{kan} promoter by the unique-restriction-site elimination procedure, or USE [23] creating pUP25. To create pUP27, pUP25 was digested with *Xba* I and *Nhe*I (New England Biolabs, Beverly, MA) and the sticky ends created by endonucleases were filled in by T4 DNA polymerase.

Table 1
Bacterial strains

Strain	Source	Construction[a]	Relevant markers
AB1157	Laboratory stock	–	Wild-type for DNA repair
NR12389	R. Schaaper	–	*mutM*::miniTn*10*
NR12393	R. Schaaper	–	*mutY*::Tn*10kan*
CSH117	J.H. Miller	–	*mutY*::miniTn*10*
UNC361045	A. Sancar	–	=AB1157 *mfd-1 zcf-117*::Tn*10*
BH206	This work	P1(CSH117)×AB1157	*mutY*::miniTn*10*
BH209	This work	P1(NR12389)×AB1157	*mutM*::miniTn*10*
BH211	This work	P1(NR12393)×BH209	*mutY*::miniTn*10 mutM*::miniTn*10*
BH203	This work	Selection for tetracycline sensitivity[b]	=AB1157 *mfd-1* Tet^S
BH216	This work	P1(NR12393)×BH203	*mfd-1 mutY*::Tn*10kan*

[a] P1 transduction; the donor strain is in parenthesis and the recipient is shown after the ×.
[b] Ref. [21].