SOS induction and mutagenesis by *dnaQ* missense alleles in wild type cells

Satyendra Gautam[1], **Raju Kalidindi**[2], and **M. Zafri Humayun**[*]

University of Medicine and Dentistry of New Jersey – New Jersey Medical School, Department of Microbiology and Molecular Genetics, 225 Warren Street, ICPH-E450V, Newark, NJ 07101-1709

Abstract

Mistranslation leads to elevated mutagenesis and replication arrest, both of which are hypothesized to result from the presence of mixed populations of wild type and mistranslated versions of DNA polymerase III subunit proteins. Consistent with this possibility, expression of missense alleles of *dnaQ* (which codes for the proofreading subunit ε in wild type (*dnaQ*+) cells is shown to lead to SOS induction as well as mutagenesis. Exposure to sublethal concentrations of streptomycin, an aminoglycoside antibiotic known to promote mistranslation, also leads to SOS induction.

Keywords

DNA replication; SOS induction; Mutagenesis; Mistranslation; Streptomycin; Mutator

1. INTRODUCTION

Protein synthesis has relatively low fidelity, such that in normal cells amino acids are misincorporated at rates estimated to be in the range of 10^{-3} to 10^{-5} [1–5]. Since ribosomal mutants with increased translational fidelity ("restrictive" mutations), as well as decreased fidelity (ribosomal ambiguity or Ram mutations) can be isolated, the observed normal error rates are thought to be growth-optimized, balancing the costs of mistranslation against the costs of more stringent error-proofing [6]. At error-rates of 10^{-4}, 10% of proteins consisting of 1,000 amino acids will contain at least one error, and 50% ribosomes (7,132 amino acids) will contain one or more errors. Similarly, at background mistranslation rates of 10^{-4}, 61% of DNA polymerase III holoenzyme assemblies[$(\alpha\varepsilon\theta)_3\tau_3\delta\delta'\chi\psi(\beta_2)_3$ =9, 523 amino acids] will have at least one amino acid error. Whether this built-in protein heterogeneity has significant biological consequences has long been debated (e.g., [2, 7, 8]), and is an area of renewed interest [9, 10].

[*]To whom correspondence should be addressed at: UMDNJ-New Jersey Medical School, Department of Microbiology and Molecular Genetics, 225 Warren Street, ICPH-E450V, Newark NJ 07101-1709. Tel: 973-972-4483 Ext. 2-6396. Fax: 973-972-8982. humayun@umdnj.edu.
[1]Bhabha Atomic Research Centre, Food Technology Division, Mumbai-400085, India; sgautam@barc.gov.in.
[2]Department of Biotechnology, Gokaraju Rangaraju Institute of Engineering and Technology, Bachupally, Kukatpally, Hyderabad – 500090, India; kalidindirj@gmail.com.

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Elevated mistranslation does have a significant impact on DNA replication as demonstrated by a mutator phenotype (translational stress-induced mutagenesis, TSM) conferred by mutations in genes specifying tRNAs [11–14], tRNA-modifying enzymes [15], and ribosomal proteins that control fidelity [16], or by exposure to sublethal concentrations of streptomycin, an antibiotic that enhances mistranslation. It has been hypothesized that occasional recruitment of mistranslated dominant-negative ε (proofreading) or α (polymerase) subunits can account for the TSM phenotype [13, 17]. TSM requires RecA-dependent homologous recombination functions for the full manifestation of the mutator effect [12, 18–20]. Homologous recombination functions were proposed to be required to rescue cells from replication arrest that accompanies error-prone DNA synthesis by corrupted DNA polymerase [17]. In accordance with this hypothesis, SOS functions were shown to be spontaneously induced in TSM-constitutive (*mutA*) cells [17]. Here, in a proof-of-principle experiment, we show that expression of missense *dnaQ* (ε subunit) alleles in *dnaQ*+ cells is sufficient to trigger SOS induction as measured by a prophage induction assay. We also find that exposure to sublethal concentrations of streptomycin, an antibiotic known to promote mistranslation, also leads to SOS induction.

2. MATERIALS AND METHODS

Strains

The *E. coli* SIVET (selectable in vivo expression technology) strain SG104 (MG1655 H-19B Δ*N*::*kan cro-tnpR168* Δ*OP*::*bla galK*::*resC-tet-resC*) was derived from the SIVET strain K10449 [21] as described [17].

Plasmids

Plasmids pSGQ, pSG12G, pSG103G and pSG108G, bearing, respectively, wild type *E. coli dnaQ* allele, *dnaQ*(D12G), *dnaQ*(D103G) and *dnaQ*(D108G) were constructed in a low copy number vector plasmid as follows. A 1482 bp *Nco* I-*Nsi* I fragment containing the *lacI*q gene along with its P$_{trc}$ promoter from plasmid pSE380 [22] was cloned into the multicloning site of the pSC101-derived low copy number vector pMW119 [23] to obtain the vector plasmid pTM100. Plasmid pSGQ (*dnaQ*+ Amp^R Spc^R) was constructed by cloning a *Nco* I-*Hin*d III 981 bp PCR fragment bearing a *dnaQ*+ allele, as well as a *Sac* I-*Sph* I 1180 bp fragment bearing the *aad9*+ allele (conferring spectinomycin resistance) from the shuttle plasmid pFW5 [24] into pTM100. Plasmids pSGQ12G, pSGQ103G and pSGQ108G were similarly constructed using the corresponding alleles of *dnaQ*, generously provided as pBR329-based clones by Jeffrey H. Miller (UCLA). Plasmids pSGQ(E14A) through pSGQ(E199A) series were derived by site-directed mutagenesis from pSGQ by inverse PCR following the primer design strategy described by Zheng *et al.* [25]. Similarly *mutD5* (T15I) and *dnaQ49* (V96G) alleles were constructed by inverse PCR in the same vector on the basis of sequence information from the literature [26]. Primers used for construction of the *dnaQ* alleles used in this study are listed in Table 1. All alleles were verified by DNA sequence analysis using primer 5'-GCT GTC AAA CAT CAG GAG C.

The SIVET prophage induction assay is based on the conversion of chloramphenicol-sensitive (Cm^S) cells to a chloramphenicol-resistant (Cm^R) phenotype upon SOS induction [21], and was carried out as described previously [17, 21] and summarized below. Briefly, the *E. coli* SG104 (an MG1655-derived SIVET strain [17]) cells carrying a pSC101-derived plasmid bearing an allele of *dnaQ* (see Table 2) were grown overnight at 37°C in LB medium supplemented with tetracycline (5 μg/ml), kanamycin (20 μg/ml) and ampicillin (30 μg/ml). Five ml of fresh LB medium supplemented with 1 mM isopropyl-β-ᴅ-thiogalactopyranoside (IPTG) was inoculated with 0.1 ml of a 1:500,000 dilution of the overnight culture (estimated to have approximately 1,000 cells) and the cells were allowed

to grow overnight at 37°C (or 28°C where noted) with aeration. To determine SIVET induction (ratio of Cm^R and Cm^S cells), the cultures were plated on LB agar supplemented with kanamycin (20 μg/ml), ampicillin (30 μg/ml), and chloramphenicol (10 μg/ml). To select all SIVET lysogens, the cultures were plated on LB agar containing kanamycin (20 μg/ml) and ampicillin (30 μg/ml). Colonies were counted after incubating the plates for 48 h at 37°C. SIVET induction results (Cm^R/Cm^S ratios) were averaged from 3 to 5 replicate cultures. Experiments using minimal medium A [27] were carried out as above except that 0.1 mM IPTG was used.

To determine the effect of exposure to streptomycin on SIVET induction, 5 ml of LB medium containing either no streptomycin (mock-treatment) or 5 μg/ml streptomycin (5 replicate cultures for each condition) was inoculated with approximately 1,000 cells from a fresh overnight culture of SG104 grown in the presence of kanamycin, ampicillin and tetracycline as described above. Cells were allowed to grow overnight at 37°C with aeration before plating to determine Cm^R/Cm^S ratios as described above.

For determining rifampicin-resistant (Rif^R) mutant frequencies, 5 ml of fresh LB medium was inoculated with 1% volume of an overnight culture of SG104 cells bearing a pSC101-derived plasmid, and the cells were grown for 18 h at 37°C with aeration. To determine the number of Rif^R mutants, 0.1 ml aliquots of the culture (or appropriately diluted culture in the case of mutator strains) were plated on LB agar plates supplemented with 100 μg/ml rifampicin, followed by incubation at 37°C for 48 h. Viable cell count was determined by plating appropriate dilutions of the same culture on LB agar. Average number of Rif^R mutants (3 to 5 replicate cultures) was divided by the average number of total viable cells to derive the mutant frequency.

3. RESULTS AND DISCUSSION

E. coli DNA polymerase III holoenzyme is a multimer of 10 distinct polypeptides organized into three polymerase cores ($\alpha\epsilon\theta$), a clamp loader (τ_3-$\delta\delta'\chi\psi$) and three processivity rings (β_2) [28, 29]. The trimeric polymerase core ($\alpha\epsilon\theta$) is responsible for DNA synthesis (α subunit) and proofreading (ϵ subunit). In addition to its proofreading 3'–5' exonuclease activity, ϵ plays an important structural role in the core such that in its absence, DNA synthesis becomes less processive in addition to being highly error-prone [30–32]. The function of the θ subunit is not known, but a structural role in stabilizing ϵ is inferred [33].

To determine whether coexpression of missense mutant ϵ subunit protein in the presence of wild type ϵ can trigger SOS induction, we used prophage induction, a surrogate for SOS induction, as an assay. In the so-called SIVET assay, SOS derepression leads to the expression of a transposase that acts at a distal site to convert cells from a chloramphenicol-sensitive to chloramphenicol-resistant (Cm^R) phenotype [21], and is thus suitable for detecting transient SOS induction occurring in a fraction of cells [17]. We have previously used this sensitive assay to demonstrate that cells expressing the *mutA* tRNA (which leads to aspartate→glycine mistranslation) suffer SOS induction presumably due to the occasional recruitment of corrupted pol-III cores ($\alpha\epsilon\theta$ in which the ϵ has a missense error) to the replication fork [17]. In the current experiments, we asked if direct expression of missense mutant ϵ proteins in wild type (*dnaQ*+) cells triggers both mutagenesis and SOS induction. Because mutant tRNAs causing D→G (aspartate→glycine) and E→A (glutamate→alanine) missense substitutions are known to confer relatively strong mutator phenotypes [11–13], we chose to look at D→G and E→A *dnaQ* missense alleles in this study.

Table 2 shows that E14A, D12G, and D103G, the strongest three of the tested mutator *dnaQ* alleles, also show the highest SIVET induction (24-, 12- and 11-fold, respectively). D12G and E14A affect two essential and highly conserved amino acids in the Exo I motif of ε (Fig. 1), whereas the D103G affects the Exo II motif. The strongest mutator allele (E14A) is also the strongest SIVET inducer, and alleles that did not elevate mutagenesis did not elevate SIVET induction, consistent with a linkage between error-prone DNA synthesis and replication arrest. An exception is provided by the E32A allele, which shows moderate SIVET induction (6-fold) with no increase in mutagenesis. Interestingly, the data show that E14A is a stronger mutator under these conditions than *mutD5* (T15I), the best known dominant-negative *dnaQ* allele that specifies a mutant protein that retains about 2% of the nuclease activity of wild type ε [34]. Increased mutagenesis by E14A may be due to a total loss of editing nuclease activity due to its critical role at the catalytic center [35, 36], or due to other factors such as stability leading ultimately to an increased ability to displace the wild type protein from polymerase cores. SIVET induction in cells expressing the *mutD5 allele* was modest, even though a significant >300-fold elevation in mutagenesis was observed. Because we have used a co-expression system in which a mutant *dnaQ* allele is expressed in the background of a wild type allele, the absence of an effect for a majority of the E→A alleles shown in Table 2 by itself does not necessarily imply that the corresponding mutant proteins have normal proofreading function because they could be recessive or unstable.

We also found that *dnaQ49* (V96G), a recessive allele known to have a strong mutator phenotype at 37°C but a moderate mutator effect at a lower temperature [37], confers a moderate mutator phenotype (20-fold; Table 2) at 28°C, indicating that the mutant ε protein must participate in replication to an appreciable extent in the presence of wild type ε at the lower temperature. As expected for a recessive allele, at 37°C *dnaQ49* (V96G) barely elevated mutagenesis (<4 fold) in the *dnaQ*+ background, and had no effect on SIVET induction (data not shown).

The linkage between mutagenesis and SOS (SIVET) induction raises the question whether SOS polymerases IV and V make a contribution to mutagenesis. While such a contribution cannot be ruled out, the dominant contributor to mutagenesis is likely to be defective DNA polymerase III proofreading as evidenced by data on *mutD5* (significant mutagenesis without SIVET induction), and E32A (SIVET induction without mutagenesis). Thus defective DNA synthesis (DNA elongation) leading to SIVET induction, and defective proofreading leading to mutagenesis, may be separable, but apparently occur together in cells expressing D12G, D103G or E14A alleles. The data in Tables 2 are consistent with the hypothesis that elevated mistranslation, by increasing the levels of dominant-negative missense mutant versions of critical DNA polymerase subunit proteins, leads to increased mutagenesis as well as replication arrest [17].

Defective DNA synthesis by a corrupted DNA polymerase III was previously proposed to lead to an accumulation of single-stranded DNA (ssDNA) gaps at replication forks, which in turn led to double-stranded breaks and arrested replication [17]. If so, slower growth in minimal medium might ameliorate the replication defect by allowing more time for the repair of ssDNA gaps, as previously proposed to explain the rich medium-sensitivity of *polA* cells defective for DNA polymerase I [38]. Table 3 shows that in minimal medium, expression of D12G or D103G alleles continues to confer a robust mutator phenotype (albeit not as strong as in rich medium), but very weak SIVET induction.

The aminoglycoside streptomycin binds to ribosomes, leading initially to codon misreading, and later to a complete inhibition of protein synthesis. In *E. coli*, aminoglycoside exposure leads to rapid cell killing, and lethality is linked to mistranslation rather than inhibition of

protein synthesis *per se* because non-aminoglycoside inhibitors of protein synthesis such as chloramphenicol or tetracycline are bacteriostatic rather than bactericidal. Since mistranslation can corrupt DNA replication and lead to replication arrest, it is interesting to ask if exposure to sublethal concentrations of streptomycin also leads to SIVET induction. Table 4 shows that exposure to 5 μg/ml streptomycin does lead to appreciable (5-fold) SIVET induction, consistent with the possibility that mistranslation in streptomycin-exposed cells may lead to replication arrest. Recent findings suggest that oxygen radicals make a contribution to aminoglycoside lethality [39, 40], and cells defective for *alkB* and other DNA repair genes are peculiarly sensitive to an aminoglycoside [41], suggesting an additional possible mechanism for SIVET induction. AlkB is capable of efficiently repairing ssDNA lesions, acting on a variety of alkylated bases as well as cyclic base lesions induced by lipid peroxidation [42–44]. Since mistranslation leads to misreplication, and replication arrest [17], it is possible that generation of ssDNA gaps by mistranslationally corrupted DNA polymerase is critical to aminoglycoside lethality. In this scenario, cell killing results from overwhelming the cell's capacity to repair oxidative, as well as other types of damage processes that target ssDNA gaps generated by a corrupted replication apparatus.

Acknowledgments

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  12                       103           162   167
   |                        |             |     |
--DtEt-------------D---------------HgallD--
   Exo I                   Exo II        Exo IIIε
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Catalytic core   **Flexible Linker** ← **α binding**

Fig. 1.
Domain structure of ε protein showing exonuclease motifs, with uppercase letters showing highly conserved amino acid residues.

Table 1

Partially overlapping inverse PCR Primers used for mutant *dnaQ* allele construction[a]

Allele	Forward Primer	Reverse Primer	Site
E14A	5' CTC GAT ACC GcA ACC ACC **GGT ATG AAC CAG ATT GGT GC**	5' **GGT GGT TgC GGT ATC GAG** AAC GAT CTG GCG TGT AAT TGC	None
E26A	5' GCG CAC TAT Gc<u>C</u> GGC CAC AAG ATC ATT GAG ATT G	5' GAT CTT GTG GCC <u>G</u>gC ATA GTG CGC ACC AAT CTG	*Nae* I
E32A	5' CAA GAT CAT TGc GAT TGG <u>C</u>GC CGT TGA AGT GGT G	5' CGG CGC AAC TCg CAA TGA TCT TGT GGC CTT CAT AG	*Hae* II
E37A	5' GAG ATT GG<u>C</u> GCC GTT GcA GTG GTG **AAC** CGT CGC CTG	5' GTT CAC CAC TgC AAC GGC <u>G</u>CC AAT CTC AAT GAT CTT G	*Hae* II
E71A	5' GCC GAT GcA TTT TT<u>T</u> CT<u>A</u> GAT AAG CCC ACG TTT G	5' CTT ATC <u>T</u>AG <u>A</u>AA AAA TgC ATC GGC AAT ACC ATG	*Xba* I
E81A	5' CAC GTT TGC <u>T</u>Gc AGT AGC CGA TG<u>A</u> GTT CAT GG	5' CAT CGG CTA CTg CA<u>G</u> CAA ACG TG<u>G</u> GCT TAT CG	*Pst* I
E94A	5' CGG CGC <u>C</u>Gc GTT GGT GAT CCA TAA C	5' ACC AAC gC<u>G</u> GCG CCG CGA ATA TAG TC	*Kas* I
E110A	5' GGA CTA TGc <u>A</u>TT TTC GTT GCT TAA GCG C	5' GCA ACG AAA ATg CAT AGT CCA TAA AGC C	*Nsi* I
E173A	5' GAT CCT TGC GGc <u>C</u>GT TTA TCT G**GC GAT GAC CG**	5' CAG ATA AAC Gg<u>C</u> CGC AAG GAT C**TG GGC ATC GAG**	*Nae* I
E199A	5' CAA CAA GGC Gc<u>C</u> GCA ACA ATT CAG **CGC ATT G**TA CGT CAG G	5' CAA TGC GCT GAA TTG TTG CGg C<u>G</u>C **CTT GTT G**CT GTT GTG	*Kas* I
T15I (*mutD5*)	5' ATC GTT CT<u>A</u> GAT ACC GAA AtC ACC **GG**T ATG AAC CAG	5' CCG GTG aTT TCG GTA TC<u>T</u> AGA ACG AT**C** TGG CGT G	*Xba* I
V96G (*dnaQ49*)	5' CGG CGC <u>C</u>GA GTT GGg GAT CCA TAA CGC AGC G	5' ATG GAT CcC CAA CTC <u>G</u>GC GCC G<u>C</u>G AAT ATA G	*Kas* I

[a]Sequence change leading to the amino acid substitution is indicated by the lowercase letter in each forward and reverse primer set. Silent sequence change(s) for creating the indicated restriction site is (are) shown by underlined letters in each primer set. Sequence overlap within each set of primers is shown in boldface.

Table 2

Effect of plasmid-borne *dnaQ* alleles on SIVET induction and Rif^R mutant frequency in LB medium[a]

dnaQ allele on plasmid	SIVET Induction (Cm^R/Cm^S)		Mutation Frequency (Rif^R/Rif^S)	
	Frequency (SD) [× 10^{-4}]	Ratio (*dnaQ*/WT)	Frequency (SD) [× 10^{-7}]	Ratio (*dnaQ*/WT)
Wild Type(*dnaQ*+)	2.4 (0.71)	1	0.85 (0.34)	1
D12G	29 (7.7)	12	1,441 (195)	1,695
D103G	27 (6)	11	942 (150)	1,108
D108G	2 (0.64)	<1	ND	ND
E14A	57.4 (19.7)	24	4,845 (1435)	5,700
E26A	3.5 (1.3)	2	0.33 (0.12)	<1
E32A	15.2 (9)	6	1.1 (0.49)	1
E37A	2.7 (1.3)	1	0.67 (0.4)	<1
E71A	1.5 (0.39)	<1	0.14 (0.05)	<1
E81A	2.4 (2.4)	1	0.67 (0.48)	<1
E94A	2.1 (1.4)	<1	0.08 (0.03)	<1
E110A	2.3 (0.74)	1	0.15 (0.06)	<1
E173A	2.8 (1.4)	1	0.87 (0.25)	1
E199A	2.1 (1.4)	1	0.16 (0.03)	<1
V96G (*dnaQ49*)	1.4 (0.32)	<1	17 (12)	20
T15I (*mutD5*)	2.8 (0.7)	1	265 (27)	312

[a]SIVET induction (Cm^R/Cm^S ratio) was assayed as described in the text after growth at 37°C for all alleles except for *dnaQ49* and *mutD5*, which were grown at 28°C. SD, Standard deviation; WT here refers to basal SIVET induction and mutation frequency values shown in the first row of data; ND, not determined. Data were averaged from 3–5 replicate cultures.

Table 3

Effect of plasmid-borne *dnaQ* alleles on SIVET induction and Rif^R mutant frequency in minimal medium[a]

dnaQ allele on plasmid	SIVET Induction (Cm^R/Cm^S)		Mutation Frequency (Rif^R/Rif^S)	
	Frequency (SD) [$\times 10^{-4}$]	Ratio (*dnaQ*/WT)	Frequency (SD) [$\times 10^{-7}$]	Ratio (*dnaQ*/WT)
Wild Type (*dnaQ*+)	1.6 (0.4)	1	0.92 (0.81)	1
D12G	2.6 (1.1)	<2	418 (78)	454
D103G	2.8 (0.94)	<2	426 (56)	463

[a]SIVET induction was assayed after growth at 37°C in minimal medium A as described in the text. SD, Standard deviation; WT here refers to basal SIVET induction and mutation frequency values shown in the first row of data; Data were averaged from 3–5 replicate cultures.

Table 4

Effect of exposure to sublethal concentrations of streptomycin on SIVET induction

Experiment[a]	Streptomycin (µg/ml)	SIVET Induction (Cm^R/Cm^S)	
		Frequency(SD) [× 10^{-4}]	Ratio (Str+/Str−)
Experiment 1	0	1.3 (0.57)	-
	5	7.8 (6.5)	6
Experiment 2	0	0.84 (0.18)	-
	5	4.1 (5.9)	5

[a]Data represent averages from five replicate cultures in each experiment. SIVET induction was assayed as described in the text. SD, standard deviation; Str, streptomycin.