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Media-dependent mutator activity of the dnaQ49 allele of Escherichia coli

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**Media-dependent mutator activity of the *DnaQ49* allele of
*Escherichia coli***

Krishnaswamy, Shalini, M.A.

San Jose State University, 1990

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Ann Arbor, MI 48106

**MEDIA-DEPENDENT MUTATOR ACTIVITY OF THE *DNAQ49* ALLELE
OF *ESCHERICHIA COLI***

A Thesis

Presented to

**The Faculty of the Department of Biological Sciences
San Jose State University**

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

By

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December, 1990

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Abstract

Media-Dependent Mutator Activity Of The *dnaQ49* Allele

Of Escherichia coli

Shalini Krishnaswamy

Escherichia coli carrying the temperature dependent *dnaQ49* allele are strong mutators in rich medium. *DnaQ49* maps at the *dnaQ* locus and encodes a defective epsilon subunit of DNA polymerase III. The impairment in proofreading is thought to contribute to errors during DNA replication. At 37°C mutation and Trp⁺ reversion frequencies were higher in rich than in minimal media. In the latter, frequencies were higher than in coisogenic wild type strains and the mutational spectrum was altered; transversions occurred more than transitions. This media-dependent mutator activity is very similar to that of *mutD5*, another mutator allele at the *dnaQ* locus. Unlike *mutD5*, thymidine did not stimulate mutator activity of *dnaQ49*. It is suggested that at 37°C, in rich medium, *dnaQ49* results in defective proofreading and postreplicative mismatch repair while in minimal medium mismatch repair is restored thereby accounting for the differences in mutator activity and mutational spectra.

To my son, Sanjay

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Introduction

Faithful replication of DNA is an amazingly accurate process which is ensured by the activity of several cellular mechanisms which have apparently evolved to correct errors during replication. Strains of the bacterium *Escherichia coli* carrying mutant genes that alter normally accurate DNA replication have been useful tools in elucidating these processes. *DnaQ49* and *mutD5* are two such mutant alleles of the *dnaQ* gene which encodes the ϵ subunit of DNA polymerase III holoenzyme; ϵ upon binding to the α subunit catalyzes the exonuclease proofreading activity that removes nucleotide misincorporation at the replication fork (Scheuermann *et al.*, 1983; Scheuermann and Echols, 1984; Maki and Kornberg, 1987). The resulting defect in proofreading is thought to be partially responsible for the enhanced mutation frequencies exhibited by these so called mutator alleles (Horiuchi *et al.*, 1978; Degnen and Cox, 1974; Erlich and Cox, 1980). *DnaQ49* is a temperature sensitive mutant exhibiting enhanced mutator activity at 37°C compared with more modest mutation rates at 30°C (Horiuchi *et al.*, 1978). At the restrictive temperature of 44.5°C *dnaQ49* exhibits defective DNA synthesis resulting in lethality (Horiuchi *et al.*, 1978). In contrast the mutator activity of *mutD5* is not temperature dependent (Degnen and Cox, 1974) but is however media dependent, demonstrating 10^4 - 10^5 fold increases in mutator activity above wild type in rich medium and about 100 fold less activity in minimal medium (Degnen and Cox, 1974; Erlich and Cox, 1980). Strong mutator activity has been demonstrated with the *mutD5* strain in minimal medium supplemented with thymidine as compared with more moderate mutator activity in unsupplemented minimal medium (Degnen and Cox, 1974; Erlich and Cox, 1980), leading to the inference that thymidine could be one of the effector molecule(s) in rich medium that enhances mutator activity. No medium effect on the behavior of the *dnaQ49* allele has been reported.

The purposes of this study were:

- Compare the mutator activity of *dnaQ49* in rich and minimal media at 37°C and 30°C.
- Examine the mutational specificity of *dnaQ49* in rich and minimal media.
- To investigate the effect of thymidine on the mutator activity of *dnaQ49*.

Literature Review

Replication of DNA in a normal *E.coli* cell is faithful with errors at any particular site occurring only about one out of a billion times per generation (Drake, 1969). In order for almost error free DNA replication to take place, the cell has developed a number of error correcting mechanisms, the interplay of which is not totally understood. Loeb and Kunkel (1982) and Echols (1982) proposed a multistage mechanism of error prevention: (1) during incorporation, selection by the polymerase of the correct base complementary to the one on the parental strand, (2) prior to elongation, exonucleolytic editing of the newly added base which would result in removal of an incorrect 3' terminal nucleotide which is mispaired with the template strand and (3) post replicative scanning for mismatched bases followed by mismatch repair.

Several gene products are known to contribute to the high fidelity of DNA replication by possible involvement at the various stages. For example, at the incorporation stage the MutT protein of the *mutT* gene of *E.coli* appears to monitor dGTP allowing the correct configuration access to the polymerizing strand (Schaaper and Dunn, 1987; Bhatnagar and Bessman, 1988; Akiyama *et al.*, 1989). The *mutT* mutant enhances A:T->C:G transversions by about 1000 fold over wild type frequencies. The MutT protein was demonstrated to have dGTPase activity which serves to degrade the specific form of dGTP that can pair with dA on the template, thus diminishing the possibility of dG.dA mispair and therefore of A:T->C:G transversions. When the MutT gene product was added to a system synthesizing deoxynucleotide homopolymer *in vitro*, it suppressed the formation of A.G mispairs (Akiyama *et al.*, 1989). Recent work by Bhatnagar *et al.* (1990) has revealed by nucleotide sequence analysis that the mutator defect in the *mutT* gene is most likely due to an IS1 insertion into the wild type gene.

At the stage of post replicative scanning of newly replicated DNA, mismatch repair mechanisms play a role in rectifying incorrect base-pairing which might have remained after proofreading. After polymerization has taken place, but before the daughter strand is methylated, the DNA is subject to repair by products of the *mutH*, *mutL*, *mutR* and *mutS* genes which probably together examine the newly synthesized DNA (Radman and Wagner, 1986; Modrich, 1987), correct mispairings, deletions or insertions via excision and repair synthesis and restore complementarity to the old strand.

The major determinant of fidelity of DNA replication in *E.coli* seems to be DNA polymerase III holoenzyme which is responsible for chain elongation. It has in addition to the 5'->3' polymerase activity, a 3'->5' and 5'->3' exonuclease activity. Genetic loci for at least seven of the polypeptides which constitute this multisubunit enzyme have been identified (McHenry and Crow, 1979). The α subunit is a product of the *dnaE* gene (McHenry and Crow, 1979) and ϵ is a product of the *dnaQ* gene (Scheuermann *et al.*, 1983). The smallest subassembly of polymerase III holoenzyme is the core enzyme which contains α , ϵ and ϕ subunits (McHenry and Crow, 1979). The core enzyme executes both the polymerase and the 3'->5' exonuclease activity of the holoenzyme (McHenry and Crow, 1979). Enzyme assays with the subunit indicated that α has the polymerase activity (Spanos *et al.*, 1981) and ϵ has the 3'->5' exonuclease activity. The latter conclusion was inferred from observations that mutations in the *dnaQ* gene render the polymerase III enzyme defective in proofreading or editing functions (Echols *et al.*, 1983; DiFrancesco *et al.*, 1984). Scheuermann and Echols (1984), utilizing ϵ overproducing plasmids carrying a strong promoter and efficient translation sequence, demonstrated that ϵ by itself and the core enzyme had indistinguishable 3'->5' exonuclease activity, thereby concluding that ϵ is the editing subunit of polymerase III holoenzyme and that base selection and 3'->5' exonuclease activity are carried out by distinct subunits, allowing for regulation of the editing function independent of

polymerization. However, later work by Maki and Kornberg (1987) demonstrated that ϵ is much less active in proofreading alone than together with α in the core. Stimulation of the editing function is due mainly to a greatly increased affinity of the ϵ subunit for the 3' hydroxyl terminus, resulting from DNA binding by the α subunit. They suggested that α might indirectly play a role in proofreading and that fidelity might be influenced by relative levels of α and ϵ subunits in the cell.

The *dnaQ* gene has been identified by two mutations, *dnaQ49* and *mutD5*, that confer a strong mutator phenotype (Degnen and Cox, 1974; Horiuchi *et al.*, 1978). Mapping studies have demonstrated that the *dnaQ49* mutation is distinct from the *dnaE* gene (Horiuchi *et al.*, 1978) and that it maps at 5 minutes at the same locus as the *mutD5* mutation previously defined by Degnen and Cox (1974). *DnaQ49* and *mutD5* have both been sequenced and shown to result from base-pair substitutions in the *dnaQ* gene (Horiuchi *et al.*, 1981; Takano *et al.*, 1986). However, the two mutations differ in several respects; *mutD5* is dominant to the wild type allele while *dnaQ49* is recessive (Cox and Horner, 1982; Maruyami *et al.*, 1983). *DnaQ49* strains show defective DNA synthesis at 44.5°C (Horiuchi *et al.*, 1978) while *mutD5* demonstrates no such lethality at the restrictive temperature (Cox and Horner, 1982). *DnaQ49* shows temperature dependent mutator activity resulting in higher mutation rates at 37°C than at 30°C (Horiuchi *et al.*, 1978) while temperature has no such effect on *mutD5* (Degnen and Cox, 1974). They result from different base-pair substitutions within the *dnaQ* gene, *dnaQ49* resulting from a single amino acid substitution (₉₆Val(GTG)->Gly(GGG)) and *mutD5* resulting from two amino acid substitutions (₇₃Leu(TTG)->Trp(TGG)) and (₁₆₄Ala(GCA)->Val(GTA)) (Takano *et al.*, 1986). *MutD5* strains demonstrate enhanced mutator activity in rich medium or thymidine supplemented minimal medium and modest mutator activity in unsupplemented minimal medium (Degnen and Cox, 1974; Erlich and Cox, 1980). No media effect for *dnaQ49* has been reported.

Piechocki *et al.*, (1986) and Schaaper (1988) have pointed out that the extremely high mutation frequencies (upto 10,000 fold) of *mutD5* strains might be due to mechanisms in addition to defective editing since the *in vitro* experiments of Fersht *et al.* (1982) showed that proofreading can only contribute to fidelity by a factor of 100 to 200. Recent studies (Schaaper, 1988, 1989) have shown that *mutD5* strains in rich media are defective in methyl-directed mismatch repair as well as proofreading. In minimal media mismatch repair is restored. Schaaper and Radman (1989) have shown that in *mutD5* strains mismatch repair is saturated by an excess of DNA replication errors.

Studies on the mutational specificities of *mutD5* have shown that in rich medium, 95% of the mutational events were single base-pair substitutions (Fowler *et al.*, 1974, 1986; Schaaper, 1988) with the remaining mutations being simple frameshifts. The base-pair substitutions were mostly transitions. In minimal medium *mutD5* has an altered mutational specificity with transversions becoming the most common mutation sequenced (Schaaper, 1988). This change in the mutational spectrum is consistent with an active mismatch repair system (Schaaper, 1988) since transitions are a major component of the mutational spectrum in strains defective in mismatch repair (Choy and Fowler, 1985; Leong *et al.*, 1986; Schaaper and Dunn, 1987).

The mutational specificity of *dnaQ49* strains at 37°C and 30°C in rich media was studied by Isbell and Fowler (1989) by using the *trpA* system developed and characterized by Yanofsky *et al.* (1966). Isbell and Fowler (1989) demonstrated that in addition to reduced mutator activity at 30°C compared with 37°C, *dnaQ49* showed an obvious change in mutational specificity. While transitions were enhanced at 37°C, transversions constituted a greater proportion of the Trp⁺ revertants at 30°C. They suggested that full restoration of mismatch repair and partial restoration of the proofreading function could explain the reduced mutator activity and altered mutational

specificity at 30°C. As previously mentioned transition mispairs are more often corrected by mismatch repair systems than are transversions (Choy and Fowler, 1985; Leong *et al.*, 1986; Schaaper and Dunn, 1987).

The media effect on mutational frequency in *mutD5* strains has been studied by Degnen and Cox (1974) and Erlich and Cox (1980). Based on the hypothesis that a particular effector molecule(s) contributes to the enhanced mutation rates of *mutD5* in rich medium, Degnen and Cox (1974) demonstrated that thymidine added to minimal broth enhances the mutation rate and it was later shown that thymidine needs to be phosphorylated in order to function as an effector molecule (Erlich and Cox, 1980). Erlich and Cox (1980) concluded, however, that thymidine alone did not restore full mutator activity to *mutD5* strains in minimal media and suggested that an additional effector(s) is present in rich media.

Materials and Methods

Bacterial Strains

The *trpA* alleles (A58, A78, A9813) of the KH116 (*dnaQ49*) strain were constructed as described by Isbell and Fowler (1989). The co-isogenic *mut*⁺ strains have been previously constructed (Degnen and Cox, 1974; Fowler *et al.*, 1974). Strain NR8093 (*mutD5*) has been described (Fowler *et al.*, 1986).

Media

Minimal salts medium supplemented with 0.2% glucose, 1 µg/ml thiamine-hydrochloride, 10 mg/ml tryptophan and an amino acids mixture containing histidine, arginine, leucine, and threonine (50 µg/ml) was used to grow cultures in minimal broth (MB). L-broth (LB) was prepared with 1% tryptone, 0.5% yeast extract and 0.5% NaCl. MB without tryptophan solidified with 1.5% agar was used to prepare plates (MM) (Vogel and Bonner, 1956). Tryptone plates consisted of 1% tryptone and 0.5% NaCl solidified with 1.5% agar. Nal^R and Str^R mutants were selected on tryptone plates containing 50 µg/ml nalidixic acid and 150 µg/ml streptomycin respectively. Saline (0.85%) was utilized for washing and diluting the cultures. A stock solution of thymidine (100 µg/ml) was used to provide 20 µM, 10 µM, 1 µM concentrations in thymidine-supplemented MB as required.

Sterilization

Glucose, agar, amino acids (with the exception of tryptophan), salts, tryptone, yeast extract and NaCl were autoclaved before use at standard pressure and temperature. Thiamine hydrochloride, thymidine, tryptophan, nalidixic acid, and streptomycin were filter sterilized using a 0.22 micron filter and refrigerated in sterile jars.

Mutagenesis Procedures

Trp⁺ reversion frequencies were measured by growing duplicate or triplicate inoculates of all mutator and non mutator strains to saturation in MB at 37°C. The cultures were diluted to approximately 1000 cells and were used to initiate three to four second day overnight cultures in LB and MB at 37°C and 30°C. The cultures in LB grew to saturation in 24 hours, those in MB at 37°C grew to saturation in about 48 hours and those in MB at 30°C took about 48 to 72 hours to reach saturation. The cultures were then washed with saline and 100 µl of the appropriate dilutions were plated on duplicate MM plates supplemented with tryptophan to titer for total cells and on duplicate MM plates to select for Trp⁺ revertants. Cultures were also plated on duplicate tryptone media to titer for total cells and on duplicate tryptone-nalidixic acid and tryptone-streptomycin to select for Nal^R and Str^R mutants respectively. Reversion frequencies were calculated by dividing the average number of Trp⁺ revertants by the average total cells on tryptophan-supplemented MM plates. Mutation frequencies to nalidixic acid and streptomycin resistance were calculated by dividing the average number of Nal^R and Str^R mutants by the total cells on tryptone plates respectively. The effect of thymidine on the mutation and reversion frequencies of mutator strains, *dnaQ49* and *mutD5* was measured using the same protocol.

Classification of Revertants

Trp⁺ revertants were classified using two tests. First purified revertants were tested for the accumulation of indole glycerol phosphate (IGP) which is an intermediate in tryptophan metabolism. Full revertants do not accumulate IGP while partial revertants do (Allen and Yanofsky, 1963). In order to determine this, all revertants from a plate of MM were individually grown in 3 ml of MB with shaking at 37°C. To 1 ml of each saturated culture was added 1.5 ml of ferric chloride reagent (1 ml of 0.5 M ferric

chloride, 30 ml concentrated sulphuric acid and 50 ml water) and the resulting color change was observed. A negative test (lack of color change) indicated a full revertant, while varying shades of pink indicated that the culture was either a partial revertant or a suppressed mutant (Allen and Yanofsky, 1963). Known full and partial revertants served as controls.

Partial revertants can be further classified by their sensitivity to 5-methyl tryptophan (5-MT) which is a false feedback inhibitor (Lester and Yanofsky, 1961). The test was performed by placing 2 μ g of 5-MT on a sterile paper disc centered on a top layer soft agar MM containing approximately 10^6 cells from a revertant culture. Plates were incubated at 37°C and zones of inhibition were observed at 24 and 48 hours. Partial revertants or suppressed mutants showed much larger zones of inhibition than did full revertants (Cox *et al.*, 1972; Fowler *et al.*, 1974).

Results

Effect of media on the mutator activity of *dnaQ49*

Three *trpA* strains with *dnaQ49* (A58, A78 and A9813) were examined for changes in mutator activity under varying conditions of media (LB and MB) and temperature (37°C and 30°C). Mutation frequencies to nalidixic acid and streptomycin resistance are shown in Table 1 while Trp⁺ reversion frequencies for two *trpA* base-pair substitution mutants and one *trpA* frameshift mutant are shown in Table 2. Enhancement levels of mutation and reversion frequencies for *dnaQ49* compared to *mut*⁺ and that for LB compared to MB were calculated and are shown in Table 3. *DnaQ49* is a stronger mutator at 37°C than at 30°C. At 37°C, in LB, mutation frequencies to Nal^R demonstrated 900-70000 fold increases over *mut*⁺ strains (Tables 1 and 3). *DnaQ49* mutation frequencies to Str^R increased 9000-62000 times over *mut*⁺ levels (Tables 1 and 3). At 30°C in LB the increase in mutation frequency of *dnaQ49* over *mut*⁺ (upto 88 fold) was moderate (Tables 1 and 3).

At 37°C *dnaQ49* still shows obvious mutator activity in MB although Nal^R and Str^R mutation frequencies are much lower than in LB (Tables 1 and 3). Comparison of *dnaQ49* mutation frequencies in LB at 37°C with those in MB showed 14-170 fold increases to Nal^R and 800-1000 fold increases to Str^R (Tables 1 and 3). At 30°C the enhancement in LB over MB for *dnaQ49* frequencies though present (~2-9 fold) was less dramatic (Tables 1 and 3).

Reversion frequencies to Trp⁺ showed a similar trend. The *dnaQ49* frequencies were enhanced 600-2000 fold over *mut*⁺ levels in LB at 37°C and less so (upto 1.7 fold) at 30°C (Tables 2 and 3). Reversion frequencies for *dnaQ49* were reduced in MB compared to LB but were still higher than *mut*⁺ values at 37°C (Tables 2 and 3).

Table 1

Mutation frequencies in *mut*⁺ and *cha249* strains in LB^a and MB^a, at 37°C and 30°C.

<i>trpA</i> allele	Strain	Mutants per 10 ⁸ cells							
		NaIR				StrR			
		37°C		30°C		37°C		30°C	
		LB	MB	LB	MB	LB	MB	LB	MB
<i>A58</i>	<i>cha249</i>	1640	120	15	7.3	1400	1.7	1.0	0.93
	<i>mut</i> ⁺	1.7	0.2	0.4	0.3	0.15	0.01	0.02	0.07
<i>A78</i>	<i>cha249</i>	4900	29.5	10.6	1.25	3100	3.55	0.016	0.3
	<i>mut</i> ⁺	0.07	0.22	0.12	0.47	0.05	0.01	0.11	0.02
<i>A9813</i> ^b	<i>cha249</i>	1880	96	1.3	2.1	1660	1.7	0.01	0.02
	<i>mut</i> ⁺	0.28	0.01	2.1	0.13	0.07	0.001	0.17	0.01

^a LB (rich broth) and MB (minimal broth).^b Frameshift mutation.

Table 2

Reversion of *trpA* base-pair substitutions and frameshift mutations in *mut*⁺ and *dnaQ49* strains in LB^a and MB^a, at 37°C and 30°C.

<i>trpA</i> allele	Strain	Revertants per 10 ⁸ cells			
		37°C		30°C	
		LB	MB	LB	MB
A58	<i>dnaQ49</i>	1030	1.7	1.3	0.9
	<i>mut</i> ⁺	0.5	0.25	1.4	0.9
A78	<i>dnaQ49</i>	4100	47	4.8	4.0
	<i>mut</i> ⁺	2.67	1.3	2.8	6.3
A9813 ^b	<i>dnaQ49</i>	1220	6.6	2.8	1.3
	<i>mut</i> ⁺	2.0	0.47	11	6.7

^a LB (rich broth) and MB (minimal broth).

^b Frameshift mutation.

Table 3
Mutation and reversion frequency enhancement of *trpA* alleles of *chaQ49* in LB^a and MB^a at 37°C.

Marker scored	<i>trpA</i> allele	Frequency enhancement ^b					
		37°C			30°C		
		<i>Q49</i> (LB) <i>mut</i> ⁺ (LB)	<i>Q49</i> (MB) <i>mut</i> ⁺ (MB)	<i>Q49</i> (LB) <i>Q49</i> (MB)	<i>Q49</i> (LB) <i>mut</i> ⁺ (LB)	<i>Q49</i> (MB) <i>mut</i> ⁺ (MB)	<i>Q49</i> (LB) <i>Q49</i> (MB)
NaI ^R	A58	965	600	14	38	24	2
	A78	70,000	134	166	88	2.7	8.5
	A9813 ^c	6,714	9,600	20	0.6	16	0.6
Str ^R	A58	9,333	170	824	50	13	1.1
	A78	62,000	355	873	0.15	15	0.05
	A9813 ^c	23,714	1,700	976	0.06	2	0.5
Trp ⁺ Revertants	A58	2,060	6.8	605	0.9	1	1.4
	A78	1,536	36	87	1.7	0.6	1.2
	A9813 ^c	610	14	185	0.25	0.2	2.2

^a LB (rich broth) and MB (minimal broth)

^b Frequency enhancement was calculated from data in Tables 1 and 2.

^c Frameshift mutant

LB growth conditions resulted in enhanced *dnaQ49* reversion frequencies (80-600 fold) over those in MB at 37°C (Table 3). The enhancement, if any, was minimal (> 1-2 fold) at 30°C (Tables 2 and 3).

In summary, in addition to enhanced mutator activity at 37°C compared to 30°C, *dnaQ49* shows increased mutation rates in rich medium as compared to minimal medium. The medium effect is stronger at 37°C than at 30°C (Table 3).

Effect of media on the reversion spectrum of *dnaQ49*

The mutator activity of *dnaQ49* was further explored by determining the reversion spectrum of *trpA* base-pair substitutions (A58, A78) and frameshift mutant A9813 (Yanofsky *et al.*, 1966; Brammer *et al.*, 1967; Berger *et al.*, 1968). Information regarding the type of reversion event occurring at the base-pair level can be obtained directly from the IGP and 5-MT phenotypes of the Trp⁺ revertants, independent of amino-acid sequence data.

The characterization and distribution of the Trp⁺ revertants of the *trpA* mutants in LB and MB at 37°C is presented in Table 4. Reversion frequencies for specific base-pair substitutions, shown in Table 5, have been calculated by multiplying the distribution proportion from Table 4 with the Trp⁺ reversion frequency from Table 2. Full revertants (IFR) of *trpA58* result from A:T->G:C transitions (Yanofsky *et al.*, 1966). At 37°C in LB *dnaQ49* enhances the occurrence of this event 7000-8000 fold as compared with *mut*⁺ strains, while in MB the increase (< 2-10 fold) was less dramatic (Table 5). A class of partial revertants of *trpA58* (IIIPR) resulting from G:C->A:T transitions at a suppressor site (Squires and Carbon, 1971) was enhanced (> 1000 fold) by *dnaQ49* in LB at 37°C compared to the *mut*⁺ strain (Table 5).

Table 4

The characterization and distribution of *dnaQ49* - induced revertants of *trpA* base-pair substitution and frameshift mutants in LB^a and MB^a at 37°C.

<i>trpA</i> allele	Revertant class ^c	Distribution ^b				Inferred base-pair substitution
		<i>mut</i> ⁺ (37°C)		<i>Q49</i> (37°C)		
		LB	MB	LB	MB	
<i>A58</i>	I FR	.16(31)	0(7)	.61(76)	.04(24)	A:T-->G:C
	II PR	.19(31)	.43(7)	.07(76)	.125(24)	A:T-->C:G
	IIIPR ^d	.65(31)	.57(7)	.33(76)	.83(24)	G:C-->A:T
<i>A78</i>	I FR	.093(107) ^f	.417(24)	.03(77)	.01(80)	A:T-->C:G
<i>A9813</i> ^e	I FR	.033(30) ^f	.595(37)	.03(39)	.09(11)	-----
	II PR	.967(30) ^f	.405(37)	.97(39)	.9(11)	

^a LB (rich broth) and MB (minimal broth).

^b Distribution is the decimal fraction of the Trp⁺ revertants which, based upon IGP and 5-MT tests, can be placed into discrete revertant classes. Number in parentheses is the total number of revertants analyzed.

^c Revertants have been placed into discrete classes FR = full revertant, PR = partial revertant on the basis of the IGP accumulation and 5-MT inhibition tests as described in Materials and Methods.

^d This class results from intergenic suppression of the *trpA58* allele (Squires and Carbon, 1971).

^e Frameshift mutant

^f Data are from Isbell and Fowler (1989).

Table 5
Enhancement of *trpA* base-pair substitutions by *dnaQ49* in LB^a and MB^a at 37°C.

Inferred base-pair substitution	<i>trpA</i> allele	Revertant class ^c	Revertants per 10 ⁸ cells ^b <i>dnaQ49</i>				Frequency enhancement		
			LB	MB	LB	MB	$\frac{Q49(LB)}{mut^+(LB)}$	$\frac{Q49(MB)}{mut^+(MB)}$	$\frac{Q49(LB)}{Q49(MB)}$
Transitions									
A:T-->G:C	A58	I FR	.08	.025	628	.07	7854	2.72	9240
G:C-->A:T	A58 ^d	III PR	.33	.14	340	1.4	1046	10	241
Transversions									
A:T-->C:G	A58	II PR	.10	.11	72	.20	759	1.96	343
A78		I FR	.25	.54	123	.47	496	.87	262

^a LB (rich broth) and MB (minimal broth)

^b Calculated by multiplying the distribution proportion from Table 3 times the Trp⁺ reversion frequency from Table 2.

^c Revertants were placed into discrete classes: FR = full revertant, PR = partial revertant based on IGP and SMT tests as described in Materials and Methods.

^d This class results from intergenic suppression of the *trpA58* allele (Squires and Carbon, 1971)

In MB the enhancement was far less (10 fold) (Table 5).

Transversions (A:T->C:G) occur at the *trpA58* (IIPR) and *trpA78* (IFR) sites (Yanofsky *et al.*, 1966). Transversions at the *trpA58* site showed a 700-800 fold increase at 37°C in LB with *dnaQ49* compared to the *mut⁺* strain while in MB the enhancement was only about two fold (Tables 4 and 5). At the *trpA78* site A:T->C:G transversions (IFR) were enhanced about 500 fold by *dnaQ49* over *mut⁺* values at 37°C in LB.

For the base-pair substitutions analyzed LB growth produced stronger *dnaQ49* mutator activity at 37°C than did MB. At the *trpA58* site the enhancement decrease in MB was less for the A:T->C:G transversions than for the A:T->G:C transitions, that is transversions were a larger proportion of the reversion spectrum in MB as compared with LB.

The *trpA9813* mutation apparently resulted from a single adenine deletion and therefore full revertants probably result from a single base addition at that site (Brammer *et al.*, 1967). The partial revertants (IIPR) presumably resulted from a single base addition occurring close to the original adenine deletion (Brammer *et al.*, 1967). *DnaQ49* stimulated mostly partial revertants of *A9813* in LB and MB at 37°C.

Some of the *mut⁺* strains show possible media and temperature effects in frequencies and mutational specificity (Table 4 and 5) but the sample sizes are too small to make any conclusions.

Effect of thymidine on the mutator activity of *dnaQ49*

The effect of thymidine on the mutation and reversion frequencies of the mutant strains *dnaQ49* and *mutD5* are shown in Table 6. *MutD5* strains have been shown to respond to thymidine in MB with enhanced mutator activity (Degnen and Cox, 1974).

Table 6

Mutator activity of *chaQ49* and *mutD5 trpA 58* strains in MB^a, thymidine- supplemented MB and LB^a at 37°C.

Mutator strain	Media conditions	Mutants per 10 ⁸ cells		Trp ⁺ Revertants per 10 ⁸ cells
		Nal ^R	Str ^R	
<i>chaQ49</i>	MB	54	2.0	1.5
	MB + thymidine:			
	20uM "	56	1.5	1.3
	10uM "	40	2.0	3.0
	1uM "	45	1.6	4.0
	LB	1390	1500	1160
<i>mutD5</i>	MB	130	19	11
	MB + thymidine			
	20uM "	800	220	190
	LB	19000	240	2800

^a LB (rich broth) and MB (minimal broth).

The data clearly demonstrates that thymidine at 20 μ M, 10 μ M and 1 μ M concentrations does not influence the mutator activity of *dnaQ49* in MB. A definite increase is observed in mutation and reversion frequencies with *mutD5* when 20 μ M thymidine was added to MB (Table 6).

Discussion

The mutator activity of several *trpA* alleles of *dnaQ49* in rich (LB) and minimal (MB) media, at 37°C and 30°C was compared by determining spontaneous mutation frequencies to nalidixic acid and streptomycin resistance and by the Trp⁺ reversion frequencies. The mutational specificity under the same conditions was investigated utilizing the *trpA* reversion system (Yanofsky *et al.*, 1966). It was found that *dnaQ49* strongly increased mutation and reversion frequencies in LB compared with more modest mutator activity in MB at 37°C. Both base-pair substitutions and frameshifts were lowered in MB. At 30°C the media-dependent differences were smaller. In comparison the co-isogenic wild type (*mut*⁺) strains showed low mutation and reversion frequencies in both media and at both temperatures.

The mutational specificity of *dnaQ49* was considerably different in the two media at 37°C. Reversion analysis of the *trpA* alleles revealed base-pair substitutions and frameshifts to be enhanced in LB at 37°C with transitions being favored over transversions, although only a few sites were analyzed. In a previous study where the reversion patterns of a larger number of *trpA* alleles were analyzed, Isbell and Fowler (1989) reached the same conclusion.

In contrast the mutational spectrum is different in minimal medium for *trpA58*. Here transversions become a significantly larger fraction of the base-pair substitutions. While in LB, A:T→G:C transitions are most strongly induced by *dnaQ49*; A:T→C:G transversions are favored in MB. This is a particularly meaningful comparison because the same base-pair is mutated in both cases (Yanofsky *et al.*, 1966), eliminating possible complicating effects contributed by differences in neighboring bases. Previous studies by Piechocki *et al.* (1986) using a genetic system based upon fine structure analysis of the *lacI* gene indicated that *dnaQ49* preferentially induced transversions to AT base-pairs in

minimal medium.

The medium-dependent mutator activity of *mutD5*, another mutator allele of the *dnaQ* gene, has been clearly demonstrated by a number of studies (Degnen and Cox, 1974; Erlich and Cox, 1980; Schaaper 1988). In LB *mutD5*, like *dnaQ49*, raises spontaneous mutation frequencies to 10^3 to 10^5 times wild type levels (Degnen and Cox, 1974) whereas in minimal medium without thymidine mutation frequencies are much lower although still 50 to 100 times wild type frequencies. In LB a number of investigations have concluded that *mutD5* mainly induces base-pair substitutions with transitions occurring more frequently than transversions (Fowler *et al.*, 1974, 1986; Schaaper, 1988). In minimal medium, however, transversions, particularly A:T→T:A events, dominate the mutational spectrum (Schaaper, 1988).

The *dnaQ* gene encoded protein is the ϵ subunit of DNA polymerase III which catalyzes the 3'→5' exonuclease (proofreading) activity of the enzyme (Scheuermann *et al.*, 1983; Scheuermann and Echols, 1984). The mutator alleles *dnaQ49* and *mutD5* have been found to be defective in this activity (Echols *et al.*, 1983; DiFrancesco *et al.*, 1984). Based on the *in vitro* observations by Fersht *et al.* (1982) that proofreading could contribute only upto 200 fold increase in the fidelity of DNA replication, it has been suggested that mutator activity of *dnaQ49* in LB which is at least an order of magnitude higher than 200 fold is due to functions in addition to proofreading that might be defective (Echols *et al.*, 1983; Piechocki *et al.*, 1986; Isbell and Fowler, 1989).

Schaaper (1988, 1989) and Damagnez *et al.* (1989) demonstrated that methyl-directed post replicative mismatch repair (Modrich, 1987) is defective in *mutD5* strains grown in rich medium, while no defect in mismatch repair was found with minimal medium. Schaaper and Radman (1989) have recently shown that the mismatch repair deficiency of *mutD5* strains results from a saturation of the *mutHLS*-mismatch

repair system by an excess of DNA replication errors due to defective proofreading. Restoration of mismatch repair in minimal media would explain the medium effect on mutational specificity. Transition mispairings are more readily corrected than transversion mispairings (Choy and Fowler, 1985; Schaaper and Dunn, 1987) and transitions are poorly represented in the *mutD5* minimal medium mutational spectrum (Schaaper 1988).

Experiments have not yet been conducted to directly observe whether or not mismatch repair is deficient with *dnaQ49* strains in rich medium. However, given the similarities in mutational spectra between *mutD5* and *dnaQ49* strains in rich and minimal medium, it seems reasonable to suggest that mismatch repair in *dnaQ49* is saturated in the former and active in the latter. In support of this suggestion are the observations of Fowler and Rogers (1989) that *mutLdnaQ49* double mutant strains where the *mutL* mutation results in defective mismatch repair (Modrich, 1987) have similar mutation frequencies to single *dnaQ49* strains in rich medium.

Analysis of *dnaQ49* is complicated by its temperature dependency (Horiuchi *et al.*, 1978). Compared to 37°C, *dnaQ49* at 30°C has lower levels of mutator activity in both rich and minimal media (Isbell and Fowler, 1989; this study). Possibly the proofreading activity of ϵ is partially restored at 30°C which results in active mismatch repair and therefore lower mutation frequencies. Consistent with this suggestion is the observation that *mutLdnaQ49* double mutator strains have significantly higher mutation frequencies than single *dnaQ49* strains at 30°C (Fowler and Rogers, 1988).

The interaction between the various gene products participating in DNA replication is yet to be clearly understood. Maki and Kornberg (1987) demonstrated an increase in 3'->5' exonuclease activity of the core enzyme (α , ϵ and θ subunits) as compared with that of the free ϵ subunit. They arrived at the conclusion that the α subunit is involved in proofreading by stimulating the 3'->5' exonuclease activity of ϵ .

They implied that the enzymatically deficient ϵ in *mutD5* has an increased affinity for the α subunit. Takano *et al.* (1986) sequenced the DNA of the dominant *mutD5* and the recessive *dnaQ49* alleles and proposed the existence of two functional domains on the ϵ subunit; a 3'->5' exonuclease domain and an α subunit binding domain. They suggested that the ϵ produced by the *dnaQ49* allele has a decreased affinity for α while the *mutD5* encoded ϵ lacks exonuclease activity.

Photoaffinity labeling studies by Biswas and Kornberg (1984) demonstrated that photolinking of dTTP to the ATP activated holoenzyme was exclusively to the ϵ subunit suggesting that dTTP may have a regulatory influence over ϵ . In an earlier study, Degnen and Cox (1974) found that thymidine stimulated the mutator activity of *mutD5* in minimal media. Erlich and Cox (1980) showed that thymidine must be phosphorylated to act as an effector. While thymidine was stimulatory in minimal media, it did not increase *mutD5* mutation frequencies to the levels found in LB, and it was suggested that an additional effector(s) is present in rich media (Erlich and Cox, 1980).

In this study, thymidine was found to have no effect on the mutator activity of *dnaQ49* strains in minimal media even though the media effect is as great for *dnaQ49* as it is for *mutD5*. This clearly shows that rich media has components in addition to thymidine that enhance mutator activity for *dnaQ49*. Preliminary observations in this laboratory suggest that the effector(s) for enhanced *mutD5* mutator activity is a small heat resistant molecule (Bhamre, unpublished data). An ammonium sulphate precipitation of LB proteins left a supernatant able to stimulate full *mutD5* mutator activity in MM without decreasing generation time (Bhamre, unpublished data). Experiments are in progress to determine if this effector will also stimulate *dnaQ49* mutator activity.

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