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Characterization of suppressors of the dna Q49 mutator allele in Escherichia coli

Sangeeta Rane
San Jose State University

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Rane, Sangeeta S., M.A.

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U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

Characterization of suppressors of the *dnaQ49* mutator allele in *Escherichia coli*

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Sangeeta Rane

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(Dr. Robert Fowler)

(Dr. John Boothby)

(Dr. Pamela Stacks)

APPROVED FOR THE UNIVERSITY

ABSTRACT

CHARACTERIZATION OF SUPPRESSORS OF THE *dnaQ49* MUTATOR ALLELE IN *ESCHERICHIA COLI*

by Sangeeta Rane

Three intergenic suppressors of the *dnaQ49* mutator allele of *Escherichia coli* that confer nalidixic acid resistance (Nal^R) were isolated and designated Nal^R-4, Nal^R-9, and Nal^R-15 . They decrease *dnaQ49* mutation frequencies about 100X. These suppressors map in the *gyrA* gene and show mutator activity themselves in the absence of the *dnaQ49* allele. These are the first reported mutator alleles at the *gyrA* locus. DNA sequencing will be necessary to determine the relationship among these three alleles.

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Introduction

The *dnaQ* gene of *Escherichia coli* encodes the ε subunit of the DNA polymerase III holoenzyme. This subunit when bound to the α subunit encoded by the *dnaE* gene provides the 3' -> 5' exonuclease activity and corrects erroneously inserted nucleotides during replication. Mutations in the *dnaQ* gene can cause large increases in spontaneous mutation frequencies without causing lethality. Two known mutator alleles of the *dnaQ* gene are *mutD5* and *dnaQ49*. *DnaQ49* is a temperature sensitive mutant that shows a high frequency of mutations at 37°C and lethality at 44.5°C. However, it shows a lower frequency of mutations at 30°C (Horiuchi et al., 1978). *MutD5* has a mutation rate dependent on the growth media. It causes a high frequency of mutations in rich medium, but a lower frequency of mutations in minimal medium (Degnen and Cox, 1974; Erlich and Cox, 1980). *DnaQ49* has also been shown to be a media-dependent mutator (Krishnaswamy et al., submitted for publication).

We have isolated putative suppressors of the *dnaQ49* mutator allele which cause lowered mutation frequencies. These suppressors were first selected as nalidixic acid resistant mutants. This study further attempts to:

a) Map these putative suppressors on the *E.coli* chromosome.

b) Characterize the possible effects these suppressors might have on spontaneous mutation frequencies in a wild type (*mut*$^+$) background.

c) Transfer and confirm the suppressor phenotype, by placing an active *dnaQ49* mutator allele into strains with a suppressor allele in a *mut*$^+$ background, using suppression of *dnaQ49* expression as an indicator.

Literature Review

Genetic information is encoded in a linear sequence in the molecules of deoxyribonucleic acid (DNA), and the sequence directly determines the linear sequence of amino acid units in molecules of proteins (Yanofsky et al., 1964). A change in the DNA sequence can result in a change in the amino acid sequence of the protein molecule, causing it to be either nonfunctional or to have an altered function. These heritable changes in the DNA sequence are known as mutations.

Fidelity during DNA synthesis is important, even vital, since organisms must replicate and repair their DNA accurately to maintain their genetic identity. However, species evolution requires the presence of mutations (Loeb and Kunkel, 1982). Alteration of the template due to alkylation, the presence of apurinic or apyrimidinic (AP) sites, and the alteration of the normal function of DNA polymerases may cause decreased fidelity that can lead to spontaneous mutations (Loeb and Kunkel, 1982). Variations in cellular nucleotide pools, keto-enol and amino-immino tautomerization, and anti-syn orientation about the glycosidic bond of the nucleotides can cause misinsertion during DNA replication (Loeb and Kunkel, 1982; Topal and Fresco, 1976).

DNA synthesis in *E. coli* is a two step process involving an incorporation reaction and a checking or proofreading reaction (Kornberg and Baker, 1992) which both act to promote a high fidelity of replication. Before polymerization occurs, the nucleoside triphosphate is paired with its template partner, and if the correct base pair has not been produced the nucleotide is rejected as a triphosphate. After polymerization, DNA polymerases can check the accuracy of base pairing between the 3' nucleotide on the nascent DNA strand and its template base. If they are unpaired or frayed, the primer nucleotide will be excised by 3'->5' exonuclease (proofreading) activity of the DNA

polymerase, thereby preventing a possible mutation. A "cost" of proofreading occurs when properly paired nucleotides are excised and polymerization is delayed.

Proofreading in *E. coli* is carried out by the DNA polymerase III holoenzyme and involves the 3'-> 5' exonuclease activity of the enzyme. DNA polymerase III of *E. coli* has ten subunits (O'Donnell and Studwell, 1990). The α,ε and θ subunits form the core enzyme (McHenry and Crow, 1979; O'Donnell and Studwell, 1990), which is the smallest unit of the holoenzyme. A five protein complex (γ, δ, δ', χ, ψ) along with the accessory protein β acts as the preinitiation complex. The preinitiation complex is a tight, ATP-activated protein clamp on primed ssDNA (Biswas and Kornberg, 1984; O'Donnell and Studwell, 1990). The τ subunit binds the core polymerase and accelerates its assembly with the preinitiation complex to form the fully assembled holoenzyme (O'Donnell and Studwell, 1990). The interactions of all these subunits with each other determine the rate, processivity and the thermostability of the enzyme (Lancy et al., 1989a). The α-subunit of the polymerase III holoenzyme encoded by the *dnaE* gene and the ε subunit of the polymerase III holoenzyme encoded by the *dnaQ* gene carry the catalytic site for polymerization and the 3'->5' exonuclease activity of the enzyme, respectively (Echols, 1982; McHenry and Crow, 1979; Scheuermann et al., 1983; Spanos et al., 1981; Maki and Kornberg, 1987). The latter conclusion was reached from the fact that mutations in the *dnaQ* gene cause polymerase III to be defective in proofreading and editing (Echols et al., 1983; DiFrancesco et al., 1984).

There is a special class of mutations that increase or decrease the frequency of spontaneous alterations of many genes. Genes with mutations which decrease the spontaneous mutability of other genes are called antimutator genes (Quinones and Piechozki, 1985). Antimutators are apparent only when they act on a major source of spontaneous mutations. They are subdivided into three groups: (a) Mutants where the

level of spontaneous mutations is decreased due to the increased efficiency of an error-correcting mechanism; (b) Mutants which are deficient in a pathway of error-prone DNA repair; (c) Mutants where the antimutator effect is a result of auxotrophy (Quinones and Piechozki, 1985). Some antimutators not only show reduced spontaneous mutability, but are also defective in the repair of ultraviolet (UV) and methyl methanesulfonate (MMS) induced damage and are unable to induce error-prone polymerase activity.

Geiger and Speyer (1977) characterized an antimutator allele located at the *purB* gene of *E. coli*. This antimutator was named *mud* for mutation defective and was tested for its effect on the induction of the SOS response (Witkin, 1976). The SOS response is the induction of enzymes to repair damaged DNA in *E. coli*. The response involves activation of an enzyme that cleaves a repressor, activating a series of genes involved in DNA repair. The *mud* mutation had no effect on the error-prone SOS response. Thus, the antimutator phenotype is not likely to be due to the repression of mutagenic repair (Lyons et al., 1985). DNA from normal *purB*$^+$ cells show a certain methylation pattern which causes a certain amount of base substitution mutations at methylated cytosine residues. Since the methylation pattern of *mud* DNA is similar to DNA from normal *purB*$^+$ cells, the antimutator effect is not due to lesser amounts of hotspot base substitution mutations (Lyons et al., 1985). A longer lag in post replicative DNA methylation gives an extended time window for strand discrimination during methyl-directed mismatch repair (Modrich, 1987, 1989). The *mud* antimutator has a normal lag period thereby indicating that there is no extended time window for strand discrimination. However, a measurement of nucleotide pools in an *in vitro* experiment showed the elevation of dCTP levels above all other nucleotide phosphates in *mud* strains. Enhanced replication fidelity in an *in vitro* system through

changes in relative dNTP concentrations make such a base selection model for *mud* action attractive (Lyons et al., 1985). Reduction in mutation rate has not yet been achieved *in vivo* in *mud* strains due to a variation of dNTPs.

Mutations which increase the mutation frequency of other genes are collectively termed mutators and are useful tools in elucidating cellular mechanisms involved in replication and preservation of genetic information. These mutators enhance spontaneous mutation rates beyond wild type levels. Some of these mutators involve genes whose products have a role in mutation avoidance during replication. The proteins encoded by these mutator genes have a direct effect on DNA metabolism, especially precursor synthesis, DNA repair, DNA recombination, and replication. Many such mutators in *E. coli* have been isolated and characterized by genetic means. Two such mutator alleles are designated *dnaQ49* and *mutD5* (Degnen and Cox, 1974; Horiuchi et al., 1978).

The *dnaQ49* mutator allele was isolated while attempting to find a new type of mutator (Horiuchi et al., 1978). Prior to this discovery, *mutT* and *mutD5* were the strongest mutators of *E. coli* (Cox and Yanofsky, 1969; Degnen and Cox, 1974). The *dnaQ49* allele is temperature sensitive and maps at the *dnaQ* locus. The *dnaQ* gene has been cloned and its product identified. It was suggested that it might be the ε subunit of the DNA polymerase III holoenzyme (Horiuchi et al., 1981). Subsequently, Echols and associates found that the *dnaQ* gene product is indeed the ε subunit and it carries the 3'->5' exonuclease activity of the DNA polymerase III holoenzyme (Echols et al., 1983; Scheuermann and Echols, 1984). They further demonstrated that enzyme preparations derived from the *dnaQ49* and the *mutD5* mutants are defective in the 3'->5' exonuclease activity. Since the *dnaQ49* allele and the *mutD5* allele are located closely at five minutes on the linkage map (Degnen and Cox, 1974; Horiuchi et al., 1978), the *dnaQ49* and the *mutD5* mutators are probably mutations in the *dnaQ* gene. Unlike *mutD5*,

dnaQ49 is temperature-dependent and is lethal at 44.5°C when grown in salts-free L-broth (Horiuchi et al., 1978; Degnen and Cox, 1974). Hybrid plasmids and phages carrying *dnaQ49* or *mutD5* were constructed using *in vivo* and *in vitro* recombination techniques. The phenotype of the wild type or the mutant bacteria then indicated that the *mutD5* mutator is dominant over the wild type allele, whereas the *dnaQ49* mutator is recessive (Maruyama et al., 1983).

The nucleotide sequences of the recessive *dnaQ49* and the dominant *mutD5* mutator were determined. The *dnaQ49* mutation is caused by a transversion (conversion of T:A to G:C) base pair mutation. This causes one amino acid change, $_{96}$Val (GTG) to Gly (GGG), in the *dnaQ* protein. The *mutD5* mutator has two base substitutions, T:A to G:C and C:G to T:A . This causes a change in two amino acids, $_{73}$Leu (TTG) to Trp (TGG), and $_{164}$Ala (GCA) to Val (GTA) (Takano et al., 1986). The transversion mutation in *dnaQ49* may have been caused by another mutator, *mut21*, present in an original *dnaQ* mutant and its parental strain (Takano et al., 1986).

Schaaper (1988) sequenced the mutations generated by *mutD5* in the N-terminal part of the *lacI* gene. Since the mutator strength of this strain depends on the medium in which it grows, mutations were analyzed in both minimal medium for moderate mutator activity and in rich medium for high mutator activity. In either case 95% of all mutations were base substitutions and 5% were single base deletions. However, the nature and site distributions of the base substitutions differed dramatically for the two conditions. In minimal medium a majority (62%) were transversions, notably A:T to T:A at three 5'-GTGG-3' sequences. Most (64%) of the transitions under this condition occured at specific sequences that are suggestive of a "dislocation" type of mutagenesis. In rich medium, 90% of the base substitutions were transitions. These observations suggest that different modes of mutagenesis operate

under the two conditions. Isbell and Fowler (1989) have determined the mutational specificity for *dnaQ49* for strains grown in L-broth at 37°C using the *trpA* reversion system (Yanofsky et al., 1966). It was found to be almost identical to that of *mutD5* strains grown in L-broth (Fowler et al., 1974).

The *dnaQ49* and the *mutD5* strains are defective in 3'->5' exonuclease activity. *In vitro* data (Fersht et al., 1982; Echols et al., 1983; Piechocki et al., 1986; Schaaper, 1988) suggest that proofreading should contribute a maximum of a 200-fold increase to the fidelity of DNA replication. While much of the mutator activity of both alleles results from the lack of proofreading, mutational levels of both *dnaQ49* and *mutD5* are orders of magnitude higher and may suggest that additional functions may be altered in these strains. Schaaper (1988) has recently shown that methyl-directed post replicative mismatch repair is defective in *mutD5* cells grown in rich media, and it may also be altered in a *dnaQ49* strain growing at 37°C in L-broth (Krishnaswamy et al., submitted for publication). The lack of both proofreading and mismatch repair activities would account for the high level of mutation in *mutD5* and *dnaQ49* strains, and provide an explanation for their apparent similar mutational specificities. Mutator activity of *dnaQ49* is dramatically reduced at 30°C as compared to 37°C and its specificity is altered (Isbell and Fowler, 1989). One possibility is that mismatch repair activity in *dnaQ49* strains is fully or almost fully regained at 30°C while proofreading is only partially restored (Krishnaswamy et al., submitted for publication). This would explain the preferential lowering of transition frequencies at 30°C from values obtained at 37°C in *dnaQ49* strains (Isbell and Fowler, 1989); transition mispairings are generally more often corrected than transversion mispairings by mismatch repair (Choy and Fowler, 1985; Leong et al., 1986; Schaaper and Dunn, 1987).

It has also been suggested that the *dnaQ49* and *mutD5* mutator alleles may lead to a misincorporation of nucleotides during replication (Echols et al., 1983; Piechocki et al., 1986). Although there is no published data to support this suggestion, Maki and Kornberg have suggested that the α subunit, which provides for the polymerase activity, may bind to the ε subunit encoded by the *dnaQ* gene (Maki and Kornberg, 1987). An altered ε could affect the incorporation of nucleotides by α.

Mutations in the *dnaQ* gene which encodes the ε subunit of DNA polymerase III are known to cause a strong mutator effect, but initially mutators recovered in the *dnaE* gene, encoding the α subunit were all weak (Cox, 1976). Recent evidence has shown that a mutation in the *dnaE* gene results in an amino acid change in the α-subunit [$_{612}$Glu (GAA) to Lys (AAA)] and causes a high level of mutator activity (Maki et al., 1991). This was attributed to decreased proofreading capacity of DNA polymerase III, as determined *in vitro* by 'turnover assay' experiments, rather than insertional errors and is consistent with the observation that ε must bind to α to carry out its proofreading activity (Maki and Kornberg, 1987).

The ε subunit may also interact with the SOS response elements of *E.coli*. The *umuC122* allele suppresses the mutator activity of *dnaQ49* while the *recA730* allele, which results in constitutive expression of the activated *recA* protein (Mount, 1977), enhances *dnaQ49* mutator expression (Foster and Sullivan, 1988).

Lancy et al. (1989a) recovered viable deletions of the *dnaQ* gene in *Salmonella typhimurium* which are defective in growth as well as proofreading function. It is assumed that the replication genes in *E. coli* and *S. typhimurium* are highly analogous (Kornberg and Baker, 1992). These null *dnaQ* mutations are strictly dependent for viability on a functional DNA polymerase I. Suppressors were recovered that compensate

for the growth ability but not the mutator activity of the *dnaQ* mutants. These intergenic suppressors have been mapped at the *dnaE* locus (Lancy et al., 1989b).

Schaaper and Cornacchio (1992) isolated a suppressor of *mutD5* which greatly suppresses mutability of the strain in minimal medium, but only weakly suppresses in rich medium. It also causes a suppression of mutator activity in *mutT*, *mutL* and *dnaQ49* backgrounds. The suppressor, designated *dnaE910*, mapped at the *dnaE* locus and also conferred an increased growth rate to *mutD5* strains.

We have isolated certain strains of *dnaQ49* with a reduced mutator activity possibly due to intergenic suppressors. These putative suppressors show a 10-1000 fold decrease in mutation frequencies to streptomycin and rifampicin resistance in a *dnaQ49* background (Fowler et al., unpublished data) The present study describes the characterization and mapping of these suppressors.

Materials and Methods

Bacterial strains

The bacterial strains used are listed in Table 1.

Media

The cultures were grown in L-broth containing 1% tryptone, 0.5% yeast extract, and 0.5% sodium chloride. The tryptone plates used to determine total cell counts contained 1% tryptone, 0.5% sodium chloride, and 1.5% agar for solidification. Nalidixic acid resistant (Nal^R), streptomycin resistant (Str^R), and rifampicin resistant (Rif^R) mutants were selected on tryptone plates containing 50 µg/ml nalidixic acid, 150 µg/ml streptomycin, and 100 µg/ml rifampicin, respectively. Tetracycline resistant (Tet^R) transductants were selected on tryptone plates supplemented with 21 µg/ml tetracycline. GlpT media contained 4 mg/ml α-glycerol phosphate, 20 ml/liter Vogel and Bonner minimal salts (Vogel and Bonner, 1956), 1 µg/ml thiamine hydrochloride, and 50 µg/ml of tryptophan, leucine, histidine, arginine, and threonine. The glpT plates were solidified with 1.5% agar. Trp^+ revertants were selected on minimal plates supplemented with 0.2% glucose, Vogel and Bonner minimal salts, 1 µg/ml thiamine hydrochloride, and an amino acid mixture containing arginine, histidine, threonine, and leucine at 50 µg/ml and solidified with 1.5% agar. The LCTG plates for P1 phage lysate production contained 1% tryptone, 0.5% yeast extract, 0.5% sodium chloride, 0.1% glucose, and 1.5% agar for solidification. The media had 2 ml 1M $CaCl_2$, and 5 ml 0.2 mg/ml glucose added per liter after autoclaving and before solidification. The soft agar used for lysate production contained 0.2% glucose, 0.2% NaCl, 0.5% yeast extract, 1% tryptone, and 0.7% agar. The Mix B used for transductions contained

Table 1

Bacterial strains used:

Strain	Relevant genotype	References/ source
KH1116	Hfr (cavalli type), *metB1*, *proA3*, *lac-3*, *tsx-76*, *relA1*, *dnaQ49*	Dr. H. Maki, Kyushu Univ School of Medicine, Fukuoka, Japan.
C G S C 5182	*tonA22*, *phoA8*, *ompF627*, *glpT13*, *fadL701*, *relA1*, *pit-10*, *spoT1*	Hayashi et al. (1964); Dr. B. Bachmann(*E.coli* Genetic stock center)
SJ1033	*dnaQ49*, *zae502*::Tn*10*, *thr*$^-$, *leu*$^-$, *his*$^-$, *arg*$^-$, *trpA58*	B. Bond (Dept. of Biol. Sci., SJSU, San Jose, CA.)
KD1090	*mut*$^+$, *trpA23*	Degnen and Cox (1974); Fowler et al. (1974).
Nal^R-4	Same as KD1090 except Nal^R, *trpA58*, *dnaQ49*	Degnen and Cox (1974); Fowler et al. (1974)
Nal^R-9	Same as KD1090 except Nal^R, *trpA58*, *dnaQ49*	Degnen and Cox (1974); Fowler et al. (1974)
Nal^R-15	Same as KD1090 except Nal^R, *trpA58*, *dnaQ49*	Degnen and Cox (1974); Fowler et al. (1974)
KD1079	*proA*$^-$, *thr*$^-$,*leu*$^-$,*his*$^-$, *arg*$^-$	Degnen and Cox (1974)

0.015M $CaCl_2$, and 0.03M $MgSO_4$. Sterile 0.85% saline was used for washing and diluting the cultures.

Sterilization

The media were sterilized before use by autoclaving at a standard temperature and pressure. The antibiotics were added to the media after cooling. The amino acids and Vogel and Bonner minimal salts media were filter sterilized and stored at room temperature. Thiamine hydrochloride, Mix B, tetracycline and streptomycin were filter sterilized and stored at 4°C.

Mutation Frequencies

To determine mutation frequencies, putative strains were grown in L-broth for approximately 24 hours at 37°C in a shaking waterbath. The saturated overnight cultures were then diluted to 10^{-6} (approximately 10^3 cells/ml) in saline and 0.1 ml of the final cell dilution was added to tubes containing 5 ml of L-broth.

After incubating them in a shaking waterbath at 37°C for 24 hours the saturated cells were washed and either the concentration was increased 10 fold by resuspending in 0.5 ml of saline or the cells were resuspended in 5 ml of saline. Appropriate dilutions were plated onto media containing rifampicin and streptomycin for two to three days at 37°C to select for Rif^R and Str^R mutants, respectively, and onto media containing tryptone for two to three days at 37°C for total cell counts. Mutation frequencies for each culture were calculated by dividing the average number of mutant cells by the average total number of cells.

Lysate Production and Transduction

Genes were transferred by P1 phage transduction (Miller, 1992). The two steps of the process were production of the phage lysate and the actual transduction. To produce the lysate 5 ml of L-broth was innoculated with the donor strain and then incubated at 37°C in the waterbath shaker overnight. The phage stock was diluted to a final dilution of 10^{-2} and 10^{-3} pfu/ml. The next day, 0.5 ml of the donor overnight culture was mixed with 0.1 ml of the dilute P1 phage and with 3 ml of molten phage soft agar. This mixture was then poured onto a LCTG plate and incubated overnight at 37°C. The plate was retrieved after 24 hours and the phage overlay was scraped off aseptically into a sterile centrifuge tube containing 0.5 ml of chloroform. The plate was washed with 5 ml of sterile L-broth which was transferred to the centrifuge tube. The L-broth was mixed with the phage overlay and the tube was centrifuged at 1000-2000 g in a table top centrifuge for 15 minutes. The supernatant was carefully pipetted out into another tube containing 0.5 ml of chloroform yielding the lysate.

Transductions were carried out by innoculating the recipient strain in 5 ml of L-broth and incubating overnight at 37°C in a shaking waterbath. The saturated, overnight culture was centrifuged for 12 minutes at 1000-2000 g in a table top centrifuge. The supernatant was discarded and the cells were resuspended in 0.5 ml of sterile L-broth thus concentrating the culture 10-fold. A 0.2 ml aliquot was transferred into a sterile tube along with 0.5 ml of the dilute donor lysate and 0.5 ml of Mix B. The tube was then incubated at 37°C in a waterbath for 20 minutes without shaking. After 20 minutes, the tube was centrifuged for 12 minutes at 2000 g in a table top centrifuge. The supernatant was discarded and the cells were resuspended in 1 ml of

sterile minimal broth. The selective plates were innoculated with 0.1ml of the resuspended cells. A control was made using the same procedure but by using sterile L-broth instead of the phage. The plates were then incubated at 37°C from two to seven days.

Reversion Frequencies:

To determine reversion frequencies, strains were inoculated in L-broth and incubated for 24 hours at 37°C. The cultures were diluted to about 1000 cells/ml and 0.1 ml was used to innoculate second day overnight cultures. These were also grown to saturation for 24 hours at 37°C. These cultures were then washed with saline and 0.1 ml of the appropriate dilutions were plated onto minimal plates for Trp^+ reversion frequencies and onto minimal plates supplemented with tryptophan for total cell counts. Reversion frequencies were calculated by dividing the average number of colonies on minimal agar (Trp^+ revertants) by the average total number of cells on the minimal plates supplemented with tryptophan (total cells).

Results

Characterization of the Suppressors in a *dnaQ49* Background

Three putative suppressor strains, originally isolated as *dnaQ49* induced Nal^R mutants (Rane et al, unpublished data) were designated Nal^R-4, Nal^R-9, and Nal^R-15. Str^R and Rif^R mutation frequencies and Trp^+ reversion frequencies were measured for Nal^R-4, Nal^R-9 and Nal^R-15 strains (Table 2). At 37°C *dnaQ49* mutator activity of Nal^R-4 and Nal^R-15 strains was suppressed 10-1000 fold compared to *dnaQ49* frequencies in a wild type background. Based upon Str^R mutation and Trp^+ reversion frequencies, the Nal^R-9 strain shows the greatest degree of suppression. At 30°C the mutation frequencies were reduced about 10 fold or less in Nal^R-4 and Nal^R-15 strains compared to *dnaQ49* values.

Characterization of the *dnaQ49* Allele in Suppressor Strains

The putative suppressor strains were tested for the alteration of the *dnaQ49* mutator allele itself. P1 phage lysates were made from Nal^R-4, Nal^R-9, and Nal^R-15 mutant strains and the *dnaQ49* mutator allele in each strain was transferred to a mut^+ background via P1 phage transduction. KD1079*proA*$^-$ was used as the recipient mut^+ strain since *proA* and *dnaQ49* are closely linked (Horiuchi et al., 1978). Pro^+ transductants were selected on minimal media and tested for mutator activity. Transductants that showed enhanced mutagenesis expressed the full mutator activity of an unaltered *dnaQ49* allele (Table 3).

Table 2

Mutation and reversion frequencies of *mut*$^+$, *dnaQ49*, Nal^R-4, Nal^R-9, and Nal^R-15 strains.

Strain	Mutants per 10^8 cells				Trp$^+$ revertants per 10^8 cells	
	Rif^R		Str^R			
	30°C	37°C	30°C	37°C	30°C	37°C
KD1090 (*mut*$^+$)	-[a]	6.6	<.02	<.02	0.4	0.85
SJ1033 *(dnaQ49)*	220	49000[b]	1.9	980	1.8	447
Nal^R-4	160	25	0.19	53	<.04	44
Nal^R-9	-	-	-	0.2[c]	-	1.5[c]
Nal^R-15	-	13	0.4	5.6	2.6	80

[a] Frequencies not determined

[b] Value taken from Horiuchi et al. (1978)

[c] Frequencies taken from Le (unpublished data)

Table 3

Mutation frequencies of *dnaQ49* alleles, transduced into a *mut*$^+$ background from Nal^R-4, Nal^R-9, and Nal^R-15 strains.

		Mutants per 10^8 cells	
Strain		Rif^R	Str^R
KD1090 (*mut*$^+$)		3.53	0.71
SJ1033(*dnaQ49*)		49000[b]	2100
Nal^R-4	Trans-1	13030[c]	1198[c]
	Trans-2	7300[c]	3690[c]
Nal^R-9	Trans-1	17600	888
	Trans-2	23500	2050
	Trans-3	20000	2250
Nal^R-15	Trans-1	9160[c]	1450[c]

[a] The *dnaQ49* gene was transduced from Nal^R-4, Nal^R-9 and Nal^R-15 donors into a KD1079 *proA*$^-$ recipient strain. The mutation frequencies of the Pro$^+$ transductants were determined and several showing *dnaQ49* mutator activity are displayed in the table.

[b] Value taken from Horiuchi et al. (1978)

[c] Values taken from Fowler et al. (unpublished data).

Mapping the Suppressors

Since the three *dnaQ49* suppressors were selected as Nal^R mutants, experiments were done to determine if they map at the *gyrA* locus. The *glpT* locus is adjacent to the *gyrA* locus and *glpT* $^+$ transductants can be selected for with a *glpT*$^-$ recipient strain. P1 phage lysates of Nal^R-4, Nal^R-9, and Nal^R-15 suppressor strains were prepared. The *glpT*$^+$ gene from the suppressors was transduced into a *glpT*$^-$ background via P1 phage transduction. The transductants were isolated on *glpT* media containing α-glycerol phosphate as the sole source of carbon, allowing the growth of *glpT*$^+$ but not *glpT*$^-$ strains. Colonies growing on these *glpT* plates were transferred onto nalidixic acid plates to check for Nal^R phenotypes. The cotransduction frequency for Nal^R-4, Nal^R-9, and Nal^R-15 with *glpT* was 62.71%, 10.7%, and 28.57%, respectively. This compares to the published value of 65% for *glpT* and *gyrA* (Fuchs and Karlstrom, 1976). Therefore, Nal^R-4, Nal^R-9, and Nal^R-15 mutations map at or near the *gyrA* locus.

Characterization of the Suppressors In a *mut*$^+$ Background

The mutation frequencies for the three suppressors were determined in a wild type (*mut*$^+$) background. Lysates were made of Nal^R-4, Nal^R-9, and Nal^R-15 suppressors using P1 phage. The suppressor alleles were transduced into a *trpA23* (*mut*$^+$) strain (KD1090) and the Nal^R transductants were selected for on nalidixic acid plates.

The Nal^R-4, Nal^R-9, and Nal^R-15 mutations when transferred to a *mut*$^+$ background form two different types of colonies, large and small. Several transductants

frequencies for three to four transductants determined (Table 4). Nal^R-4, Nal^R-9, and Nal^R-15 mutants showed elevated mutation frequencies in the *mut*$^+$ background.

The mutator activity of the transductants was further explored by determining Trp$^+$ reversion frequencies of the *trpA23* allele which is known to revert by several types of base-pair substitutions (Yanofsky *et al.*, 1966). The selective plates showed several sizes of revertant colonies. Some revertants formed large colonies while others were small and evident after five or more days of incubation. Trp$^+$ reversion frequencies of these transductants indicated some mutator activity (Table 4).

Transfer of an Active *dnaQ49* Mutator Allele into Nal^R-4, Nal^R-9, and Nal^R-15 *mut*$^+$ Backgrounds

An active *dnaQ49* allele was transferred into Nal^R-4, Nal^R-9 and Nal^R-15 suppressor strains in a *mut*$^+$ background via P1 phage transduction. The donor was a *dnaQ49 zae-502* :: Tn*10* strain (SJ1033) where the transposon Tn*10* is closely linked to *dnaQ49* and confers resistance to tetracycline. Tet^R transductants were selected for on tetracycline plates and colonies were further tested to insure they still possessed the Nal^R phenotype.

Rif^R and Str^R forward mutation frequencies and Trp$^+$ reversion frequencies were determined for 14 to 16 transductants for each suppressor strain (Table 5).

The *dnaQ49* allele typically showed a 100 fold decrease in Rif^R and a 10-1000 fold decrease in Str^R mutation frequencies when transferred into a suppressor background. Reversion frequencies to Trp$^+$ were similar.

Table 4

Typical mutation and reversion frequencies of the Nal^R-4, Nal^R-9, and Nal^R-15 alleles transduced into a *trpA23 mut*$^+$ strain.

Strain	Nal^R Transductant [a]		Mutants per 10^8 cells		Trp^+ Revertants per 10^8 cells
			Rif^R	Str^R	
KD1090*trpA23* (*mut*$^+$)			4.0	0.3	1.91
Nal^R-4	Large -	1	694	50.8	27.7
		2	525	19.3	- b
	Small -	1	1442	11.5	0.48
		2	836	0.5	-
Nal^R-9	Large -	1	608	19.3	12.38
		2	424	23	-
	Small -	1	968	1.1	22.22
		2	643	2.1	-
Nal^R-15	Large -	1	428	10.2	91.71
		2	485	6.5	-
	Small -	1	244	-	1.8

[a] Two types of Nal^R transductant colonies were observed, large and small. Examples of each were tested for spontaneous mutation and reversion frequencies.

[b] Not done

Table 5

Mutation and reversion frequencies of *dnaQ49* transductants into Nal^R-4, Nal^R-9, and Nal^R-15 *mut*$^+$ backgrounds.

Strains		Mutants per 10^8 cells		Trp$^+$ Revertants per 10^8 cells
		Rif^R	Str^R	
SJ1033		49000[a]	980	447
(dnaQ49)				
Nal^R-4	1[b]	500	25	156
	2	493	2.03	51.7
Nal^R-9	1	326	.65	2.4
	2	895	.15	2.3
Nal^R-15	1	590	31	34
	2	622	6.2	12.4

[a] Value taken from Horiuchi et al. (1978).

[b] Two transductants are shown for each Nal^R allele.

Discussion and Conclusions:

The *E.coli dnaQ* gene encodes the ε subunit of DNA polymerase III which catalyses the 3'->5' exonuclease (proofreading) activity of the enzyme (Scheuermann et al., 1983; Scheuermann and Echols, 1984). There are two known strong mutator alleles for this gene, a dominant *mutD5* (Degnen and Cox, 1974; Horiuchi et al., 1983) and a recessive *dnaQ49* (Horiuchi et al., 1978; Horiuchi et al., 1983) allele. High mutation rates for both these alleles is thought to be due to faulty proofreading (Echols et al., 1983; Difrancesco et al., 1984). Fersht et al. (1982) observed that proofreading could contribute at most a 200 fold increase in fidelity of DNA replication *in vitro* which suggests that additional functions may be defective (Echols et al., 1983; Piechocki et al., 1986; Isbell and Fowler, 1989).

Schaaper (1988, 1989) and Damagnez et al. (1989) demonstrated that *mutD5* strains when grown in rich media are also defective in methyl-directed post replicative mismatch repair (Modrich, 1987). Schaaper and Radman (1989) showed that saturation of this mismatch repair system by an excess of unaltered DNA replication errors causes a mismatch repair deficiency in *mutD5* strains. Studies with single and double *dnaQ49 mutL* mutator strains, where the *mutL* allele results in defective mismatch repair, have shown that *dnaQ49* strains are also defective in mismatch repair when grown in rich media (Krishnaswamy et al., submitted for publication).

The interaction between the several gene products necessary for DNA replication is not yet clearly understood. The ε subunit, along with the α (polymerase) subunit (Maki and Kornberg, 1985) and the θ subunit, form the core enzyme of DNA polymerase III. This core has a polymerase as well as a proofreading function. Maki and Kornberg (1987) observed an increase in the 3'->5' exonucleolytic activity of the ε subunit when

it complexed with the α subunit as compared to the free ε subunit itself. This led to the conclusion that the α subunit of DNA polymerase III is also involved in the proofreading function by virtue of its binding to the ε subunit or by causing a conformational change in ε which orients it appropriately.

Lancy *et al.* (1989a) recovered growth and proofreading defective deletion mutations of the *dnaQ* gene in *S. typhimurium* . Intergenic suppressors located in the *dnaE* gene were strongly selected for in these deletion strains. These suppressors compensated for the growth defect, but not the proofreading defect of the deletions. Schaaper and Cornacchio (1992) have isolated a mutation localized to the *dnaE* gene which greatly suppresses the mutability of *mutD5* strains in a minimal medium.

The three Nal^R mutations characterized in this study seem to produce a strong suppression of *dnaQ49* mutator activity as measured by Str^R and Rif^R forward mutation and Trp^+ reversion frequencies. Suppression of mutator activity at 30°C is less intense compared to 37°C.

When the *dnaQ49* allele was transferred from the suppressor backgrounds into a *mut* $^+$ background, *dnaQ49* expressed full mutator activity indicating that the reduction of mutator activity was not due to a change in the *dnaQ49* allele itself. Some *dnaQ49* transductants did show slightly reduced mutator activity but this is typical of passage of *dnaQ49* by transduction.

A reciprocal experiment using an active *dnaQ49* allele transduced into Nal^R-4, Nal^R-9, and Nal^R-15 strains in a *mut* $^+$ background resulted in severe suppression of the *dnaQ49* allele, indicating that Nal^R-4, Nal^R-9, and Nal^R-15 are intergenic suppressors of the *dnaQ49* allele.

Although the cotransduction frequencies for Nal^R-9 and Nal^R-15 with *glpT* $^+$ were considerably below the reported cotransduction frequency of 65% (Fuchs and

Karlstrom, 1976) of the *glpT* + locus and the *gyrA* locus, cotransduction values are known to be quite variable (Miller,1992). When grown in the absence of nalidixic acid these strains often became sensitive to nalidixic acid indicating strong selective pressure away from the Nal^R phenotype. Since the initial selection for *glpT* + transductants was performed on medium without nalidixic acid, it is quite possible that the number of Nal^R cotransductants was underestimated. In addition the *gyrA* gene is the only known locus in this region of the *E.coli* chromosome that confers resistance to nalidixic acid (Bachmann, 1990). All three suppressors probably map at the *gyrA* locus.

The *gyrA* and the *gyrB* loci encode the A and B subunits respectively of DNA gyrase (Cozzarelli, 1980) which is thought to act as a swivel, allowing progress of the fork during replication (Kornberg and Baker, 1992). The popular interpretation is that gyrase is active ahead of the replication complex removing positive supercoils that would otherwise prevent progress of the fork. However, Maki *et al.* (1989) reported that the mutant *pcbA1,* an allele of *gyrB,* can suppress certain *dnaE* nonsense mutations in the presence of an active DNA polymerase I. This suggests a possible direct or indirect interaction between gyrase and the α subunit of DNA polymerase III, a central component of the replication complex.

The data from this study suggest an interaction between gyrase and the *dnaQ49* encoded ε subunit of DNA polymerase III. If this is a direct physical interaction, it would imply a physical presence of the gyrase at the replication fork. Possibly the swivel activity of the gyrase is carried out at the replication fork rather than ahead of it as is currently thought.

It was surprising to detect mutator activity attributed to the Nal^R-4, Nal^R-9, and Nal^R-15 alleles. It was expected that the suppressors would have no effect on

spontaneous mutation frequencies or might possibly act as antimutators in a *mut*$^+$ background. Instead they raised Str^R and Rif^R mutation frequencies about 100X above *mut*$^+$ levels, and Trp$^+$ reversion frequencies somewhat less. These values may be underestimates because the Nal^R strains show poor growth on minimal media and certain Trp$^+$ revertants may have been unable to form colonies under these conditions. The Rif^R, and Str^R mutations and Trp$^+$ revertants scored in this experiments resulted exclusively from base-pair substitutions (Miller, 1992; Yanofsky et al., 1966).

These Nai^R mutants are the first reported recoveries of mutator alleles at the *gyrA* locus. Miller *et al.* (1992) recently reported a mutator allele, *mutR*, that maps at *topB*, the structural gene for topoisomerase III. The *mutR* allele causes an increase in deletion mutations which probably occur through changes in chromosomal supercoiling (Miller et al., 1992). The Nal^R-4, Nal^R-9, and Nal^R-15 mutators cause base-pair substitutions. Possibly these suppressors act by affecting the degree of supercoiling and this affects proofreading efficiency or they could have a direct interaction where the gyrase directly interacts with the α or ε subunits. If gyrase is indeed a part of the replication complex, perhaps these mutant forms are affecting the functioning of the α or ε subunits of DNA polymerase III in accurate polymerization or proofreading during replication.

Since the Nal^R-4, Nal^R-9, and Nal^R-15 mutations were isolated independently, it is not known if any are identical *gyrA* alleles. Phenotypically Nal^R-9 appears to be different from the other two. It has a slower generation time (data not shown) and seems to be a stronger suppressor of *dnaQ49* mutator activity. Eventually DNA sequencing will be needed to determine the relationship between the three alleles.

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