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By

Roberta J. Isbell

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APPROVED FOR THE DEPARTMENT OF BIOLOGICAL SCIENCES

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ABSTRACT

TEMPERATURE-DEPENDENT MUTATIONAL SPECIFICITY OF AN *ESCHERICHIA COLI* MUTATOR, *DNAQ49*, DEFECTIVE IN 3' --->5' EXONUCLEASE (PROOFREADING) ACTIVITY

by Roberta J. Isbell

Escherichia coli strains carrying the temperature-dependent *dnaQ49* allele are strong mutators at 37°C. Since the *dnaQ49* gene encodes the epsilon subunit of DNA polymerase III, it is thought that the large number of errors result in part from impaired proofreading activity during DNA replication. *DnaQ49* -induced reversion patterns of defined *trpA* alleles have been examined to determine the kinds of errors produced by *dnaQ49* at 30°C and 37°C. It was found that at 37°C *dnaQ49* produced all types of base-pair substitutions in addition to frameshifts with transitions generally occurring more frequently than transversions. This generalized mutator activity is very similar to that displayed in rich medium by *mutD5*, another mutator allele at the *dnaQ* locus. However, when *dnaQ49* strains were cultured at 30°C, not only were reversion frequencies much lower than at 37°C, but in addition, the spectrum was altered. Transversions became proportionally more prevalent in the reversion spectra at the lower temperature. It is possibile that at 37°C *dnaQ49* results in defective proofreading and methyl-directed postreplicative mismatch repair, while at 30°C mismatch repair is fully and proofreading partially restored.

Introduction

Replication of DNA in the normal *E. coli* cell is an amazingly accurate process. Errors occur at a given site about one out of a billion times per generation. To ensure such faithful replication, the cell has evolved an overlapping array of protective mechanisms. While a picture of the intricacies and interdependencies of the entire system is not yet clear, several gene products are known to contribute to mutation avoidance. For example, *mut T* appears to monitor dGTP, allowing the correct configuration access to the polymerizing strand and keeping out G-A mispairs which would lead to transversions if incorporated (Bhatnagar and Bessman, 1988; Schaaper and Dunn, 1987). The *dnaQ (mut D)* product, epsilon, is referred to as the proofreading subunit of DNA polymerase holoenzyme III since it works on the polymerizing strand to immediately excise incorrectly incorporated nucleotides (Scheuermann and Echols, 1984). After polymerization but before the new strand has been methylated, *mutH, mutL, mutS* and *mutU* presumably work in concert to scrutinize the new strand (Modrich, 1987; Radman and Wagner, 1986). Mispairings, deletions and insertions are corrected in favor of the methylated, old strand via excision of several nucleotides around the erroneous site. Subsequently a corrected insert is synthesized and ligated in place.

These are only three of the mechanisms that the cell employs to avoid mutation from generation to generation. The processes involved have been elucidated largely by investigating defective gene products noted for their propensity to increase spontaneous mutation rates over that expected in a normal cell. Such defective alleles, whether of *dnaQ*, *mut T*,

or of one of the genes that codes for a protein involved in mismatch repair, are known as mutator alleles. Comparing the frequencies and types of mutations created in mutator backgrounds with those seen in normal cells should ultimately reveal how the cell normally avoids mutation.

One such mutator allele, designated *dnaQ49*, has been shown to result from a base-pair substitution within the *dnaQ (mutD)* gene (Horiuchi et al., 1981; Takano et al., 1986) that encodes the epsilon subunit of DNA polymerase III holoenzyme which catalyses 3' --->5' exonuclease proofreading activity (Scheuermann et al., 1983; Scheuermann and Echols, 1984). *DnaQ49* strains have been shown to be defective in 3'--->5' exonuclease activity (Echols et al., 1983) and this is assumed to be at least in part responsible for the high spontaneous mutation rates associated with such cells (Horiuchi et al., 1978).

In addition to *dnaQ49* a second *dnaQ* gene mutator allele, *mutD5*, has been intensely studied (Degnen and Cox, 1974; Takano et al., 1986). The *mutD5* allele also results in impaired 3'--->5' exonuclease proofreading activity (Scheuermann et al., 1983; Scheuermann and Echols, 1984; DiFrancesco et al., 1984) with resulting high mutation rates (Degnen and Cox, 1974; Fowler et al., 1974). However the *dnaQ49* and *mutD5* alleles demonstrate the following differences: (1) *dnaQ49* strains have defective DNA synthesis at an elevated temperature (44.5^{o}C) (Horiuchi et al., 1978) while *mutD5* does not exhibit lethality at higher temperatures (Cox and Horner, 1982) (2) *dnaQ49* shows temperature dependency for mutator activity with mutation frequencies higher at 35°C than at 30°C (Horiuchi et al., 1978), while temperature has no effect on *mutD5* mutation frequencies (Degnen and Cox, 1974) (3) the *mutD5* allele is dominant to

the wild type (*mut*$^+$) allele (Cox and Horner, 1982), while *dnaQ49* is recessive (Maruyama et al., 1983) (4) *mutD5* and *dnaQ49* result from different base-pair substitutions within the *dnaQ* gene (Takano et al., 1986). The high mutation frequencies (10^4 to 10^5 above wild type levels) associated with *mutD5* are found only when strains are grown in a rich medium, whereas mutations frequencies when cells are grown in minimal medium are about 100- fold less (Degnen and Cox, 1974; Erlich and Cox, 1980).

The mutational specificity of *mutD5* has been the subject of several studies (Fowler et al., 1974; Fowler et al., 1986; Schaaper, 1988). *MutD5* is a generalized mutator usually producing single base-pair substitutions of all types (95%). In rich medium most substitutions (90%) are transitions while in minimal medium the majority (62%) are transversions, particularly AT--->TA events (Schaaper, 1988). In contrast to *mutD5*, only one study has examined the mutational specificity of *dnaQ49*. Piechocki et al. (1986) selected *dnaQ49*-induced suppressor mutations on minimal medium and, after classification of the mutational events, concluded that *dnaQ49* enhanced mainly transversions to AT base pairs. In this study the well-defined *trpA* reversion system is used to determine the mutational events caused by *dnaQ49* in rich medium at 37°C and 30°C. It is shown that the temperature-dependent mutational potency of *dnaQ49* is accompanied by a difference in mutational specificity at the two temperatures and that at 37°C the specificity of *dnaQ49* appears to be nearly identical to that of *mutD5*. A portion of this study has already been published (Fowler and Isbell, 1988).

Materials and Methods

Bacterial strains

Strain KH1116 (*dnaQ49*) was obtained from H. Maki (Horiuchi et al., 1978), KD1079 *proA* Δ(*tonB-trpA,B*) from E. Cox (Degnen and Cox, 1974) and the tryptophan synthetase A gene mutants from C. Yanofsky (Yanofsky et al., 1966). The *dnaQ49* allele was P1 transduced as described by Miller (1972) into KD1079 using Pro^+ selection. Pro^+ transductants were checked for mutator activity by plating 0.1 ml of an overnight L-broth culture on tryptone-nalidixic acid plates; *dnaQ49* clones gave one hundred to several hundred colonies per plate while mut^+ gave none to few. One *dnaQ49* Δ(*tonB-trpA,B*) clone, designated SJ1001, was selected and used as the recipient for transductions with *trpA* donor strains. The *trpA* alleles were transduced into SJ1001 with selection for the $trpB^+$ allele on indole plates as previously described (Degnen and Cox, 1974). The coisogenic mut^+ *trpA* strains have been previously constructed (Degnen and Cox, 1974; Fowler et al., 1974). Strain NR8093 (*mutD5*) has been previously described (Fowler et al., 1986).

Media

VB medium is the minimal salts medium of Vogel and Bonner (1956). Minimal plates contained minimal medium solidified with 1.5% agar and supplemented with 0.2% glucose and 1ug/ml thiamine. Required amino acids were added at 50 ug/ml to liquid and solid media; and indole was added to solid media at 10 ug/ml. Tryptone plates consisted of 1% tryptone and 0.5% NaCl solidified with 1.5% agar. Mutation to nalidixic acid and streptomycin resistance was measured on tryptone plates

containing 50 ug/ml nalidixic acid and 150 ug/ml streptomycin respectively. L-broth was 1% tryptone, 0.5% yeast extract and 0.5% NaCl. The saline for dilutions and washing was 0.85% NaCl.

Mutagenesis procedures

For each *dnaQ49 trpA* strain a saturated culture was diluted, and approximately 1000 cells were used as inocula to initiate 60 to 80 new cultures. One half of these cultures were grown to saturation (24 hrs.) in L-broth at 37°C while the other half were grown to saturation (36-48 hrs) in L-broth at 30°C. Three cultures, randomly selected for each temperature, were used to determine mutation and reversion frequencies. Such cultures were washed in saline, diluted appropriately and plated on minimal media with tryptophan to titer for total cells and on minimal media to select for Trp^+ revertants. The plates, spread with 37°C or 30°C culture cells, were incubated at 37°C or 30°C so that total cells (48 hrs.) and Trp^+ revertants (five days) could be counted. Reversion frequencies for each *trpA* strain were calculated by dividing the average number of Trp^+ revertants by the average total cell titer.

The remaining 37°C and 30°C cultures were washed in saline, plated on minimal media without tryptophan, and incubated for 5 days at 37°C or 30°C to select for Trp^+ revertants for classification. In order to insure selection of independent Trp^+ revertants, one colony was randomly picked per culture, with the exception of one or two cultures at each temperature where every Trp^+ colony was utilized per plate. When no differences were found in the reversion spectra between the two sources of Trp^+ revertants, the data were pooled. Reversion frequencies at 37°C for *mut*$^+$

trpA strains were determined as previously described for *dnaQ49* strains. Nal^R and Str^R mutation frequencies were determined by plating saturated *dnaQ49* and *mut*$^+$ cultures on tryptone media to titer for totals cells and on tryptone-nalidixic acid and tryptone- streptomycin media to select for Nal^R and Str^R cells respectively.

Classification of Trp$^+$ revertants.

The classification of Trp$^+$ revertants of *trpA* strains was based upon two physiological tests. First, purified revertants were tested for indole glycerol phosphate (IGP) accumulation. Full revertants do not accumulate IGP; partial revertants do (Allen and Yanofsky, 1963). All Trp$^+$ revertants were tested for IGP accumulation. Second, various partial revertants can often be distinguished quantitatively by their sensitivity to 5-methyl tryptophan (5- MT) (Lester and Yanofsky, 1961). Partial or suppressed revertants growing on agar plates show much larger zones of inhibition than full revertants around disks impregnated with 5-MT (Cox et al., 1972; Fowler et al., 1974). The 5-MT inhibition test was used to classify all partial revertants of *trpA* alleles *23, 46*, and *58*.

Results

Mutation frequencies for *mut*$^+$, *dnaQ49*, and *mutD5* strains are shown in Table 1. Consistent with earlier reports (Degnen and Cox, 1974; Horiuchi et al., 1978) both *dnaQ49* and *mutD5* are strong mutators at 37°C; however, while *mutD5* remains potent at 30°C, *dnaQ49* loses much of its mutator activity at the lower temperature. Mutation frequencies for the *mut*$^+$ strain are similar for both temperatures.

The mutator activity of *dnaQ49* was further explored by determining the reversion spectrum of *trpA* base-pair substitution and frameshift mutants (Yanofsky et al., 1966; Drapeau et al., 1968; Brammar et al. 1967; Berger et al., 1968). The reversion pattern of most *trpA* alleles can be deduced from IGP accumulation and 5-MT inhibition tests independently of amino acid sequence data; and, thus, base-pair changes can often be determined directly from the IGP and 5-MT phenotypes of the Trp$^+$ revertants. The Trp$^+$ reversion frequency for each *trpA* mutant in *mut*$^+$ and *dnaQ49* strains is shown in Table 2. At 37°C the data indicate that the Trp$^+$ reversion frequency for both base- pair substitution and frameshift mutants is greatly increased by *dnaQ49*. In comparison, at 30°C *dnaQ49* displayed much more modest mutator activity which for some *trpA* alleles approached *mut*$^+$ levels.

The characterization and distribution of Trp$^+$ revertants of the *trpA* mutants, based upon the IGP accumulation and 5-MT inhibition tests, is shown in Table 3. The *trpA* mutants, revertants and inferred base-pair substitutions are taken from data of Yanofsky and co-workers (Yanofsky et al., 1966; Drapeau et al., 1968). Reversion frequencies for particular base-pair substitutions, shown in Table 4, have been calculated by

Table 1

Mutation frequencies in *mut* $^+$, *mutD5,* and *dnaQ49* strains at 30°C and 37°C.

Strain	Mutants per 10^9 cells			
	Nal^R		Str^R	
	30°C	37°C	30°C	37°C
KD1092 (*mut*$^+$)	2.01	3.61	.756	.842
NR8093 (*mutD5*)	10300	32600	3720	3410
KH1116 (*dnaQ49*)	91.7	3333	4.57	68100

Table 2. Reversion of *trpA* base-pair substitution and frameshift mutations in *mut* $^{+}$ and *dnaQ49* strains

trp A allele	Mutator allele (^{o}C)	Revertants per 10^{8} cells	*dnaQ49* / *mut* $^{+}$
A3	*mut* $^{+}$(37°C)	1.28	
	dnaQ49 (30°C)	11.0	8.59
	dnaQ49 (37°C)	57.8	45.2
A11	*mut* $^{+}$(37°C)	.251	
	dnaQ49 (30°C)	.984	3.92
	dnaQ49 (37°C)	521	2080
A23	*mut* $^{+}$(37°C)	2.40	
	dnaQ49 (30°C)	81.1	33.8
	dnaQ49 (37°C)	7860	3280
A46	*mut* $^{+}$(37°C)	.138	
	dnaQ49 (30°C)	112	812
	dnaQ49 (37°C)	8900	64500
A58	*mut* $^{+}$(37°C)	2.67	
	dnaQ49 (30°C)	75.0	28.1
	dnaQ49 (37°C)	3020	1130
A78	*mut* $^{+}$(37°C)	19.6	
	dnaQ49 (30°C)	118	6.02
	dnaQ49 (37°C)	7510	383

A88	*mut*$^{+}$(37°C)	.269	
	dnaQ49 (30°C)	.506	1.88
	dnaQ49 (37°C)	32.8	121
A223	*mut*$^{+}$(37°C)	3.50	
	dnaQ49 (30°C)	163	46.6
	dnaQ49 (37°C)	7950	2270
A446[a]	*mut*$^{+}$(37°C)	.898	
	dnaQ49 (30°C)	4.47	4.98
	dnaQ49 (37°C)	1700	1890
A540 (fs)[b]	*mut*$^{+}$(37°C)	5.23	
	dnaQ49 (30°C)	3.52	.673
	dnaQ49 (37°C)	63.3	12.1
A9813 (fs)[b]	*mut*$^{+}$(37°C)	3.09	
	dnaQ49 (30°C)	8.76	2.83
	dnaQ49 (37°C)	3870	1250

[a] *A446* revertants were plated on minimal media plates containing 0.1 *u* g/ml 5-methyl tryptophan to control leakiness of this allele.

[b] Frameshift mutation

Table 3

The characterization and distribution of *dnaQ49* -induced revertants of *trpA* base-pair substitution and frameshift mutants[a]

trp A allele	Revertant class[c]	Distribution[b] *mut*$^{+}$(37°C)	*Q49* (30°C)	*Q49* (37°C)	Inferred base-pair substitution
A3	I FR	.985 (67)	.986(70)	.716(95)	A:T-->T:A
A11	I FR	1.00(10)	1.00(35)	.563(71)	G:C-->C:G
A23	I FR	.750(96)	.322(90)	.640(86)	A:T-->G:C A:T-->C:G A:T-->T:A
	II PR	.167(96)	.100(90)	.174(86)	G:C-->C:G
	IIIPR	.052(96)	.567(90)	.163(86)	G:C-->T:A
A46	I FR	.586(88)	.831(59)	.772(114)	A:T-->G:C A:T-->C:G
	II PR	.195(88)	.102(59)	.026(114)	A:T-->T:A
	IIIPR	.218(88)	.068(59)	.202(114)	A:T-->G:C
A58	I FR	.127(63)	.035(57)	.653(72)	A:T-->G:C
	II PR	.063(63)	.140(57)	.069(72)	A:T-->C:G
	IIIPR[d]	.810(63)	.825(57)	.278(72)	G:C-->A:T
A78	I FR	.093(107)	.551(107)	.014(145)	A:T-->C:G
A88	I FR	1.00(14)	.973(37)	.784(88)	A:T-->C:G
A223	I FR	.603(63)	.667(87)	.447(85)	A:T-->G:C A:T-->C:G
A446	I FR	.140(57)	.889(54)	.842(76)	G:C-->A:T
A540 [e]	I FR	.458(59)	.731(67)	.486(70)	-------
	II PR	.542(59)	.269(67)	.514(70)	
A9813 [e]	I FR	.033(30)	.129(70)	.194(67)	-------
	IIPR	.967(30)	.871(70)	.806(67)	

[a] Some *dnaQ49* -induced revertants could not be placed into well defined classes and are not included in this table.

[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

Table 4

Enhancement of *trpA* base-pair substitutions by *dnaQ49* at 30°C and 37°C.

Inferred base-pair substitution	*trpA* allele	Revertant class	Revertants per 10^8 cells			Frequency enhancement		
			mut⁺ (37°C)	*dnaQ49* (30°C)	*dnaQ49* [b] (37°C)	*Q49* (30°C) / *mut*⁺	*Q49* (37°C) / *mut*⁺	*Q49* (37°C) / *Q49* (30°C)
Transitions								
A:T-->G:C	*A58*	I FR	.339	2.63	1970 (1370)	7.76	5810	749
	A46	III PR	.030	7.62	1800 (1630)	254	60000	236
G:C-->A:T	*A58* [c]	III PR	2.16	61.9	840 (475)	28.7	389	13.6
	A446	I FR	.126	3.97	1430 (1930)	31.5	11300	360
Transversions								
A:T-->C:G	*A88*	I FR	.296	.492	25.7 (4.44)	1.66	86.8	52.2
	A58	II PR	.168	10.5	208 (57)	62.5	1238	19.8
	A78	I FR	1.82	65.0	105 (30)	35.7	57.7	1.62
G:C-->T:A	*A23*	III PR	.125	46.0	1280 (186)	368	10200	27.7
A:T-->T:A	*A46*	II PR	.027	11.4	231 (260)	422	8560	20.3
	A3	I FR	1.26	10.8	41.4 (232)	8.57	32.9	3.84
G:C-->C:G	*A11*	I FR	.251	.984	293 (285)	3.92	1170	297
	A23	II PR	.401	8.11	1370 (279)	20.2	3420	169

[a] Revertants were placed into discrete classes FR = full revertant, PR = partial revertant based on the IGP and 5MT tests as described in Materials and methods.

[b] Data for *mutD5* shown in parentheses for comparison is from Fowler et al. (1974).

[c] This class results from intergenic suppression of the *trpA58* allele (Squires and Carbon, 1971)

multiplying the distribution proportion from Table 3 times the Trp$^+$ reversion frequency from Table 2 for the particular *trpA* allele involved. At 37°C *dnaQ49* enhanced over *mut$^+$* levels all base-pair substitutions that can be identified with the *trpA* sites available (Table 4). Reversion patterns at two sites provide unambiguous data on AT--->GC transitions. Full revertants of *trpA58* and a partial revertant class of *trpA46* result from these events (Yanofsky et al., 1966). The data of Tables 3 and 4 clearly show that these base-pair substitutions are strongly enhanced by *dnaQ49.* Likewise AT--->GC transitions produce the full revertant classes of *trpA23, trpA46* and *trpA223* (Table 3); and these revertants are also increased by *dnaQ49.* In these cases, however, full revertants may result not only from AT--->GC transitions but also from other substitutions, thus allowing it to be concluded only that transitions or other events or both are *dnaQ49* - stimulated at these sites.

GC--->AT transitions can also be detected at two sites. Full revertants of *trpA446* and suppressed mutants of *trpA58* result from such events (Yanofsky et al., 1964) and again are strongly enhanced by *dnaQ49* at 37°C (Tables 3 and 4).

Transversions are also induced by *dnaQ49* at 37°C at several sites (Tables 3 and 4). Each of the four transversion types was increased, although the frequency for a particular transversion event varied from site to site (Table 4). In addition the *dnaQ49* -induced reversion frequencies for transversions were generally less than those for transitions at 37°C. This is most clearly demonstrated by the *trpA58* allele where full revertants result from AT--->GC transitions and a class of partial revertants (II PR) arise from AT--->CG transversions at the same

nucleotide position (Yanofsky et al., 1966). At 37°C *dnaQ49* induced the transition almost 10 times more frequently that it did the transversion event (Tables 3 and 4). For comparison Trp$^+$ reversion frequencies at 37°C enhanced by *mutD5*, another mutator allele at the *dnaQ* locus, are taken from Fowler et al. (1974) and presented in Table 4.

The Trp$^+$ reversion frequencies of two *trpA* frameshift mutants, *trpA540* and *trpA9813*, were also increased by *dnaQ49* at 37°C (Table 2). The *trpA540* allele originally arose by either a single or double base deletion and the full revertants that have been characterized arose from single or double additions at the site of the original deletion (Berger et al., 1968). Berger et al. (1968) also concluded that partial revertants of *trpA540* occurred by base deletions near the original mutant site. The *trpA9813* allele apparently arose via a single adenine deletion; and full revertants presumably result from a single base addition at that site. The partial revertants that have been characterized resulted from subsequent single base additions occurring near the original adenine deletion (Brammar et al., 1967). While *dnaQ49* stimulated both full and partial revertants of *trpA540*, mostly partial revertants of *trpA9813* were created, (Table 3). It cannot be determined if the revertant classes for *trpA540* and *trpA9813* resulted from the same events as those previously described; but it does seem reasonable to conclude that most, if not all, did result from frameshift events which are enhanced by *dnaQ49*.

At 30°C the mutator activity of *dnaQ49* was markedly reduced compared to that at 37°C (Table 4). In addition, there was an obvious change in mutational specificity at 30°C. As shown in Table 3, transitions no longer dominated the Trp$^+$ revertant classes as was the case at 37°C.

Transitions were still stimulated by *dnaQ49* at 30°C; however, their enhancement, only 200-fold or less over *mut*$^{+}$ values, was far less compared to 37°C values (Table 4). The proportion of transversions among Trp^{+} revertants was higher at 30°C than at 37°C for most *trpA* alleles (Table 3). Whereas at 37°C full revertants of *trpA58* resulting from AT--->GC transitions were almost ten times as frequent as AT--->CG transversions (II PR) at the same AT site, the latter event was four times as frequent as the former at 30°C (Tables 3 and 4).

Discussion

The mutational specificity of the *dnaQ49* mutator allele in rich medium at 37°C and 30°C has been determined using the *trpA* reversion system. It has been found that *dnaQ49* is a generalized mutator causing all six classes of base-pair substitutions and frameshift mutations. (Complex mutations are not detectable with the *trpA* reversion system.) Within the resolution of the *trpA* system it appears that at 37°C the specificity and potency of *dnaQ49* is nearly identical to that of *mutD5,* a second mutator allele of the *dnaQ* gene (Fowler et al., 1974). A previous study of the mutational specificity of *dnaQ49* (Piechocki et al., 1986) indicated that *dnaQ49* induced mainly transversions to AT base pairs. The difference in results from this previous study and the present data may not be contradictory since the mutational events occurred in minimal medium in the former study while L-broth was used here. Although a medium effect on mutator activity and mutational specificity has not yet been clearly demonstrated for the *dnaQ49* allele, the amount of mutator activity and mutational specificity is definitely medium-dependent with the *mutD5* allele (Degnen and Cox, 1974; Erlich and Cox, 1980; Schaaper, 1988). In minimal medium *mutD5* induces mainly AT--->TA transversions based upon DNA sequence analysis of *lacI* $^{-d}$ mutations (Schaaper, 1988).

The *dnaQ* -encoded protein is the epsilon subunit of DNA polymerase III which catalyzes the 3'--->5' exonuclease (proofreading) activity of the enzyme (Scheuermann et al., 1983; Scheuermann and Echols, 1984); *dnaQ49* and *mutD5* strains are defective in this activity (Echols et al., 1983; DiFrancesco et al., 1984). At the same time it has been pointed out by several investigators (Echols et al., 1983; Piechocki et al., 1986;

Schaaper, 1988) that *in vitro* data (Fersht et al., 1982) 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 probably results from the lack of proofreading, mutational levels of both *dnaQ49* and *mutD5* are orders of magnitude higher and suggest that functions in addition to proofreading may be altered in *dnaQ49* and *mutD5* strains. It has also been suggested that the *dnaQ49* and *mutD5* alleles may lead to misincorporation of nucleotides during replication (Echols et al., 1983; Piechocki et al., 1986). Although there are no published data to support this suggestion, it is a logical possibility since the alpha subunit of DNA polymerase III, which provides the polymerase activity, binds to epsilon in the core enzyme (Maki and Kornberg, 1987).

Schaaper (1988) has recently shown that methyl-directed postreplicative mismatch repair, reviewed by Radman and Wagner (1986) and Modrich (1987), is defective in *mutD5* cells grown in rich medium and may also be altered in a *dnaQ49* strain grown at 37°C (Schaaper, personal communication). 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. Consistent with this possibility is the observation that *dnaQ49mutL* double mutator strains, where the *mutL* mutation results in defective mismatch repair (Modrich, 1987), have mutation frequencies at 37°C similar to *dnaQ49* strains (Fowler, unpublished data).

Mutator activity of *dnaQ49* is dramatically reduced at 30°C compared to 37°C. However simply assuming that the activities impaired at 37°C

are partially and proportionally restored at 30°C does not explain the phenomenon because the mutational spectrum is decidedly altered. 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. This would explain the preferential lowering of transition frequencies at 30°C from values obtained at 37°C; transition mispairings are generally more often corrected through mismatch repair than transversion mispairings (Choy and Fowler, 1985; Leong *et al.*, 1986; Schaaper and Dunn, 1987). It is interesting to note that the transversion events that become significant in the *dnaQ49 trpA* reversion spectra at 30°C are the very ones that are seldom found among the *trpA* revertants in *mutL* strains (Choy and Fowler, 1985). Also consistent with this possibility, it has been found that *dnaQ49 mutL* double mutators have mutation frequencies at 30°C intermediate between *dnaQ49* strains at 37°C and at 30°C (Fowler, unpublished data).

To conclude, the mutator allele *dnaQ49* provides some insight into the mechanisms that contribute to the great degree of fidelity of replication seen in normal cell systems. Nevertheless, more study is needed to uncover how various gene products work together to avoid mutation.

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