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Isolation of Conditional Lethal Mutator Mutants of *Escherichia coli* by Localized Mutagenesis

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By using localized mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, we isolated 39 temperature-sensitive growth mutants that exhibited high mutability when the bacteria were grown at the permissive temperature. Two of the mutations, *dnaQ186* and *dnaQ231*, were shown to be new alleles of the *dnaQ* gene by genetic mapping and complementation tests with the *dnaQ49* mutation previously isolated. They shared common properties with the *dnaQ49* strain, but their mutator activity was not temperature dependent. The *dnaQ* mutants exhibited increased sensitivity to inhibitors of DNA gyrase and to DNA intercalating and alkylating agents.

Organisms appear to possess elaborate mechanisms to keep their mutation rate at certain low levels. To understand the mechanisms for the control of the spontaneous mutation rate of a cell, we have initiated genetic and biochemical studies on mutator genes of *Escherichia coli*. Recently, we isolated a conditional lethal mutator mutant, *dnaQ49*, that exhibits defective DNA synthesis at the restrictive temperature and a strong mutator activity at the permissive temperature (9). By in vitro recombination, the *dnaQ* gene has been cloned and its gene product identified (8). It has been shown that certain mutations in the *dnaE* gene, the structural gene for DNA polymerase III, also cause the conditional lethal mutator phenotype (7, 11, 16).

The *dnaQ49* mutant, the only *dnaQ* mutant thus far isolated, possesses peculiar properties. Its mutator activity is temperature dependent, and growth inhibition at high temperatures is partially suppressed by the addition of salts to the medium (9). To determine whether these are allele or gene specific and to obtain further insight into this interesting gene function, it was necessary to isolate more *dnaQ* mutants. This paper describes the isolation and genetic analyses of new *dnaQ* mutants.

MATERIALS AND METHODS

Bacteria, phages, and plasmids. The bacterial strains used in this study are derivatives of *E. coli* K-12 and are listed in Table 1. Plasmid pMM5 carries a 1.5-kilobase *E. coli* DNA fragment containing the *dnaQ* and *rnh* genes (8). pMM5::$\gamma\delta$ (*dnaQ*$^-$) carries the $\gamma\delta$ sequence in the midst of the *dnaQ* gene and does not complement the *dnaQ49* mutation (8).

Media and chemicals. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was purchased from Tokyo Kasei Kogyo Co. All media and other chemicals have been described previously (9).

Mutagenesis and isolation of mutants. Mutagenesis was accomplished with MNNG by the method of Cerda-Olmedo et al. (2). A culture (5 ml) of MK183 was grown to a cell density of about 3×10^8/ml, washed twice by centrifugation, and then resuspended in 4 ml of Eagle (E) medium. A newly prepared MNNG solution was added to the cell suspension to give a final concentration of 100 μg/ml, and the mixture was incubated at 37°C. Incubation was terminated at 40 min, at which time survival of the cells was about 50%. To select MetD$^+$ revertants, appropriate dilutions were spread on E medium plates supplemented with D-methionine, L-proline, and thymine (25 μg of each per ml), and the plates were incubated for 3 days at 30°C.

Detection of mutator activity. To determine quickly whether bacteria had mutator activity, the following semiquantitative method was used. Purified single colonies were picked with toothpicks and inoculated into 0.5-ml portions of Luria (L) broth. The cultures were incubated at 30°C for 24 h. A loopful of the cultures was patched on L broth plates containing rifampin (100 μg/ml), nalidixic acid (50 μg/ml), or streptomycin (100 μg/ml), and the plates were incubated at 30°C for 2 days. The mutator mutants we isolated usually produced 20 to several hundred Rif^r (rifampin-resistant) colonies, several to 20 Nal^r colonies, and 20 to 100 Str^r colonies on these plates, whereas the parent strain produced no colonies or only a few colonies on such plates. By using three kinds of antibiotic plates in each experiment, it was possible to distinguish mutator clones (Mut$^-$) from nonmutator clones (Mut$^+$).

Mutation frequency. Cells were grown in L broth at 30°C, and portions containing about 150 cells were transferred to fresh L broth. Triplicate cultures were grown to saturation at an appropriate temperature. After appropriate concentration or dilution, the bacteria were plated on L broth plates containing rifampin (100 μg/ml) or streptomycin sulfate (100 μg/ml). The plates were incubated at 30°C for 2 days, and colonies

TABLE 1. Bacterial strains used

Strain	Genotype[a]	Source
MK180	Hfr (Cavalli type Hfr) *metB1 proA3 metD88 lac-3 tsx76 relA1 sup126*	This paper
MK183	Same as MK180, except *thyA*	MK180, trimethoprim selection
MK184	Same as MK183, except *metD*$^+$ *dapD4*	This paper
MK186	Same as MK183, except *metD*$^+$ *dnaQ186*	MK183, mutagenized with MNNG
MK231	Same as MK183, except *metD*$^+$ *dnaQ231*	MK183, mutagenized with MNNG
KH1161	F$^-$ *metB1 metD88 proA3 thr leu his phi rpsL ara xyl galK2 lac supE mtl-1*	This paper
MK304	Same as KH1161, except *metD*$^+$ *leu*$^+$ *proA*$^+$ *dnaQ186*	MetD$^+$ Leu$^+$ Pro$^+$ Str^r recombinant from MK186 × KH1161
MK317	Same as MK184, except *proA*$^+$ *dnaQ186*	P1(MK304)→MK184, Pro$^+$ selection
MK345	Same as MK183, except *metD*$^+$ *proA*$^+$ *dnaQ186*	P1(MK317)→MK183, MetD$^+$ selection
MK352	Same as MK183, except *metD*$^+$ *dnaQ186*	P1(MK345)→MK183, MetD$^+$ selection
MK248	Same as MK183, except *proA*$^+$ *val*^{r}	This paper
MK297	Same as MK248, except *metD*$^+$ *dnaQ231*	P1(MK231)→MK248, MetD$^+$ selection
MK344	Same as MK180, except *proA*$^+$ *dnaQ231*	P1(MK297)→MK180, MetD$^+$ selection
MK363	Same as MK183, except *metD*$^+$ *dnaQ231*	P1(MK297)→MK183, MetD$^+$ selection
KH1113	Same as MK180, except *sup*$^+$ *metD*$^+$	T. Horiuchi et al. (9)
KH1116	Same as MK180, except *sup*$^+$ *metD*$^+$ *dnaQ49*	T. Horiuchi et al. (9)
KH1145	Same as KH1116, except *dnaQ*$^+$	Spontaneous temperature-resistant revertant of KH1116
KH1171	F$^-$ *thr leu tonA21 proA supE rpsL xyl mtl recA1*	This paper
KH1172	Same as KH1171, except *dnaQ49*	This paper
MK364	Same as KH1171, except *dnaQ186*	This paper
MK366	Same as KH1171, except *dnaQ231*	This paper
CSH62	HfrH *thi*	J. H. Miller (14)
KH1228	HfrH *metB1 metD88 dnaQ49 lac-3 val*^{r}	This paper

[a] For gene symbols, see Bachmann and Low (1). Relevant strains used in the present experiments are listed, but most derivatives made during strain construction are omitted.

formed were scored as resistant mutants. The number of viable cells was determined by plating portions of the cultures on L broth plates and incubating at 30°C for 1 day. The mutation frequency was expressed as the number of mutants per total number of viable cells.

Sensitivity to chemicals. A gradient plate was prepared according to the method of Szybalski and Bryson (18) with a square dish (Niplon Products Ltd.; no. 106; 138 by 98 by 13 mm) with two layers of agar. The lower layer consisted of 50 ml of L broth agar and was allowed to harden with the plate slanted just sufficiently to cover the entire bottom. After placing the dish in the normal horizontal position, another 50 ml of agar containing a certain concentration of chemical was added. During subsequent incubation, the downward diffusion of drug results in the dilution being proportional to the thickness ratio of agar layers, establishing a uniform concentration gradient. Overnight cultures grown in L broth at 30°C were diluted 100-fold and streaked on the gradient plate. The plate was incubated at an appropriate temperature for 24 h.

Other methods. Transduction with P1*vir* and bacterial matings were done as described by Miller (14). F′ factors carrying *dnaQ*$^+$ or *dnaQ49* were constructed according to the method of Low (12). To prevent segregation of the F′ factor, minimal medium E was used for the growth of these F′ strains. DNA transformation was performed according to the method described by Cohen et al. (3), except that the heat shock step was omitted (15).

RESULTS

Isolation of *metD*-linked conditional lethal mutator mutants. The *dnaQ* gene has been mapped between the *metD* and *proA* genes on the *E. coli* linkage map (1, 9). Since it is known that the mutagen MNNG often induces multiple, closely linked mutations (2), we can expect that some MetD$^+$ revertants from MNNG-treated cultures of the *metD*$^-$ strain would have mutations in the *dnaQ* and/or neighboring genes.

E. coli MK183 *metD*$^-$ was treated with MNNG, and MetD$^+$ revertants were isolated. With the MNNG treatment, the reversion frequency to MetD$^+$ increased about 200-fold. Among 2,050 MetD$^+$ revertants examined, 922 clones exhibited the temperature-sensitive growth characteristic; they were unable to grow at 44.5°C on a salt-free L broth plate. These clones were purified and tested for high mutability to Rif^r, Nal^r, and Str^r at 30°C by using the

TABLE 2. Genetic mapping of *dnaQ186* and *dnaQ231* by P1 transduction

Donor	Recipient	Selected marker	Genotype of transductants[a]			No. of transductants (%)
			metD	*dnaQ*	*proA*	
MK304 (*dnaQ186*)	MK180	Pro+	0	0	1	44 (88.0)
			0	1	1	2 (4.0)
			1	1	1	3 (6.0)
			1	0	1	1 (2.0)
MK317 (*dnaQ186*)	MK183	MetD+	1	0	0	49 (62.8)
			1	1	0	15 (19.2)
			1	1	1	14 (18.0)
			1	0	1	0 (0.0)
MK297 (*dnaQ231*)	MK184	Pro+	0	0	1	41 (82.0)
			0	1	1	9 (18.0)
MK297 (*dnaQ231*)	MK180	MetD+	1	0	0	77 (79.4)
			1	1	0	16 (16.5)
			1	1	1	4 (4.1)
			1	0	1	0 (0.0)

[a] 0, Allele of recipient; 1, allele of donor.

semiquantitative test. As the result, we obtained 39 conditional lethal mutator mutants.

The newly isolated mutator strains were examined to determine whether their mutator mutations are linked to *metD*. In Hfr crosses and P1 transduction experiments, two mutants, MK186 and MK231, exhibited a close association of the Mut$^-$ character with the MetD$^+$ phenotype, suggesting that their mutations may be closely linked to the *metD* gene. These two mutants were further analyzed.

Genetic analyses of mutants. Since four mutator genes (*mutT*, *dnaE*, *mutD*, and *dnaQ*) are known to be located near the *metD* gene on the *E. coli* linkage map (1, 4), more detailed genetic mapping of the newly isolated mutations was performed. Table 2 shows the results of three-factor crosses achieved by P1 transduction. These results suggested that both mutations of strains MK186 and MK231, designated *dnaQ186* and *dnaQ231*, respectively, were located between *metD* and *proA*. Figure 1 summarizes the order of genes and the approximate distance between the genes, computed from the data in Table 2.

It must be emphasized that the two phenotypes, high mutability and conditional lethality, have never been separated in these crosses, suggesting that both *dnaQ186* and *dnaQ231* are single mutations causing the pleiotropy. This

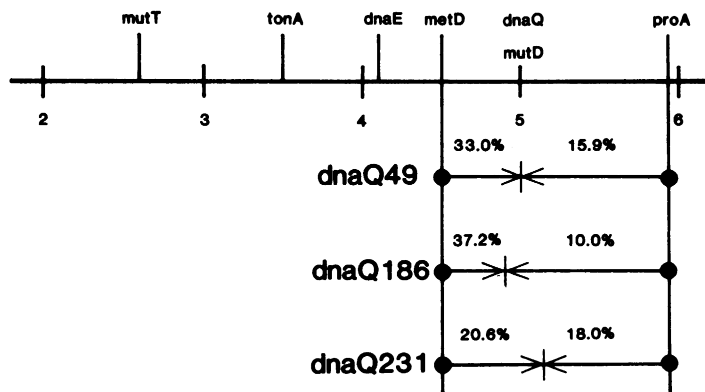

FIG. 1. Location of *dnaQ* mutations on the *E. coli* linkage map by P1 transduction. Closed circles and arrows represent selective and nonselective markers, respectively. Percentages between genes indicate cotransduction frequencies of the two markers shown in Table 2 and by Horiuchi et al. (9). The position of each *dnaQ* mutation was computed from the ratio of cotransduction frequency with the selective markers indicated on the right and left side of each allele.

TABLE 3. Mutation frequency of temperature-resistant revertants of MK352 *dnaQ186* and MK297 *dnaQ231*

Strain	Rifampin resistant		Streptomycin resistant	
	Mutation frequency[a]	Relative value[b]	Mutation frequency	Relative value
MK183 *dnaQ*$^+$	1.6×10^{-8}	1.0	3.6×10^{-10}	1.0
MK352 *dnaQ186*	8.6×10^{-5}	5,500	1.1×10^{-5}	30,000
MK352 R-1	1.8×10^{-8}	1.1	2.6×10^{-9}	7.2
MK352 R-2	5.2×10^{-8}	3.3	3.5×10^{-10}	1.0
MK297 *dnaQ231*	2.9×10^{-5}	1,800	6.3×10^{-6}	18,000
MK297 R-1	8.6×10^{-8}	5.4	4.6×10^{-10}	1.4
MK297 R-2	4.3×10^{-8}	2.7	4.6×10^{-11}	0.1

[a] Number of mutants per total number of viable cells.
[b] Mutation frequency of each strain was divided by that of MK183.

was confirmed by other experiments in which temperature-resistant revertants of *dnaQ186* and *dnaQ231* mutants were obtained and their mutator phenotype was examined. Samples of cultures of MK352 and MK297 were plated on salt-free L broth plates. After incubation at 44.5°C overnight, normal-sized colonies of both strains, which appeared at frequencies of 10^{-6} to 10^{-7}, were picked as spontaneous revertants. As shown in Table 3, all the temperature-resistant revertants (MK352 R-1, MK352 R-2, MK297 R-1, MK297 R-2) examined exhibited low mutator activity.

To obtain further genetic evidence to show that *dnaQ186* and *dnaQ231* are alleles of the *dnaQ* gene, complementation tests were performed (Table 4). Although some mutability remained in *dnaQ186*/F′ *dnaQ*$^+$ and *dnaQ231*/F′ *dnaQ*$^+$ merodiploids, most of mutator activity of *dnaQ186* and *dnaQ231* was suppressed by F′ *dnaQ*$^+$. Thus, *dnaQ186* and *dnaQ231* mutations are recessive, as is *dnaQ49* (8). When F factor carrying *dnaQ49* was introduced into strain MK364 *dnaQ186* and MK366 *dnaQ231*, the mutator activity of neither strain was suppressed. It was observed that the mutator activity of *dnaQ231* and *dnaQ186* was rather enhanced by the introduction of F′ *dnaQ49*. From these results, it is evident that *dnaQ49*, *dnaQ186*, and *dnaQ231* are alleles of the same gene.

This was confirmed by the finding that the mutator phenotype of the *dnaQ231* strain was completely suppressed by the *dnaQ*$^+$ plasmid, pMM5, which carries only the *dnaQ* and *rnh* genes (8), but not by pMM5::$\gamma\delta$ (dnaQ$^-$) (Table 5). The presence of pMM5 but not pMM5::$\gamma\delta$ (dnaQ$^-$) conferred the ability to grow at high temperature (44.5°C) on strain MK366. This supports the view that the mutator and temperature-sensitive growth characteristics are due to mutation in the same gene.

Effect of temperature on growth and mutator activity of *dnaQ* strains. We examined the effect of temperature on the colony-forming ability of *dnaQ186* and *dnaQ231* mutants. These two mutants produced a normal number of colonies at temperatures below 42°C, but a greatly reduced number of colonies at 44.5°C on salt-free L broth plates. Growth inhibition at the high temperature was suppressed by the addition of 85 mM

TABLE 4. Complementation test of *dnaQ* mutations by F′ *dnaQ49* factor[a]

Strain	Genotype	Rifampin resistant[b]	
		Mutation frequency	Relative value
KH1171	*dnaQ*$^+$	7.1×10^{-9}	1.0
KH1172	*dnaQ49*	3.6×10^{-6}	507
MK364	*dnaQ186*	2.9×10^{-6}	408
MK364 (F′ *dnaQ*$^+$)	*dnaQ186*/*dnaQ*$^+$	1.2×10^{-7}	16.9
MK364 (F′ *dnaQ49*)	*dnaQ186*/*dnaQ49*	2.0×10^{-5}	2,816
MK366	*dnaQ231*	2.5×10^{-6}	352
MK366 (F′ *dnaQ*$^+$)	*dnaQ231*/*dnaQ*$^+$	3.7×10^{-8}	5.2
MK366 (F′ *dnaQ49*)	*dnaQ231*/*dnaQ49*	9.0×10^{-5}	12,676

[a] F′ *dnaQ* and F′ *dnaQ49* were introduced to recombination-deficient female strains by mating with HfrH type strains CSH62 or KH1228, respectively, and Thr$^+$ Leu$^+$ Pro$^+$ Str^r bacteria were selected.
[b] For mutation frequency and relative value, see footnotes *a* and *b* of Table 3.

TABLE 5. Complementation test of *dnaQ231* with multicopy *dnaQ*$^+$ plasmids[a]

Strain	Genotype		Rifampin resistant[b]	
	Chromosome	Plasmid	Mutation frequency	Relative value
KH1171	*dnaQ*$^+$	None	2.4×10^{-8}	1.0
MK366	*dnaQ231*	None	3.4×10^{-5}	1,417
MK366(pMM5)	*dnaQ231*	*dnaQ*$^+$	2.9×10^{-8}	1.2
MK366(pMM5::γδ[*dnaQ*$^-$])	*dnaQ231*	*dnaQ*$^-$	4.2×10^{-5}	1,750

[a] The procedure was the same as that described in the text, except that overnight cultures were grown at 30°C in L broth containing ampicillin (50 μg/ml) and tetracycline (35 μg/ml), and the secondary cultures were grown at 30°C in the same medium.

[b] For mutation frequency and relative value, see footnotes *a* and *b* of Table 3.

NaCl to the growth medium, as previously found in some temperature-sensitive growth mutants (M. Richard and Y. Hirota, Ann. Microbiol. [Paris] **124A**:29, 1973). As observed with the *dnaQ49* mutant (10), DNA synthesis, but not protein synthesis, of the *dnaQ186* and *dnaQ231* mutants was suppressed under the restrictive temperature (data not shown).

It has been shown that the mutator activity of the *dnaQ49* mutant is temperature dependent (8). Therefore, we examined the effect of temperature on the frequency of rifampin- and streptomycin-resistant mutations (Fig. 2). The mutator activity of the *dnaQ49* mutant was enhanced with increasing incubation temperature, confirming the previous result. The mutator activity of the *dnaQ186* and *dnaQ231* mutants did not show such a striking temperature dependence; they exhibited a considerably high mutator activity even at 25°C.

Sensitivity to chemicals. In the course of studies on the mutator activity of mutant cells, we noticed that the *dnaQ49* mutant is unusually sensitive to nalidixic acid; the background growth of the *dnaQ* mutant on nalidixic acid-containing plates was rather thin as compared with that of wild-type strain. This unusual sensitivity to nalidixic acid was checked by the gradient plate method. Figure 3 shows that the *dnaQ* mutants were more sensitive to nalidixic acid than their isogenic wild-type strain, Mk183, and their temperature-resistant revertants.

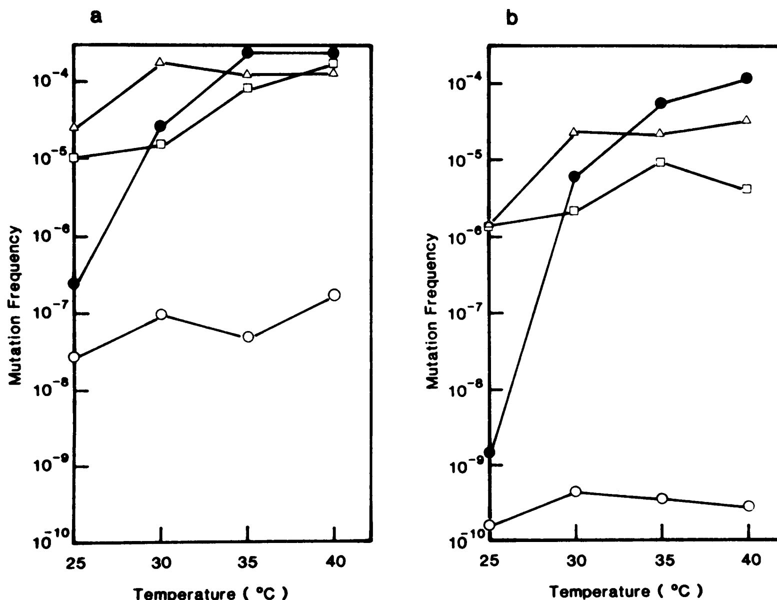

FIG. 2. Effect of temperature on mutator activity of the *dnaQ* mutants. (a) Frequency of spontaneous mutation to rifampin resistance. (b) Frequency of spontaneous mutation to streptomycin resistance. The procedure is described in the text. Symbols: ○, MK183 *dnaQ*$^+$; □, MK352 *dnaQ186*; △, MK344 *dnaQ231*; ●, KH1116 *dnaQ49*.

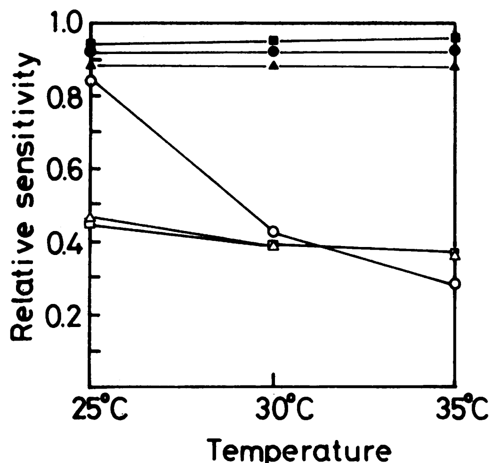

FIG. 3. Effect of temperature on the sensitivity to nalidixic acid of *dnaQ* mutants. Bacteria were streaked on gradient plates containing nalidixic acid (0 to 4.0 μg/ml), and the plates were incubated at the temperatures indicated. Other procedures were as described in the text. Relative sensitivity was expressed by dividing the length of growth zone of each mutant by that of the corresponding isogenic wild-type strain. Symbols: ○, KH1116 *dnaQ49*; △, MK352 *dnaQ186*; □, MK344 *dnaQ231*; ●, KH1145 (temperature-resistant revertant of KH1116); ▲, MK352 R-1; ■, MK344 R-1.

One interesting feature that emerged from this experiment is that the sensitivity of the *dnaQ49* mutant is temperature dependent. The *dnaQ49* strain showed nearly the same sensitivity as the wild-type strain at 25°C but was as sensitive as other *dnaQ* strains at 35°C. No such temperature dependence was observed with the *dnaQ186* and *dnaQ231* strains. Thus, two seemingly unrelated phenotypes, increased sensitivity to nalidixic acid and mutator activity, may be caused by the same mechanism.

The sensitivity of the *dnaQ* mutant to other chemicals was investigated next. As shown in Table 6, the *dnaQ49* strain exhibited increased sensitivity to several groups of chemicals that interact with DNA or inhibit DNA synthesis (5). These included inhibitors of DNA gyrase (novobiocin, coumermycin, and nalidixic acid) (6, 13, 17), DNA intercalating agents (acridine dyes and basic dyes), and alkylating agents (methyl methane sulfonate and mitomycin C). On the other hand, the mutant exhibited no increased sensitivity to chemicals that inhibit RNA synthesis (rifampin), protein synthesis (chloramphenicol and streptomycin), and cell wall biosynthesis (ampicillin). It is unlikely, therefore, that the hypersensitivity of *dnaQ* mutants to nalidixic acid and other inhibitors of DNA synthesis is due to the general increase in permeability of the cell.

DISCUSSION

We isolated 39 conditional lethal mutator mutants by using MNNG localized mutagenesis. Two of these mutations, *dnaQ186* and *dnaQ231*, were closely linked to the *metD* gene. They seem to be new alleles of the *dnaQ* gene according to the following genetic criteria (i) they were mapped at the same position as the *dnaQ49* mutation, and (ii) they were suppressed by the *dnaQ*+ but not by the *dnaQ49* allele.

The *dnaQ186* and *dnaQ231* mutants shared common features with the *dnaQ49* mutant, whose properties have been studied previously (8–10). They were unable to produce colonies at 44.5°C on NaCl-free L broth plates and exhibited strong mutator activity under permissive conditions. They also showed a decreased rate of DNA synthesis under restrictive conditions and enhancement of thermosensitivity of *dnaE* (Ts) mutants (10) and were more sensitive to DNA intercalating reagents or inhibitors of DNA gyrase as compared with wild-type cells. These properties of the mutants suggested that the *dnaQ* gene product might be a component of the cellular DNA synthesizing machinery or a factor stabilizing the chromosome structure and that its

TABLE 6. Sensitivity of *dnaQ49* strain to various chemicals[a]

Chemical	Concentration	Length of growth zone (cm)		
		dnaQ+	*dnaQ49*	Ratio
Nalidixic acid	0–4.0 μg/ml	4.9	2.5	0.51
Novobiocin	0–2.0 mg/ml	2.5	1.5	0.60
Coumermycin	0–40 μg/ml	9.8	3.2	0.32
5-Aminoacridine	0–60 μg/ml	4.8	2.0	0.42
Acriflavin	0–1.0 mg/ml	3.5	1.7	0.49
Acridine orange	0–1.0 mg/ml	5.6	3.4	0.61
Crystal violet	0–200 μg/ml	2.8	1.0	0.36
Methyl green	0–5.0 mg/ml	8.2	4.5	0.55
Ethidium bromide	0–1.0 mg/ml	4.7	2.2	0.47
Methyl methane sulfonate	0–8.0 mM	4.5	2.8	0.62
Mitomycin C	0–100 mg/ml	4.0	2.5	0.62
Rifampin	0–20 μg/ml	5.1	4.8	0.94
Chloramphenicol	0–10 μg/ml	3.2	3.2	1.0
Streptomycin	0–5.0 μg/ml	3.1	3.1	1.0
Ampicillin	0–10 μg/ml	3.3	3.3	1.0

[a] Bacteria were streaked on gradient plates containing the chemicals, and the plates were incubated at 35°C for 24 h. The length of growth zone of KH1113 *dnaQ*+ and KH1116 *dnaQ49* were measured, and their ratio (*dnaQ49*/*dnaQ*+) was calculated. The length of the whole range of streak was 9.8 cm.

defectiveness may cause a decrease in the fidelity of DNA replication.

There is a difference in the temperature dependence of mutator activity of various *dnaQ* strains. The mutator activity of the *dnaQ49* strain was as low at 25°C as that of the wild-type strain and increased with increasing temperature. On the other hand, mutator activity of the *dnaQ186* and *dnaQ231* strains was constantly high over a wide range of temperatures. Such a difference was observed in the temperature dependence of the unusual sensitivity to nalidixic acid. These differences might reflect the degree of thermosensitivity of the *dnaQ* protein in these mutant cells. It can be supposed that the *dnaQ49* protein may function almost normally at low temperature, whereas the *dnaQ186* and *dnaQ231* proteins may be partially inactive even at low temperature. Despite these differences, these *dnaQ* mutants exhibited growth inhibition at nearly the same high temperature. This implied that the high mutability is not the direct cause of growth inhibition.

All the *dnaQ* mutants thus far isolated exhibited their growth inhibition only under certain restricted conditions: in salt-free medium at 44.5°C. It is possible that even the mutant form of *dnaQ* protein might undergo a conformational change that leads to complete loss of activity. However, it is still possible that the *dnaQ* protein may not be essential for DNA replication at normal growth temperature but may be required under very severe conditions. To distinguish these possibilities, it may be worthwhile to isolate amber *dnaQ* mutants. Such attempts are now under way in this laboratory.

In the course of complementation studies, we found that the mutation frequencies of *dnaQ186*/F′ *dnaQ49* and *dnaQ231*/F′ *dnaQ49* merodiploids were 10- to 100-fold higher than that of each single mutant, *dnaQ186*, *dnaQ231*, or *dnaQ49*. This phenomenon implies that the *dnaQ* gene product might carry out its function in a multimeric form rather than in a monomeric form. Coexistence of different types of mutant proteins might enhance the effect of each defectiveness.

The other temperature-sensitive mutants isolated in the present studies also possessed strong mutator activity in addition to the temperature-sensitive growth characteristic. These mutations were not linked to the *metD* gene, suggesting that they are not *dnaQ* or *dnaE* mutants. Since no other *dna* mutant with such a phenotype is known, it is possible that they may include some new "replicative" mutator mutants. Further analyses of this type of mutant should be profitable.

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