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Isolation and Characterization of Mutants with Deletions in *dnaQ*, the Gene for the Editing Subunit of DNA Polymerase III in *Salmonella typhimurium*

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***dnaQ* (*mutD*) encodes the editing exonuclease subunit (ε) of DNA polymerase III. Previously described mutations in *dnaQ* include dominant and recessive mutator alleles as well as leaky temperature-sensitive alleles. We describe the properties of strains bearing null mutations (deletion-substitution alleles) of this gene. Null mutants exhibited a growth defect as well as elevated spontaneous mutation. As a consequence of the poor growth of *dnaQ* mutants and their high mutation rate, these strains were replaced within single colonies by derivatives carrying an extragenic suppressor mutation that compensated the growth defect but apparently not the mutator effect. Sixteen independently derived suppressors mapped in the vicinity of *dnaE*, the gene for the polymerization subunit (α) of DNA polymerase III, and one suppressor that was sequenced encoded an altered α polypeptide. Partially purified DNA polymerase III containing this altered α subunit was active in polymerization assays. In addition to their dependence on a suppressor mutation affecting α, *dnaQ* mutants strictly required DNA polymerase I for viability. We argue from these data that in the absence of ε, DNA replication falters unless secondary mechanisms, including genetically coded alteration in the intrinsic replication capacity of α and increased use of DNA polymerase I, come into play. Thus, ε plays a role in DNA replication distinct from its known role in controlling spontaneous mutation frequency.**

The ε subunit of DNA polymerase III is a fidelity factor for DNA replication by virtue of its ability to catalyze excision of erroneously inserted nucleotides at the growing point of a new chain (32, 33). The antimutagenic effect of ε is not essential for cell viability, since *Escherichia coli* readily tolerates a wide range of spontaneous mutation rates. Indeed, the *mutD5* mutation of *dnaQ* increases the spontaneous mutation frequency at some loci as much as 10^5-fold without causing general lethality (7). None of the *dnaQ* mutations described in the literature is an unambiguous null, however, and for this reason the cellular requirement for ε for viability has remained an open question.

In this paper, we report the construction and properties of null mutations in *dnaQ*. We first constructed defined mutations (deletion substitutions) in cloned *dnaQ* and then used these constructs to replace chromosomal *dnaQ*. Our main finding was that loss of *dnaQ* caused a severe growth defect, but this phenotype was well suppressed by a class of mutations in *dnaE*, the gene for the polymerization subunit (α) of DNA polymerase III (42). Survival in the absence of ε required DNA polymerase I in addition to the genetically altered DNA polymerase III. Our evidence also suggested that the suppressor mutations were specific for the growth phenotype of Δ*dnaQ* and not for its mutator phenotype. On the basis of these and other observations, we argue that *dnaQ* normally plays an important if not essential role in promoting successful DNA replication and that this role is formally separable from the nonessential role of *dnaQ* in promoting faithful DNA replication.

MATERIALS AND METHODS

Abbreviations. Abbreviations used are as follows: Tn*10*dTc, Tn*10*dKm, and Tn*10*dCm, derivatives of Tn*10* conferring resistance to tetracycline, kanamycin, and chloramphenicol, respectively; EGTA, ethyleneglycol-bis(β-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetic acid; bp, base pairs; SDS, sodium dodecyl sulfate; kDa, kilodaltons; Tc^r and Tc^s, tetracycline resistance and sensitivity; Km^r and Cm^r, kanamycin and chloramphenicol resistance.

Bacterial strains and media. Bacterial strains used are described in Table 1. Generalized transduction was carried out by using bacteriophage P22 *int* HT12/4 (34). General bacteriological media and procedures were as described previously (23). Drugs at the following concentrations (micrograms per milliliter) were added to plates as needed: tetracycline hydrochloride, 25; kanamycin, 50; chloramphenicol, 20; rifampin, 100; and nalidixate, 50. Tetracycline plates contained, in addition, 10 mM EGTA to prevent reinfection of transductants by P22.

***dnaQ* constructs.** Plasmids and λ phages carrying mutated *dnaQ* genes of *Salmonella typhimurium* may be considered derivatives of pFF16 (Fig. 1) and λRM354 (23), respectively. Insertions of Tn*10*dTc in λRM354 were isolated as described previously (23); among these, insertions specifically in *dnaQ* were identified by a red-plaque test (23). As judged by restriction enzyme analysis (not shown), the most widely separated Tn*10*dTc insertions in *dnaQ* having the same orientation differed by about 300 bp in their sites of insertion; these insertions were found in λRM81 and λRM164. To create the *dnaQ200* allele in which Tn*10*dTc exactly replaced these ~300 bp, a fragment from λRM81 containing one end of *dnaQ* and the adjacent portion of Tn*10*dTc was joined to a fragment from λRM164 containing the rest of Tn*10*dTc and the other end of *dna*Q, all in plasmid pUC8 (39). Neither fragment contained the ~300-bp region between the two sites of insertion. In effect, an unequal crossover between the two transposons was produced; this was plasmid pFF16.

Further manipulations of pFF16 enlarging the extent of

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TABLE 1. *S. typhimurium* strains used

Strain	Genotype or description[a]	Source or reference[b]
RM10 (=DB9005)	*thyA deo*	23
RM97	RM311[c] *zag-1256*::Tn*10*dKm[d]	This laboratory
RM238	RM10 *bio-101*::Tn*10 hisC527*(Am)	This laboratory[e]
RM311 (=DB4673=TS736)	λ-sensitive strain	23, 29
RM708 to -717, RM816, and RM812 to -815	RM97 *dnaQ200*::Tn*10*dTc carrying *spq-2* through *spq-16*, respectively	Tc^r survivors of infection of RM97 with λRM612[f]
RM1655, -821, -824, -827, -830, -833, -836, -839, -842, -845, -1657, -848, -1659, -1661, -1663, and -1665	RM10 *zag-1256*::Tn*10*dKm carrying *spq-1* through *spq-16*, respectively	Transduction of RM10 to Km^r by using P22 grown on RM708, etc.
RM745	RM311 *zag-1256*::Tn*10*dKm	RM311 + P22(RM708)
RM746	RM311 *zag-1256*::Tn*10*dKm *spq-2*	RM311 + P22(RM708)
RM749	RM311 *zag-1256*::Tn*10*dKm *spq-3*	RM311 + P22(RM709)
RM820	RM10 *zag-1256*::Tn*10*dKm (*dnaE*$^+$)	RM10 + P22(RM708)
RM822	RM821 *dnaQ200*::Tn*10*dTc	RM821 + P22(RM708)
RM976	RM745 *dnaQ201*::Tn*10*dTc	Transduction of RM745 to Tc^r with λRM780
RM978	RM746 *dnaQ201*::Tn*10*dTc	Transduction of RM746 to Tc^r with λRM780
RM1674	*leu-485 polA2 zie-3024*::Tn*10*dTc	C. Miller (TN2847); 15
RM1763	*leu-485 polA*$^+$ *zie-1260*::Tn*10*dCm (Tc^s)	Transductant of RM1674 obtained from a pool of random Tn*10*dCm insertions[g]
RM1778	*leu-485 polA2 zie-1260*::Tn*10*dCm (Tc^s)	RM1674 + P22(RM1763)
RM1835	RM10 *polA2 zie-1260*::Tn*10*dCm	RM10 + P22(RM1778)
RM1836	RM821 *polA2 zie-1260*::Tn*10*dCm	RM821 + P22(RM1778)
RM1837	RM824 *polA2 zie-1260*::Tn*10*dCm	RM824 + P22(RM1778)
RM1838	RM833 *polA2 zie-1260*::Tn*10*dCm	RM833 + P22(RM1778)
RM2685	RM10 *polA*$^+$ *zie-1260*::Tn*10*dCm	RM10 + P22(RM1778)
RM2686	RM821 *polA*$^+$ *zie-1260*::Tn*10*dCm	RM821 + P22(RM1778)
RM2687	RM824 *polA*$^+$ *zie-1260*::Tn*10*dCm	RM824 + P22(RM1778)
RM2688	RM833 *polA*$^+$ *zie-1260*::Tn*10*dCm	RM833 + P22(RM1778)

[a] All strains are F$^-$ (see reference 23 for comments on the sex of RM311 and its derivatives).
[b] Generalized transductions are indicated as recipient + P22(donor). Selection was always for a drug marker: Tc^r for *dnaQ* alleles, Km^r for *spq* alleles, and Cm^r for *polA*. Unselected markers were scored as indicated in Materials and Methods for *spq* and by sensitivity to methyl methanesulfonate for *polA2*.
[c] All markers of the strain are included in the genotype.
[d] *zag-1256* is an insertion in a nonessential site near *dnaE*.
[e] *bio101*::Tn*10* was obtained from J. Roth in strain TT401.
[f] The *spq-1* strain of this series was not kept.
[g] *zie-1260* is approximately 4% cotransducible with *polA*.

DNA replaced by Tn*10*dTc are illustrated in Fig. 1. Plasmid pFF73 was derived from pFF16 by filling in the *Hin*dIII site near the 5′ end of *dnaQ* with the Klenow fragment of DNA polymerase I. Plasmid pFF73 was linearized by cutting within Tn*10*dTc with *Xba*I. The linearized DNA was digested to various extents with *Bal* 31 to produce a series of deletion endpoints within *dnaQ* from digestion at one end and deletion endpoints within Tn*10*dTc from digestion at the other end. The ends were ligated to *Hin*dIII linkers (duplex DNA having the sequence 5′-GCAAGCTTGC on both strands; Bethesda Research Laboratories, Inc.), and the DNA was then digested with *Hin*dIII. This procedure had the effect of reducing all of the deletion endpoints on one side to the *Hin*dIII site near the end of Tn*10*dTc. The digested DNA was circularized to yield plasmids pFF113 to pFF124, which conferred resistance to ampicillin but not to tetracycline. In the final step, the intact Tn*10*dTc was restored by inserting, in the *Hin*dIII site of these plasmids, a *Hin*dIII fragment obtained from λRM637, a *dnaC* clone containing an insertion of Tn*10*dTc.

In plasmids pFF113 to pFF124 (Fig. 1), a unique portion of Tn*10* abutted different parts of *dnaQ*. Using an oligonucleotide primer corresponding to the Tn*10* sequence (5′-GATC CAAGAGAACCAAC; nucleotides 1550 to 1534 as numbered in reference 35), we sequenced the adjoining *dnaQ* segments. The information obtained from this sequence (Fig. 2) was sufficient to establish several points. First, the target of the transposon insertions was the authentic *dnaQ* gene, as judged by its extensive homology to *E. coli dnaQ*. Second, the ~300-bp deletion in *dnaQ200* affected the 3′ portion of *dnaQ*. Third, the more extensive deletions (*dnaQ201* to *dnaQ206*) removed additional portions of *dnaQ* toward the 5′ end of the gene. The sequence information shown in Fig. 2 will appear in the EMBL, GenBank, and DDBJ nucleotide sequence data bases under the accession number M26045.

Each mutant *dnaQ* allele formed in a plasmid was transferred to λRM354 by homologous recombination. This was accomplished by propagating λRM354 through an *E. coli* strain harboring the particular plasmid and selecting a Tc^r λ recombinant (23). The expected physical map of each recombinant was verified by restriction enzyme analysis.

Formation of chromosomal *dnaQ*::Tn*10* strains. The first chromosomal *dnaQ*::Tn*10* strains were made by infecting a λ-sensitive *Salmonella* strain with λ*dnaQ*::Tn*10*dTc and selecting for Tc^r survivors (23). For the experiments reported in this paper, we used λ*dnaQ* derivatives in which Tn*10*dTc replaced part of *dnaQ*, in contrast to the simple Tn*10*dTc insertion used earlier. We shall use the generic designation *dnaQ*::Tn*10* to refer to both insertion and substitution alleles. Regardless of the exact *dnaQ* allele used, introduction of *dnaQ*::Tn*10* into the bacterial chromosome always resulted in the isolation of survivors bearing a suppressor mutation linked to *dnaE* (see next paragraph and Results). In subsequent steps, the *dnaQ*::Tn*10* allele was moved to other strains by P22-mediated transduction, again with selection for Tc^r. *dnaQ*::Tn*10* strains with a known suppressor mutation were constructed in two steps. First, the suppressor mutation was moved into a *dnaQ*$^+$ strain by

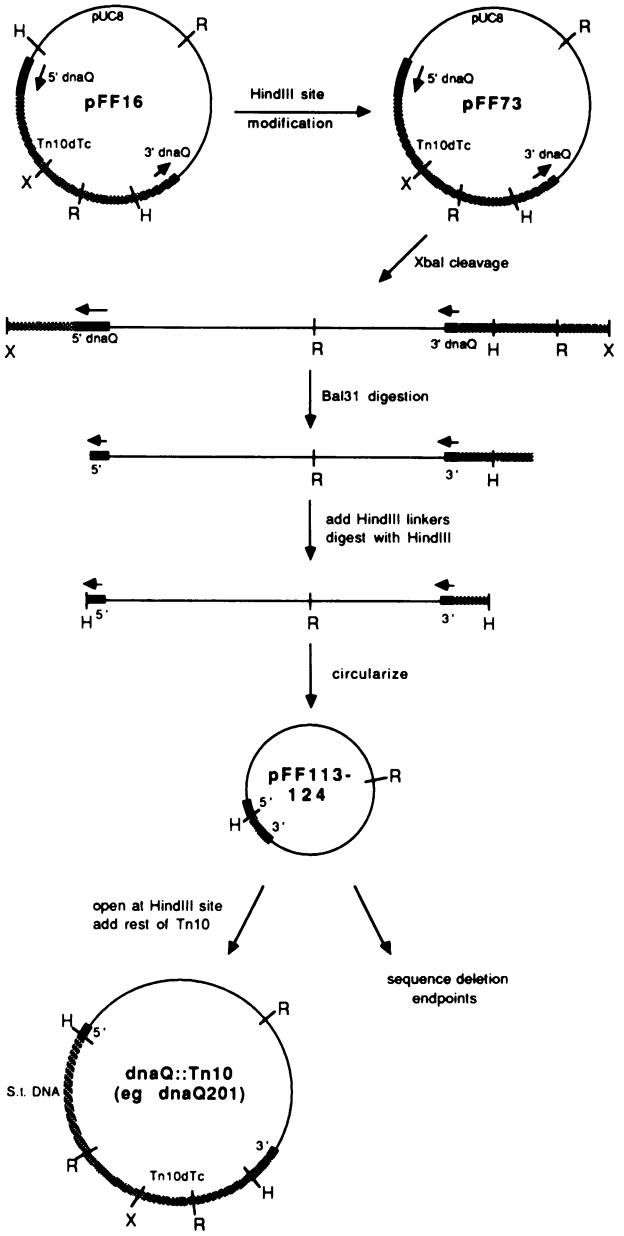

FIG. 1. *dnaQ* constructs (see text).

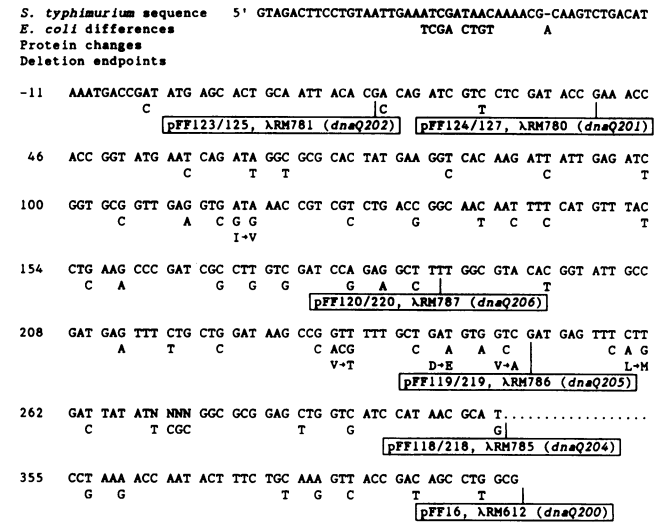

FIG. 2. Partial *dnaQ* sequence and deletion endpoints. The main sequence was obtained from a series of *Bal* 31 deletions derived from plasmid pFF16 (see Materials and Methods and Fig. 1). Except for a one-base deletion in the 5'-flanking region (hyphen at −24), the sequence aligns precisely with that of *dnaQ* from *E. coli* (6); differences at the nucleic acid and protein levels are shown. Numbering is with reference to the beginning of the protein-coding sequence. A few bases that could not be read because of a compression artifact are shown as N; a region corresponding to 53 bases in the *E. coli* sequence that was not read because of its distance from the nearest deletion endpoint is indicated (....). The base shown as each *Bal* 31 deletion endpoint is the terminal deleted base. The plasmids and λRM354 derivatives in which each deletion can be found, as well as the *dnaQ* allele number, are boxed. Additional deletions not shown ended in Tn*10*dTc itself. The largest such deletion removed all but 218 bp from the end of the transposon. Sequencing on this template allowed unambiguous determination of the position of transposon interruption of *dnaQ* in pFF16 (*dnaQ200*). Since *Salmonella* ε is similar in size to *E. coli* ε on Western blots (not shown) and the latter is 243 amino acid residues in length, it follows that *dnaQ200* eliminates the C-terminal half (approximately) of ε.

P22 transduction, using selection for a linked Km^r marker (see below for scoring of the suppressor genotype). In the second step, a *dnaQ*::Tn*10* allele was introduced by an additional round of transduction.

Mapping of suppressor genes. Mapping of suppressor genes was facilitated by a correct hunch that *dnaE* was the suppressor locus. The suppressors characterized in this study were isolated in strain RM97, in which the vicinity of *dnaE* was marked by a transposon insertion conferring Km^r. Each of the immortal survivors of *dnaQ200*::Tn*10*dTc insertion of RM97, carrying a presumptive suppressor, was used to propagate phage P22. These lysates were used to transduce strain RM10 to Km^r. Ten colonies from each transduction, in most cases, were scored for the suppressor genotype by judging colony appearance after transduction to Tc^r, using the same lysate a second time. Typically, cotransduction of suppressor and Km^r was about 50%, but this number should be considered a rough estimate in view of the small sample size and occasional difficulty in judging the suppressor phenotype. In a few cases, more than 10 transductants had to be tested to find one that clearly carried the suppressor mutation. The cotransduction frequency of Km^r and *dnaE698*(Ts) (23), measured in a separate experiment, was 68%; *dnaE698* maps to the same half of *dnaE* as does *spq-2* in marker rescue experiments (18).

Molecular cloning of suppressor genes. Genomic libraries of strains RM746, RM812, RM813, and RM815 were prepared in the vector λ1059 as described previously (23). *dnaE* clones from these libraries were identified by red-plaque complementation of *dnaE*(Ts) strains (23). Presence of a functional suppressor gene in these clones was assayed by a red-plaque suppression test (22). This suppression test determined the ability of a λ clone to restore growth at 40.5°C of *S. typhimurium* RM858, a temperature-sensitive survivor of Tn*10*dTc insertion in *dnaQ*. Control experiments showed that *dnaE*$^+$ clones from wild type did not restore growth to RM858, whereas *dnaQ*$^+$ clones did. Thus, the failure of RM858 to grow at high temperature resulted from failure to suppress the growth defect conferred by *dnaQ*::Tn*10*dTc rather than from an ordinary temperature-sensitive mutation in *dnaE*.

Nomenclature. Although all suppressors mapped to the

TABLE 2. DNA polymerase III activity from RM822

Fraction	Protein (mg)	Activity (10^3 U)	Sp act (10^3 U/mg)
I (lysate supernatant)	3,600	840	0.23
II (ammonium sulfate)	390	200	0.51
III (Heparin-agarose)	46	210	4.6
IV (Sephacryl S-300)	2.3	81	35
V′ (Mono Q, peak I)	0.015	2.2	150
V″ (Mono Q, peak II)	0.007	1.8	260

vicinity of *dnaE*, only one suppressor mutation has been explicitly identified as a *dnaE* allele. For this reason, and for clarity, in this paper we have designated all of the suppressors as *spq* (suppressor of *dnaQ*).

Determination of mutation rates. On several widely separated occasions, one or more cultures of each strain to be examined were grown to saturation in 5 ml of LB broth plus thymine (10 μg/ml). A mutant-free inoculum (typically a few thousand cells) from each culture was used to seed a subculture, which was also allowed to grow to saturation. The total titer and the titer of drug-resistant mutants in each subculture were determined by plating samples on plates with no drug, nalidixate, or rifampin. Colonies were counted after 1 day except for the rifampin-resistant colonies, which were counted after 2 days. The raw data from all subcultures were pooled and used to estimate mutation rate by the method of maximal likelihood (19).

Purification of DNA polymerase III. DNA polymerase III activity from strain RM822 was prepared by modification of procedures of Maki and Kornberg (20) and McHenry and Crow (25; Table 2). A cell lysate was prepared by incubating 200 g of cell paste at 0°C for 60 min in 200 ml of 50 mM Tris hydrochloride (pH 7.5)–10% (wt/vol) sucrose–5 mM dithiothreitol–10 mM spermidine hydrochloride–0.05% (wt/vol) lysozyme. The lysate was cleared of cell debris by centrifugation at 31,700 × *g* for 60 min to give fraction I. Fraction I was treated with ammonium sulfate (0.25 g/ml of fraction I) for 30 min with stirring at 0°C. The precipitate was collected by centrifugation at 31,700 × *g* for 45 min, dissolved in 20 ml of buffer A (50 mM Tris hydrochloride [pH 7.5], 20% [vol/vol] glycerol, 1 mM EDTA, and 10 mM β-mercaptoethanol), and dialyzed overnight against 2 liter of buffer A plus 20 mM NaCl to yield fraction II. Fraction II was diluted with buffer A plus 20 mM NaCl until its conductivity matched that of the diluent and was then applied to a 100-ml column (5 by 5 cm) of heparin-agarose equilibrated with diluent. The column was washed with 5 column volumes of diluent, and activity was eluted with a 20-column-volume linear salt gradient (20 to 400 mM NaCl in buffer A) at a flow rate of 70 ml/h. Peak fractions were pooled to yield fraction III. Protein was precipitated from fraction III by addition of 0.4 g of ammonium sulfate per ml of fraction. The suspension was stirred for 30 min and held at 0°C for an additional 12 h. The precipitate was collected by centrifugation at 12,250 × *g* for 30 min at 0°C, dissolved by 1 ml of buffer A, and gel filtered on a 350-ml column (2.5 by 72 cm) of Sephacryl S-300 equilibrated in buffer A plus 40 mM NaCl. Fractions were collected at a flow rate of 20 ml/h, and peak fractions were pooled to yield fraction IV. Fraction IV was applied to a 1-ml Mono Q HR 5/5 anion-exchange column equilibrated in buffer A plus 40 mM NaCl. The column was washed with 2 ml of equilibration buffer, and activity was eluted by a 20-column-volume linear salt gradient (40 to 400 mM NaCl in buffer A) at a flow rate of 12 ml/h. Activity eluted in two peaks, fractions V′ and V″.

Enzyme assays. DNA polymerase activity was monitored by incorporation of [^{3}H]dTTP into gapped salmon sperm DNA (25). One unit of activity is the amount catalyzing incorporation of 1 pmol of deoxynucleoside triphosphate per min at 30°C. Exonuclease activity (8) was determined by using a 3′ mismatched substrate, $(dT)_{17}$-([^{3}H]dC)$_{1.8}$/$(dA)_{1500}$, prepared by the terminal transferase method (2).

Other biochemical methods. Standard procedures were followed for SDS-polyacrylamide gel electrophoresis (16), silver staining (45), and determination of protein concentration (using bovine serum albumin as standard) by the method of Bradford (1). Western blots (immunoblots) were prepared by electrophoretic transfer of proteins from 6% SDS-polyacrylamide gels (38) and stained with a Vecta-stain ABC kit (Vector Laboratories).

RESULTS

Null phenotype of *dnaQ*. Results of the reconstruction experiment (Fig. 3) illustrate the null phenotype of *dnaQ*. When wild-type cells were transduced to Tc^r by using a donor strain with Tn*10* in *dnaQ* or a control donor with Tn*10* in the nonessential *bio* genes, the *dnaQ*::Tn*10* transductant colonies differed from the controls in three visible features: they were smaller, they had an irregular border, and they had a mottled surface appearance (compare Fig. 3A and B). The control colony morphology was stable, as shown by replating (Fig. 3D). The "sick" *dnaQ*::Tn*10* colony morphology, in contrast, was unstable, since *dnaQ*::Tn*10* colonies gave rise to some colonies of wild-type appearance upon replating (Fig. 3E). We deduced that the sick cells gave rise to the healthy ones, and not vice versa, because primary transductants replated at earlier times had few if any healthy cells in them and because primary transductants allowed to grow on plates for 72 h formed sick colonies in which several papillae or outgrowths with wild-type morphology could be seen in each colony. The healthy cells exhibited the expected mutant structure of the *dnaQ* region (Fig. 4), and we show below that these cells also contained a suppressor mutation. Therefore, the sick cells represented the unaltered original transductant genotype, and they define the *dnaQ* null phenotype as "poor growth" under the conditions illustrated. There was some variability in the severity of the growth defect among many repetitions of this experiment, the precise cause of which we do not understand.

Although the reconstruction experiment shown in Fig. 3 used a single *dnaQ* allele, a single host strain, and a single method of strain construction, the growth defect was not specific to these conditions. We observed similar behavior in λ-sensitive *Salmonella* cells transduced to *dnaQ*::Tn*10* via λ infection. Moreover, the same phenotype was produced by every insertion or substitution allele of *dnaQ* in our collection. These included (i) simple insertions of Tn*10*Δ16Δ17 near the middle of *dnaQ* or near its 3′ end and (ii) internal substitutions encompassing the 3′ half of *dnaQ* and extending to various degrees toward the 5′ end (Fig. 1 and 2). All of these *dnaQ* alleles shared the feature that expression of the carboxyl portion of ε was disrupted. Thus, the carboxyl portion of ε appears to be necessary for ε to carry out its growth-promoting function.

Suppressors of *dnaQ*::Tn*10*. The observations on the appearance and behavior of freshly made *dnaQ* null mutants suggested the presence of a suppressor mutation in their healthy progeny. This suggestion was confirmed by showing that the factor responsible for the improved growth of these cells was a genetic element that could be transduced from

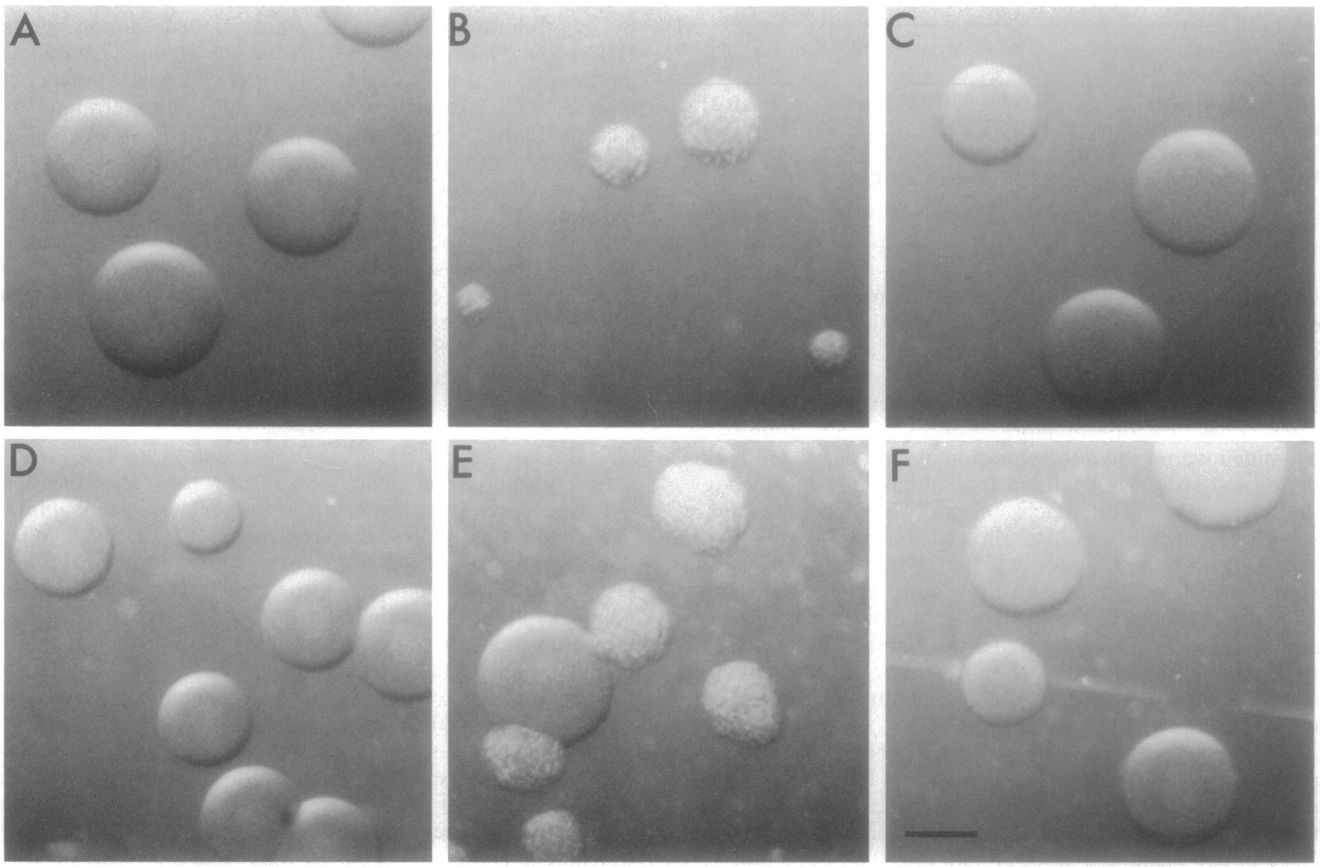

FIG. 3. *dnaQ* null phenotype. Freshly growing cells were transduced at a multiplicity of 0.1 PFU per cell with P22 lysates propagated on strain RM238 (*bio*::Tn*10*) or RM978 (*dnaQ201*::Tn*10*). After 15 min of incubation at 37°C, the transduction mixes were diluted with an equal volume of LB broth containing 20 mM EGTA to prevent reinfection of transductants. The mixes were then incubated with aeration at 37°C for 2 h to allow expression of Tc^r and plated on LB plates containing tetracycline and EGTA. After 24 h of incubation at 37°C, the plates were photographed, and then incubation was continued for an additional 24 h. After the additional incubation, single colonies from each plate were resuspended, diluted, and replated on the identical medium. These plates were also photographed after 24 h of incubation at 37°C. (A) RM820 (*dnaE*$^+$) × *bio*::Tn*10*; (B) RM820 × *dnaQ201*::Tn*10*; C, RM821 (*spq-2*) × *dnaQ201*::Tn*10*; D to F, replated transductants from panels A to C, respectively. Bar, 0.5 mm.

one cell to another by phage P22. When a cell harbored such a suppressor, it could tolerate the subsequent introduction of a *dnaQ* null allele, as evidenced by the formation of transductant colonies of normal and stable morphology (Fig. 3C and F). In the best-studied case, three lines of evidence agree that one of these suppressors, called *spq-2*, is a mutation of *dnaE*, the gene for the polymerization subunit (α) of DNA polymerase III. These lines of evidence include transductional linkage of *spq-2* to a Km^r marker near *dnaE*, molecular cloning of *spq-2* on a fragment of DNA that also includes *dnaE*, and marker rescue of the *spq-2* phenotype by a fragment of DNA whose only difference from wild type (determined by complete DNA sequence of the fragment) encodes a valine → glycine change in α (18).

The presence of two cell types, one sick and without a suppressor and the other healthy and having a suppressor, in each *dnaQ*::Tn*10* transductant colony exemplified in Fig. 3B implied that the suppressors arose after the time of plating and therefore must have arisen independently in each colony. In addition to characterizing *spq-2* as described above, we partially characterized 15 other suppressors obtained from different primary transductant colonies. All of these suppressors were linked to *dnaE* by P22 cotransduction with a Km^r marker near *dnaE* (see RM1655 et seq. in Table 1), and *spq-13*, *-14*, and *-16* were, in addition, molecularly cloned in phage λ on a fragment of DNA that also included *dnaE*. These data are not sufficient to prove that the additional suppressors are in fact alleles of *dnaE*, but the example of *spq-2* makes this likely. In a few cases (*spq-11*, *-13*, *-14*, and *-16*), the cotransduction frequency of suppressor with Km^r was suspiciously low (<10% cotransduction versus 68% cotransduction of Km^r with *dnaE698*), perhaps indicating a second locus in the *dnaE* vicinity that can give rise to suppressor mutations. However, other possible explanations for the low cotransduction frequency, such as error or ambiguity in scoring the suppressor phenotype, have not been ruled out, and the cloning results mentioned above imply that *spq-13*, *-14*, and *-16* are closer to *dnaE* than is indicated by their cotransduction frequency.

All of the suppressors were generated with the *dnaQ200* allele, in which the 3′ portion of *dnaQ* was substituted whereas the 5′ portion of *dnaQ* encoding the N-terminal 132 amino acid residues of ε remained intact (Fig. 2). When tested, however, all 16 suppressors were able to suppress the more inclusive *dnaQ201* substitution allele encoding only 13 N-terminal residues (Fig. 2). This observation was consistent with the apparent equivalence of the various *dnaQ* alleles in growth phenotype.

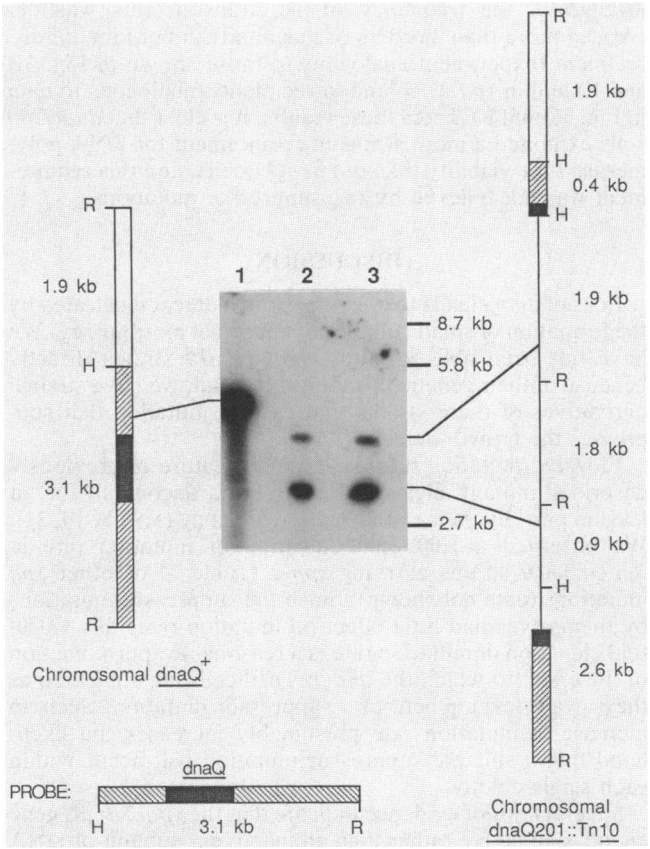

FIG. 4. Southern analysis of *dnaQ* mutants. *Eco*RI-digested genomic DNA was probed with a plasmid containing the indicated 3.1-kilobase (kb) *dnaQ*$^+$ restriction fragment. Lanes: 1, *dnaQ*$^+$ (RM749); 2, *dnaQ201*::Tn*10* (RM976); 3, *dnaQ201*::Tn*10* (RM978). The latter two strains differ in that RM976 presumably contains an uncharacterized suppressor arising after introduction of the *dnaQ* mutation, whereas RM978 was constructed by introducing the *dnaQ* mutation into an *spq-2* strain. The blot shows that the *dnaQ* gene in the wild-type strain resides on a single *Eco*RI fragment, whereas in the *dnaQ201*::Tn*10* strains two bands are detected as expected, since the deletion substitution in *dnaQ* introduces two new *Eco*RI sites. H, *Hin*dIII; R, *Eco*RI.

Additional properties of *spq-2* and *dnaQ200 spq-2* strains. We examined several aspects of the physiology of strains RM821 (*spq-2*) and RM822 (*spq-2 dnaQ200*) as exemplars of the consequences of loss of *dnaQ* and its suppression. These two strains grew in LB broth with a doubling time of 35 to 40 min at 37°C, the same as their wild-type ancestor, RM10 (data not shown). Strain RM822 was a mutator by a factor of about 10^3 at two loci tested, but strain RM821 exhibited nearly wild-type spontaneous mutation rate (Table 3; other suppressors tested behaved similarly). Strains RM821 and RM822 were not more sensitive than RM10 to UV light; in fact, *dnaQ200* afforded some degree of protection from UV killing (S. Slater and R. Maurer, manuscript in preparation).

Active DNA polymerase III in a *dnaQ* null strain. A biochemical analysis of DNA polymerase III was undertaken to test two opposing hypotheses about the nature of the *spq-2* suppressor mutation. According to one hypothesis, in the absence of ε the remaining DNA polymerase III components interfere with some alternative mechanism of DNA replication. The *spq-2* mutation in α inactivates DNA polymerase III, potentiating the alternative mechanism. According to the opposing hypothesis, the *spq-2* mutation

TABLE 3. Mutation rates

Strain	Relevant genotype[a]	No. of cultures	Mutations/10^8 cells per generation ± variance	
			Rifampin resistance	Nalidixate resistance
RM820	*dnaE*$^+$ (*spq*$^+$) *dnaQ*$^+$	15	0.19 ± 0.06	0.09 ± 0.04
RM821	*spq-2 dnaQ*$^+$	15	0.58 ± 0.13	0.06 ± 0.03
RM822	*spq-2 dnaQ200*::Tn*10*	19	375 ± 22	127 ± 7

[a] The strains are otherwise isogenic.

restores some critical property of DNA polymerase III that is lost when ε is missing. An active DNA polymerase III species was isolated from strain RM822 (*dnaQ200 spq-2*) (Fig. 5 and 6). The polymerase activity of fraction IV was decreased 5-fold in 150 mM KCl and 10-fold in *N*-ethylmaleimide. This pattern of sensitivity is characteristic of DNA polymerase III and not of DNA polymerase I (14). Fraction V″ consisted of four major polypeptides, one of which was identified as the α subunit of DNA polymerase III on the basis of its apparent molecular size, 128 kDa, and its cross-reactivity on Western blots to a polyclonal antibody specific to purified *E. coli* α (Fig. 5). No 3′→5′ exonuclease activity was detectable in fraction V″, although such activity was readily detected in a comparable preparation of wild-type enzyme (Fig. 6). Use of up to five times more units of

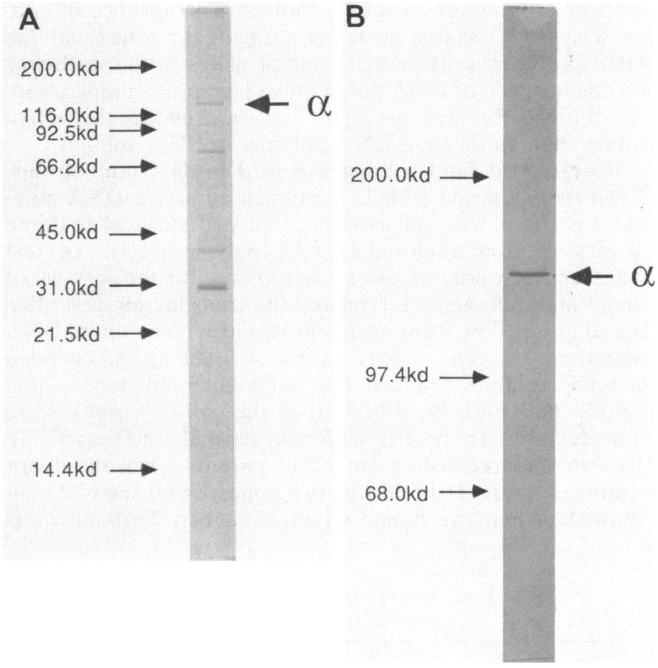

FIG. 5. DNA polymerase III from strain RM822. (A) Fraction V″ (1 μg) was electrophoresed through a 10 to 15% SDS-polyacrylamide gradient gel and visualized by silver staining. Marker proteins (Bio-Rad Laboratories) were myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase *b* (92.5 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa). (B) Fraction V″ (1 μg) was electrophoresed through a 6% SDS-polyacrylamide gel and visualized by staining with polyclonal antibody to *E. coli* α subunit. Predyed protein standards (Bio-Rad) and their approximate molecular weights were myosin (200 kDa), phosphorylase *b* (97.4 kDa), and bovine serum albumin (68 kDa). Additional marker proteins were not resolved in the 6% gel.

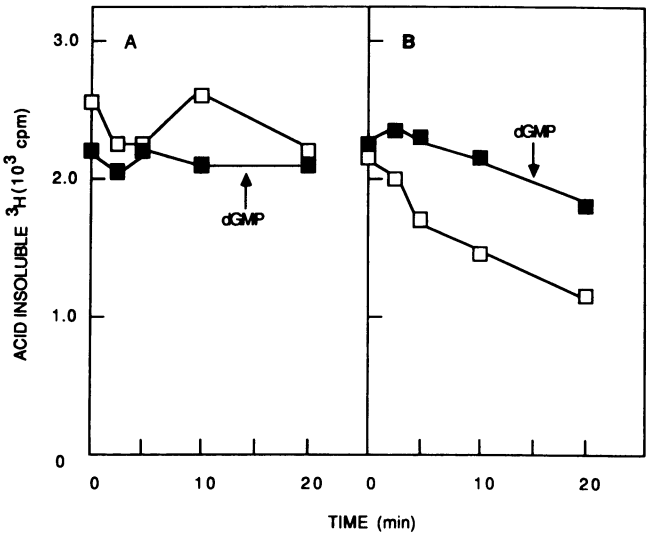

FIG. 6. 3′→5′ exonuclease activity in DNA polymerase III preparations. (A) *spq-2* polymerase (fraction V″; 6.5 U); (B) wild-type polymerase (fraction V; 6.5 U). This enzyme was prepared from RM10 by the procedure used for *spq-2* enzyme except that fraction V was prepared by DEAE chromatography. dGMP (5 mM) is a specific inhibitor of ε exonuclease (33).

mutant enzyme than was used for Fig. 6 failed to reveal any evidence of exonuclease (not shown). The absence of exonuclease suggests that no active ε fragment purified with the DNA polymerase III activity and provides further evidence for the absence of DNA polymerase I from this preparation. All of these observations argue that *spq-2* encodes an active rather than an inactive DNA polymerase III α subunit.

Requirement for DNA polymerase I in *dnaQ* null strains. Even though strain RM822 contained an active DNA polymerase III, it was still possible that cell survival required assistance from additional DNA polymerase(s). To test whether DNA polymerase I was required for the survival of *dnaQ* null mutants, we repeated the transduction test illustrated in Fig. 3 by using recipient strains with a mutant DNA polymerase I gene, *polA2* (Table 4). The *polA2*-encoded enzyme exhibits reduced but detectable polymerase and editing activities (9, 43). All of the *polA2* strains were transduced to Tc^r by P22 with near-normal efficiency (50 to 100%) compared with their *polA*$^+$ parents when the donor strain contained Tn*10* located in a nonessential site (data not shown). When the donor strain contained Tn*10* in *dnaQ* (*dnaQ200*), the frequency of Tc^r transductants was decreased more than 2 orders of magnitude in both the *dnaE*$^+$ recipient (experiment analogous to those shown in Fig. 3B and E) and in *spq-2*, *-3*, and *-6* recipients (analogous to data in Fig. 3C and F). From these results, it is clear that *dnaQ200* cells exhibited a more stringent requirement for DNA polymerase I for viability than did *dnaQ*$^+$ cells, and this requirement was not relieved by *spq* suppressor mutations.

TABLE 4. Requirement for DNA polymerase I for *dnaQ*::Tn*10* viability

Strain	Relevant genotype	No. of *dnaQ*::Tn*10* transductants[a]
RM2685	*dnaE*$^+$ (*spq*$^+$) *polA*$^+$	665
RM1835	*dnaE*$^+$ (*spq*$^+$) *polA2*	0
RM2686	*spq-2 polA*$^+$	630
RM1836	*spq-2 polA2*	0
RM2687	*spq-3 polA*$^+$	597
RM1837	*spq-3 polA2*	0
RM2688	*spq-6 polA*$^+$	409
RM1838	*spq-6 polA2*	1

[a] Number of Tc^r transductants obtained from 5 × 10^6 PFU of P22 lysate propagated on strain RM711 (*dnaQ200*::Tn*10 spq-5*). Colonies were counted after 2 days.

DISCUSSION

Loss of *dnaQ* leads to a severe growth defect illustrated by the formation of small colonies of abnormal morphology. We have not been able to study *dnaQ*::Tn*10* strains directly because of their genetic instability. Instead, we have studied derivatives of these strains that carry a mutation that suppresses the growth defect.

Elevated mutation rate is a familiar feature of previously described mutant alleles of *dnaQ* and is accounted for, at least in part, by their exonuclease deficiency (5, 7, 8, 10, 31). We observed a 1,000-fold elevation of mutation rate in *dnaQ*::Tn*10* strains carrying *spq-2* (Table 3) or other *spq* mutations (data not shown). Since the suppressor mutations by themselves had little effect on mutation rate, this 1,000-fold elevation of mutation rate is a reasonable approximation of the effect to which the *dnaQ*::Tn*10* cells are subjected as they await development of a suppressor mutation. Such an increase in mutation rate presumably increases the likelihood that a suitable suppressor mutation will occur within each single colony.

Several lines of evidence indicate that the *spq-2 dnaE* gene encodes an active rather than an inactive α subunit of DNA polymerase III. Most significantly, an active DNA polymerase III species has been isolated from strain RM822 (Fig. 5 and 6; Table 2). Moreover, α subunit purified from an *E. coli* strain that overproduces *dnaE* (*spq-2*) is as active as wild-type α in gap-filling activity (data not shown). The interpretation of this biochemical evidence is consistent with two in vivo results as well. In *spq-2* strains, the spontaneous mutation rate varies with the *dnaQ* genotype (Table 3). Since ε acts as an intrinsic subunit of DNA polymerase III, this observation implies that DNA polymerase III, and specifically its *spq-2*-encoded α subunit, is functional. It has also been observed with *dnaQ*::Tn*10* transductants do not appear if the recipient strain carries a temperature-sensitive mutation in *dnaE*. This observation implies that the suppressor mechanism, whatever it is, can work only in the context of a functional α protein (A. Wong and R. Maurer, unpublished data).

A plausible view, then, is that ε normally provides a function critical to the operation of DNA polymerase III. When ε is lacking, a suppressor mutation operates at the level of the polymerase α subunit to restore or bypass the critical function. It appears that the critical aspect of ε is not its fidelity function, for *spq-2* by itself has little effect on the fidelity of DNA replication, as judged from mutation rates (Table 3), nor does it unveil or recruit any detectable novel exonuclease activity that purifies with the polymerase (Fig. 6).

If fidelity is not the growth-promoting function of ε, then what is? There is biochemical evidence that α is more active and more stable when bound to ε than it is alone (21) and genetic and biochemical evidence that α and ε interact (11, 21, 22, 37). Also, the full replication competence of DNA polymerase III requires several other subunits in addition to α and ε (24); conceivably, one or more of these subunit could

attach to the polymerase complex through ε and would be lost or loosely bound in the absence of ε (36). (The major peptides, other than α, that purify with our ε-free gap-filling activity have not been identified explicitly, but as judged by apparent molecular weight, they could be the β, τ, and δ subunits that have been described previously.) These considerations lead to several possible hypotheses connecting ε to a property of DNA polymerase III such as its rate, processivity, intrinsic stability, ability to dimerize, ability to extend a mismatched primer terminus, or ability to participate in methyl-directed mismatch repair (17). Deterioration of any of these characteristics of the polymerase might impair the ability of a cell to complete DNA replication yet could be remedied, in principle, by a modification of α. Analysis of possible alteration in such properties of α by *spq* mutations will require the application of more specific tests of polymerase activity than the general gap-filling assay we have used. This work is currently in progress.

Role of DNA polymerase I. *dnaQ* null mutants, even after acquisition of a suppressor mutation, exhibit a more stringent requirement for DNA polymerase I than do *dnaQ*$^+$ strains. Elucidation of the physiological basis of this requirement will require further investigation because polymerase I possesses three distinct activities (13), any of which (or some combination thereof) could be required, depending on the actual nature of the growth function of ε. The polymerase I mutant used in observing this requirement has reduced polymerization and editing capacities, whereas the effect of the mutation on its 5′→3′ exonuclease activity is unknown (9, 43). If the loss of ε makes DNA polymerase III a less processive enzyme, this could lead to a higher demand for DNA polymerase I activity fo fill additional gaps in the replicated DNA. If such gaps are characterized by RNA primers on one side (as in normal Okazaki fragment synthesis), the additional demand would include the 5′→3′ exonuclease activity. Note that the *dnaQ200*::Tn*10* allele used in the DNA polymerase I experiments leaves enough of *dnaQ* intact to ensure normal promoter activity of the divergently oriented RNase H (*rnh*) gene (6, 27). This may be important if RNase H contributes to the removal of RNA primers of DNA synthesis. The editing exonuclease activity of DNA polymerase I could be used to erase a fraction of nucleotides misinserted by DNA polymerase III and resistant to further extension. However, the elevated mutation frequency of *dnaQ*::Tn*10 spq* strain indicates that many misinsertions are not corrected. Finally, another possible source of increased demand for DNA polymerase I would be in postreplication repair of mismatches that will be more frequent in the absence of editing at the replication fork. In the specific case of the *mutHLS* methyl-directed mismatch repair system, which normally utilizes DNA polymerase III (17), involvement of DNA polymerase I might arise if the absence of ε or the suppressor-encoded alteration in α interfered with participation by DNA polymerase III.

The requirement for DNA polymerase I in *dnaQ*::Tn*10* strains differs in two respects from findings of another report of a special situation when DNA polymerase I becomes essential for replication. Niwa et al. (26) found that DNA replication strictly depends on DNA polymerase I in *dnaE*(Ts) mutants at high temperature and in unsuppressed *dnaE*(Am) mutants (3), provided that a mutant allele of a poorly characterized gene, *pcbA*, is present. In the *dnaQ*::Tn*10* strains, in contrast, the requirement for *dnaE* is not bypassed; indeed, survival appears to depend on an alteration in some property of the *dnaE* product. Moreover, wild-type *S. typhimurium* clearly exhibits the PcbA$^+$ phenotype since *dnaE*(Ts) mutations are temperature-sensitive lethals (23). *Salmonella* cells that were once *dnaQ*::Tn*10* and then later transduced to *dnaQ*$^+$ are also PcbA$^+$ (data not shown), showing that survival after introduction of *dnaQ*::Tn*10* does not require induction of a mutation in *pcbA* such as described by Bryan and Moses (in *E. coli*, *pcbA* is not linked to *dnaE* or *dnaQ* [4]).

No requirement for RecA. When normal DNA replication in *E. coli* is blocked or slowed, either by treatment with DNA-damaging agents or by raising the temperature in certain temperature-sensitive replication mutants, a complex response (SOS) involving induction of a set of genes ensues. In a variety of ways, the induced genes aid the cell in recovering from the inducing condition. A key positive regulatory element in the response is the RecA protein, whose activation leads to proteolysis of LexA protein, the common repressor of the induced SOS genes (41). *S. typhimurium* clearly possesses a similar system under analogous control (28). The fact that we (S. Slater and R. Maurer, unpublished data) have been able to make a Δ*recA* derivative of an *spq-2 dnaQ200*::Tn*10* strain argues that survival of this strain does not require chronic induction of any SOS proteins. However, this result does not address whether transient induction of SOS might play a role in promoting the persistence of freshly made *dnaQ*::Tn*10* transductants until the development of a suppressor mutation.

Other roles for ε? The isolation of strains devoid of ε subunit opens up the opportunity to investigate the role, if any, of ε in several aspects of cell physiology. For example, the role of ε in inhibiting or possibly promoting DNA synthesis on UV-damaged DNA is controversial (12, 30, 36, 40, 44). Whether any SOS-induced proteins needed for UV mutagenesis have ε as their primary target is also unknown. It will also be of interest to study the structure and genetic requirements of DNA polymerase III holoenzyme in *dnaQ*::Tn*10* strains to learn whether any holoenzyme subunits use ε as the site of attachment to the polymerase.

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