JOURNAL OF BACTERIOLOGY, Nov. 1992, p. 6965–6973
0021-9193/92/216965-09$02.00/0
Copyright © 1992, American Society for Microbiology
Vol. 174, No. 21

DNA Replication Defect in *Salmonella typhimurium* Mutants Lacking the Editing (ε) Subunit of DNA Polymerase III

MIRIAM R. LIFSICS, EDWARD D. LANCY, JR.,† AND RUSSELL MAURER*

Department of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, 10900 Euclid Avenue, Cleveland, Ohio 44106-4960

Received 24 June 1992/Accepted 4 September 1992

In *Salmonella typhimurium*, *dnaQ* null mutants (encoding the ε editing subunit of DNA polymerase III [Pol III]) exhibit a severe growth defect when the genetic background is otherwise wild type. Suppression of the growth defect requires both a mutation affecting the α (polymerase) subunit of DNA polymerase III and adequate levels of DNA polymerase I. In the present paper, we report on studies that clarify the nature of the physiological defect imposed by the loss of ε and the mechanism of its suppression. Unsuppressed *dnaQ* mutants exhibited chronic SOS induction, indicating exposure of single-stranded DNA in vivo, most likely as gaps in double-stranded DNA. Suppression of the growth defect was associated with suppression of SOS induction. Thus, Pol I and the mutant Pol III combined to reduce the formation of single-stranded DNA or accelerate its maturation to double-stranded DNA. Studies with mutants in major DNA repair pathways supported the view that the defect in DNA metabolism in *dnaQ* mutants was at the level of DNA replication rather than of repair. The requirement for Pol I was satisfied by alleles of the gene for Pol I encoding polymerase activity or by rat DNA polymerase β (which exhibits polymerase activity only). Consequently, normal growth is restored to *dnaQ* mutants when sufficient polymerase activity is provided and this compensatory polymerase activity can function independently of Pol III. The high level of Pol I polymerase activity may be required to satisfy the increased demand for residual DNA synthesis at regions of single-stranded DNA generated by ε-minus pol III. The emphasis on adequate polymerase activity in *dnaQ* mutants is also observed in the purified α subunit containing the suppressor mutation, which exhibits a modestly elevated intrinsic polymerase activity relative to that of wild-type α.

DNA polymerase III (Pol III), the replicative polymerase of *Escherichia coli* and *Salmonella typhimurium*, is a multisubunit enzyme whose polymerase and editing exonuclease activities reside on different subunits, designated α and ε, respectively (for a review, see reference 22). Null mutations in *dnaQ*, the gene encoding ε, confer an elevated mutation rate and feeble growth on *S. typhimurium* (17). The growth defect may be compensated by suppressor mutations (designated *spq*) located in *dnaE*, the gene encoding the α subunit (17, 18). Mutations capable of suppressing the growth defect appear to be limited; 4 of 15 independently obtained suppressors have the same single-base change in the 3,480-bp gene (16a, 18). This suppressor α subunit (Val-832→Gly) is active in simple gap-filling assays (17, 18), but its mechanism of suppression is not understood.

Ordinarily, the single-subunit DNA Pol I, encoded by *polA*, plays a subsidiary role in DNA replication through repair synthesis that replaces RNA primers (e.g., those used to initiate Okazaki fragments on the discontinuously synthesized strand [12]). This role of Pol I is essential during growth on rich medium but can be satisfied by a level of Pol I activity much lower than that provided by the $polA^+$ gene (11). Pol I is also involved in a variety of nonessential DNA repair reactions. In *dnaQ* null mutants, whether suppressed by an *spq* mutation or not, reduced Pol I activity such as that provided by the *polA2* allele is not sufficient for growth (17).

In this paper, we report on our investigations into the reasons for the poor growth of *dnaQ* mutants and the mechanisms whereby suppressor mutations in Pol III and provision of adequate Pol I activity bring about better growth of such mutants. Our results suggest a model in which the total DNA replication capacity of the cell becomes limiting and cell survival is threatened by the formation of single-stranded gaps in replicated DNA. The mechanisms which suppress the growth defect appear to ensure the availability of adequate polymerase activity.

MATERIALS AND METHODS

General microbiological methods. The bacterial strains and plasmids used are described in Table 1. General bacteriological media and procedures were as described previously (21). Generalized transductions were carried out with phage P22 *int* HT12/4 (27). Bacterial matings were performed as outlined by Miller (23). The following drugs (concentrations in micrograms per milliliter) were added to the plates as needed: tetracycline-HCl, 25; kanamycin, 50; chloramphenicol, 20; streptomycin, 25; and fusaric acid, 12. The tetracycline plates contained, in addition, 10 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA) to prevent reinfection of transductants by P22.

Assay for viability of *dnaQ* null mutants. A transductional assay has been described in detail elsewhere (17). Briefly, a strain to be tested is transduced to tetracycline resistance with a P22 lysate prepared on a *dnaQ*::Tn*10* (deletion-substitution alleles of *dnaQ* [10]) donor strain or with a control lysate prepared on a strain with Tn*10* in a nonessential gene. With the control lysate, the number of transductants obtained was the same (within a factor of 2) for all recipient strains. The results of transductions with the *dnaQ*::Tn*10* donor lysate were determined after 36 to 48 h of incubation.

***E. coli* DNA Pol I F′ plasmids.** The F′ plasmids used in

* Corresponding author.
† Present address: Division of Biological Sciences, University of Missouri, 105 Tucker Hall, Columbia, MO 65211.

TABLE 1. Bacterial strains and plasmids used

Bacterial strain or plasmid	Genotype or description[b]	Source or reference
Strains[a]		
RM820	*dnaE*$^+$ (*spq*$^+$) *polA*$^+$ *zag-1256*::Tn*10*dKm	17
RM1734[c]	*dnaE*$^+$ *polA*$^+$	G. Weinstock
RM1835	*polA2 zie-1260*::Tn*10*dCm	17
RM1836	*spq-2 polA2 zag-1256*::Tn*10*dKm *zie-1260*::Tn*10*dCm	17
RM2685	*dnaE*$^+$ (*spq*$^+$) *polA*$^+$ *zie-1260*::Tn*10*dCm	17
RM2686	*spq-2 polA*$^+$ *zag-1256*::Tn*10*dKm *zie-1260*::Tn*10*dCm	17
RM2779	*spq-2 dnaQ201*::Tn*10* Δ(*recA*)*496 zag-1256*::Tn*10*dKm	29
RM2852	*dnaE*$^+$ (*spq*$^+$) *polA2 zag-1256*::Tn*10*dKm	Derived from RM1835[d]
RM2853	*spq-2 polA2 zag-1256*::Tn*10*dKm	Derived from RM1836[d]
RM3709	*mutH*::Tn*5 dnaE*$^+$ *polA2 zie-1260*::Tn*10*dCm	This laboratory[e]
RM3710	*mutH*::Tn*5 dnaE*$^+$ *polA*$^+$ *zie*-1260::Tn*10*dCm	This laboratory[e]
RM3784	*ung spq-2 polA2 zie-1260*::Tn*10*dCm/pCJ100	This laboratory[f]
RM3788	*ung spq-2 polA2 zie-1260*::Tn*10*dCm/pCJ105	This laboratory[f]
Plasmids[g]		
pCJ100	F′ *polA*$^+$; Cm^r	11
pCJ102	F′ 5′→3′ exo; Cm^r	11
pCJ103	F′ Klenow; Cm^r	11
pCJ105	F′ Cm^r (no *polA*)	C. Joyce
pCJ145	F′ *polA* D355A, E357A; Cm^r	C. Joyce
pβL	Rat Pol β; Cm^r	J. Sweasy[h]

[a] All strains are *Salmonella typhimurium*, except where noted. All *Salmonella* strains contain, in addition to the markers listed, mutations in *thyA* and *deo*. *zag-1256*::Tn*10*dKm is a transposon insertion in a nonessential site linked to *dnaE*. *zie-1260*::Tn*10*dCm is a transposon insertion in a nonessential site linked to *polA*. *spq-2* is an allele of *dnaE*.

[b] Tn*10*dTc, Tn*10*dKm, and Tn*10*dCm, derivatives of Tn*10* conferring resistance to tetracycline, kanamycin, and chloramphenicol, respectively.

[c] Wild-type strain of *E. coli* K-12.

[d] The related parental strains (17) carry a chloramphenicol resistance marker (*zie1260*::Tn*10*dCm) linked to *polA2*. Each strain was rendered Cm^s by transduction to tetracycline resistance with a linked marker, *zie-3024*::Tn*10*dTc (13). These Tc^r strains were then made Tc^s by selection on fusaric acid (1). A stable isolate ($<10^{-8}$ reversion to Tc^r) was kept in each case. Since the P22 transductional linkage of *zie-3024* to *polA* is only 4%, it is unlikely that fusaric acid resistance mutations (presumably deletions) extend to *polA*. Finally, *zag-1256*::Tn*10*dKm was introduced into the *dnaE*$^+$ member of this pair of strains to make it isogenic with the *spq-2* strain.

[e] These strains were made by P22 transduction of *mutH*::Tn*5* from SL4213 (25) with selection for Km^r. The *mutH* mutants exhibited elevated mutagenesis to rifampin resistance.

[f] In outline, the *ung* mutants were made by transducing the *E. coli ung-152*::Tn*10* mutation (6) into *Salmonella* strains. The insertion was then moved into strains containing pCJ plasmids as indicated. Finally, the strains were made Tc^s by selection on fusaric acid (1). Strain 3784 is able to be transformed by uracil-containing single-stranded plasmid DNA, whereas transformation of an *ung*$^+$ strain is reduced by a factor of 10^4 with the same DNA. Strain RM3788 could not be verified by this test because of the *polA* mutation; however, its *ung-152*::Tn*10* ancestor could donate the insertion to a *polA*$^+$ recipient which then exhibited the Ung$^-$ phenotype. Additional details of these strain constructions can be obtained from the authors.

[g] These plasmids, obtained in *E. coli* backgrounds, were transferred by two sequential matings into strains RM2852 and RM2853. The intermediate host was the nonrestricting *S. typhimurium* LB5010 (4).

[h] The pβL plasmid contains the pSC101 origin of replication and is present at about five copies per cell. Transcription is regulated by the *lac* promoter.

polA complementation experiments contain various alleles of the *E. coli polA* gene. Plasmid pCJ100 contains the entire *polA* gene and thus encodes all three activities of Pol I: polymerase, 3′→5′ exonuclease, and 5′→3′ exonuclease. Plasmid pCJ102 contains the *polA1* amber allele and produces a 341-amino-acid fragment, encoding only the 5′→3′ exonuclease. The possibility that a readthrough product would be formed by this construct is eliminated by deletion of the coding sequence for the carboxy-terminal 413 amino acids of the native polypeptide. Plasmid pCJ103 contains the 5′-truncated *polA* gene which encodes the Klenow fragment (polymerase and 3′→5′ exonuclease), amino acids 324 to 928 of the 928-amino-acid native polypeptide. The full-length *polA* gene on pCJ145 contains two point mutations, D355A and E357A, which eliminate the 3′→5′ exonuclease activity (5). The *polA* alleles in pCJ100, pCJ102, and pCJ145 are expressed from the natural *polA* promoter, whereas in pCJ103 the Klenow fragment is expressed from the *lacUV5* promoter. pCJ105, which does not contain any *polA* gene, serves as a control. All of the plasmids are marked by a nontransposing chloramphenicol resistance determinant which provided the basis for selection of exconjugants in strain constructions.

Rat DNA Pol β. Rat DNA polymerase β (Pol β) was expressed in vivo from a cDNA fused to the *lac* promoter. The plasmid bearing this construct, pβL, contains a pSC101 origin of replication (33a). The polymerase contains no intrinsic nuclease activities (35).

Determination of RecA/LexA ratios. The concentration of cells in each sample was determined by measuring the A_{600}. Samples containing 10^5 to 10^8 cells were lysed in Laemmli sample buffer and applied to 12% sodium dodecyl sulfate (SDS)-polyacrylamide gels. Electrophoretic transfer to nitrocellulose membranes was performed at 4°C for 4 h in 25 mM Tris–192 mM glycine–20% methanol (pH 8.3) (36). The membranes were incubated with LexA or RecA antibodies (1:2,000 dilution) and then with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (Zymed Laboratories, Inc.). Rabbit polyclonal antibody against *E. coli* RecA was provided by Jeffrey Roberts (Cornell University), and rabbit polyclonal antibody against *E. coli* LexA was provided by John Little (University of Arizona). Bands were visualized on X-ray film by chemiluminescence after enzymatic activation of the substrate, AMPPD [3-(2′-spiroadamantane)-4-methoxy-4-(3″-phosphoryloxy)phenyl-1,2-dioxetane], according to the instructions of the manufacturer

(Tropix, Inc.). Total band intensity was quantitated with a Sciscan 5000 automated scanning system (United States Biochemical). For each genotype to be assayed, a sample concentration range was determined in which the intensities of both the RecA and the LexA bands varied in linear fashion with the amount of sample loaded, within 15%. The ratio of RecA to LexA was calculated for each lane within the range. These ratios were averaged to obtain the reported values, which have a standard deviation of 9 to 29%.

Overexpression of the α subunit. A 7.0-kb *Eco*RI-*Bam*HI fragment encompassing *spq-2* (17) was subcloned into the pBlueScript II SK(−) phagemid vector (Stratagene). An *Nde*I-site was created at the initiator methionine codon by oligonucleotide-directed mutagenesis (14). In addition, a comparable clone for wild-type *dnaE* was generated by restoring the original valine codon at position 832. The sequence of each clone was confirmed over an ~200-bp interval around codon 832. The *Nde*I-*Bam*HI fragments were then subcloned into the pET-3a expression plasmid (31) for overproduction, which was carried out in a BL21 (λDE3) host harboring a pLysS plasmid. No overexpression was seen in the absence of pLysS. Expression was induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) and carried out for 4 h at 18°C to increase the solubility of α.

Purification of the α subunit. Wild-type and *spq-2* α protein were purified by the same procedures and behaved identically throughout purification. All procedures were carried out at 4°C. Cells from a 2-liter overproduction were lysed in 50 mM Tris (pH 7.5)–10% (wt/vol) sucrose–2 mM EDTA–100 mM thioglycolate–20 mM NaCl–1 mM dithiothreitol by the action of the pLysS-encoded lysozyme after a single freeze-thaw cycle. The lysate supernatant was made 20 mM spermidine-hydrochloride and cleared of cellular debris by centrifugation at 98,500 × *g* (average) for 30 min and further clarified by ammonium sulfate precipitation at 15% saturation. α protein was then precipitated by increasing ammonium sulfate to 40% saturation. The pellet was desalted and applied at a flow rate of 1 column volume per h to a 10-ml column of DEAE-Sephacel (Pharmacia) equilibrated in buffer A (50 mM Tris-HCl [pH 7.5], 0.1 mM EDTA, 100 mM thioglycolate, 1 mM dithiothreitol, 20% glycerol) containing 20 mM NaCl. The column was washed with 5 column volumes of buffer A–20 mM NaCl and developed with a 10-column volume linear gradient, 20 to 400 mM NaCl in buffer A. Fraction composition was analyzed by SDS-polyacrylamide electrophoresis. Fractions containing α were pooled, and the protein was precipitated by the addition of ammonium sulfate to 40% saturation. The pellet was resuspended in buffer A–100 mM NaCl, and the suspension was applied to a 44.5-ml (0.9-cm-diameter) Sephacryl S-200 (Pharmacia) gel filtration column equilibrated in buffer A–100 mM NaCl and eluted at a flow rate of 3 ml/h. Fractions containing α were pooled, desalted, applied to a Mono-Q HR 5/5 (Pharmacia) column in buffer A–20 mM NaCl, and eluted with a linear gradient, 20 to 500 mM NaCl in buffer A. α fractions lacking other detectable bands were assayed for polymerase and single-stranded exonuclease activity. Fractions lacking detectable single-stranded exonuclease activity were pooled and concentrated by filtration (Amicon) and stored at −80°C.

Enzyme assays. Polymerase assays on gapped DNA templates were performed as described elsewhere (17). The oligonucleotide (snapback) template was essentially identical to the misinsertion target 1 described by Sloane et al. (30), except that the 3′ end of the template strand was joined to the 5′ end of the primer strand by a $(pT)_3$ linker to allow formation of the duplex region within a single oligonucleotide. Polymerase assays with the oligonucleotide template were performed as described by Maki and Kornberg (20). Single-stranded exonuclease assays were performed as described by Brenowitz et al. (2), except that a 24-base single-stranded oligomer, labeled with ^{32}P at the 5′ end, was used as the substrate.

Other biochemical methods. SDS-polyacrylamide gel electrophoresis was performed as described elsewhere (17), except that gels were stained in Coomassie brilliant blue. Protein concentrations were determined by the Bradford assay as described elsewhere, with bovine serum albumin as the standard (17).

RESULTS

Evidence for exposure of single-stranded DNA. One obvious way in which a partially defective DNA polymerase can fail is by premature termination of synthesis. Even if synthesis subsequently resumes (e.g., by the Okazaki fragment initiation pathway [39]), a stretch of single-stranded DNA (a gap) will be produced. Unless all gaps are converted to double-stranded DNA in a timely fashion, they will prevent the successful completion of a subsequent round of DNA replication. Because of its cellular abundance, DNA Pol I is the most likely enzyme to fill in gaps (12). Because of the nearly lethal phenotype of *dnaQ* mutants and the exaggerated dependence on DNA Pol I, we sought evidence that DNA replication in the absence of Pol III ε creates gaps in replicated DNA. Several attempts to measure single-stranded regions in purified chromosomal DNA by either nucleotide incorporation or nuclease digestion assays yielded inconsistent results, which we attribute to the difficulty of preparing and using intact, nuclease-free chromosomal DNA. Instead, we used the approach of Sassanfar and Roberts, who provided persuasive evidence that exposure of single-stranded DNA in vivo in the vicinity of a replication fork leads to the activation of the RecA protein and cleavage of the LexA protein (10, 26). This in turn leads to increased expression of several proteins, including RecA and LexA (SOS response [39]). Because the newly made LexA protein continues to be turned over while the SOS signal persists, the ratio of RecA to (intact) LexA increases during SOS. Accordingly, the absence of ε, if it leads to the repeated formation of transient gaps, should produce a chronic SOS signal and an increased RecA/LexA ratio. Figure 1 shows that this prediction is met and provides other significant details.

Figure 1 records Western blots of total cell lysates from various *Salmonella* strains which were obtained with antibodies against *E. coli* RecA and LexA. The *Salmonella* RecA and LexA proteins were identified by several criteria, including the similarity of their mobilities to those of the corresponding proteins of *E. coli* (data not shown), their change in ratio as expected upon SOS induction (Fig. 1, lane c), and the absence of the putative RecA protein from the extract of a *recA* deletion mutant (Fig. 1, lane d).

With one minor exception, the results in the remaining lanes of Fig. 1 can be summarized by the rule that any genotype that supported healthy strain growth did not exhibit chronic SOS induction whereas genotypes that did not support healthy strain growth showed distinct induction. Thus, introduction of a *dnaQ* deletion into a wild-type background produced transductants in which induction was evident (Fig. 1, lane e). It should be noted that the figure probably understates the degree to which the ratio was

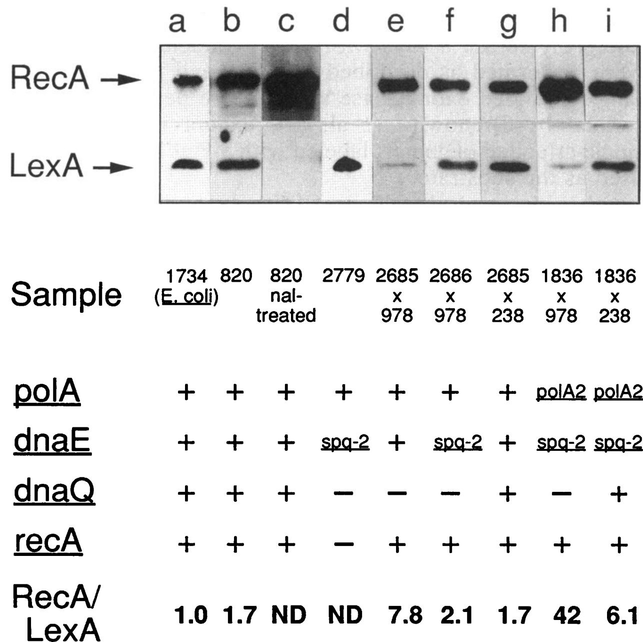


FIG. 1. SOS induction in *dnaQ* null mutants. Western blots were prepared and quantitated as described in Materials and Methods. The figure is a composite showing only the RecA and LexA bands. The lane chosen to illustrate each genotype was from the linear-response range for that genotype. Since the amount of sample loaded in each lane is unique, the intensity of a band in one lane is not directly comparable to its intensity in another lane. The ratio of the intensity of RecA to that of LexA is independent of the concentration of the sample. Lanes: a to d, cells were obtained from mid-log phase cultures; c, SOS was induced for 1 h with 100 μg of nalidixic acid (nal) per ml; e to g, cells were obtained by scraping transductant colonies from plates 48 h after transduction; h to i, cells were scraped from plates 96 h after transduction. (We have previously reported that *dnaQ polA2* cells are not viable. However, by plating the transductants on minimal agar, transductants were obtained. The lower rate of growth on this type of agar probably diminished the extent of incomplete replication by allowing more time for gap filling.) ND, not determined.

elevated in cells with the $dnaE^+$ $polA^+$ *dnaQ* genotype because the source material for the extract was a heterogeneous population of cells and colonies collected from a transduction plate. As has been reported previously, the transductant colonies were heavily papillated with suppressor (*spq*) strains (17). Figure 1, lane f, shows that a pure suppressor strain gave no evidence of induction. In addition, the source material for Fig. 1, lane e, contained approximately 10^7 untransduced tetracycline-sensitive cells. A control experiment (data not shown) showed that these cells contributed no detectable RecA or LexA. Finally, the fact that these cells were recently transduced did not by itself cause SOS induction, as shown by a control transduction to *bio*::Tn*10* in which the transductant colonies were treated similarly (Fig. 1, lane g).

The *spq-2*-dependent avoidance of SOS induction seen in Fig. 1, lane f, required intact DNA Pol I. When a *polA2 spq-2* recipient cell was transduced to mutant *dnaQ*, the RecA-to-LexA ratio in the collected transductants was again increased (Fig. 1, lane h). Figure 1, lane i, shows that the *polA2* recipient strain used for the experiment depicted in lane h was itself experiencing a degree of SOS induction, but not to the extent observed in the transductants.

TABLE 2. The role of DNA repair in the mutant *dnaQ* phenotype

Genotype (strain)	Tc^r transductants/10^7 PFU[a]
Mismatch repair	
$dnaE^+$ $polA^+$ $mutH^+$ (RM2685)	7,440
$dnaE^+$ *polA2* $mutH^+$ (RM1835)	22
$dnaE^+$ $polA^+$ *mutH* (RM3710)	7,960
$dnaE^+$ *polA2 mutH* (RM3709)	36
Uracil repair[b]	
spq-2 $polA^+$ ung^+ (RM2899)	1,168
spq-2 polA2 ung^+ (RM2902)	7
spq-2 $polA^+$ *ung* (RM3784)	1,268
spq-2 polA2 ung (RM3788)	11

[a] The donor strain was RM978 (*dnaQ201*::Tn*10 spq-2* [17]). The results of single measurements are shown. All of the numbers in excess of 1,000 were obtained by normalizing the numbers of transductants obtained from fewer than 10^7 PFU, either by plating a portion of the transduction mix or by counting only a portion of some plates.

[b] The uracil repair series of experiments was performed with strains encoding a chromosomal *polA2* gene. Strains designated $polA^+$ contain plasmid pCJ100, whereas strains designated *polA2* carry pCJ105 (see Table 1). Strains RM2899 and RM2902 are the ung^+ ancestors of RM3784 and RM3788, respectively.

Taken together, these results indicate that the *spq-2* allele for α, wild-type ε, and wild-type DNA Pol I can each provide some function that helps avoid the exposure of single strands of DNA and consequent SOS induction. The absence of one of these elements does not lead to SOS induction (Fig. 1, lanes b and f) or leads to slight induction (lane i), but the absence of two of these elements potentiates induction (lanes e and h), clearly indicating that the effects of the different elements are additive.

Replication and repair. In normal cells, DNA replication is an essential function, whereas a variety of DNA repair pathways are dispensable. However, it is not difficult to imagine that the physiological importance of repair could change when DNA replication is disrupted by a *dnaQ* mutation. On the one hand, some repair pathway could become essential; on the other hand, a repair pathway could become growth inhibitory if it sequesters some component needed more urgently to carry out replication. We examined the effects of two major cellular repair pathways on the physiology of a *dnaQ* mutant. The results shown in Table 2 indicate that methyl-directed mismatch repair had no effect either on the viability of the *dnaQ* mutant or on its dependence on DNA Pol I for survival. In addition, elimination of uracil repair did not alter the dependence of an *spq-2 dnaQ* mutant on Pol I for survival.

Although these experiments were not an exhaustive survey of the known repair pathways, they examined the pathways that are probably the most active in undamaged cells. The results provide evidence that the limited growth of *dnaQ* mutants does not require repair activity and that it is not the result of an adverse effect of continuing repair activity upon growth due to restricted polymerase availability. Instead, we conclude that the growth defect is most likely independent of repair.

Pol I complementation studies. The requirement for Pol I in *dnaQ* null mutants was originally observed as an ≈100-fold reduction in transducing efficiency of *dnaQ*::Tn*10* when the recipient strain was *polA2* versus $polA^+$ and transductants were selected on rich medium (17). Subsequently, it has become evident that the growth of *polA2 dnaQ* transductants

TABLE 3. Transduction of *dnaQ*::Tn*10* into *polA2* strains

Chromosomal genotype	Complementing polymerase activity	Colony appearance of Tc^r transductants (no. Tc^r colonies/10^8 PFU)[a]
polA2 dnaE$^+$ (*spq*$^+$)	None (no plasmid)	Microscopic[b]
	None (pCJ105)	Microscopic[b]
	Pol I 5'→3' exonuclease (pCJ102)	Microscopic[b]
	Pol I polymerase and 3'→5' exonuclease (pCJ103)	Microscopic[b]
	Pol I polymerase and 5'→3' exonuclease (pCJ145)	Small, papillated (6,320)[b]
	Pol I wild type (pCJ100)	Small, papillated (6,800)[b]
	Rat Pol β (pβL)	Heterogeneous sizes, papillated (4,480)
polA2 spq-2	None (no plasmid)	Microscopic[b]
	None (pCJ105)	Microscopic[b]
	Pol I 5'→3' exonuclease (pCJ102)	Microscopic[b]
	Pol I polymerase and 3'→5' exonuclease (pCJ103)	Normal (6,340)
	Pol I polymerase and 5'→3' exonuclease (pCJ145)	Normal (7,600)
	Pol I wild type (pCJ100)	Normal (4,960)
	Rat Pol β (pβL)	Normal (9,580)

[a] The donor strain was RM978 (*dnaQ201*::Tn*10 spq-2* [17]). The results of single measurements are shown. Colonies were described and counted after 2 days of incubation. Colony appearance was designated normal if it was similar to that obtained with control transductants of Tn*10* in a nonessential gene (e.g., Fig. 2 in reference 17). The appearance was designated microscopic if colonies were difficult to see with the unaided eye. Because of their size, microscopic colonies were not counted, but it was apparent that their numbers were approximately the same as the numbers of more readily visualized transductants seen in experiments in which the *polA2* mutation was effectively complemented. All of the recipient strains were transduced to Tc^r by P22 with similar efficiency (the number of transductants varied by no more than a factor of 2) when the donor strain contained Tn*10* in a nonessential site.

[b] In addition to many microscopic colonies, this transduction produced a small number of colonies (10 to 60 colonies per 10^8 PFU) in which size and appearance were normal.

is sensitive to the composition of the selection medium. The use of minimal medium allows *dnaQ*::Tn*10* to be transduced into *polA2* recipients with approximately the same efficiency as that for *polA*$^+$ recipients. On some batches of rich (Luria-Bertani) medium, *dnaQ*::Tn*10* transductants of *polA2* recipients form microscopically visible colonies at high efficiency in addition to the macroscopically visible colonies formed at low efficiency which we observed previously. This behavior was evident in the experiments presented in this section, in which the requirements for the individual activities of Pol I were examined by introducing F' plasmids containing various *E. coli polA* alleles into *S. typhimurium polA2* strains (*dnaE*$^+$ or *spq-2*) and then observing the outcome of transduction to *dnaQ*::Tn*10* when these merodiploids were used as recipients. The results of these experiments are shown in Table 3.

Requirement for Pol I in a *dnaE*$^+$ strain. The *dnaE*$^+$ *polA2* strains containing pCJ100 (*polA*$^+$) or pCJ145 (5'→3' exonuclease plus polymerase activity) yielded *dnaQ*::Tn*10* transductants roughly as efficiently as did the *polA*$^+$ parent strain carrying no plasmid. The colonies were easily detected with the unaided eye but were smaller when the functional *polA* gene was located on a plasmid than when it was chromosomally located. This difference may indicate plasmid instability in the *dnaE*$^+$ *dnaQ*::Tn*10* genotype. Like transductants of the parent strain, transductants of the complemented *polA2* strain were heavily papillated (i.e., with presumptive *spq* mutants), as expected. Strains lacking any complementing *polA* activity (no plasmid or pCJ105) or containing truncated Pol I polypeptides (pCJ102 [5' exonuclease only] and pCJ103 [Klenow fragment]) generated numerous irregularly shaped microscopic transductants and a few (10 to 60) macroscopically visible colonies, as previewed above. These results suggest that the alleles of *polA* which promote growth require an intact Pol I polypeptide with polymerase activity. The 5'→3' exonuclease activity alone cannot complement the *polA2* allele, and the 3'→5' exonuclease activity appears to be dispensable.

Requirement for Pol I in an *spq-2* strain. When *spq-2 polA2* strains containing the various *E. coli polA* alleles were used as recipients, results similar to those observed with *dnaE*$^+$ *polA2* strains were obtained, with one exception. As before, plasmid pCJ100 (*polA*$^+$) or pCJ145 (deficient in 3'→5' exonuclease) satisfied the requirement for Pol I. In addition, so did plasmid pCJ103 (Klenow fragment). Recipients lacking the complementing activity or harboring pCJ102 (5'→3' exonuclease only) did not satisfy the requirement. These results indicate that Pol I polymerase activity is pivotal to the growth of *dnaQ* mutants whereas the exonuclease activities are not required to complement the *polA2* mutation in an *spq-2* strain (see Discussion).

Rat Pol β complementation studies. To test more directly whether the Pol I requirement in *dnaQ*::Tn*10* strains is targeted at the polymerization activity, the capacity of a eucaryotic polymerase to support growth in *polA2 dnaQ*::Tn*10* strains was determined. We used rat DNA Pol β, which lacks intrinsic nuclease activity (35), because when overexpressed in *E. coli* it can complement the lethality of a *polA*(Ts) mutation in a *recA* mutant background (34). Table 3 shows that when overexpressed in *S. typhimurium*, rat Pol β complemented the *polA2* mutation in *dnaQ*::Tn*10* transductants. In both *dnaE*$^+$ and *spq-2* recipients containing pβL, transduction efficiency to *dnaQ*::Tn*10* was comparable to that obtained in the *polA*$^+$ parent strain carrying no plasmid. In addition, the colony morphology of the transductants exhibited the expected variation with the *dnaE* genotype; in the *spq-2* background, the morphology was wild type, whereas in the *dnaE*$^+$ background colonies were small, irregular, and heavily papillated. These results suggest that *polA2 dnaQ* mutants lack sufficient polymerase activity for growth on rich medium. Moreover, the complementing polymerase activity can function independently of Pol III.

Polymerase activity of the *spq-2* α subunit. We now turn to the other feature found in *dnaQ* mutants that grow normally, namely, the suppressor mutation in *dnaE*. In view of the results described above emphasizing the importance of Pol I

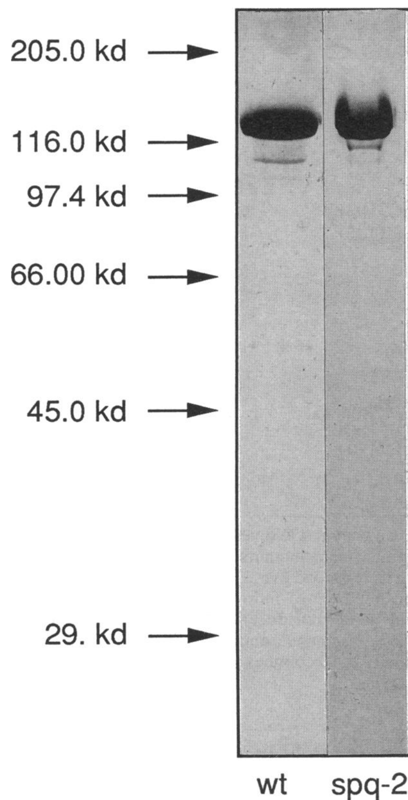
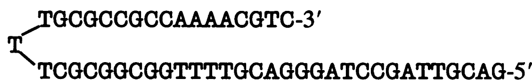


FIG. 2. Purification of wild-type (wt) and *spq-2* α subunits of DNA Pol III. The α subunit was overproduced and purified as described in Materials and Methods. Protein (13.4 μg) was applied to each lane. The principal minor band has been judged to be a proteolytic fragment of α (data not shown).

polymerase activity in supporting normal growth of *dnaQ* mutants and the SOS induction data showing that *polA*$^+$ and *dnaE* (*spq-2*) are similarly effective in avoiding SOS induction, a reasonable hypothesis about *spq-2* would be that it affects the polymerization capacity of Pol III. This hypothesis also dovetails with reconstitution studies showing that ε affects the polymerization activity of α (as well as other of its properties, such as thermal stability). To evaluate whether the *spq-2* mutation mimics either of these effects of ε on α, we determined the levels of intrinsic polymerase activity and thermal stability of purified wild-type and *spq-2* α subunits. Both wild-type α and *spq-2* α were overproduced in the pET expression system and purified to near homogeneity as described in Materials and Methods (Fig. 2). The level of polymerase activity was determined on two different templates: gapped salmon sperm DNA and a snapback oligonucleotide template with a 14-base single-stranded 5′ tail with the following sequence:



The suppressor α subunit exhibited slightly greater levels of activity (three- to fivefold) than the wild-type subunit on both templates. The wild-type α subunit exhibited (in 10^3 U/mg, where 1 U is the incorporation of 1 pmol of nucleotide per min) specific activities of 1,140 and 163 on gapped DNA and snapback DNA, respectively, whereas the *spq-2* α subunit exhibited specific activities of 5,390 and 473, respectively. This is similar to the stimulation of α activity by the ε subunit. The mechanism of this modest stimulation has not been determined. In contrast, the results of thermal-stability assays indicated that the mutation reduces the thermal stability of the α subunit. Figure 3 shows that the mutant α lost approximately 50% of its activity in 8 min at 40°C while the wild-type subunit required 16 min. Consequently, it is unlikely that the *spq-2* mutation stimulates activity by increasing the level of thermal stability of the isolated subunit.

DISCUSSION

Evidence of defective DNA synthesis in *dnaQ*::Tn*10* strains was obtained from analysis of SOS induction. We observed elevated RecA/LexA ratios for two *dnaQ* mutant genotypes: *dnaQ dnaE*$^+$ *polA*$^+$ and *dnaQ dnaE* (*spq-2*) *polA2*. For the first of these genotypes, the extent of elevation was moderate (about fivefold), but this number undoubtedly underestimated the real extent of SOS induction. This is because the sampled population was unavoidably and heavily contaminated with subpopulations containing new *spq* mutations. In these subpopulations, Rec/Lex ratios were not elevated. For the second genotype, the Rec/Lex ratio was greatly elevated over that observed in the wild type, but a portion of this effect was attributable to the *polA2* allele (Fig. 1, lane i). In this background, the change in ratio attributable specifically to *dnaQ* was about sevenfold. Not all *dnaQ* mutant genotypes led to SOS induction. Either a wild-type *dnaE* allele (i.e., not *spq*) or a defective *polA* allele had to be present in addition. Thus, *spq* α and normal Pol I activity levels were physiological antagonists of the loss of ε.

There was a striking correlation between SOS induction and defective growth in the various strains we studied. This correlation could have two explanations: either the same underlying events brought on by the absence of ε lead independently to SOS induction and poor growth or the absence of ε leads to SOS induction which in turn leads to poor growth. In *E. coli*, one of the genes induced during SOS is *sulA*, whose product is an inhibitor of cell division (9). Cells in which *sulA* is expressed chronically are unable to form colonies on plates. A related gene has been identified in *S. typhimurium* (8), suggesting a straightforward way in which SOS induction in *S. typhimurium* could lead to inhibition of growth. If this were the complete story, however, null mutations in *sulA* would be expected to restore the normal growth of *dnaQ* mutants. No such mutations have been found in *S. typhimurium* among suppressors of the *dnaQ* mutant defective-growth phenotype. In addition, an *E. coli sulA* mutant exhibits defective growth when *dnaQ* is deleted, as does an *E. coli lexA3* mutant, in which SOS is noninducible (28a). Thus, the growth defect directly reflects defective DNA metabolism and, more specifically, defective DNA replication, as discussed below. We take SOS induction as an indicator of underlying events, not as the primary cause of the growth phenotype.

The role of DNA repair in the growth phenotype. Both Pol I and Pol III have roles in DNA repair as well as DNA replication. Our experiments show that both Pol I and the *spq-2* allele of *dnaE* are required for normal growth of *dnaQ* mutants, and the demand for each of these enzymes is in a sense intensified compared with the demand observed in wild-type cells. For Pol I, a reduced-activity allele that is fully viable in combination with *dnaQ*$^+$ is nearly inviable in combination with the *dnaQ* mutation in rich medium. (The

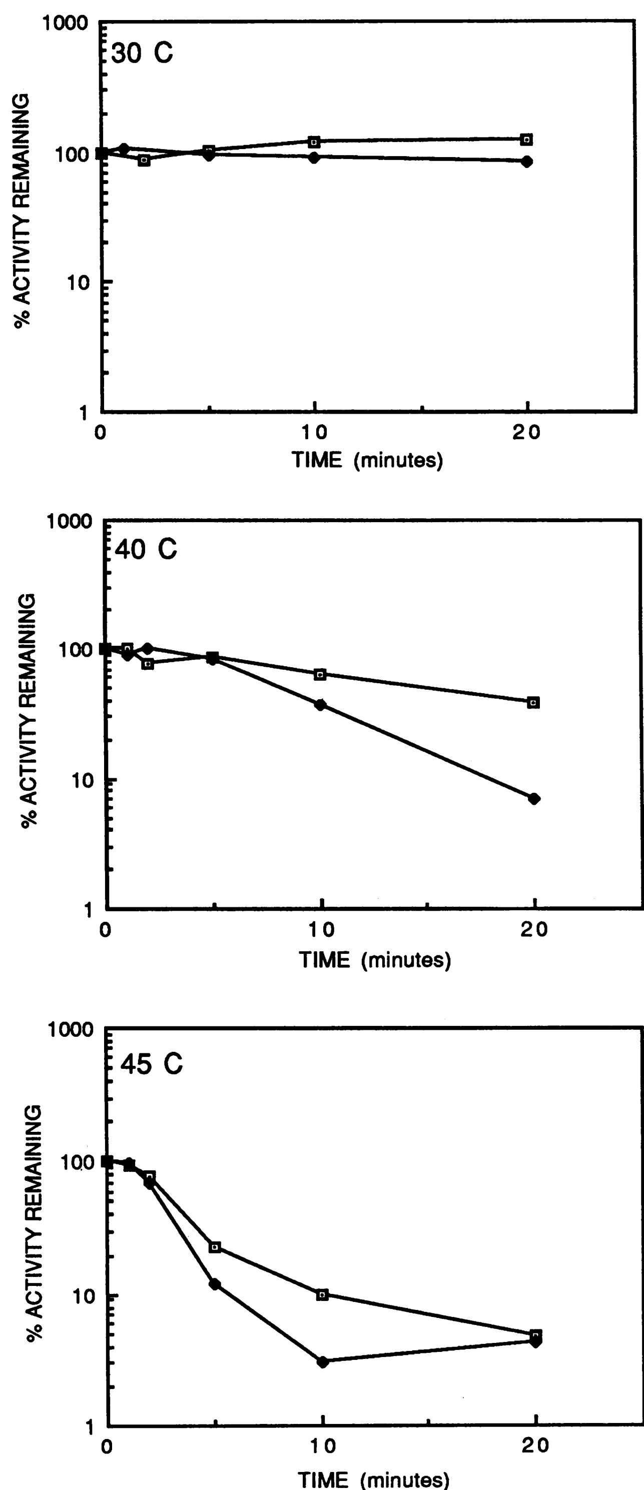


FIG. 3. Thermal stability assays of wild-type and *spq-2* α subunits. Aliquots of each protein, incubated at the indicated temperatures, were withdrawn at various times and assayed for levels of polymerase activity on gapped templates as described in Materials and Methods. Wild-type α, ⊡; *spq-2* α, ◆.

polA2 dnaQ double mutant grows better on minimal medium presumably because the slower growth rate permits sufficient time for the single-stranded gaps in the DNA to be converted to double-stranded DNA.) For Pol III, only an *spq* allele of *dnaE*, not the wild type, yields normal growth in combination with the *dnaQ* mutation. We examined whether, in the face of intensified demand for these enzymes, repair activity would affect the growth phenotype of the *dnaQ* mutant.

The requirement for an altered Pol III α subunit for restoration of normal growth did not appear to be related to methyl-directed mismatch repair. Mismatch repair is targeted to DNA replication errors and in normal cells accounts for the preponderance of repair of such errors (15). This repair process entails a substantial tract of Pol III-dependent synthesis extending, at a minimum, from the site of the replication error to the site of potential methylation, GATC (24). Genetic blockage of mismatch repair at the incision step (the *mutH* mutant) had no effect either on the formation of *dnaQ* mutants or on the growth of their stable (i.e., *spq*) derivatives. Clearly, this mismatch repair pathway is not essential for growth of *dnaQ* mutants. Furthermore, the growth of *dnaQ* mutants was similar whether the genotype was $mutH^+$ or *mutH*, suggesting that any sequestration of Pol III in active mismatch repair complexes in a $mutH^+$ strain was of little consequence.

Similarly, the requirement for Pol I did not appear to be related to repair of uracils in DNA. Uracil in DNA is repaired by cleavage of the N-glycosylic bond to yield an apyrimidinic site (AP site), which is locally excised to a short gap that is filled by Pol I (6, 37). Because of the high rate of incorporation of dUMP in place of dTMP during DNA replication, the repair of uracil is probably the source of the majority of AP site repair in growing cells that have not been deliberately exposed to DNA-damaging treatments. Genetic blockage of uracil repair at the glycosylase step (the *ung* mutant) had no effect on the requirement for Pol I in stable (i.e., *spq*) derivatives of *dnaQ* mutants. Thus, as for mismatch repair, repair of uracils was not essential for normal growth of *dnaQ* mutants and when uracil repair was present, there was no adverse effect on the growth phenotype. There can be little effective sequestration of Pol I into uracil repair activity.

Together, these results provide no evidence of any physiological effect of major DNA repair pathways on the growth of *dnaQ* mutant cells. Although these experiments are not adequate to rule out every possible model involving repair activities, they most simply support the view that the growth defect of *dnaQ* mutant cells is the direct result of defective DNA replication rather than defective repair or interference between replication and repair. This conclusion is further reinforced by the observation that the *dnaQ* mutant genotype is compatible with the *recA* mutation (29), because the *recA* mutant genotype blocks a variety of repair pathways that either involve the RecA protein directly or whose expression is dependent on functional RecA (SOS genes).

Aborted DNA synthesis occurs, presumably, because DNA Pol III holoenzyme lacking the ε subunit is deficient in some aspect(s) of its normal function. Cells compensate for the defective DNA Pol III in at least two ways: by recruiting DNA Pol I to assume a greater role in DNA replication and by acquiring alterations in Pol III α. These observations appear to be unrelated to a *gyrB*-dependent phenomenon, in which Pol I can replace Pol III for DNA replication (3, 17). To derive a coherent understanding of the role played by each of these polymerases in *dnaQ* mutant cells, we have

analyzed both the Pol I requirement and the altered Pol III α subunit in more detail.

The requirement for Pol I. The Pol I requirement was examined both in *dnaE*$^+$ strains and in *spq* mutants, with generally similar results. *dnaQ polA2* mutants in both backgrounds formed microscopic colonies on rich medium, and the triple mutants exhibited chronic SOS induction when growing on minimal medium. It appeared that the chromosomal *polA2* gene provided inadequate polymerase activity, since a eucaryotic DNA polymerase, rat Pol β, which has no intrinsic exonuclease activity (35), was able to complement the *polA2* mutation in either a *dnaE*$^+$ or an *spq-2* strain. The level of polymerase activity of the *polA2* mutant enzyme is approximately 10% of that of the wild-type enzyme (7). The mutant Pol I has 3'→5' exonuclease activity, although it is poorly characterized, while the presence of 5'→3' exonuclease has not been established. Apparently, the exonuclease functions of Pol I either are not required for growth or are provided in sufficient amounts by the *polA2* allele.

Complementation of *spq-2 polA2* mutants by plasmids encoding alleles of *polA* which eliminated one or two Pol I activities produced results fully consistent with the rat Pol β results. Any plasmid encoding a polymerase domain complemented the *polA2* mutation.

A more complex situation was observed with *dnaE*$^+$ strains. Only plasmids encoding a full-length Pol I polypeptide (i.e., the wild-type enzyme and the D355A, E357A mutant) complemented the *polA2* mutation. In contrast, the Klenow fragment, which complemented in an *spq-2* strain, failed to do so in a *dnaE*$^+$ strain. Several alternative explanations may account for this observation. The most obvious is that the *dnaE*$^+$ strain requires the 5'→3' exonuclease in addition to Pol I polymerase. However, the observed requirement for the 5'→3' exonuclease domain does not necessarily imply an involvement of this activity in complementation. The inability of the Klenow fragment to support growth could reflect inadequate synthesis or stability of the truncated enzyme leading to a lower effective level of Pol I polymerase activity in vivo. Although the Klenow fragment has high intrinsic polymerase activity and complements a *polA* null mutation in an otherwise wild-type background, its activity in vivo has been poorly characterized (11).

In addition, there is the possibility that the F' factors bearing the complementing *polA* genes were slightly unstable in the *dnaQ dnaE*$^+$ background. If this instability was exaggerated in the Klenow strain, it would lead to a negative complementation test. We note that in a *dnaQ*$^+$ background, the replication of F is known to depend exclusively on Pol III and was not seen to be affected by *polA2* in our experiments (28, 38).

The role of the suppressor mutation in the α subunit. We initially assumed that the *spq-2* mutation mimics some function normally provided by ε and were guided by previous biochemical investigations of Pol III, which have identified a number of effects of ε apart from editing per se. These include the following: (i) the intrinsic polymerase activity of α is approximately threefold higher in the presence of ε than in its absence (20); (ii) ε (or ε and θ) increases the thermal stability of α (19); (iii) Pol III holoenzyme reconstituted without ε has substantially reduced processivity (32); (iv) compared with α alone, an α-ε complex is better able to form a productive initiation complex with preinitiation proteins (γ complex plus β) bound at a primer terminus (32). Each of these activities is a plausible target of the *spq* suppressor mutation; the first two can be readily analyzed by comparing levels of activity and stability of the purified wild-type and suppressor α subunits. Our experiments do show a slight elevation of polymerization activity in the purified *spq-2* α, similar in magnitude to the increase reported in α activity upon binding to ε. On the other hand, we did not observe an increase in thermal stability of the *spq-2* α (in fact, we saw the opposite), and preliminary data indicate no alteration in the intrinsic processivity of α (data not shown). We did not test initiation complex formation.

Whether the modest increase in α polymerization activity in the *spq-2* mutant determines or is even relevant to the in vivo phenotype requires further investigation. Our observations to date have been obtained with the isolated α subunit rather than a holoenzyme assembly. Other holoenzyme subunits contribute substantially to the activity of wild-type α, and they may do so in a way that overcomes differences in the levels of intrinsic activity of different α subunits. Nonetheless, the apparent increase in the activity of the mutant α is consistent with two (not necessarily mutually exclusive) general mechanisms by which the suppressor mutation could act. In one, the suppressor α subunit could increase the activity of Pol III lacking ε, thus allowing replication to be more nearly complete at the fork. Second, the mutant α could work as a single-subunit enzyme, helping to ensure complete replication by repairing residual gaps in replicated DNA.

In addition, other important hypotheses remain to be tested. For example, the single most significant element providing processivity to the holoenzyme is not the intrinsic processivity of α but the β subunit sliding clamp. This subunit is thought to bind α (16, 33). In the absence of ε, an allosteric effect on α might cause a reduction in its affinity for β, leading to reduction in processivity of the holoenzyme. This could be remedied by a mutation that compensated for the allosteric effect or otherwise increased the affinity of the mutant α for β. This property of the mutant protein would not be revealed by the tests we have performed.

In conclusion, loss of the ε subunit of DNA Pol III holoenzyme appears to severely compromise DNA replication. Mutants lacking ε compensate for the defective holoenzyme by altering the α polymerase subunit of the enzyme and recruiting Pol I to assume a greater role in DNA synthesis. The suppressor mutation in the α subunit does not appear to restore Pol III activity to the wild-type level, since even suppressed strains require an elevated level of Pol I polymerase activity.

ACKNOWLEDGMENTS

We thank Catherine Joyce for providing the *polA* plasmids, Jeffrey Roberts for RecA and LexA antibodies, John Little for LexA antibody, and Joann Sweasy and Lawrence Loeb for pβL. We also thank Steve Slater for helpful discussion.

This work was supported by Public Health Service grants AI19942 and GM39419 from the National Institutes of Health.

REFERENCES

1. **Bochner, B. R., H.-C. Huang, G. L. Schieven, and B. N. Ames.** 1980. Positive selection for loss of tetracycline resistance. J. Bacteriol. **143:**926–933.
2. **Brenowitz, S., S. Kwack, M. Goodman, M. O'Donnell, and H. Echols.** 1991. Specificity and enzymatic mechanism of the editing exonuclease of *E. coli* DNA polymerase III. J. Biol. Chem. **266:**7888–7892.
3. **Bryan, S. K., and R. E. Moses.** 1984. Map location of the *pcbA* mutation and physiology of the mutant. J. Bacteriol. **158:**216–221.
4. **Bullas, L. R., and J.-I. Ryu.** 1983. *Salmonella typhimurium* LT2 strains which are r$^-$ and m$^+$ for all three chromosomally located

systems of DNA restriction and modification. J. Bacteriol. 156:471–474.
5. Derbyshire, V., P. S. Freemont, M. R. Sanderson, L. Beese, J. M. Friedman, C. J. Joyce, and T. A. Steitz. 1988. Genetic and crystallographic studies of the 3′,5′-exonucleolytic site of DNA polymerase I. Science 240:199–201.
6. Duncan, B. K. 1985. Isolation of insertion, deletion, and nonsense mutations of the uracil-DNA glycosylase (*ung*) gene of *Escherichia coli* K-12. J. Bacteriol. 164:689–695.
7. Engler, M. J., and M. J. Bessman. 1979. Characterization of a mutator DNA polymerase I from *Salmonella typhimurium*. Cold Spring Harbor Symp. Quant. Biol. 43:929–935.
8. Freudl, R., G. Braun, N. Honoré, and S. T. Cole. 1987. Evolution of the enterobacterial *sulA* gene: a component of the SOS system encoding an inhibitor of cell division. Gene 52:31–40.
9. Gottesman, S. 1987. Regulation by proteolysis, p. 1308–1312. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
10. Higashitani, N., A. Higashitani, A. Roth, and K. Horiuchi. 1992. SOS induction in *Escherichia coli* by infection with mutant filamentous phage that are defective in initiation of complementary-strand DNA synthesis. J. Bacteriol. 174:1612–1618.
11. Joyce, C. M., and N. D. Grindley. 1984. Method for determining whether a gene of *Escherichia coli* is essential: application to the *polA* gene. J. Bacteriol. 158:636–643.
12. Kornberg, A. 1980. DNA replication. W. H. Freeman & Co., San Francisco.
13. Kukral, A. M., K. L. Strauch, R. A. Maurer, and C. G. Miller. 1987. Genetic analysis in *Salmonella typhimurium* with a small collection of randomly spaced insertions of transposon Tn*10*Δ16Δ17. J. Bacteriol. 169:1787–1793.
14. Kunkel, T. A. 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA 82:488–492.
15. Kushner, S. R. 1987. DNA repair, p. 1044–1053. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
16. Kuwabara, N., and H. Uchida. 1981. Functional cooperation of the *dnaE* and *dnaN* gene products in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 78:5764–5767.
16a. Lancy, E. D. 1990. Ph.D. thesis. Case Western Reserve University, Cleveland, Ohio.
17. Lancy, E. D., M. R. Lifsics, D. G. Kehres, and R. Maurer. 1989. Isolation and characterization of mutants with deletions in *dnaQ*, the gene for the editing subunit of DNA polymerase III in *Salmonella typhimurium*. J. Bacteriol. 171:5572–5580.
18. Lancy, E. D., M. R. Lifsics, P. Munson, and R. Maurer. 1989. Nucleotide sequences of *dnaE*, the gene for the polymerase subunit of DNA polymerase III in *Salmonella typhimurium*, and a variant that facilitates growth in the absence of another polymerase subunit. J. Bacteriol. 171:5581–5586.
19. Maki, H., and A. Kornberg. 1985. The polymerase subunit of DNA polymerase III of *Escherichia coli*. II. Purification of the α subunit, devoid of nuclease activities. J. Biol. Chem. 260:12987–12992.
20. Maki, H., and A. Kornberg. 1987. Proofreading by DNA polymerase III of *Escherichia coli* depends on cooperative interaction of the polymerase and exonuclease subunits. Proc. Natl. Acad. Sci. USA 84:4389–4392.
21. Maurer, R., B. C. Osmond, and D. Botstein. 1984. Genetic analysis of DNA replication in bacteria: *dnaB* mutations that suppress *dnaC* mutations and *dnaQ* mutations that suppress *dnaE* mutations in *Salmonella typhimurium*. Genetics 108:25–38.
22. McHenry, C. S. 1988. DNA polymerase III holoenzyme of *Escherichia coli*. Annu. Rev. Biochem. 57:519–550.
23. Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. Modrich, P. 1991. Mechanisms and biological effects of mismatch repair. Annu. Rev. Genet. 25:229–253.
25. Rayssiguier, C., D. S. Thaler, and M. Radman. 1989. The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch repair mutants. Nature (London) 342:396–400.
26. Sassanfar, M., and J. W. Roberts. 1990. Nature of the SOS-inducing signal in *Escherichia coli*. The involvement of DNA replication. J. Mol. Biol. 212:79–96.
27. Schmieger, H. 1972. Phage P22-mutants with increased or decreased transduction abilities. Mol. Gen. Genet. 119:75–88.
28. Scott, J. R. 1984. Regulation of plasmid replication. Microbiol. Rev. 48:1–23.
28a. Slater, S., and R. Maurer. Unpublished observations.
29. Slater, S. C., and R. Maurer. 1991. Requirements for bypass of UV-induced lesions in single-stranded DNA of bacteriophage φX174 in *Salmonella typhimurium*. Proc. Natl. Acad. Sci. USA 88:1251–1255.
30. Sloane, D. L., M. F. Goodman, and H. Echols. 1988. The fidelity of base selection by the polymerase subunit of DNA polymerase III holoenzyme. Nucleic Acids Res. 16:6465–6475.
31. Studier, F. W., A. H. Rosenberg, J. J. Dunn, and J. W. Dubendorf. 1990. Use of T7 RNA polymerase to direct the expression of cloned genes. Methods Enzymol. 185:60–88.
32. Studwell, P. S., and M. O'Donnell. 1990. Processive replication is contingent on the exonuclease subunit of DNA polymerase III holoenzyme. J. Biol. Chem. 265:1171–1178.
33. Stukenberg, P. T., P. S. Studwell-Vaughan, and M. O'Donnell. 1991. Mechanism of the sliding β-clamp of DNA polymerase III holoenzyme. J. Biol. Chem. 266:11328–11334.
33a. Sweasy, J. Personal communication.
34. Sweasy, J. B., and L. A. Loeb. 1992. Mammalian DNA polymerase beta can substitute for DNA polymerase I during DNA replication in *Escherichia coli*. J. Biol. Chem. 267:1407–1410.
35. Tanabe, K., E. W. Bohn, and S. H. Wilson. 1979. Steady-state kinetics of mouse DNA polymerase β. Biochimie 18:3401–3406.
36. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA 76:4350–4354.
37. Warner, H. R., B. F. Demple, W. A. Deutsch, C. M. Kane, and S. Linn. 1980. Apurinic/apyrimidinic endonucleases in repair of pyrimidine dimers and other lesions in DNA. Proc. Natl. Acad. Sci. USA 77:4602–4606.
38. Willets, N., and R. Skurray. 1987. Structure and function of the F factor and mechanism of conjugation, p. 1110–1133. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
39. Witkin, E. W. 1991. RecA protein in the SOS response: milestones and mysteries. Biochimie 73:133–141.