JOURNAL OF BACTERIOLOGY, Feb. 1994, p. 815–821
0021-9193/94/$04.00+0

Vol. 176, No. 3

holE, the Gene Coding for the θ Subunit of DNA Polymerase III of *Escherichia coli*: Characterization of a *holE* Mutant and Comparison with a *dnaQ* (ε-Subunit) Mutant

STEVEN C. SLATER,[1]† MIRIAM R. LIFSICS,[1] MIKE O'DONNELL,[2,3] AND RUSSELL MAURER[1]*

Department of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106-4960,[1] and Department of Microbiology, Cornell University Medical College,[2] and Howard Hughes Medical Institute,[3] New York, New York 10021

Received 4 October 1993/Accepted 2 December 1993

DNA polymerase III holoenzyme is a multiprotein complex responsible for the bulk of chromosomal replication in *Escherichia coli* and *Salmonella typhimurium*. The catalytic core of the holoenzyme is an αεθ heterotrimer that incorporates both a polymerase subunit (α; *dnaE*) and a proofreading subunit (ε; *dnaQ*). The role of θ is unknown. Here, we describe a null mutation of *holE*, the gene for θ. A strain carrying this mutation was fully viable and displayed no mutant phenotype. In contrast, a *dnaQ* null mutant exhibited poor growth, chronic SOS induction, and an elevated spontaneous mutation rate, like *dnaQ* null mutants of *S. typhimurium* described previously. The poor growth was suppressible by a mutation affecting α which was identical to a suppressor mutation identified in *S. typhimurium*. A double mutant null for both *holE* and *dnaQ* was indistinguishable from the *dnaQ* single mutant. These results show that the θ subunit is dispensable in both *dnaQ*$^+$ and mutant *dnaQ* backgrounds, and that the phenotype of ε mutants cannot be explained on the basis of interference with θ function.

A goal of research on DNA polymerase III (Pol III) holoenzyme is the elucidation of the precise role of each of its 10 or more distinct subunits. Research thus far has identified the α subunit as the polymerase proper (22), the ε subunit as the proofreading exonuclease (34), and the β subunit as a doughnut-shaped clamp that topologically tethers the polymerase to the DNA and makes it highly processive (15, 29, 41). In addition, the τ subunit dimerizes the holoenzyme to allow coordinated leading- and lagging-strand synthesis by twin polymerases (27, 39). Most of the remaining subunits are involved in controlling the assembly of β with the template-primer, but the individual subunit roles in this function are not yet clear.

At least some of the subunits of the holoenzyme are multifunctional. ε, for example, affects the rate, processivity, and thermal stability of the enzyme, not just its fidelity (22, 23, 38). Null mutants affected in ε, obtained in *Salmonella typhimurium*, exhibit both an elevated mutation rate and a severe growth defect (16). These phenotypes are experimentally distinct because the growth defect can be specifically suppressed by mutations affecting α (16, 17). Genetic and biochemical analysis suggests that the ε mutants suffer from insufficient polymerization capacity and that the suppressors increase this capacity (16, 19).

To date, the role of the θ subunit has remained elusive. Early efforts to define its role biochemically were frustrated by the scarcity of the protein and by the difficulty of preparing other core subunits free of θ. Conversely, the lack of θ mutants has prevented genetic analysis. Recently, however, the gene for θ has been identified by reverse genetics (5, 40), making it possible to overproduce and purify θ and begin its characterization. Initial biochemical analysis shows that θ binds tightly to ε, but not to α, and has no detectable effect on the polymerase activity of α or an αε complex. The only activity so far identified for θ is a threefold stimulatory effect on the exonuclease activity of ε on a mispaired primer terminus (40).

Here, we report the construction of the first θ (*holE*) mutation and describe its properties, both as a single mutation and in combination with a null mutation in *dnaQ*.

MATERIALS AND METHODS

Bacterial strains and media. Bacterial strains and plasmids are listed in Table 1. Phage P1*vir* was used for transductions. Bacteria were generally maintained at 37°C on Luria-Bertani (LB) medium (36). Sucrose plates were NaCl-free LB plus 5% sucrose (3). Antibiotics were used at the following concentrations (in micrograms per milliliter): ampicillin, 100; kanamycin, 50; tetracycline, 25; chloramphenicol, 15; rifampin, 100; nalidixate, 50.

Enzymes and reagents. Restriction enzymes were from New England Biolabs, United States Biochemical, or Boehringer Mannheim. Sequenase was from United States Biochemical. GeneScreen Plus hybridization membrane was from Dupont. Radiolabelled nucleotides were from Amersham. Western blots (immunoblots) were performed with a Tropix luminescent labelling kit. SeaKem agarose was from FMC. Constituents of bacteriological media were from Difco. Other reagents were from Sigma.

***holE* constructs.** The *holE* disruption mutants were initially constructed by using plasmid pUC18-θ, which contains *holE* on a 2.7-kb *Eco*RV fragment from Kohara λ clone 336 (40). The plasmid contains a unique *Eag*I site 65 bp downstream of the *holE* initiation codon. It also contains three *Nru*I sites, all within the insert. Site 1 is located approximately 1.7 kb upstream of *holE*, very near the insert-vector junction. Site 2 is within *holE*, 156 bp downstream of the *holE* initiation codon. Site 3 is approximately 270 bp downstream of the *holE* termination codon. The antibiotic marker used for disruption

* Corresponding author. Phone: 216-368-6021. Fax: 216-368-3055. Electronic mail address: ram3@po.cwru.edu.

† Present address: Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138.

TABLE 1. *E. coli* strains and plasmids

Species and strain or plasmid	Relevant characteristic(s)	Source or reference
E. coli[a]		
RM990	Δ(*lac proAB*) *thi rpsL supE endA sbcB15 hsdR4*/F′ *traD36 proAB*$^+$ *lacI*^{q} Δ(*lacZM15*)	25
RM1094	HfrH	25
RM1734 (=MG1655)	F$^-$ wild type	1
RM2541	*E. coli* C-1 (susceptible to phage φX174)	*E. coli* Genetic Stock Center
RM3979	RM1094 *dnaQ903*::*tet*	Allele exchange (pFF596)
RM3980	RM1734 *dnaQ903*::*tet spq-2*	P1 · RM3979 and spontaneous suppressor
RM4006	RM1734 *malE*::Tn*5 lexA3* (Ind$^-$)	This laboratory
RM4021	RM1094 *holE201*::*cat*	Allele exchange (pFF608)
RM4029 (=CAG18580)	RM1734 *zae-3095*::Tn*10kan*	35
RM4030	RM1734 *holE201*::*cat*	P1 · RM4021
RM4074	RM1094 *holE202*::*cat*	Allele exchange (pFF622)
RM4193	RM1734 *holE202*::*cat*	P1 · RM4074
RM4194	RM1734 *dnaQ903*::*tet spq-2 holE202*::*cat*	RM3980 + P1 · RM4193
RM4196	RM1734 *zae-3095*::Tn*10kan spq-2*	RM3980 + P1 · RM4029
RM4221 (=W1485)	F$^+$ wild type	1
Plasmid		
pBIP3	Allele replacement vector	36
pFF441	*mucA′B*	37
pFF498	*mucA′*	37
pFF499	*mucB*	37
pFF588	*dnaQ*	This laboratory
pFF589	*cat*	This laboratory
pFF590	*tet*	This laboratory
pFF596	pBIP3 derivative carrying *dnaQ903*::*tet*	This work
pFF608	pBIP3 derivative carrying *holE201*::*cat*	This work
pFF622	pBIP3 derivative carrying *holE202*::*cat*	This work
pUC18-θ	*holE*$^+$	40

[a] *E. coli* K-12 except as indicated.

(*cat*, chloramphenicol resistance) was derived from pFF589, which is pBluescript IISK$^+$ (Stratagene) carrying *cat* on a *Bam*HI fragment of pJD12 (11). *cat* was excised from pFF589 by digestion with *Eag*I and *Sma*I. Plasmid pUC18-θ was partially digested with *Nru*I, and linearized plasmid was gel purified. This DNA was then digested to completion with *Eag*I and ligated to the *cat* cassette. *Escherichia coli* RM990 was transformed with the ligation products (6). As expected, three classes of recombinants were produced, each one deleting DNA from the *Eag*I site to a different *Nru*I site. In pFF605, *cat* replaced DNA from the *Eag*I site through *Nru*I site 3, while in pFF617 the replacement interval ended at *Nru*I site 2. These alleles were designated *holE201* and *holE202*, respectively. Each disrupted allele was cloned into the polylinker of plasmid pBIP3 (36), and the resulting plasmids (pFF608 carrying *holE201* and pFF622 carrying *holE202*) were used to replace the *E. coli* wild-type allele as described below.

***dnaQ* constructs.** Plasmid pFF588 is a derivative of pBluescript II SK$^+$ that carries *E. coli dnaQ* on a 1.6-kb *Eco*RI fragment subcloned from λRM406, a *dnaQ*$^+$ clone in our collection. Plasmid pFF588 was digested to completion with *Bss*HII and *Mlu*I, deleting most of the 3′ half of *dnaQ*. The vector fragment containing the remainder of *dnaQ* was end filled (31) and gel purified.

Plasmid pFF590 contains the *tet* (tetracycline resistance) gene of Tn*10*) cloned into the *Eco*RI and *Hin*dIII sites of pBluescript IISK$^+$. A fragment containing *tet* was excised from plasmid pFF590 by complete digestion with *Bam*HI and *Hin*dIII, end filled, and gel purified. This fragment was joined with the fragment from plasmid pFF588, and *E. coli* RM990 was transformed with the ligation products. Plasmid pFF593 was isolated from one of the transformants. This plasmid contains the expected replacement of the *dnaQ Bss*HII-*Mlu*I fragment with *tet*. This deletion-insertion mutation, which we designated *dnaQ903*, leaves the *rnh* promoter intact (28) and is very similar to the *dnaQ200* allele previously constructed in *S. typhimurium* (16). The entire insert from pFF593 was cloned into the polylinker of pBIP3 (36), and the resulting plasmid (pFF596) was used to replace the *E. coli* wild-type allele as described below.

Replacement of chromosomal *dnaQ* and *holE* with disrupted alleles. Exchange of the disrupted alleles for the chromosomal wild-type alleles of strain RM1094 was performed by two-step plasmid integration-excision using the method of Slater and Maurer (36). The excision step was carried out in the presence of tetracycline (for the *dnaQ* replacement) or chloramphenicol (for the *holE* replacement) to select for the excision product preserving the disrupted allele.

Mutation frequency analysis. The number of mutation events per culture was estimated from the number of mutants observed in at least 10 independent cultures of each strain, by the method of maximal likelihood as described previously (16, 18). The standard deviation of this estimate was calculated from Fig. 3 of Lea and Coulson (18). The estimate and its associated error were converted to rates shown in Table 2 by division by $2n$, where n is the total number of cells per culture, averaged separately for each strain.

Western blot. Analysis of RecA and LexA protein levels in cell extracts was carried out by quantitative Western blots as described previously (19).

Sequencing the *spq-2* region of *dnaE*. To sequence the region of *E. coli dnaE* corresponding to the location of the *spq-2*

TABLE 2. Rates of mutation to resistance to nalidixic acid or rifampin

Strain	Relevant genotype	Mutations/10^8 cells/generation[a] to resistance to:	
		Nalidixic acid	Rifampin
RM1734	Wild type	0.057 ± 0.007	0.46 ± 0.03
RM4030	*holE201*	0.052 ± 0.006	0.99 ± 0.07
RM4193	*holE202*	0.060 ± 0.006	1.6 ± 0.1
RM4196	*spq-2*	0.093 ± 0.010	0.97 ± 0.06
RM3980	*dnaQ903 spq-2*	11 ± 1	230 ± 20
RM4193	*dnaQ903 spq-2 holE202*	26 ± 2	92 ± 6

[a] Mean ± standard deviation.

mutation in *S. typhimurium* (17), we performed asymmetric PCR (50:1 primer ratio) to produce template DNA including the *spq-2* site. The primers were removed by selective precipitation (26), and the PCR product was sequenced directly (32) by using an internal primer.

Microscopic observation of cells and nucleoids. Strain RM1734 was transduced with a P1 lysate conveying either *dnaQ903* or *holE202*, plated on LB plus tetracycline (*dnaQ903*) or chloramphenicol (*holE202*), and grown at 37°C. After 16 h for *holE202* transductants or 24 h for *dnaQ903* transductants, cells from individual transductant colonies were transferred to a microscope slide and stained by using 1 μg of DAPI (4′,6-diamidino-2-phenylindole) per ml in ethanol-H_2O (1:1). Cells were visualized with combined phase-contrast and fluorescence microscopy on a Zeiss Axiophot microscope and photographed with Kodak T-Max 400 black-and-white film.

Lesion bypass assays. The ability to perform lesion bypass was analyzed by Weigle reactivation of UV-irradiated bacteriophage φX174, essentially as previously described (37). *E. coli* RM2541 (*E. coli* C-1) and its derivatives carrying *dnaQ903* or *holE202* or both mutations were transformed with plasmid pFF441, pFF498, or pFF499 (MucA′B, MucA′, or MucB, respectively). Phage were irradiated, diluted, and plated on the various host strains, which were themselves not irradiated. Plaques were counted after overnight incubation in the dark at 37°C.

RESULTS

Disruption of *holE*. Two different deletion-substitution alleles of *holE* were constructed on plasmids and crossed into the *E. coli* chromosome. *holE201* removed about 70% of *holE*; the deletion extended into uncharacterized 3′ flanking sequence. *holE202* removed about 90 bp completely internal to *holE*. Both alleles encode only the amino-terminal 23 residues of native θ. In both constructions, the deleted material was replaced by a cassette encoding resistance to chloramphenicol. The chromosomal disruptions were obtained initially as chloramphenicol-resistant, sucrose-resistant resolution products derived from a *holE*$^+$/*holE*::*cat* heterodiploid strain in which a pBIP plasmid carrying the *holE* mutation was integrated at *holE*. The *holE*::*cat* resolution products were obtained at high frequency, suggesting that *holE* is dispensable for growth. Moreover, the colonies were indistinguishable from those of the wild type. Southern blot analysis (31) (Fig. 1) confirmed the presence of a disrupted *holE* gene and the absence of an intact *holE* gene in these resolution products. As both *holE201* and *holE202* behaved identically, we used the internal mutation, *holE202*, for the remaining studies. Strains bearing *holE202* made by conventional P1 transduction were indistinguishable from those made by resolution of the integrated pBIP.

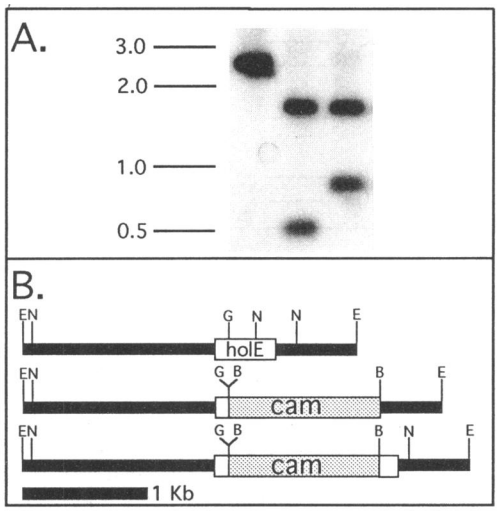

FIG. 1. Chromosomal structure of *holE*. Panel A shows Southern blots of (left to right) RM1094 (*holE*$^+$), RM4021 (*holE201*::*cat*), and RM4074 (*holE202*::*cat*), and the corresponding maps are shown in panel B from top to bottom. The positions and sizes (in kilobase pairs) of marker DNA molecules are shown to the left in panel A. The chromosomal DNAs were digested with *Eco*RV (E) and *Bam*HI (B) and probed with the wild-type *Eco*RV fragment containing *holE*. Other restriction sites indicated are *Nru*I (N) and *Eag*I (G).

Disruption of *dnaQ*. The unremarkable growth of *holE* mutants stands in marked contrast to the distinctive colony morphology of *dnaQ* mutants reported for *S. typhimurium* (16) and therefore suggests that the growth phenotype of *dnaQ* mutants cannot be explained by an associated interference with θ function in Pol III holoenzyme. This conclusion rests on the assumption that the phenotype of an *E. coli* null mutant for *dnaQ* would be similar to that of the analogous *S. typhimurium* mutant. To test this assumption and also to enable construction of double mutants of *E. coli*, we constructed and characterized an *E. coli dnaQ* null mutant. Disruption of *dnaQ* was carried out in the same fashion as disruption of *holE*. The disruption allele, *dnaQ903*::*tet*, was first constructed on a pBIP-derived plasmid which was then integrated at *dnaQ* to form a heterodiploid. *dnaQ*::*tet* resolution products were then selected. As predicted, these colonies were small and irregular and developed numerous sectors after incubation for 48 h. Stable derivatives that formed normal colonies were readily obtained. Identical behavior following P1 transduction of *dnaQ903*::*tet* into a wild-type background was observed. These results mimic those seen in *S. typhimurium dnaQ* mutants, and as shown below, this similarity extends to details of the phenotype. Disruption of the *dnaQ* gene was confirmed by Southern blot analysis (Fig. 2), and no ε was detected by Western blot analysis of an extract from a strain bearing *dnaQ903*::*tet* (8).

Suppressors of *dnaQ903*. Suppressors of *S. typhimurium dnaQ* mutations all map to the *dnaE* region, and two such suppressors, *spq-2* and *spq-16*, have been shown by DNA sequencing to lie within *dnaE* (17, 37a). Transductional analysis using several independently formed *dnaQ903* strains showed that *spq* mutations in *E. coli* also map near *dnaE* (data not shown). Transduction of *dnaQ903* into a *dnaQ*$^+$ *spq* strain yielded normal-looking colonies. In addition, we sequenced a portion of *dnaE* from several independently derived *E. coli spq* mutants. One strain, RM3980, carried a mutation identical to that found in *Salmonella spq-2* (17). Thus, the identical valine-

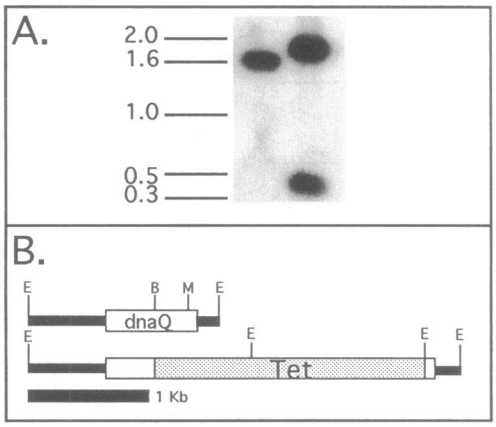

FIG. 2. Chromosomal structure of *dnaQ*. Panel A shows Southern blots of RM1734 (*dnaQ*$^+$; left) and RM3980 (*dnaQ903*::*tet*; right), and the corresponding maps are shown in panel B. The positions and sizes (in kilobase pairs) of marker DNA molecules are shown to the left in panel A. The chromosomal DNAs were digested with *Eco*RI (E) and probed with the wild-type *Eco*RI fragment containing *dnaQ*. Other restriction sites indicated are *Bst*EII (B) and *Mlu*I (M).

to-glycine substitution at residue 832 of α arises in *dnaQ* mutants of both *E. coli* and *S. typhimurium*.

Mutation rate and growth rate. Disruption of *dnaQ* produced a highly elevated spontaneous mutation rate in *E. coli* just as it did in *S. typhimurium* (Table 2). In contrast, disruption of *holE* had no detectable effect on mutation rate, as judged from forward mutation to nalidixic acid resistance, and only a slight increase in mutation rate as judged from forward mutation to rifampin resistance. Both the *dnaQ903 spq-2* mutant and the *holE202* mutant grew at rates similar to that of their wild-type parent strain (data not shown). *holE202* had a slight, paradoxical effect on the *dnaQ903* mutant, increasing the rate of mutation to Nal^r and decreasing the rate of mutation to Rif^r.

Cell and nucleoid morphology. Figure 3 illustrates the microscopic appearance of various DAPI-stained *dnaQ903* and *holE202* strains. Freshly formed (i.e., unsuppressed) *dnaQ903* strains were highly filamented (Fig. 3C), a reaction typical of SOS induction following disruption of DNA synthesis. Although analysis of RecA and LexA protein levels (below) showed that these strains were indeed induced for SOS, the

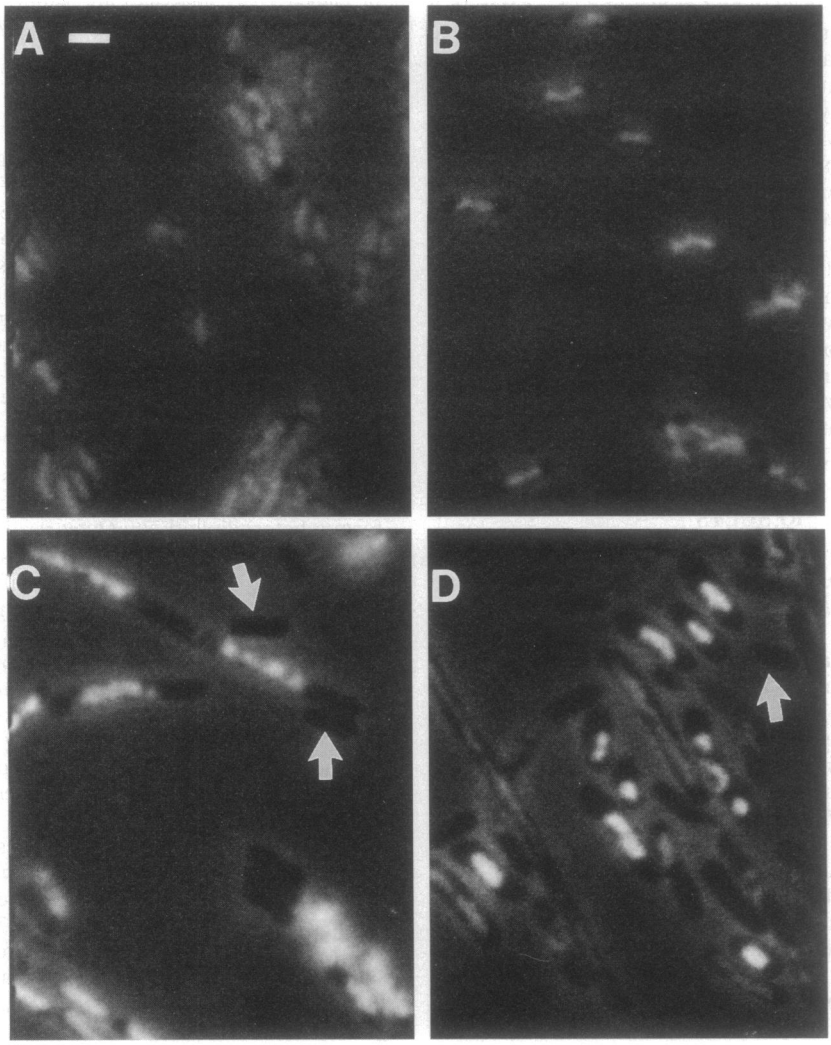

FIG. 3. DAPI staining of strain RM1734 (wild type [A]), fresh transductants of strain RM1734 with *holE202*::*cat* (B) or *dnaQ903*::*tet* (C), and fresh transductants of a *lexA3* derivative of strain RM1734 with *dnaQ903*::*tet* (D). The bar in panel A represents 1 μm. Arrows (C and D) indicate anucleate cells.

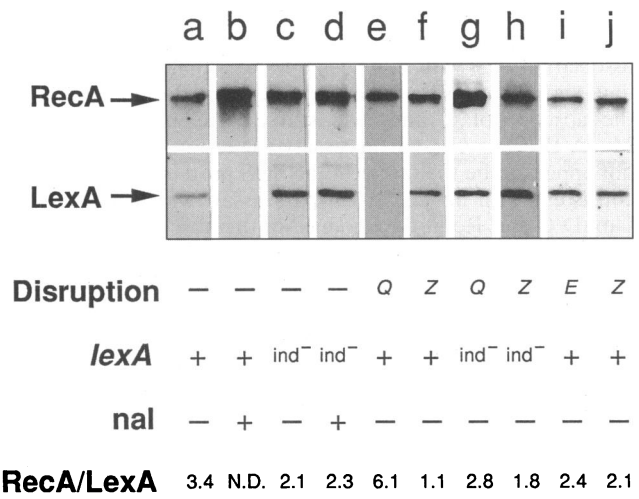

FIG. 4. SOS induction in *holE* and *dnaQ* null mutants. Western blots were prepared and quantitated as described elsewhere (19). The figure is a composite showing only the RecA and LexA bands. The lane chosen to illustrate each genotype was from the linear-response range for that genotype. Since the amount of sample loaded in each lane is unique, the intensity of a band in one lane is not directly comparable with its intensity in another lane. The ratio of the intensity of RecA to that of LexA (bottom line) is independent of the concentration of the sample. We noted that the *lexA3* gene product exhibited reduced mobility relative to wild-type LexA on sodium dodecyl sulfate-polyacrylamide gels as described by Little and Harper (20); however, in the composite all the LexA bands have been aligned for simplicity. Lanes: a to d, cells were obtained from mid-log-phase cultures; b and d, SOS was induced for 1 h with 100 μg of nalidixic acid (nal) per ml. In the experiments shown in lanes e to j, pBIP3-derived heteroallelic diploids for *lacZ*, *dnaQ*, or *holE* were plated under conditions (i.e., sucrose plus the appropriate drug [36]) that allow colony formation only by the haploid resolution product retaining the disrupted allele. Cell extracts were prepared from colonies collected after 48 h of growth on the plates. N.D., not determined; Q, *dnaQ*; Z, *lacZ*; E, *holE*; ind⁻, *lexA3*.

filamentation was SOS independent because it occurred equally in *lexA3* (Fig. 3D) or *sulA* (not shown) strain backgrounds, in which SOS-dependent filamentation is suppressed. Filamentation was accompanied by nucleoid irregularities. Nucleoids were heterogeneous in size, number per cell, and degree of condensation, and they were positioned irregularly, often bunching together near the cell center. In addition, anucleate cells were apparent (Fig. 3C and D, arrows).

In contrast, *holE202* cells (Fig. 3B) were indistinguishable from the wild type (Fig. 3A), and they exhibited normal nucleoid staining patterns. No anucleate cells were seen.

SOS induction. Upon disruption of *dnaQ* in wild-type *S. typhimurium*, the SOS regulon becomes induced, as judged from an increase in the ratio of RecA to LexA in cell extracts (19, 33). The presence of an *spq* mutation is sufficient to block this induction (19). Figure 4 summarizes the ratio of RecA to LexA in cell extracts of various *E. coli dnaQ* and *holE* mutants. As in *S. typhimurium*, disruption of *dnaQ* in wild-type *E. coli* induced SOS (Fig. 4, lane e). That this induction occurred through the authentic SOS pathway was proven by the lack of induction in the *lexA3* mutant host (Fig. 4, lane g). In contrast, disruption of *holE* did not induce SOS (Fig. 4, lane i). In control experiments, the ratio of RecA to LexA behaved as expected in response to the SOS inducer nalidixic acid in inducible and noninducible genetic backgrounds (Fig. 4, lanes b and d). Moreover, disruption of *lacZ* by the same method used to disrupt *dnaQ* or *holE* did not induce SOS (Fig. 4, lanes f, h, and j).

TABLE 3. Reactivation of UV-irradiated bacteriophage φX174 in *holE* and *dnaQ* mutants

Chromosomal genotype[a]	Plasmid *muc* gene(s)	Slope ± SD[b] (m^2 J^{-1})
Wild type	None	−0.0337 ± 0.0002
Wild type	*A'B*	−0.0251 ± 0.0015
holE202	None	−0.0339 ± 0.005
holE202	*A'B*	−0.0256 ± 0.0018
holE202	*A'*	−0.0341
holE202	*B*	−0.0334
holE202 recA56	*A'B*	−0.0333
dnaQ903	None	−0.0344 ± 0.0005
dnaQ903	*A'B*	−0.0236 ± 0.0006
dnaQ903	*A'*	−0.0347
dnaQ903	*B*	−0.0351
holE202 dnaQ903	None	−0.0350 ± 0.0008
holE202 dnaQ903	*A'B*	−0.0252 ± 0.0013
holE202 dnaQ903	*A'*	−0.0323
holE202 dnaQ903	*B*	−0.0345

[a] The wild type is RM2541, and all the mutants were derived from RM2541 by P1 transduction. Plasmids were added last. All *dnaQ903* strains presumably carry an (uncharacterized) suppressor mutation. Since the *holE dnaQ* double mutant was made by introducing the *holE* mutation into the *dnaQ* mutant strain, the suppressor mutation should be preserved.

[b] Slope of a best-fit line for log(fractional survival) versus UV dose to the phage. Where a standard deviation for the slope is shown, the slope was determined three times by using independently irradiated phage stocks and independent bacterial cultures; otherwise, the slope was determined once.

Template lesion bypass. SOS-mediated template lesion bypass is an error-prone DNA repair pathway that requires *dnaE*, *recA*, and either *umuDC* or *mucAB*. The *dnaE* requirement suggests that RecA and/or MucA'B modify Pol III to allow polymerization opposite a template lesion, but the mechanism of lesion bypass remains enigmatic. Using reactivation of bacteriophage φX174 as an assay, we have shown that Δ*dnaQ* mutants of *S. typhimurium* perform lesion bypass (33). We have extended these studies to include *holE* and *dnaQ* mutants of *E. coli*.

In all of the phage reactivation experiments, plots of log (surviving fraction) versus dose to the phage were clearly linear (five datum points; correlation coefficient > 0.99 in all cases). Therefore, we have reported the data as the slope (Table 3). Any two conditions can be compared by calculating the repair sector (37), given by 1 − (slope J/slope K), which estimates the fraction of lethal damage observed under condition K that is repaired under condition J. For example, addition of pFF441 (MucA'B) to the wild-type strain, RM2541, changed the slope from −0.0337 to −0.0251. The corresponding repair sector is 0.25, very similar to that observed previously for MucA'B in *S. typhimurium* (37). The data in the remainder of Table 3 demonstrate that *holE* and *dnaQ* mutations, either individually or in combination, did not alter lesion bypass in *E. coli*. Moreover, removal of *holE* from Pol III did not negate the requirement for *mucA*, *mucB*, or *recA* in lesion bypass.

DISCUSSION

ε and θ are subunits of the Pol III catalytic core with dramatically different effects on polymerase function. Loss of ε leads to sufficient disruption of DNA synthesis to induce SOS, interfere with cell division, and cause segregational abnormalities. In addition, loss of ε leads to reduction in fidelity, presumably because of a lack of proofreading. In contrast, loss of θ leads to none of these phenotypes, and in fact we have

been unable to detect any consequences resulting from loss of θ.

The strongest subunit-subunit interactions between Pol III subunits have been identified through gel filtration analysis. This kind of analysis showed that α binds to ε and ε binds to θ, but no binding of α to θ was detected in the absence of ε (40). Whether this result would change in the presence of other holoenzyme subunits has not been determined. Nonetheless, these results raised the possibility that θ needs ε to carry out its function in the polymerase. Hence, holoenzyme molecules in a *dnaQ* null mutant may lack θ function, if not the θ subunit per se. The results presented here indicate that the phenotype of a *dnaQ* null mutation must be attributed to the lack of ε, not to the absence of θ function, because the *holE* null mutation produces no aspect of the *dnaQ* mutant phenotype.

Initial experiments with purified θ demonstrated that θ stimulates the exonuclease activity of ε on a mispaired (G · T) substrate approximately threefold (40). We did not detect a consistent mutator effect in the θ null mutant. One possible explanation for this difference is that the mutational assay detects a broad spectrum of base substitutions, whereas the exonuclease assay used a single substrate. If θ regulates the excision of different mispairs differentially, the effect of θ on the aggregate mutation frequency in vivo might vary in different assays depending on the particular spectrum of mutations that score in each assay. Thus, the present data cannot rule out a possible role for θ in correcting some types of errors. Alternatively, the simple εθ complex may have properties that do not carry over to the holoenzyme, the complex that presumably is more similar to that operating in vivo.

From the data in Table 2, it also appears that θ influences the mutation rate of a *dnaQ* null mutant. This result would not be expected if θ acted simply by modulating the activity of ε, and it suggests the possibility for another mode of action for θ. However, this suggestion should be treated cautiously as the effects are small (2.5-fold) and paradoxical, and mutation rate determinations are subject to several artifacts and assumptions. The presence of θ in ε^- holoenzyme has not been demonstrated.

There is conflicting evidence on the question of a role for θ in polymerase dimerization. Binary αε complexes show no tendency to dimerize, whereas core complexes have been reported to dimerize at high concentration, implicating a role for θ (24). However, this result was not confirmed with αεθ complexes reconstituted from purified subunits, and purified θ shows no tendency to dimerize (40). Dimerization itself is thought to be important for the correct functioning of the replication fork, in particular, to provide the protein-protein contacts needed to retain the lagging-strand polymerase at the fork as it cycles from a completed Okazaki fragment to the next Okazaki fragment primer. If θ does contribute to dimerization, its contribution must be minor, at least in a wild-type background, because the loss of this contribution has no detectable effect on growth. In contrast, the other dimerization agent, the τ subunit (39), is essential for growth (2).

We also tested for a role for θ in translesion bypass synthesis (SOS repair). It has long been thought that such synthesis required a modulation of Pol III activity by some combination of RecA and UmuD'C (or analogous plasmid-encoded proteins, MucA'B). The involvement of Pol III in SOS repair or in the closely related reaction SOS mutagenesis is supported by both genetic (10) and biochemical (30) evidence. It has been hypothesized that polymerase editing would interfere with lesion bypass (7, 21, 42), and indeed, overproduction of ε does inhibit SOS mutagenesis (9, 14), but the absence of ε clearly is not sufficient for lesion bypass, nor does it relieve the requirement for SOS proteins (30, 37). The precise target within Pol III for SOS proteins remains unknown. Given the intimate association of θ with ε, θ seemed like a plausible target. Our data show that lesion bypass can be carried out effectively in the absence of θ, and it is still dependent on RecA and MucA'B. Thus θ is not necessary for lesion bypass and does not appear to be the target of the SOS proteins. This finding is consistent with an in vitro bypass assay, which also exhibits UmuD'C and RecA dependence in the absence of θ (30).

The function of θ, if any, is apparently quite subtle. The idea that θ is necessary for the correct operation of some other polymerase subunit during chromosomal replication can be ruled out in almost all cases because mutations of the other subunit genes lead to a distinctive mutant phenotype. Genes for α, β, δ, δ', and τ are essential (2, 4a, 27); the genes for ε and χ are dispensable, but mutants grow slowly (16, 37a). Only the gene for γ can be eliminated without untoward effect, and it has not been ruled out in this case that γ could be produced intracellularly by proteolysis of τ (2). Mutants with mutations in ψ are not yet available. It remains possible that θ makes some difference when another polymerase subunit is partially active, or when Pol III participates in some nonessential activity such as mismatch repair or recombination. Also, our data do not rule out the possibility that *E. coli* possesses a functionally redundant gene whose product substitutes for θ in a *holE* mutant. Finally, although it seems unlikely, we cannot rule out the possibility that the 23-residue amino-terminal fragment of θ, encoded by both *holE201* and *holE202*, has residual activity.

Our studies on *E. coli dnaQ* null mutants provide additional insight into the physiology of ε^- strains. From a previous study, it was known that freshly made *dnaQ* mutants undergo SOS induction which continues until an *spq* suppressor mutation develops (19). It was unclear whether SOS was necessary for cell survival in the $dnaE^+$ background. Here we have shown that a *dnaQ* mutation can be transduced into a *lexA3* genetic background, in which SOS induction does not take place. In this background, the growth of such transductants was feeble and suppressors of the growth phenotype were favored. Thus, SOS induction was not needed for survival, nor was it the cause of the poor growth phenotype.

Freshly-transduced *dnaQ903* mutants were highly filamented, even in a *lexA3* strain. SOS-independent filamentation is generally observed upon exposure of *E. coli* to replication-inhibiting treatments or after growth of temperature-sensitive replication mutants at restrictive temperature (4, 12, 13). Whereas termination of chromosomal replication and cell division are normally tightly coupled, SOS-independent filamentation results in uncoupling of these processes and production of numerous anucleate cells (13). It appears that unsuppressed *dnaQ* mutants also induce this SOS-independent filamentation pathway.

ACKNOWLEDGMENTS

This work was supported by grant NP-810 from the American Cancer Society to R.M. and by Public Health Service grant GM38839 from the National Institutes of Health to M.O.

We thank Nancy Kleckner, in whose laboratory a portion of this work was completed.

REFERENCES

1. **Bachmann, B. J.** 1987. Derivations and genotypes of some mutant derivatives of *Escherichia coli* K-12, p. 1190–1219. *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, vol. 2. American Society for Microbiology, Washington, D.C.

2. **Blinkova, A., C. Hervas, P. T. Stukenberg, R. Onrust, M. E. O'Donnell, and J. R. Walker.** 1993. The *Escherichia coli* DNA polymerase III holoenzyme contains both products of the *dnaX* gene, τ and γ, but only τ is essential. J. Bacteriol. **175:**6018–6027.
3. **Blomfield, I. C., V. Vaughn, R. F. Rest, and B. I. Eisenstein.** 1991. Allelic exchange in *Escherichia coli* using the *Bacillus subtilis sacB* gene and a temperature-sensitive pSC101 replicon. Mol. Microbiol. **5:**1447–1457.
4. **Burton, P. B., and I. B. Holland.** 1983. Two pathways of division inhibition in UV-irradiated *E. coli*. Mol. Gen. Genet. **190:**128–132.

4a. **Carter, J.** Personal communication.

5. **Carter, J. R., M. A. Franden, R. Aebersold, D. R. Kim, and C. S. McHenry.** 1993. Isolation, sequencing and overexpression of the gene encoding the θ subunit of DNA polymerase III holoenzyme. Nucleic Acids Res. **21:**3281–3286.
6. **Chung, C. T., S. L. Niemela, and R. H. Miller.** 1989. One-step preparation of competent *Escherichia coli*: transformation and storage of bacterial cells in the same solution. Proc. Natl. Acad. Sci. USA **86:**2172–2175.
7. **Fersht, A. R., and J. W. Knill-Jones.** 1983. Contribution of 3′ to 5′ exonuclease activity of DNA polymerase III holoenzyme from *Escherichia coli* to specificity. J. Mol. Biol. **165:**669–682.
8. **Foster, P. L., and M. G. Marinus.** 1992. Levels of epsilon, an essential replication subunit of *Escherichia coli* DNA polymerase III, are controlled by heat shock proteins. J. Bacteriol. **174:**7509–7516.
9. **Foster, P. L., A. D. Sullivan, and S. B. Franklin.** 1989. Presence of the *dnaQ-rnh* divergent transcriptional unit on a multicopy plasmid inhibits induced mutagenesis in *Escherichia coli*. J. Bacteriol. **171:**3144–3151.
10. **Hagensee, M. E., T. Timme, S. K. Bryan, and R. E. Moses.** 1987. DNA polymerase III of *Escherichia coli* is required for UV and ethyl methanesulfonate mutagenesis. Proc. Natl. Acad. Sci. USA **84:**4195–4199.
11. **Heath, J. D., J. D. Perkins, B. Sharma, and G. M. Weinstock.** 1992. *Not*I genomic cleavage map of *Escherichia coli* K-12 strain MG1655. J. Bacteriol. **174:**558–567.
12. **Huisman, O., R. D'Ari, and J. George.** 1980. Inducible *sfi*-dependent division inhibition in *Escherichia coli*. Mol. Gen. Genet. **177:**629–636.
13. **Jaffé, A., R. D'Ari, and V. Norris.** 1986. SOS-independent coupling between DNA replication and cell division in *Escherichia coli*. J. Bacteriol. **165:**66–71.
14. **Jonczyk, P., I. Fijalkowska, and Z. Ciesla.** 1988. Overproduction of the epsilon subunit of DNA polymerase III counteracts the SOS mutagenic response of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **85:**9124–9127.
15. **Kong, W.-P., R. Onrust, M. O'Donnell, and J. Kuriyan.** 1992. Three-dimensional structure of the beta subunit of the *E. coli* DNA polymerase III holoenzyme: a sliding DNA clamp. Cell **69:**425–437.
16. **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.
17. **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.
18. **Lea, D. E., and C. A. Coulson.** 1949. The distribution of the numbers of mutants in bacterial cultures. J. Genet. **49:**264–285.
19. **Lifsics, M. R., E. D. Lancy, Jr., and R. Maurer.** 1992. DNA replication defect in *Salmonella typhimurium* mutants lacking the editing (ε) subunit of DNA polymerase III. J. Bacteriol. **174:**6965–6973.
20. **Little, J. W., and J. E. Harper.** 1979. Identification of the *lexA* gene product of *Escherichia coli K-12*. Proc. Natl. Acad. Sci. USA **76:**6147–6151.
21. **Lu, C., R. H. Scheuermann, and H. Echols.** 1986. Capacity of RecA protein to bind preferentially to UV lesions and inhibit the editing subunit (epsilon) of DNA polymerase III: a possible mechanism for SOS-induced targeted mutagenesis. Proc. Natl. Acad. Sci. USA **83:**619–623.
22. **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.
23. **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.
24. **Maki, H., S. Maki, and A. Kornberg.** 1988. DNA polymerase III holoenzyme of *Escherichia coli*. IV. The holoenzyme as an asymmetric dimer with twin active sites. J. Biol. Chem. **263:**6570–6578.
25. **Maurer, R., and A. Wong.** 1988. Dominant lethal mutations in the *dnaB* helicase gene of *Salmonella typhimurium*. J. Bacteriol. **170:**3682–3688.
26. **McCabe, P. C.** 1990. Production of single-stranded DNA by asymmetric PCR, p. 76–83. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR protocols, a guide to methods and applications. Academic Press, San Diego, Calif.
27. **McHenry, C. S.** 1988. DNA polymerase III holoenzyme of *Escherichia coli*. Annu. Rev. Biochem. **57:**519–550.
28. **Nomura, T., H. Aiba, and A. Ishihama.** 1985. Transcriptional organization of the convergent overlapping *dnaQ-rnh* genes of *Escherichia coli*. J. Biol. Chem. **260:**7122–7125.
29. **O'Donnell, M., J. Kuriyan, X.-P. Kong, P. T. Stukenberg, and R. Onrust.** 1992. The sliding clamp of DNA polymerase III encircles DNA. Mol. Biol. Cell **3:**953–957.
30. **Rajagopalan, M., C. Lu, R. Woodgate, M. O'Donnell, M. F. Goodman, and H. Echols.** 1992. Activity of the purified mutagenesis proteins UmuC, UmuD′, and RecA in replicative bypass of an abasic DNA lesion by DNA polymerase III. Proc. Natl. Acad. Sci. USA **89:**10777–10781.
31. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
32. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
33. **Sassanfar, M., and J. W. Roberts.** 1990. Nature of the SOS-inducing signal in *E. coli*: the involvement of DNA replication. J. Mol. Biol. **212:**79–96.
34. **Scheuermann, R. H., and H. Echols.** 1984. A separate editing exonuclease for DNA replication: the ε subunit of *Escherichia coli* DNA polymerase III holoenzyme. Proc. Natl. Acad. Sci. USA **81:**7747–7751.
35. **Singer, M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel, W. Dove, K. J. Jaacks, A. D. Grossman, J. W. Erickson, and C. A. Gross.** 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. **53:**1–24.
36. **Slater, S., and R. Maurer.** 1993. Simple phagemid-based system for generating allele replacements in *Escherichia coli*. J. Bacteriol. **175:**4260–4262.
37. **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.

37a. **Slater, S. C., and R. Maurer.** Unpublished data.

38. **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.
39. **Studwell-Vaughan, P. S., and M. O'Donnell.** 1991. Constitution of the twin polymerase of DNA polymerase III holoenzyme. J. Biol. Chem. **266:**19833–19841.
40. **Studwell-Vaughan, P. S., and M. O'Donnell.** 1993. DNA polymerase III accessory proteins. V. θ encoded by *holE*. J. Biol. Chem. **268:**11785–11791.
41. **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.
42. **Villani, G., S. Boiteux, and M. Radman.** 1978. Mechanism of ultraviolet-induced mutagenesis: extent and fidelity of in vitro DNA synthesis on irradiated templates. Proc. Natl. Acad. Sci. USA **75:**3037–3041.