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Identification of the ε-subunit of *Escherichia coli* DNA polymerase III holoenzyme as the *dnaQ* gene product: A fidelity subunit for DNA replication

(mutator mutation/*mutD* gene/*dnaE* gene/exonucleolytic editing)

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ABSTRACT **Based on extensive genetic and biochemical studies, the multisubunit DNA polymerase III holoenzyme is considered responsible for the chain-elongation stage in replication of the genome of *Escherichia coli* and is thus expected to be the major determinant of fidelity as well. Previous experiments have shown that two mutations conferring a very high mutation rate on *E. coli*, *mutD5* and *dnaQ49*, decrease severely the 3′→5′ exonucleolytic editing activity of the polymerase III holoenzyme. To identify more precisely the nature of these mutations, we have carried out genetic mapping and complementation experiments. From these studies and experiments by others, we conclude that the most potent general mutator mutations in *E. coli* occur in a single gene, *dnaQ*. To define further the role of the *dnaQ* gene, we have used two-dimensional gel electrophoresis to compare the labeled *dnaQ* gene product with purified polymerase III holoenzyme. The *dnaQ* product comigrates with the ε-subunit, a 25-kilodalton protein of the polymerase III "core" enzyme. We conclude that the ε-subunit of polymerase III holoenzyme has a special role in defining the accuracy of DNA replication, probably through control of the 3′→5′ exonuclease activity.**

The duplication of a genome is an extremely accurate process; mutation rates are typically $\approx 10^{-9}$ to 10^{-10} per base replicated (1). This impressive fidelity factor for DNA replication is interesting from two points of view. First, how does the replication machinery achieve the extraordinarily low mistake level usually observed, given the rather similar structure and energetics of correct and incorrect base pairs? Second, does this "spontaneous" mutation rate represent a constant baseline or is mutation rate subject to environmental control? Echols (2, 3), Lark *et al.* (4), and Wills (5) have recently presented some biological arguments that a control mechanism might exist to provide for an increased mutation rate in a highly stressful environment.

The high fidelity of DNA replication is thought to be achieved by a multistage mechanism: (*i*) base selection in the original incorporation of the complementary nucleotide; (*ii*) exonucleolytic editing of the newly added base; and (*iii*) postreplicative scanning of the DNA for mismatched bases. The summation of these processes can yield the observed accuracy of genome duplication (3, 6, 7). For *Escherichia coli*, the major determinant of replication fidelity is likely to be the polymerase III (pol III) holoenzyme because this multisubunit enzyme in considered to be responsible for elongation of DNA chains during chromosome duplication (6). For this reason, we have focused our study of replication fidelity on this enzyme.

To search for *E. coli* proteins with a major role in defining the accuracy of DNA replication, we have studied mutations that greatly increase the mutation rate for all types of mutations (general "mutator" mutations). We have previously shown that two such mutations, *mutD5* (8) and *dnaQ49* (9), lead to a defective 3′→5′ exonuclease activity for polymerase III holoenzyme (10). This work has raised two questions. Do these two mutations and other high level mutators affect the same gene, indicating a possible fidelity gene? If so, is the gene product a subunit of polymerase III holoenzyme? In the work reported here, we present evidence that a single gene, *dnaQ*, is the major site of high level mutators and that the *dnaQ* gene product is the ε-subunit of polymerase III holoenzyme. Because the ε-subunit probably does not itself carry the 3′→5′ exonuclease activity of polymerase III, ε may be a regulatory subunit with a special role in fidelity.

MATERIALS AND METHODS

Bacterial Strains. The *E. coli* strains used and their relevant markers are LE30*mutD5* (8), KH1116*dnaQ49* (9), SG175*dnaE175* and SG902*dnaE902* (11), CD4*metD88proA3* (9), and R5230, a *recA*$^-$ derivative of AD5230 (λ*N7N53cI857ΔcII-J*) (12).

Materials. M9 minimal medium and LB broth were the standard recipes (13). The minimal medium was supplemented with 0.2% glucose/vitamin B1 (20 mg/liter)/amino acids (20 mg/liter). Where needed, antibiotics were added as follows: tetracycline (10 mg/liter), ampicillin (50 mg/liter), rifampicin (100 mg/liter), and chloramphenicol (20 mg/liter). Solid medium contained agar at 15 g/liter. Antibiotics were from Sigma. Ampholines pH3–10 and pH5–7 were from LKB. [^{35}S]Methionine and ^{35}S-labeled protein molecular weight standards were from New England Nuclear.

Enzymes. Restriction enzymes were from New England BioLabs. Polymerase III holoenzyme was prepared as described (14).

Transduction Experiments. Growth of phage Plkc and transduction experiments were carried out at 30°C as described (13). To prepare more isogeneic mutator strains, the *mutD5*, *dnaQ49*, and *dnaE902* alleles were transduced from their original strains to CD4. After selection for *proA*$^+$*metD*$^+$ recombinants, the recipient cells were scored for mutator phenotype by plating 0.1 ml of an overnight culture ($\approx 4 \times 10^8$ cells) on LB plates with rifampicin. The resultant strains, TAM12*mutD5*, TAM21*dnaQ49* and TAM30*dnaE902*, were used in the mapping and complementation experiments. For mapping, TAM12, TAM21, and

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Abbreviations: kb, kilobase(s); kDa, kilodalton(s); pol III, polymerase III.

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TAM30 were used as donors and CD4 as recipient. Selection was for either $proA^+$ or $metD^+$, and the presence or absence of the mutator allele was scored on LB plates with rifampicin as noted above. About 150 isolated recombinant colonies were scored for mutator phenotype for each mutator allele mapped. A culture from a mut^+ transductant gave ≤5 rifampicin-resistant colonies per plate, whereas *mutD5* gave >1,000, *dnaQ49* gave ≥200, and *dnaE902* gave ≥50 colonies per plate.

Preparation of Plasmid DNA and Transformation. For cloning experiments, plasmid DNA was isolated by the procedure of Clewell and Helinski (15) and then sedimented to equilibrium in a CsCl/ethidium bromide density gradient. Alternatively, plasmid DNA for restriction analysis was extracted by the alkaline miniscreen procedure of Birnboim and Doly (16). Bacteria were transformed with plasmid DNA by a modification of the Hanahan procedure (17).

Complementation Tests. The TAM12, TAM21, and TAM30 mutator strains were transformed by plasmid DNA carrying the wild-type *dnaQ* gene (pNS121) or *dnaE* gene (pMWE303) (18), with selection for tetracycline or ampicillin, respectively. A purified, rifampicin-sensitive colony of each strain was suspended in 0.5 ml of 0.15 M NaCl, and 0.1 ml of the suspension was added to each of four tubes containing 2.5 ml of LB broth with ampicillin or tetracycline. After overnight growth at 37°C, the fraction of rifampicin-resistant mutants was scored by plating on LB plates with or without rifampicin.

Plasmid Constructions. The *dnaQ* gene was previously identified on a 1.5-kilobase (kb) *Eco*RI fragment of the plasmid pLC28-22 (19). We transferred the *dnaQ* gene to the plasmid pMOB45 (20) by cleavage of both plasmids with *Eco*RI followed by ligation and transformation of KH1116 *dnaQ49*, using selection for the tetracycline resistance of pMOB45. Transformants were screened for sensitivity to chloramphenicol, which indicates an insert at the *Eco*RI site of pMOB45 (20), and for loss of mutator activity. The resultant plasmid pNS121 carried the expected 1.5-kb insert.

To obtain overproduction of the *dnaQ* gene product, we first prepared a convenient cloning vector, pNS2, carrying the p_L promoter and the *N* gene of λ phage, positioned to give *N*-activated transcription from p_L through a nearby *Eco*RI site. This was done by insertion of a 1.2-kb *Bam*HI/*Bgl* II fragment of λ DNA carrying p_L and *N* into the *Bam*HI site of pBR322 (21).

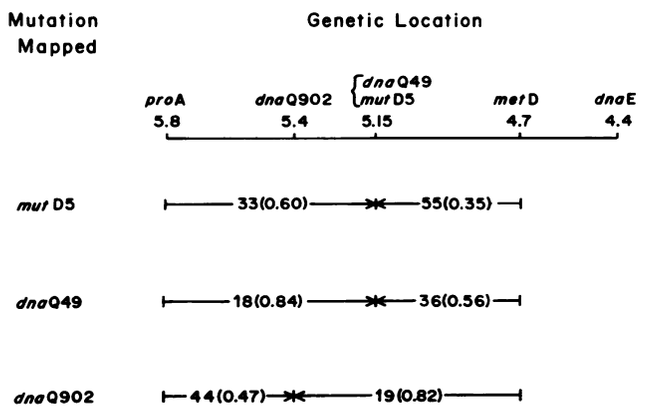

FIG. 1. Mapping of mutator mutations. The first set of numbers gives the percent co-transduction of the mutator mutation with $proA^+$ or $metD^+$ in an experiment in which $proA^+$ or $metD^+$ was the selected marker and the mutator phenotype was scored by the frequency of rifampicin-resistant cells after overnight growth of a recombinant colony. The numbers in parentheses are map distances (in min) calculated from the co-transduction frequencies by using the Wu formula (26). The mutator mutations were located by normalizing the data to a total map distance of 1.1 min from *proA* to *metD*.

Table 1. Complementation of mutator phenotype by plasmid-carried *dnaQ* gene

Mutator mutation	Relative mutation rate in the presence of: No plasmid	$dnaQ^+$ plasmid	$dnaE^+$ plasmid
Wild type	1	9	1
dnaQ49	1,400	8	3,800
dnaQ902	150	7	330
mutD5	3,300	180	2,900

Mutation rate was estimated by the frequency of mutation to rifampicin resistance in an overnight culture. Numbers represent mean of four determinations. The data are normalized by setting the mutation frequency of 5×10^{-9}, found for the wild-type no plasmid case, equal to 1.

Plasmids with the appropriate insert were identified by resistance to ampicillin and sensitivity to tetracycline and for capacity to complement an N^- mutant of λ phage. The *Eco*RI fragment of pNS121 carrying *dnaQ* was then inserted into the *Eco*RI site of pNS2, and the resultant plasmids were analyzed for production of the *dnaQ* gene product.

Protein Labeling and Gel Electrophoresis. The procedures for pulse-labeling and one-dimensional gel electrophoresis were essentially those described (22, 23). The *E. coli* strain R5230 carrying a defective λ prophage was used to repress the p_L promoter of the plasmid until activated by a temperature increase to 42°C. Two-dimensional gel electrophoresis was carried out by the O'Farrell procedure (24). The two-dimensional gels were silver stained, essentially as described by Oakley *et al.* (25).

RESULTS

Evidence for *dnaQ* as a Fidelity Gene. Three high level mutator mutations in the same general region of the *E. coli* genome were independently isolated several years ago. We wanted to determine whether these mutations, originally designated *mutD5* (8), *dnaQ49* (9), and *dnaE902* (11), were all in the same gene. For this purpose, we used transduction with phage P1 to measure genetic distance and used complementation with the cloned *dnaQ* gene to assess functional equivalence.

To map the three mutations, we measured the co-transduction frequency of the mutator alleles with the nearby markers *proA3* and *metD88*. The donor strain was $proA^+ metD^+$ and mutator, and the recipient was *proA3metD88* and nonmutator. The percentage of mutators among $proA^+$ or $metD^+$ recombinants is given in Fig. 1. From the mapping data, we conclude that all three mutator mutations are closely linked, consistent with a single fidelity gene in this region. The mutation originally designated *dnaE902* was provisionally assigned to the *dnaE* gene because it conferred a temperature-sensitive phenotype for DNA replication, and the only replication gene known to be in this general region was *dnaE* (11). We have redesignated the mutation *dnaQ902* based on the mapping data and on the complementation data presented below. To confirm the location of *dnaE*, we have mapped a separate *dnaE* mutation, *dnaE175* (11), to the previously assigned *dnaE* locus at 4.4 min (9, 27) (data not shown).

To study the functional relationship between the three mutator mutations, we transferred into the mutator strains a wild-type *dnaQ* or *dnaE* gene cloned on a plasmid vector and measured mutation frequency. The 1.5-kb *dnaQ* segment from *E. coli* carries the *dnaQ* and *rnh* (RNase H) genes and has no space for additional nonoverlapping genes (19); the 4.6-kb *dnaE* segment from *E. coli* has very little space for additional genes besides *dnaE* (18). The $dnaQ^+$ plasmid restored the mutation fre-

quency to near wild-type levels for *dnaQ902* and *dnaQ49*; for *mutD5* the mutation frequency of the haploid strain was decreased ≈20-fold (Table 1).

From the combined mapping and complementation data, we conclude that *dnaQ49*, *dnaQ902*, and *mutD5* are probably all alleles of the same gene, *dnaQ*. The partial dominance of *mutD5* to the multicopy *dnaQ*$^+$ plasmid is not surprising, because *mutD5* is dominant to wild type in 1:1 partial diploids with a wild-type gene carried by F′ or λ phage (28, 29). Cox and Horner have also found that *mutD5* is partially dominant to a wild-type gene on a multicopy plasmid (30). From an extensive genetic analysis involving mapping and complementation studies, Maruyami *et al.* (29) have also concluded that *dnaQ49* and *mutD5* are alleles of the same gene.

Identification of the *dnaQ* Gene Product as the ε-Subunit of pol III Holoenzyme. To discern whether the *dnaQ* gene product is a subunit of pol III holoenzyme, we have identified the plasmid-encoded *dnaQ* product as an overproduced ^{35}S-labeled protein. We have then compared the labeled *dnaQ* gene product to the subunits of purified pol III holoenzyme by two-dimensional gel electrophoresis.

To overproduce the *dnaQ* product, we used a plasmid vector carrying the λ p_L promoter and *N* gene, which provides efficient transcription of genes downstream from p_L (22). The p_L promoter is controlled by a temperature-sensitive cI protein encoded by a defective λ prophage, so that genes transcribed from p_L are thermally inducible. The synthesis of the λ N protein prevents termination of RNA chains prior to a downstream gene. We placed the 1.5-kb *Eco*RI fragment carrying *dnaQ* adjacent to the λ p_L promoter in each of the two possible orientations of *dnaQ*. Horiuchi *et al.* have previously identified the product of the *dnaQ* gene as a protein of ≈25 kilodaltons (kDa) and the product of the adjacent *rnh* gene as a 21-kDa protein (19). Cox and Horner have assigned the *mutD* gene product a similar molecular mass to *dnaQ*, ≈28 kDa (30). In the correct orientation for *dnaQ* expression (orientation 1), we expect a protein of 25–28 kDa to be overproduced when the highly efficient p_L promoter is activated; in the opposite orientation (orientation 2), we should find no increase in *dnaQ* over that provided by its *E. coli* promoter (or possibly underproduction because of converging transcription).

Two plasmids in each orientation, as determined by analysis with restriction enzymes, were subjected to thermal induction. Total protein was pulse-labeled with [^{35}S]methionine, and the ^{35}S-labeled proteins were fractionated by electrophoresis in acrylamide gels in the presence of $NaDodSO_4$ (Fig. 2). The two plasmids in orientation 1 produced a protein in the 25- to 28-kDa range that is not seen for the induced parental plasmid (lanes D and F); in contrast, the two plasmids with orientation 2 produced a protein of about 20 kDa (lanes E and G). We presume that the larger protein is the *dnaQ* gene product, and that the smaller protein, produced in the opposite orientation, is RNase H. The heavily labeled band just above *dnaQ* is β-lactamase (29 kDa) encoded by the plasmid *bla* gene (31–33). To indicate the specificity of the system, a p_L-controlled segment carrying the *dnaE* gene and several adjacent genes is also shown (lane C).

To identify the dnaQ protein in the pattern of proteins produced by two-dimensional gel electrophoresis, we compared

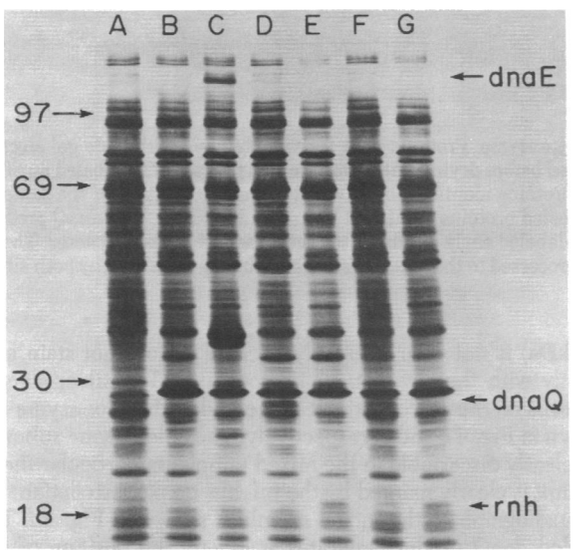

FIG. 2. Identification of the *dnaQ* product. After thermal induction of the plasmid p_L promoter, protein was pulse-labeled with [^{35}S]-methionine; the ^{35}S-labeled proteins were fractionated by acrylamide gel electrophoresis in the presence of $NaDodSO_4$ and visualized by autoradiography. Lanes: A, no plasmid; B, p_LN only (pNS2); C, p_LN and *dnaE*; D–G, plasmids with p_LN and the *dnaQ* segment in either orientation 1 or 2. Lane D, pNS221 (1); E, pNS222 (2); F, pNS224 (1); and G, pNS225 (2). The arrows on the left give the migration positions of marker proteins: phosphorylase B, 97 kDa; bovine serum albumin, 69 kDa; carbonic anhydrase, 30 kDa; and lactoglobulin A, 18 kDa. The arrows on the right give the migration positions of plasmid-encoded proteins presumed to be the *dnaE*, *dnaQ*, and *rnh* gene products.

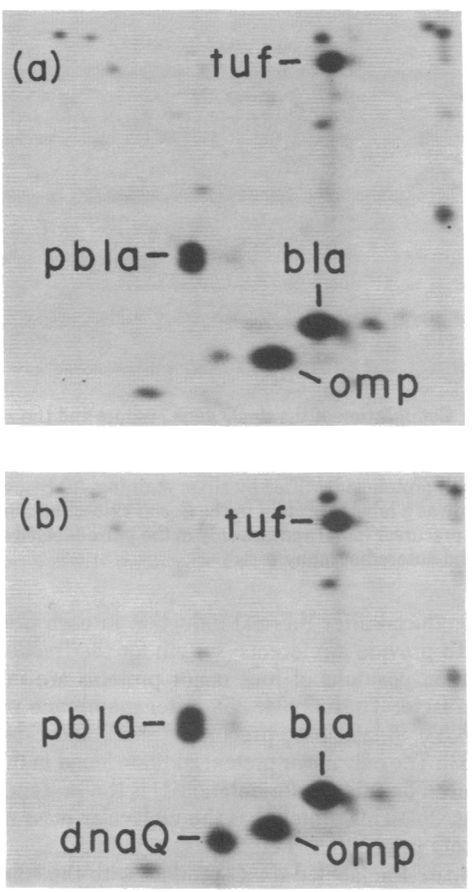

FIG. 3. Identification of the *dnaQ* product in a two-dimensional gel. Proteins were labeled with [^{35}S]methionine as for Fig. 2, and the ^{35}S-labeled proteins were fractionated by acrylamide gel electrophoresis in two dimensions, as described by O'Farrell (24). (*a*) Pattern of labeled proteins from a plasmid strain, pNS222, that does not overproduce dnaQ protein. (*b*) Pattern from a plasmid strain, pNS221, that does overproduce dnaQ protein. The protein inferred to be dnaQ is indicated, as are other major identifiable proteins, the products of the *tufAB*, *ompA*, and *bla* genes.

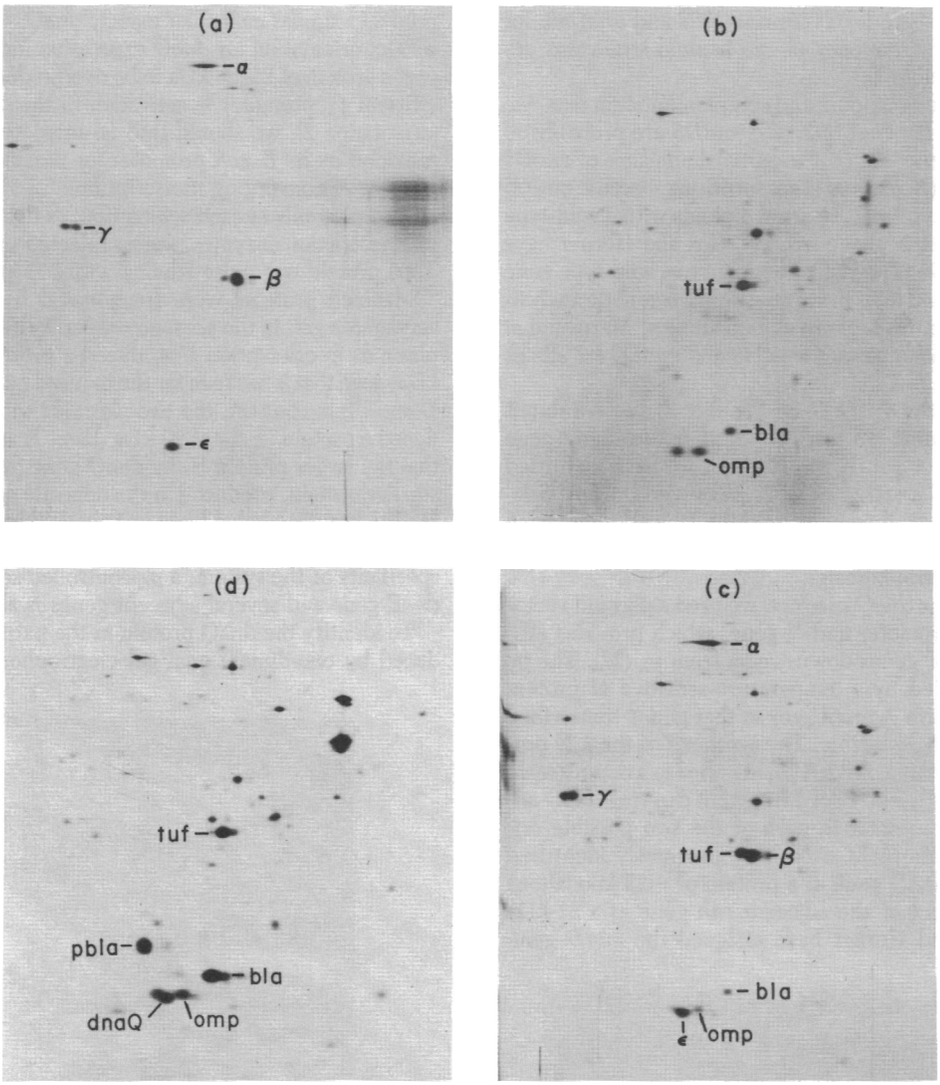

FIG. 4. Comigration of the *dnaQ* gene product and the ε-subunit of pol III holoenzyme. Proteins were fractionated by acrylamide gel electrophoresis in two dimensions, as for Fig. 3. Gels were silver stained and photographed before drying. (*a*) Silver-stained pattern of unlabeled purified holoenzyme only. (*b*) ^{35}S-Labeled *E. coli* extract from induction of pNS221 with proteins identified by silver staining. (*c*) Mixture of the samples *a* and *b*, with proteins identified by silver staining. (*d*) Same gel as *c*, with ^{35}S-labeled proteins identified by autoradiography. The *dnaQ* product appears only as a labeled protein in the *E. coli* extract because proteins are pulse-labeled early (5 min) after activation of the p_L promoter. The β-lactamase precursor (pbla) appears only in the pulse-labeled sample because it is processed to the mature form (bla) that is visualized by both silver staining and autoradiography.

extracts produced after thermal induction in each orientation (Fig. 3). To provide a reference system for the fractionations, the migration positions of four major proteins are indicated: elongation factor Tu (tuf) (34, 35), outer membrane protein d (omp) (34, 35), β-lactamase precursor (pbla), and β-lactamase (bla) (31–33). The only major protein addition found in the *dnaQ*-overproduced orientation (orientation 1) is the protein marked dnaQ (Fig. 3*b*). This protein has the molecular mass expected for the dnaQ product (cf. Fig. 2).

To compare the labeled *dnaQ* product with the subunits of purified pol III holoenzyme, we repeated the two-dimensional fractionation, using a mixture of the labeled *E. coli* extract of Fig. 3*b* and purified pol III holoenzyme. The unlabeled pol III subunits were identified by silver staining, and the position of the *dnaQ* product was determined by autoradiography. The silver-stained pattern of pol III holoenzyme alone is shown in Fig. 4*a*; the α (140 kDa)-, γ (52 kDa)-, β (40 kDa)-, and ε (25 kDa)-subunits (14, 36) are marked. The τ-subunit (83 kDa) is probably the spot near the left (basic) edge of the gel; the δ-subunit (32 kDa) is not seen, probably because it does not stain efficiently with the silver-staining procedure. The silver-stained patterns of the *E. coli* extract alone and with holoenzyme are shown in Fig. 4 *b* and *c*, respectively. The holoenzyme subunits are clearly discernible in the mixed sample; in particular the ε-subunit is clearly marked by the tuf–bla–omp constellation. An autoradiograph of the mixed sample is shown in Fig. 4*d*. The labeled *dnaQ* product migrates at exactly the position of the unlabeled ε-subunit.

From the data presented in Figs. 2, 3, and 4, we conclude that the *dnaQ* gene product is the ε-subunit of pol III holoenzyme. E. C. Cox and D. L. Horner (personal communication) have also derived a similar conclusion from a two-dimensional gel analysis.

DISCUSSION

The *dnaQ* Gene and pol III Fidelity. We believe that the *dnaQ* gene product, the ε-subunit of pol III holoenzyme, has a special role in the fidelity of DNA replication for two reasons:

(*i*) The most efficient general mutator mutations occur repetitively in this gene; (*ii*) with the exception of *dnaE*, other genes involved in DNA replication do not exhibit a mutator phenotype (11). To explore the idea of a fidelity gene, we selected the three most potent general mutator mutations in *E. coli* known at the time we began this work, and we asked whether they represented different mutations in the same gene. We have found that the mutator mutations are indeed alleles of *dnaQ*. Other high level mutators have also recently been mapped to the *dnaQ* region (28, 37). Several additional mutator loci are known in *E. coli* (38); however, most of these—*mutS*, *mutR*, *mutL*, and *mutU* (*uvrD*)—probably represent genes involved in postreplicative repair (3, 39). The *mutT* gene has not been associated with a function, but the *mutT* mutator gives only a special class of mutation, unidirectional transversion (38, 40). Thus *dnaQ* is likely to be unique among genes identified by mutator mutations for its general involvement in accuracy of the replication process.

The Role of the ε- and α-Subunits of pol III. The α-subunit of pol III holoenzyme is the *dnaE* gene product, a 140-kDa protein (14, 18, 36, 41, 42). Enzyme assays with subunits separated by gel electrophoresis have indicated that the α-subunit has the polymerization and 3′→5′ exonuclease activities of pol III holoenzyme (42). Thus the α-subunit polypeptide appears by itself to be similar to other prokaryotic DNA polymerases. From this point of view, it is not surprising that some *dnaE* mutations are mutators (11, 43, 44). However, the extraordinarily high mutation rate conferred by *dnaQ* mutations, far greater than that of any *dnaE* mutations, is remarkable given that the ε-subunit appears to be an accessory protein for DNA replication.

We believe that the ε-subunit affects the fidelity of DNA replication through control of 3′→5′ exonuclease activity because two *dnaQ* mutations, *dnaQ49* and *mutD5*, drastically reduce the 3′→5′ exonuclease of pol III holoenzyme *in vitro* without marked effects on capacity to replicate DNA (10). Because the ε-subunit polypeptide probably does not carry the catalytic site for 3′→5′ exonuclease (42), the function of ε in the multisubunit holoenzyme may be primarily to regulate the 3′→5′ exonuclease. However, ε-subunit may also contribute to the polymerization reaction in a major way. A clear assessment of the functions of the ε- and α-subunits is difficult at present, because the two proteins are tightly associated in the pol III core and have been separated only under denaturing conditions.

If indeed ε is a regulatory subunit, cellular signals might be transmitted through it to alter the fidelity of replication, allowing for an enhanced mutation rate under stress conditions. As noted previously, the enhanced mutation rate found under conditions of SOS induction might be one manifestation of such a control mechanism (2, 3). From the point of view of cellular control, we find it intriguing that *mutD5* is a very high-level mutator only if thymidine is present in the growth medium (38, 45). Although a function for the ε-subunit in cellular control of mutation rate remains to be established, we believe that the results presented here clearly define a central role for ε-subunit in the fidelity of replication.

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