A separate editing exonuclease for DNA replication: The ϵ subunit of *Escherichia coli* DNA polymerase III holoenzyme

(protein overproduction/fidelity of DNA replication/*dnaQ* gene)

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Communicated by I. Robert Lehman, August 20, 1984

ABSTRACT DNA polymerase III (polIII) holoenzyme of *Escherichia coli* has 3' $\rightarrow$ 5' exonuclease ("editing") activity in addition to its polymerase activity, a property shared by other prokaryotic DNA polymerases. The polymerization activity is carried by the large α subunit, the product of the *dnaE* gene. Mutations affecting the fidelity of DNA replication *in vivo* and the activity of 3' $\rightarrow$ 5' exonuclease assayed *in vitro* are found in the *dnaQ* gene, which specifies the ϵ subunit. To determine whether ϵ carries the 3' $\rightarrow$ 5' exonuclease activity, we have used an overproduction protocol to purify ϵ separately from the other subunits of polIII holoenzyme. We find that ϵ has 3' $\rightarrow$ 5' exonuclease activity indistinguishable from that of polIII core, the subassembly of polIII holoenzyme consisting of the α , ϵ , and θ subunits. We conclude that the editing and polymerization activities of polIII holoenzyme reside on distinct subunits, in contrast to DNA polymerase I of *E. coli* and DNA polymerase of phage T4. This functional separation may provide for regulation of exonucleolytic editing independently of polymerization, allowing cellular control of replication fidelity.

Duplication of the *Escherichia coli* genome is an extremely accurate process: error frequencies are typically $\approx 10^{-9}$ – 10^{-10} per base replicated (1). This high fidelity is thought to occur through a multistage process: (i) selection of the complementary base in the initial 5' $\rightarrow$ 3' incorporation, (ii) exonucleolytic 3' $\rightarrow$ 5' editing of a noncomplementary base at the growing point, and (iii) postreplicative mismatch repair. The summation of these steps could achieve the accuracy observed (2–4). DNA polymerase III (polIII) holoenzyme is the primary enzyme involved in chain elongation in *E. coli* and is, therefore, likely to be the major determinant of fidelity (2, 3). To study fidelity mechanisms in DNA replication and explore the possibility that fidelity might be regulated, we have been seeking to assess the contribution of polIII subunits to base selection and editing.

The polIII holoenzyme has at least seven subunits: α , ϵ , θ , τ , γ , δ , and β (2, 5). The smallest subassembly of polIII holoenzyme prepared in native form is polIII core, which contains the α , ϵ , and θ subunits (6); α is the *dnaE* gene product (7), and ϵ is the *dnaQ* gene product (8). polIII core carries both the polymerase and the 3' $\rightarrow$ 5' exonuclease activities of polIII holoenzyme (6). Enzyme assays with subunits separated by NaDodSO₄/PAGE have indicated that the large (α) subunit has the polymerase activity and might also carry the 3' $\rightarrow$ 5' exonuclease activity (9). However, an important role for ϵ in 3' $\rightarrow$ 5' exonuclease (and replication fidelity) has been inferred from the observation that mutator mutations in the *dnaQ* gene render polIII holoenzyme defective in the editing exonuclease (10, 11).

To determine the role of ϵ in exonucleolytic editing by polIII, we wanted to study ϵ separately from the other subunits

of the multisubunit polIII holoenzyme. To provide an excess of ϵ and facilitate its purification, we used an overproducing strain that expresses the *dnaQ* gene under the control of a strong promoter, P_L of phage λ , and an efficient ribosome-binding region. We have purified the ϵ subunit of polIII to homogeneity. We have found that ϵ carries a 3' $\rightarrow$ 5' exonuclease activity with characteristics closely similar to those of purified polIII core enzyme. Thus, the editing and polymerization activities of polIII holoenzyme reside on distinct subunits.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The *E. coli* strains used are MC1000 *lac* deletion (12) and C600 (13). The plasmids used are pRK248-*clts2-tet* (14), pMC1403-*lac'Z-amp* (15), pRC23- λP_L (16), pNS121-*dnaQ* and pNS221- λP_L -*dnaQ* (8), and pMD1, a pBR322 derivative containing the 1.6-kilobase-pair (kbp) *EcoRI dnaQ* fragment.

Materials. M9 minimal medium and LB broth were the standard recipes (17). The minimal medium was supplemented with 0.2% glucose, thiamine (20 mg/liter), and amino acids (20 mg/liter). Where needed, ampicillin (100 mg/liter) and tetracycline (15 mg/liter) were added. Minimal plates for the screening of Lac⁺ colonies were spread with 5-bromo-4-chloro-3-indolyl- β -D-galactoside (Sigma) (20 mg/ml in *N,N*-dimethylformamide) prior to use. Antibiotics and ADP were from Sigma. *EcoRI* linkers were from New England Biolabs. [³H]dCTP (58 Ci/mmol; 1 Ci = 37 GBq), [³H]dTTP (108 Ci/mmol), and [γ -³²P]ATP (>5000 Ci/mmol) were from Amersham. (dT)₁₈, poly(dA), and all other nucleotides were from P-L Biochemicals. Guanidine-HCl was from Whittaker (Delaware Water Gap, PA). DEAE-Sephacel was from Pharmacia. Gel matrix Blue A was from Amicon.

Enzymes. *E. coli* polIII core fraction VI (6) was generously provided by C. McHenry. Calf thymus terminal deoxynucleotidyl transferase was from P-L Biochemicals. T4 polynucleotide kinase was from Boehringer Mannheim. All restriction enzymes, T4 DNA ligase, BAL-31, *E. coli* DNA polymerase I (polI), and polI Klenow fragment were from New England Biolabs.

Preparation of Plasmid DNA and Transformation. Plasmid DNA isolation and bacterial transformation were carried out as described (8).

Plasmid Constructions. To obtain overproduction of the ϵ protein, we first prepared a convenient derivative of the cloning vector pRC23 (16); this was done by replacing the 760-base-pair (bp) *Pst* I/*EcoRI* fragment of pMC1403 with the 1-kbp *Pst* I/*EcoRI* fragment of pRC23. The resultant plasmid pNS3 carries the P_L promoter of phage λ , a consensus Shine-Dalgarno sequence for efficient translation, and *EcoRI*, *Bam*HI, and *Sma* I sites preceding a truncated *lacZ* gene. Thus, insertion of a properly aligned *dnaQ* gene fragment within the polylinker sequence generates a β -galacto-

sidase fusion protein containing the NH₂ terminus of ϵ protein subject to the efficient transcription and translation signals of the plasmid vector. Once efficient expression of the fusion gene was achieved, as judged by β -galactosidase assays, the intact *dnaQ* gene was reconstructed by inserting the 3' end of the gene at the fusion site.

Plasmid DNA containing the *dnaQ* gene, pNS121 (8), was digested to completion with *EcoRI* and *Acc I* and subjected to agarose gel electrophoresis, and the 1069-bp *Acc I/EcoRI* fragment was isolated (18). From sequence analysis, this *Acc I* site lies ≈ 60 bp upstream from the ATG translational start signal of the *dnaQ* gene (19). The purified *Acc I/EcoRI* fragment was treated with BAL-31 nuclease, after which the ends were filled in with polI Klenow fragment, and *EcoRI* linkers were added by T4 DNA ligase (20). After treatment with *EcoRI* and removal of excess linkers, the fragments were cleaved with *EcoRI* and *BamHI* and inserted into the *EcoRI* and *BamHI* sites of pNS3. The desired plasmids were selected as Lac⁺ Amp^r Tet^r transformants (at 30°C) of the *lac* deletion strain MC1000 carrying pRK248-*clts-tet*. To ensure that the resultant plasmids carried the 5' end of the *dnaQ* gene, plasmid DNA was checked for the presence of an *HgiAI* site that overlaps the translational start codon of *dnaQ* (19). Strains that met this criterion were then examined for inducible expression of the fusion protein after a shift to 42°C to derepress the *P_L* promoter. For the highest overproducers, the *dnaQ* gene was reconstructed by replacing the *BamHI/Cla I lacZ* fragment with a *BamHI/Cla I* fragment from pMD1 containing the 3' end of *dnaQ*. The resultant plasmid, pNS360, carries an intact *dnaQ* gene by two criteria: (i) conversion of a *dnaQ*49 mutator phenotype to wild-type; (ii) production of an inducible protein that comigrates with the normal *dnaQ* gene product in the two-dimensional gel experiment described previously (8).

Exonuclease Assay. Reaction mixtures (0.05 ml) contained 20 mM Tris-HCl buffer (pH 8.1), 8 mM dithiothreitol, 4 mM MgCl₂, 4% glycerol, bovine serum albumin (80 μ g/ml), and 1.2 pmol of 3' ends of the appropriate DNA substrate. Reactions were started by the addition of enzyme at an appropriate dilution. After incubation at 34°C, samples were removed, spotted on DE-51 paper, and processed as described (10). One unit of exonuclease activity is defined as the amount catalyzing the removal of 1 pmol of nucleotide per min under the above assay conditions.

Preparation of DNA Substrates. The substrates (dT)₁₈-([³H]dC)_{2.8} and (dT)₁₈-([³H]dT)_{2.6} were prepared by extension of (dT)₁₈ with [³H]dCTP or [³H]dTTP, respectively, by terminal transferase (10, 21). These oligonucleotides were hybridized to (dA)₁₅₀₀ in a 20:1 molar ratio of adenine to thymine at 37°C for 45 min in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 0.1 M NaCl. 5'-³²P-labeled poly(dA) was prepared by a modification of the kinase exchange reaction (22).

Purification of the ϵ Subunit of polIII. The bacterial strain MC1000 with plasmids pRK248-*clts* and pNS360-*dnaQ* was grown at 30°C in 100 liters of LB broth containing 0.2% glucose, ampicillin (100 mg/liter), and tetracycline (15 mg/liter); when the culture density reached $\approx 5 \times 10^8$ cells/ml the temperature was elevated to 42°C, and after 90 min the cells were harvested by centrifugation. At the time of harvest, ϵ represented $\approx 10\%$ of the total cellular protein.

The wet cell paste (100 g) was suspended and then lysed by a lysozyme procedure previously described (23). All subsequent steps were carried out at 4°C unless otherwise noted. Lysed cells were centrifuged for 60 min at $23,000 \times g$ in a GSA rotor, after which $>95\%$ of the ϵ protein remained associated with the cell-debris pellet. The supernatant was discarded, and the cell debris was homogenized with a blender after the addition of 50 ml of buffer S (20% glycerol/25 mM Tris-HCl, pH 7.5/1 mM EDTA/5 mM dithiothreitol/10 mM NaCl). The resultant cell debris suspension was then soni-

cated for 2 min to reduce the viscosity. After centrifugation for 30 min at 20,000 rpm in a Spinco type 30 rotor, the pellet was resuspended and washed twice with 100 ml of buffer S containing 1.0 M NaCl, washed once with 100 ml of buffer S, and resuspended with 25 ml of buffer S. This washed pellet fraction contained $>95\%$ of the ϵ present in the harvested cells and only small amounts of other cellular proteins. However, ϵ remained in an insoluble form.

The ϵ protein in the washed pellet fraction was converted to soluble form by a denaturation-renaturation protocol with guanidine-HCl. A portion (1.0 ml) was diluted 1:10 with buffer S, and an equal volume of 6 M guanidine-HCl in buffer S was added. After the solution cleared, it was warmed to room temperature and slowly diluted to 1 M guanidine-HCl by the addition of buffer S. The resultant solution was dialyzed twice against buffer S containing 0.5 M NaCl and then against buffer S containing 50 mM NaCl. After this procedure, $\approx 40\%$ of the ϵ in the washed pellet was soluble and remained so during subsequent manipulations. This soluble fraction was assayed for the presence of 3' $\rightarrow$ 5' exonuclease activity for mispaired bases by the copolymer assay described above; potent exonuclease activity was found (53,000 units/mg of protein).

The soluble ϵ fraction was diluted with buffer S to a conductivity equivalent to 50 mM NaCl and applied to a DEAE-Sephacel column. The column was washed with buffer S containing 50 mM NaCl, and ϵ was eluted with a linear gradient of 50–150 mM NaCl in buffer S; ϵ eluted at 100 mM NaCl. Nearly all of the exonuclease activity eluted with ϵ . Fractions with $\geq 50\%$ of the peak activity were combined; the specific activity of the pooled DEAE fraction was 51,000 units/mg of protein. The pooled DEAE fraction was applied to an agarose Blue A column, the column was washed with buffer S containing 150 mM NaCl, and ϵ protein and exonuclease activity were eluted together with buffer S containing 300 mM NaCl. The peak fraction (specific activity 110,000 units/mg of protein) was used for the characterization of ϵ as an exonuclease.

Other Methods. The procedures for NaDodSO₄/PAGE and Coomassie-blue staining were essentially as described (24, 25). Protein was determined by the method of Bradford (26) using bovine serum albumin as a standard.

RESULTS

Overproduction and Purification of the ϵ Subunit of polIII Holoenzyme. To purify ϵ separately from the other subunits of polIII holoenzyme, we needed to produce the protein in excess. Moreover, lacking an obvious enzyme assay for ϵ , we sought very large overproduction of the protein to allow ready detection of ϵ by PAGE. In earlier experiments, a substantial rate of ϵ synthesis was achieved by placing the *dnaQ* gene downstream of the λP_L promoter (8). However, the rate of ϵ synthesis (ϵ was $\approx 0.2\%$ of total cellular protein) was considerably less than that possible from maximal translation of a λP_L transcript; thus the expression of the *dnaQ* gene from its own ribosome-binding region appears to be translationally limited.

To achieve better translational expression of *dnaQ*, we used gene-trimming techniques to place the *dnaQ* gene next to a highly efficient ribosome-binding region carrying a consensus Shine-Dalgarno sequence. To assay for maximal overproduction, we used an intermediate construction in which the NH₂-terminal end of ϵ was fused to the COOH-terminal end of β -galactosidase, allowing for a simple enzymatic assay of gene expression. The complete *dnaQ* gene was reconstructed for the purification of ϵ .

With the optimized overproduction scheme, $\approx 10\%$ of the cellular protein is ϵ (Fig. 1, lanes U and I). However, the ϵ protein is not present in soluble form. This precipitation phenomenon has been found with some other overproduced pro-

FIG. 1. Purification of ϵ protein. Proteins were fractionated by NaDodSO₄/PAGE and visualized by staining with Coomassie blue. Lanes: U and I, total cellular protein from lysed uninduced or induced cells, respectively; P, pellet from low-speed centrifugation after cell lysis with lysozyme; WP, pellet from low-speed centrifugation after washes with high salt (0.5 M NaCl); S, soluble fraction obtained after denaturation and renaturation of the WP fraction; D, fraction obtained by chromatography of S on DEAE-Sephacel. Procedures are described in *Materials and Methods*. The position of ϵ is indicated on the right; the positions and apparent molecular masses (in kDa) of marker proteins are given on the left.

teins in *E. coli* (27–30); the insolubility may result from formation of a micelle-like structure at very high concentrations of rather hydrophobic polypeptides. The complex is not simply a loose membrane association because it is not disrupted by nonionic detergents and/or high salt concentrations. To convert ϵ to soluble form, we employed a denaturation–renaturation protocol (see *Materials and Methods*). The insolubility of ϵ allows for a substantial purification by washing the precipitate (Fig. 1, lanes P and WP). The soluble fraction achieved by renaturation is >95% ϵ (Fig. 1, lane S). Once present in soluble form, ϵ behaves as a typical protein in chromatography on DEAE-Sephacel (Fig. 1, lane D) and the dye-matrix Blue A.

Exonuclease Activity of the ϵ Protein. Since mutations in the *dnaQ* gene produce a defective 3' → 5' exonuclease (10, 11), we expected ϵ either to carry the 3' → 5' exonuclease activity of polIII holoenzyme or to control such an activity in the α subunit. Using the mispaired copolymer substrate poly(dA)-[(dT)₁₈-(³H)dC]_{2.8}, we found a potent 3' → 5' exonuclease activity in the first soluble ϵ fraction. Nearly all of this activity comigrated with ϵ during chromatography on DEAE-Sephacel (Fig. 2). Since the DEAE fraction is ≈99% ϵ (Fig. 1, lane D), we can conclude that ϵ is very likely the 3' → 5' exonuclease. The exonuclease activity also comigrates with ϵ on a Blue A column (data not shown).

Comparison of the 3' → 5' Exonuclease Activities of ϵ and polIII Core. The polIII core polymerase, an assembly of α , ϵ , and θ subunits, carries both polymerase and 3' → 5' exonu-

FIG. 2. Exonuclease activity of ϵ protein. Fractions from DEAE-Sephacel were added to the synthetic copolymer (dA)₁₅₀₀[(dT)₁₈-(³H)dC]_{2.8}; the 3' → 5' exonuclease activity was determined by measuring the [³H]dCMP remaining in polymer form. ○, protein concentration; ●, exonuclease activity.

lease activities (6). To verify that ϵ is indeed the editing exonuclease of polIII holoenzyme, we compared the exonuclease activities of ϵ and polIII core. The 3' → 5' exonuclease activities of polIII holoenzyme and polIII core are highly selective for a mispaired base in a copolymer substrate (6, 31, 32). We have compared exonuclease activities with poly(dA)-[(dT)₁₈(dC)_{2.8}]_n (mispaired substrate) and poly(dA)-[(dT)_{20.6}]_n (paired substrate). We find that purified ϵ exhibits a marked preference for the mispaired substrate (Fig. 3). To be sure that the paired substrate is sensitive to a 3' → 5' exonuclease, we have demonstrated release of dTMP with polI (data not shown). As expected, both ϵ and polIII core are highly active with the nonhybridized oligonucleotide substrates (data not shown).

The 3' → 5' exonuclease activity of polIII holoenzyme is inhibited by deoxyribonucleoside monophosphates; a remarkable property of the inhibition pattern is preferential inhibition by dGMP (ref. 32 and our unpublished work). The

FIG. 3. Exonuclease activity of ϵ and polIII core with paired or mispaired substrates. Copolymer substrates were (dA)₁₅₀₀[(dT)₁₈-(³H)dC]_{2.8} (mispaired) and (dA)₁₅₀₀[(dT)_{20.6}-(³H)dT]_{2.6} (paired). The 3' → 5' exonuclease activity was determined by measuring the [³H]dCMP or [³H]dTMP remaining in polymer form. ○ and ●, ϵ with mispaired or paired substrates, respectively; △ and ▲, polIII core with mispaired or paired substrates, respectively.

FIG. 4. Inhibition of ϵ and polIII core by deoxyribonucleoside monophosphates. The assays were carried out as for Fig. 2, except that dGMP or dCMP were added as inhibitors of the exonuclease activity of ϵ (A) or polIII core (B). $\circ$ and $\bullet$, standard assay; Δ and $\blacktriangle$, 2 mM dGMP added; $\square$ and $\blacksquare$, 2 mM dCMP added. dAMP and dTMP gave inhibitions intermediate between those obtained with dGMP and dCMP (data not shown).

exonuclease activities of polIII core and of ϵ also show this preferential inhibition (Fig. 4). Because preferential inhibition by dGMP is likely to be a special property of polIII, we consider the data of Fig. 4 to be a strong indication that ϵ carries the 3' $\rightarrow$ 5' exonuclease. Although less diagnostic in nature, parallel inhibition of ϵ and polIII core also occurs with *N*-ethylmaleimide, NaCl, and EDTA (data not shown).

From the data presented in Figs. 2–4, we conclude that ϵ protein is the 3' $\rightarrow$ 5' exonuclease of polIII holoenzyme.

Lack of 5' $\rightarrow$ 3' Exonuclease with Purified ϵ . PolIII has been reported to carry a very weak 5' $\rightarrow$ 3' exonuclease activity specific for a single-stranded end (6, 31). We have measured release of 5' terminal dAMP from single-stranded poly(dA). As judged by this assay, the 5' $\rightarrow$ 3' exonuclease activity in the ϵ preparations is $<0.01\%$ the 3' $\rightarrow$ 5' activity (Table 1). Although detectable, the 5' $\rightarrow$ 3' exonuclease activity by preparations of two subassemblies of polIII holoenzyme

Table 1. Comparison of 3' and 5' exonuclease activities of ϵ and polIII subassemblies

Enzyme	3'-dCMP released, pmol/min	5'-dAMP released, pmol/min	Ratio of 5' activity to 3' activity
ϵ	25	$\leq 1 \times 10^{-3}$	$\leq 4 \times 10^{-5}$
polIII core	15	3×10^{-3}	2×10^{-4}
polIII*	5	10×10^{-3}	2×10^{-3}

The 3' $\rightarrow$ 5' exonuclease assay was carried out as described for Fig. 2. For the 5' $\rightarrow$ 3' assay, remaining [32 P]dAMP in polymer form was measured as radioactivity insoluble in trichloroacetic acid, in order to solubilize small oligonucleotide products that might be retained in the DEAE paper assay used for 3'-exonuclease; 2.1 pmol of 5' ends was used. By 120 min, there appeared to be a slight release (≈ 5 –10%) of 5'-dAMP by ϵ ; since this small effect was difficult to quantitate and is likely to represent extensive 3' activity in any case, the number is reported as $\leq 1 \times 10^{-3}$. polIII core consists of the α , ϵ , and θ subunits. polIII* is the subassembly of polIII holoenzyme lacking β subunit (2, 5).

zyme is extremely low. The relevance of such a low 5' $\rightarrow$ 3' exonuclease activity to the biochemistry of polIII holoenzyme is unclear.

DISCUSSION

The ϵ Subunit of polIII as an Editing Exonuclease. We purified the ϵ subunit of polIII holoenzyme to assess the role of ϵ in maintaining the fidelity of DNA replication. We wanted to determine initially whether ϵ carries the 3' $\rightarrow$ 5' editing exonuclease activity or functions as a regulatory subunit for the exonuclease activity of α (8). By cloning the gene for ϵ under control of a powerful promoter and efficient translation start signal, we have been able to purify ϵ separately from the other subunits of polIII holoenzyme. Since the purified ϵ protein has a 3' $\rightarrow$ 5' exonuclease activity with properties identical to those of the polIII core exonuclease activity, we have concluded that ϵ alone carries the 3' $\rightarrow$ 5' exonuclease. The specific activity of our purified ϵ is about 2-fold greater than that of the purified polIII core that we used for comparison. Though the molar specific activity of free ϵ is somewhat less than that of ϵ in polIII core (which contains α , ϵ , and θ), ϵ protein is clearly a highly efficient 3' $\rightarrow$ 5' exonuclease by itself. Thus ϵ appears capable of effectively functioning independently of α .

Our finding that ϵ is the 3' $\rightarrow$ 5' exonuclease of polIII surprised us for two reasons. First, for polI of *E. coli* and DNA polymerase of phage T4, the 3' $\rightarrow$ 5' exonuclease and polymerase activities reside in the same polypeptide (2). Second, the 3' $\rightarrow$ 5' exonuclease of polIII has been previously attributed to α , based on assays carried out in polyacrylamide gels with protein subunits separated by NaDodSO₄/PAGE (9). Because the gel assays are necessarily not quantitative, we attribute the discrepancy to the difficulties of the gel system, which might not have completely resolved tightly associated proteins. The first characterization of the 3' $\rightarrow$ 5' exonuclease of polIII also indicated that the exonuclease and polymerase activities might be separable (31).

Implications for Replication Fidelity of a Separate Editing Subunit. Previous work has established three major points about the role of exonucleolytic editing in the accuracy of genome duplication by *E. coli*: (i) mutations leading to the most drastic loss of fidelity are found in the *dnaQ* gene (8, 33, 34); (ii) these *dnaQ* mutator mutations confer a severe defect in the 3' $\rightarrow$ 5' exonuclease activity of polIII holoenzyme (10, 11); and (iii) the *dnaQ* gene specifies the ϵ subunit of polIII holoenzyme (8). These results indicated strongly that ϵ might serve a special role in replication fidelity by controlling the editing activity of polIII holoenzyme (8). Our present findings support this concept in a much more explicit way; we conclude that ϵ is the editing subunit of polIII holoenzyme. Moreover, because ϵ can function so effectively independently of the other subunits, the 10^4 -fold increase in mutation rate associated with *dnaQ* mutations might be largely or exclusively a consequence of a failure of editing. If this is indeed correct, the contribution of exonucleolytic editing to the accuracy of genome duplication is impressive.

The observation that base selection and exonucleolytic editing by polIII are carried out on distinct subunits has several interesting implications. First, the functional separation may provide for regulation of exonucleolytic editing independently of polymerization, allowing for cellular control of replication fidelity (3, 35). Second, the failure to find a 3' $\rightarrow$ 5' exonuclease with the major eukaryotic DNA polymerases has been interpreted as indicative of other editing mechanisms (36). However, if the editing subunit is less tightly associated with the polymerase in eukaryotes than ϵ is with α , the purification procedures used so far might have resulted in loss of the editing subunit. Third, for polIII holoenzyme, "kinetic proofreading" schemes of the type proposed so far

are probably not major contributors to editing because the mechanisms suggested involve use of the same active site for base selection and removal (36).

We thank Charles McHenry for polIII core enzyme; Robert Crowl for plasmid pRC23; Kevin Young, Joe Rosa, Leslie Berg, and Arthur Kornberg for biochemical advice; Warren Gish for computer assistance; Terri DeLuca for editorial help; and William Ricco for photography. This work was supported in part by a grant from the American Cancer Society (ACS MV-131).

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