Fidelity of Mammalian DNA Polymerases

Abstract. *The fidelity of copying natural DNA in vitro with each of the three classes of eukaryotic DNA polymerases has been determined. DNA polymerases-β and -γ are highly inaccurate, catalyzing noncomplementary single-base substitution at a frequency between 1/3000 and 1/8000. DNA polymerase-α is substantially more accurate, with an error rate of 1/30,000. When the error rates of these DNA polymerases are considered in the context of the accuracy of DNA replicative processes in vivo, it seems likely that other factors must exist in mammalian cells which are involved in the accurate replication and maintenance of the genetic information.*

In order to perpetuate species homogeneity, DNA replication must be an exceptionally accurate process. Measurements of spontaneous mutation frequencies suggest that the average frequency of base-pair substitutions is in the range of 10^{-8} to 10^{-11} misincorporations per base pair replicated (*1*). This value results from the contributions to accuracy of the primary replication events, the correction of mistakes made during replication, and the repair of spontaneous damage to the DNA. A logical first step in dissecting this process is a quantitation of the accuracy of the pivotal enzymes in DNA synthesis, the DNA polymerases themselves. Mammalian cells contain at least three classes of DNA polymerase, designated α, β, and γ (*2*). Several experimental approaches suggest a major, although not necessarily an exclusive, role for DNA polymerase-α in replication, for DNA polymerase-β in repair, and for DNA polymerase-γ in mitochondrial DNA replication (*2*). Measurements on the accuracy of DNA polymerases have been limited to quantifying the frequency of incorporation of bases that are not complementary to those in synthetic polynucleotide templates (*3*). However, we have recently observed that accuracy with prokaryotic DNA polymerases is 10- to 100-fold greater with a natural DNA template than with synthetic polynucleotides (*4*). Since there are no studies on the accuracy of mammalian DNA polymerases with natural DNA, they also may be inherently more accurate. We therefore have determined the accuracy of DNA polymerases-α, -β, and -γ in copying a natural DNA template. In the assay we use as a template a mutant single-stranded circular DNA molecule from bacteriophage φX174, containing a single nucleotide substitution, in this instance an A (adenine) for a G (guanine) at position 587. Since the mechanism of catalysis is similar for all known DNA polymerases, it seems likely that fidelity with biologically active φX174 DNA would be no different from that with eukaryotic chromosomal DNA. Synthesis is initiated at a single fixed point on the template, with a DNA restriction endonuclease fragment as primer, and continues beyond the amber mutation in vitro. The frequency of erroneous nucleotide substitutions in the daughter strand is quantified from the reversion frequency of the amber mutation to wild-type after we infect *Escherichia coli* spheroplasts and plate the bacteriophage on permissive and nonpermissive *E. coli* strains (*5*).

This assay requires that in vitro DNA synthesis proceed past the *am*3 mutation (83 nucleotides with restriction endonuclease Hae III fragment Z5) producing a viable minus strand. The synthesis requirement is easily fulfilled by certain purified DNA polymerases, such as *E. coli* DNA polymerase I (*4*). Since mammalian DNA polymerases-α and -β are inefficient in copying long stretches of single-stranded DNA in Mg^{2+}-activated reactions (*6*), we found it necessary to increase the deoxynucleoside triphosphate concentration to 500 μ*M*. Under these conditions, incorporation was several hundred nucleotides per template, suggesting that DNA synthesis proceeded well past the amber mutation. The effect of in vitro DNA synthesis by several DNA polymerases-α, -β, and -γ from divergent sources on the reversion of the amber mutation to wild-type is shown in Table 1 (unfractionated reaction). The enhancement in reversion frequency as a result of copying DNA is abolished by omitting any of the required components in the polymerase reaction (data not shown). The values obtained from transfection of unfractionated copying reactions indicate substantial differences in the accuracy of the three

Table 1. Fidelity of in vitro DNA synthesis by mammalian DNA polymerases. DNA polymerase reaction mixtures (50 μl) contained 20 m*M* tris-HCl (*p*H 8.0), 2 m*M* dithiothreitol, 10 m*M* $MgCl_2$, 500 μM deoxyadenosine triphosphate, deoxyguanosine triphosphate, and deoxycytidine triphosphate and (α-^{32}P)-labeled deoxythymidine triphosphate (500 cpm/pmole) and 0.10 μg of ^{3}H-labeled φX174 *am*3 viral DNA (26,000 cpm/μg) primed at a 5:1 molar ratio with Hae III restriction endonuclease fragment Z5. The following amounts of enzyme were used, with unit definitions corresponding to that given in the purification for each enzyme (*11*): calf thymus DNA polymerase-α, 0.64 unit; mouse myeloma DNA polymerase-α, 0.0016 unit; mouse myeloma DNA polymerase-β, 0.0016 unit; rat (Novikoff) hepatoma DNA polymerase-β, 0.76 unit, and HeLa cell DNA polymerase-γ, 0.40 unit. After incubation at 37°C for 10 minutes (γ) or 30 minutes (α and β) the reactions were terminated by the addition of EDTA to a concentration of 15 m*M* and 2 μl portions were assayed for acid-insoluble radioactivity. The remainder of the copied DNA was used to infect *E. coli* spheroplasts (*4*, *5*). Reversion frequencies were determined by plating the progeny phage on *E. coli* indicator bacteria that were either permissive or nonpermissive for the amber mutation. These reversion frequencies have been corrected for the background reversion frequency for uncopied DNA, which was 3.08 ± 1.20 for 38 determinations. The reversion frequencies listed under fractionated dsDNA were determined with the double-stranded DNA fractions obtained from the sucrose gradient centrifugation (see legend to Fig. 1). Only fractions estimated to contain less than 10 percent of uncopied DNA were included for the calculation of error rates. The numbers in parentheses indicate the number of independent determinations. The error rate is calculated by dividing the reversion frequency by 0.39, which is the estimated expression of the minus strand which contains the error (*4*).

Tissue	Unfractionated reaction		Fractionated dsDNA	
	Reversion frequency ($\times 10^{-6}$)	Error rate	Reversion frequency ($\times 10^{-6}$)	Error rate
	DNA polymerase-α			
Calf thymus	3.85 ± 0.82 (4)	1/101,000		
	7.70 (1)	1/50,600	16.4 (1)	1/23,800
	3.09 (1)	1/126,000	12.8 (1)	1/30,500
Mouse myeloma	4.12 ± 2.12 (5)	1/95,000		
	DNA polymerase-β			
Rat hepatoma	54.5 ± 2.13 (2)	1/7,160		
	39.6 (1)	1/9,850	58.6 (1)	1/6,660
Mouse myeloma	40.9 (1)	1/9,540	83.7 (1)	1/4,660
	74.0 (1)	1/5,270	133.0 (1)	1/2,930
	DNA polymerase-γ			
HeLa	23.4 ± 14.5 (6)	1/16,700		
	20.4 (1)	1/19,100	48.3 (1)	1/8,070
	28.6 (1)	1/13,600	58.6 (1)	1/6,660

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classes of DNA polymerases. Since a simple calculation from net synthesis is not a demonstration that the polymerase copied past the mutation, we analyzed the product of the copying reactions on neutral sucrose gradients (Fig. 1). In these reactions, the φX174 DNA template was labeled with ^3H and the newly synthesized strand was labeled with ^{32}P. Most of the synthesis is primer-dependent (data not shown). The position of the product peak synthesized by either DNA polymerase-α, -β, or -γ is shifted several fractions nearer to the position of completely double-stranded φX174 DNA. This, as well as the ratio of ^{32}P to ^3H in the product peak, indicates that the copied molecules are more than 15 percent double-stranded and thus synthesis has proceeded past the *am*3 site. The DNA from the peak fractions of the sucrose gradients (*p*H 7) was analyzed in the transfection assay. The reversion frequency obtained from fractions containing almost exclusively copied molecules (typically fractions 17 to 20) is much greater than that of those containing uncopied DNA. From our analysis of fractions containing predominantly copied molecules, we conclude that the error rate of DNA polymerase-α is ~ 1/30,000, and four- to tenfold more accurate than either DNA polymerase-β (1/3000 to 1/7000) or DNA polymerase-γ (1/6000 to 1/8000) (Table 1, fractionated double-stranded DNA). The DNA polymerases-β and -γ are, in fact, nearly as error prone as avian myeloblastosis virus DNA polymerase—that is, about 1/1000 (*7*) to 1/5000 (*8*). While DNA polymerase-α is significantly more accurate than -β or -γ, it is much less accurate than *E. coli* DNA polymerase I (*4*), *E. coli* DNA polymerase III (*9*), or the bacteriophage T_4 replicating complex (*10*). One possibility to account for this dichotomy is the proofreading capability of the prokaryotic DNA polymerases. Since many eukaryotic DNA polymerases lack an associated (proofreading) exonuclease activity (*2*, *11*, and references therein), the large difference between DNA polymerase-α and DNA polymerases-β and -γ must reflect a differential base selection ability of these enzymes. If the in vivo accuracy is limited by the fidelity of the DNA polymerases themselves, then DNA repair could be inherently less accurate than DNA replication. Similarly, the high error rate of DNA polymerase-γ suggests that mitochondrial replication may be inaccurate.

Since in vivo DNA synthesis involves the concerted action of several proteins in addition to the DNA polymerases themselves, it seems likely that such proteins may alleviate the high levels of substrate required for copying single-stranded DNA in vitro, while at the same time altering fidelity. Most important, all three classes of eukaryotic DNA polymerases are several orders of magnitude less accurate in copying natural DNA than the estimated accuracy for the combined DNA replication processes in vivo.

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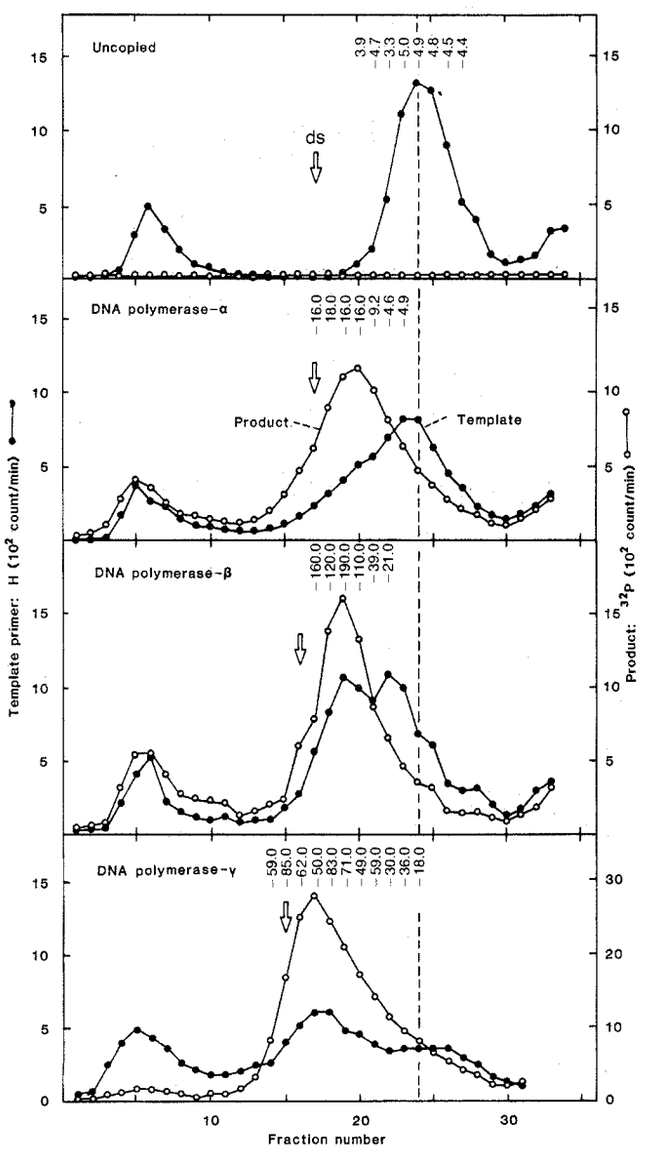

Fig. 1. DNA copying reactions were performed as described in the legend to Table 1, except that the reaction mixtures were larger (in identical proportions) in order to copy 2 μg of ^3H-labeled φX174 *am*3 template. The reactions were terminated by the addition of an equal volume of equilibrated phenol, and mixed gently for 15 minutes at 37°C. The aqueous phase was removed and the DNA was precipitated in ethanol, resuspended in 100 μl of 10 m*M* tris-HCl (*p*H 8.0) and 1 m*M* EDTA, and analyzed on neutral sucrose gradients (*p*H 7.0) (*4*). A sample (50 μl) of each fraction was used to determine acid-insoluble radioactivity, and 100 μl samples of the peak fractions were used to determine the reversion frequencies ($\times 10^{-6}$) shown above each gradient for individual fractions. The arrow designates the position of completely double-stranded φX174 DNA (replicative form II), used as a standard in a separate gradient.

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polymerase-α, 100 to 500 μ*M*; and DNA polymerase-β, 125 to 500 μ*M*; and DNA polymerase-γ, 50 to 500 μ*M*). In searching for a proofreading activity in eukaryotic cells, the requirement for a high concentration of deoxynucleotide substrates may be of concern, since this may reduce the contribution of proofreading to accuracy.
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11. This study was made possible by gifts of highly purified DNA polymerases from several investigators. We thank J. W. Hockensmith and R. A. Bambara (University of Rochester, Rochester, N.Y.) for DNA polymerase-α from calf thymus, form C, purified as described by A. M. Holmes, I. P. Hesselwood, and I. R. Johnston [*Eur. J. Biochem.* **43**, 487 (1974)]; S. H. Wilson (NIH) for DNA polymerase-α from mouse myeloma [which was fraction IV purified as per Y.-C. Chen, E. W. Bohn, S. R. Planck, S. H. Wilson, *J. Biol. Chem.* **254**, 11678 (1979)]. This fraction contains both form α_1 (containing exonuclease activity) and α_2 (devoid of exonuclease activity). The relative contribution of the two forms to activity and accuracy is not known. DNA polymerase-β from mouse myeloma, also supplied by S. H. Wilson, was the homogeneous fraction V, purified as described [K. Tanabe, E. W. Bohn, S. H. Wilson, *Biochemistry* **18**, 3401 (1980)]. For both mouse myeloma DNA polymerases, a unit is described as 1 μmole of total nucleotide incorporated per hour at 37°C. The DNA polymerase-β from rat hepatoma cells (Novikoff) was a gift of D. W. Mosbaugh and R. R. Meyer (University of Cincinnati), and was the homogeneous fraction VI, purified as described [D. M. Stalker, D. W. Mosbaugh, R. R. Meyer, *Biochemistry* **15**, 3114 (1976)]. Highly purified DNA polymerase-γ from HeLa cells (DNA-cellulose fraction) was a gift from S. Spadari (Laboratorio di Genetica Biochimica ed Evoluzionistica, Pavia, Italy).

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Radioactivity Measurements of Former Military Personnel Exposed to Weapon Debris

Abstract. *Sixteen former military personnel who were present at the "Smoky" atmospheric nuclear weapon test have been investigated for internal deposits of radioactivity. Whole-body and thorax gamma-ray measurements, thorax and skeletal actinide measurements, and urinalyses for plutonium-239 and strontium-90 indicated no evidence of radioactivity in excess of that found in the general population.*

An increase in the incidence of leukemia (9 cases observed versus 3.5 expected) has been reported in a cohort of 3224 men who were present at the "Smoky" atmospheric nuclear weapon test in 1957 (*1*). Because of the relatively low mean external dose recorded for the cohort (466 mrem), it has been suggested that additional exposure from internally deposited radioactivity may have been a significant component of the total dose received (*2*). To assess the validity of this hypothesis, a small group of men who were present at the test were examined for internal deposits of long-lived radioactivity. Nineteen men were selected by the Centers for Disease Control on the basis of high film-badge readings or their opportunity for inhalation or ingestion of weapon debris, or both; 16 of the men visited Argonne National Laboratory for measurements in 1979, and three chose not to participate. The 16 ranged in age from 40 to 60 with a mean age of 46.9 ± 5.9 years; none exhibited any clinical signs of malignancy or other radiation-induced pathology. In what follows, only the outlines of our measurement techniques and the mean results are reported; detailed results are given in (*3*).

The whole-body contents of γ-ray emitters were measured with large NaI(Tl) detectors in both reclining chair and flat bed geometries (*4*). The γ-ray spectra were analyzed by a computer method of least squares. With the exception of ^{40}K, ^{137}Cs, and short-lived radon daughters, γ-ray emitters in the 16 subjects were below the limit of detection of 0.2 to 0.5 nCi (depending on energy). The mean potassium content determined from ^{40}K was 0.20 ± 0.03 percent of body weight. [One standard deviation (σ) is given for all values in this report.] For a set of 12 control men, the value was 0.19 ± 0.03 percent, whereas that for "reference man" (*5*) is 0.20 percent. The mean $^{137}Cs/K$ ratio for the 16 subjects was 8.2 ± 2.5 pCi/g, whereas that for the controls was 8.9 ± 2.4 pCi/g. Similar values have been reported for other groups drawn from the general population (*6*). The short biological half-life of ^{137}Cs (~ 110 days) (*6*) precludes any observation of even a large intake that occurred 22 years before. However, our measurements rule out the possibility that during the weapon test period these subjects inhaled and retained highly fused particles of fallout which contained ^{137}Cs (and, by inference, other fission products) in a form insoluble in body fluids.

We determined the possible actinide (^{239}Pu and ^{241}Am) contents of these subjects by two methods: (i) external counting with a large, xenon-filled proportional counter and (ii) urinalysis for ^{239}Pu. The proportional counter is sensitive to the low-energy (13 to 60 keV) photons emitted by these isotopes after α-decay. The mean counting rate observed for the subjects in the band from 16 to 26 keV with the counter viewing the lungs was 3.67 ± 0.54 count/min, whereas that from several uncontaminated controls was 3.68 ± 0.13. Similarly, with the counter viewing the skull, the mean subject counting rate was 2.07 ± 0.52 versus 2.05 ± 0.18 for controls. None of the counting rates for individual subjects differed from the mean control rate at the 90 percent (1.64 σ) level. However, external counting is a relatively insensitive means of detecting internally deposited ^{239}Pu because of the low photon abundance and severe attenuation by bone and soft tissue. An individual could contain several times the maximum permissible lung burden of ^{239}Pu (16 nCi) and still not yield a statistically significant increase in counting rate above that for the controls. These considerations do not apply to ^{241}Am, and no evidence of the 60-keV γ-ray from ^{241}Am was observed in any of the subjects.

Because of the possibility that the results of external counting for both fission products and actinides might be negative, we also collected a 24-hour urine specimen from each subject. Samples were analyzed for plutonium by isotope-dilution α-spectrometry, and the plutonium content of each sample was below the limit of detection (4.5 fCi) of our standard method (*7*). We then estimated upper limits for the body content of plutonium by applying either Langham's power function equation (*8*) or the retention function proposed by a task group of the International Commission on Radiological Protection (ICRP) (*9*) to a urinary excretion rate of 4.5 fCi/day. From Langham's equation, which relates daily urinary excretion to systemic intake, we deduce that the systemic intake was less than 1.7 nCi in August 1957 (7900 days before the urine collections). Since the use of the equation for times much longer than 5 years is known to overestimate the systemic intake (*10*), the value of 1.7 nCi must be regarded as an extreme upper limit. A limiting value for the current body content of < 200 pCi results from the use of the retention equation suggested in ICRP Publication 19 (*9*). [The urinary value was multiplied by 1.47 to determine the total excretion rate (*10*) before application of the retention equation.] These low values for plutonium content based on excretion rates

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