Proc. Natl. Acad. Sci. USA
Vol. 78, No. 7, pp. 4251–4255, July 1981
Biochemistry

DNA polymerase accuracy and spontaneous mutation rates: Frequencies of purine·purine, purine·pyrimidine, and pyrimidine·pyrimidine mismatches during DNA replication

(DNA polymerase III holoenzyme/bacteriophage φX174/editing/transitions/transversions)

ALAN R. FERSHT AND J. W. KNILL-JONES

Department of Chemistry, Imperial College of Science and Technology, London SW7 2AY, England

Communicated by Sydney Brenner, April 13, 1981

ABSTRACT **DNA from the *am16* mutant of bacteriophage φX174 may be replicated *in vitro* and expressed *in vivo* to give five classes of revertants. Each class may be specifically induced by the appropriate biasing of the concentrations of deoxynucleoside triphosphates in a predictable manner. The frequency of each reversion follows a kinetic rate equation relating it to the concentrations of the triphosphates involved in the substitution. The reversions corresponding to TAG → GAG, AAG, CAG, TGG, and TCG are calculated to occur with frequencies of 5×10^{-7}, 4×10^{-7}, 4×10^{-7}, $\approx 2 \times 10^{-7}$, and $\approx 5 \times 10^{-9}$, respectively, at the concentration of triphosphates found *in vivo*. The frequencies are in the range found for the reversion of the phage *in vivo* and so are consistent with errors in nucleotide selection by DNA polymerase (deoxynucleosidetriphosphate:DNA deoxynucleotidyltransferase, EC 2.7.7.7) III being largely responsible for the rate of spontaneous mutation *in vivo*. The relative frequency of mispairing leading to misincorporation is: purine·purine ≈ purine·pyrimidine ≫ pyrimidine·pyrimidine, confirming predictions from model-building studies that transversions arise through purine·purine mismatches.**

The fidelity of replication of DNA and the molecular basis of mutation are now amenable to study by a combination of kinetic and genetic techniques with *in vitro* and *in vivo* methods. Bacteriophage DNA may be copied by a DNA polymerase (deoxynucleosidetriphosphate:DNA deoxynucleotidyltransferase, EC 2.7.7.7) *in vitro* under controlled conditions, and the products may be transfected into *Escherichia coli* to produce viable progeny phage (1). By using DNA containing a known point mutation, errors in nucleotide selection by the polymerase may be detected by an increase in the population of wild-type revertants (2, 3). These errors may then be compared with the reversion frequency of the particular mutation *in vivo* found from classical genetic measurements.

An *in vitro* system of exceptional utility for studying polymerase accuracy in *E. coli* is the replication of double-stranded replicative form (RF) DNA of bacteriophage φX174 to form single-stranded (ss) φX174 DNA (RF → ss). DNA polymerase III holoenzyme, in conjunction with single-stranded DNA binding protein, *rep* protein, and gene *A* protein, produces multiple copies of the single-stranded viral (+) DNA by using the minus strand of the RF duplex as template (4). The particular advantages of this system are: (*i*) it uses DNA polymerase III holoenzyme, which is thought to be the enzyme responsible for the major replication of the *E. coli* chromosome; (*ii*) many copies of synthetic DNA are produced from one parent template so that the phage produced on transfection are predominantly from the synthetic progeny; (*iii*) only one strand of the duplex DNA is copied so that kinetic analysis is greatly simplified; and (*iv*) only single-stranded DNA is produced so that there is no possibility of error correction from the mismatch repair systems now thought to check base pairing in duplex DNA (5).

By applying this procedure to the replication of DNA from the *am3* mutant of φX174, which contains the transition TGG → TAG at position 587, it was shown that the accuracy of the polymerase *in vitro* is very similar to the overall accuracy *in vivo* (6). Further, by biasing the concentrations of deoxynucleoside triphosphates (dNTPs) to favor the reversion (high dGTP and low dATP concentrations in copying -A-T-C-), back mutations can be induced at high frequency. This approach has now been successfully applied to the replication of φX174 DNA catalyzed by the T4 polymerase system (7, 8) and by DNA polymerase I from *E. coli* (9) to show that these enzymes have similar accuracies.

In the present study, we examine the reversion *in vitro* of the *am16* (gene *B*) mutant of φX174 by systematically biasing the concentrations of dNTPs to favor specific transitions and transversions in the amber codon. This mutant, which has a G → T transversion at position 5276 in a region where genes *A* and *B* overlap (10, 11), was chosen because it is known from its viability in suppressor-containing host strains (12, 13) that at least three reversion pathways give rise to acceptable amino acid substitutions because they are the same as those inserted by the suppressors (Table 1).

MATERIALS AND METHODS

Materials. Reagents and enzymes have been described (6). DNA polymerase III holoenzyme, ssDNA binding protein, *rep* protein, and dUTPase (dUTP nucleotidohydrolase, EC 3.6.1.23) were generously provided by A. Kornberg, as was gene *A* protein by S. Eisenberg. *E. coli* CQ2 (*supF*) was obtained from U. Hibner. φX174 *am16*, provided by W. E. Borrias, was plaque-purified, and high-titer stocks were produced by published procedures with *E. coli* CQ2 as the permissive host (13). φX174 *am16* RF DNA was prepared by a modification of the Hirt procedure (14, 15).

Methods. φX174 RF I DNA (1.4×10^{-2} pmol) was replicated in a 25-μl reaction mixture at 30°C for 1 hr and then isolated by procedures similar to those described by Fersht (6). The modified reaction mixture contained 50 mM Hepes·HCl (pH

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: RF, replicative form (covalently closed, circular, superhelical DNA); ss, single-stranded; dNTP, deoxynucleoside triphosphate; Pur, purine; Pyr, pyrimidine.

Table 1. Reversion pathways at the *am16* locus*

	Leu Arg		Phe Arg
wt = T-T-G-A-G-G		*am16* = T-T-T-A-G-G	
	Glu		*am*
Nucleotide in *amber* codon	Substitution	Amino acids encoded: gene *A*	gene *B*
T	G	Leu-Arg	Glu†
T	A	Leu-Arg	Lys
T	C	Phe-Arg	Gln‡
A	G	Phe-Gly	Trp
A	C	Phe-Arg	Ser§
A	T	Phe-Trp	Leu
G	A	Phe-Lys	Ochre
G	T	Phe-Met	Tyr
G	C	Phe-Thr	Tyr

wt, Wild type.
* *am16* in gene *B* caused by G → T at position 5276; gene *A* reads as in upper frame.
† Wild type.
‡ *am16* grows in *supE*-containing cells that insert Gln.
§ *am16* grows in *supD*-containing cells that insert Ser at the *amber* codon.

8.1), 10 mM $MgCl_2$, 40 mM NaCl, 0.16 mM EDTA, 5.5 mM dithioreitol, 16% (vol/vol) glycerol, 800 μM ATP, bovine serum albumin (0.15 mg/ml), 52 units of DNA polymerase III holoenzyme, 300 units of *rep* protein, 27 units of gene *A* protein, 67 units of ssDNA-binding protein, 200 units of dUTPase, and various concentrations of dGTP, dATP, dTTP, and dCTP. (This reaction mixture gave better rates and yields with less enzyme than was used previously.) The dNTPs were preincubated with the dUTPase for 2–25 min at room temperature prior to reaction. However, no radioactive triphosphates were used in the preparation of DNA destined for spheroplast assays, as the radioactive reagents caused higher background reversion frequencies. Instead, the extent of reaction (always 4 or 5 progeny strands per input RF template) was monitored in separate, parallel studies with labeled materials. The product DNA was expressed at 30°C with spheroplasts prepared from *E. coli* C600 (*supE*) as described (6). Indicator bacteria were *E. coli* C for wild type and *E. coli* CQ2 for *am16*. Plaques were scored after 3–4 hr of incubation at 37°C or 5–6 hr at 30°C and then again after overnight incubation. About 10^{-4}–10^{-3} infective centers were formed per DNA particle.

Representative plaques from each experiment were stored in 50 mM sodium tetraborate solution and later tested for growth at different temperatures.

RESULTS

Reversion of φX174 *am16* in Vivo. A single plaque of amber mutant, grown in liquid culture at 36°C to give about 10^{10} phage, produced revertants that fall into three clear classes based on their temperature sensitivity between 30 and 39°C: (*i*) vigorous wild type at a frequency of 1–2 × 10^{-7}; (*ii*) *ts* revertants at a frequency of 1.0 × 10^{-6} that do not grow at 39°C but form small plaques at 37°C and are as vigorous as wild type at 30°C (for the purpose of this study, these will be denoted as "ts_{37}"); and (*iii*) *ts* revertants at a frequency of 1.5 × 10^{-6} that form small plaques at 30°C and are not viable at 35°C (denoted as "ts_{30}"). In addition to the ts_{37} revertants at 37°C, small plaques were also observed at a frequency of 4 × 10^{-7}. However, upon plaque purification, these appeared identical to the ts_{37}. Similarly, tiny pin-point plaques were observed at a frequency of ≈5 × 10^{-7} at 30°C, which on isolation appear to be a mixture of ts_{37} and ts_{30}. Our interpretation is that these small plaques are produced by reversion on the plates, whilst the larger plaques of ts_{37} and ts_{30} are produced predominantly during the last round of replication in liquid culture.

Production of Mutants During Replication *in Vitro*. The basic strategy of these studies was to induce mutations during replication by biasing the dNTP pool to favor the replacement of a chosen nucleotide in the amber codon by a selected alternative. For example, to produce GAG by copying the sequence -A-T-C- in the complementary strand, elementary reasoning dictates increasing the concentration of dGTP and lowering the concentration of dTTP in the replication system to increase the probability of dGTP rather than dTTP pairing with dA. However, a more detailed analysis of the kinetics of mutation suggests that the concentration of the following nucleotide to be incorporated may affect the mutation frequency (6). This is because the 3′ → 5′ exonuclease activity of the polymerase acts as a proofreading function, excising a proportion of the mismatches as they are incorporated and before chain elongation can occur. Because the permanent incorporation of a mismatch requires chain elongation, and the rate of this depends to some extent on the concentration of the dNTP following the mismatch, a general expression for the reversion frequency, ν, is:

$$\nu = \frac{\alpha[\mathrm{dNTP^{Inc}}]}{[\mathrm{dNTP^{Cor}}]} \cdot \frac{[\mathrm{dNTP^{Fol}}]}{K + [\mathrm{dNTP^{Fol}}]} \quad , \qquad [1]$$

where superscripts Cor, Inc, and Fol designate the correct, incorrect, and following dNTPs. K is a complex ratio of rate constants for binding, hydrolysis, and elongation and is not the value of K_m for the normal polymerization reaction. When $K \ll [\mathrm{dNTP^{Fol}}]$, Eq. 1 reduces to the simple expression:

$$\nu = \alpha[\mathrm{dNTP^{Inc}}]/[\mathrm{dNTP^{Cor}}]. \qquad [2]$$

When $K \gg [\mathrm{dNTP^{Fol}}]$, Eq. 2 reduces to

$$\nu = \frac{\alpha[\mathrm{dNTP^{Inc}}]\,[\mathrm{dNTP^{Fol}}]}{K\,[\mathrm{dNTP^{Cor}}]}. \qquad [3]$$

[Previously (6), Eq. 3 was found to hold for TAG → TGG in the reversion of φX174 *am3*.] The analysis of, and the search for, directed mutations must involve examining the concentration dependance of ν with respect to the three dNTPs.

The RF DNA used in the replication studies *in vitro* was produced from *am16* phage containing 2.2 × 10^{-7} wild type, 1.9 × 10^{-6} ts_{37}, and 2.0 × 10^{-6} ts_{30} revertants. On replicating this DNA *in vitro* at 30°C in the presence of 40 μM each of the four dNTPs, the infective centers formed from transfecting spheroplasts at 30°C consisted of 2.3 × 10^{-6} wild type, 4.1 × 10^{-6} ts_{37}, and 1.3 × 10^{-5} ts_{30} revertants. Phage release assays,

Table 2. Revertants of φX174 *am16* produced *in vitro*

Change	Base pairing	Phenotype, plaque size* 30°	37°	40°	42°	43°	Production rate law
T → G	A:G	l	l	l	l	s	1 × 10^{-6} [dG]/[dT]
T → A	A:A	l	l	l	l	pp	3 × 10^{-7} [dA]/[dT]
T → C	A:C	l	l	l	l	s	3.6 × 10^{-7} [dC]/[dT]
A → G	T:G	s	—	—	—	—	10^{-2}[dG]²/[dA]
A → C	T:C	l	l	l	s	pp	10^{-4}[dC][dG]/[dA]
A → T	T:T						
G → C	C:C						
G → T	C:T						
(Unknown *in vivo*		l	s	—	—	—)	

* Incubation temperature is in degrees Celsius. l, Large (3–6 mm in diameter); s, small (1–2 mm); pp, pin-points on incubation overnight on L-plates with *E. coli* C as indicator.

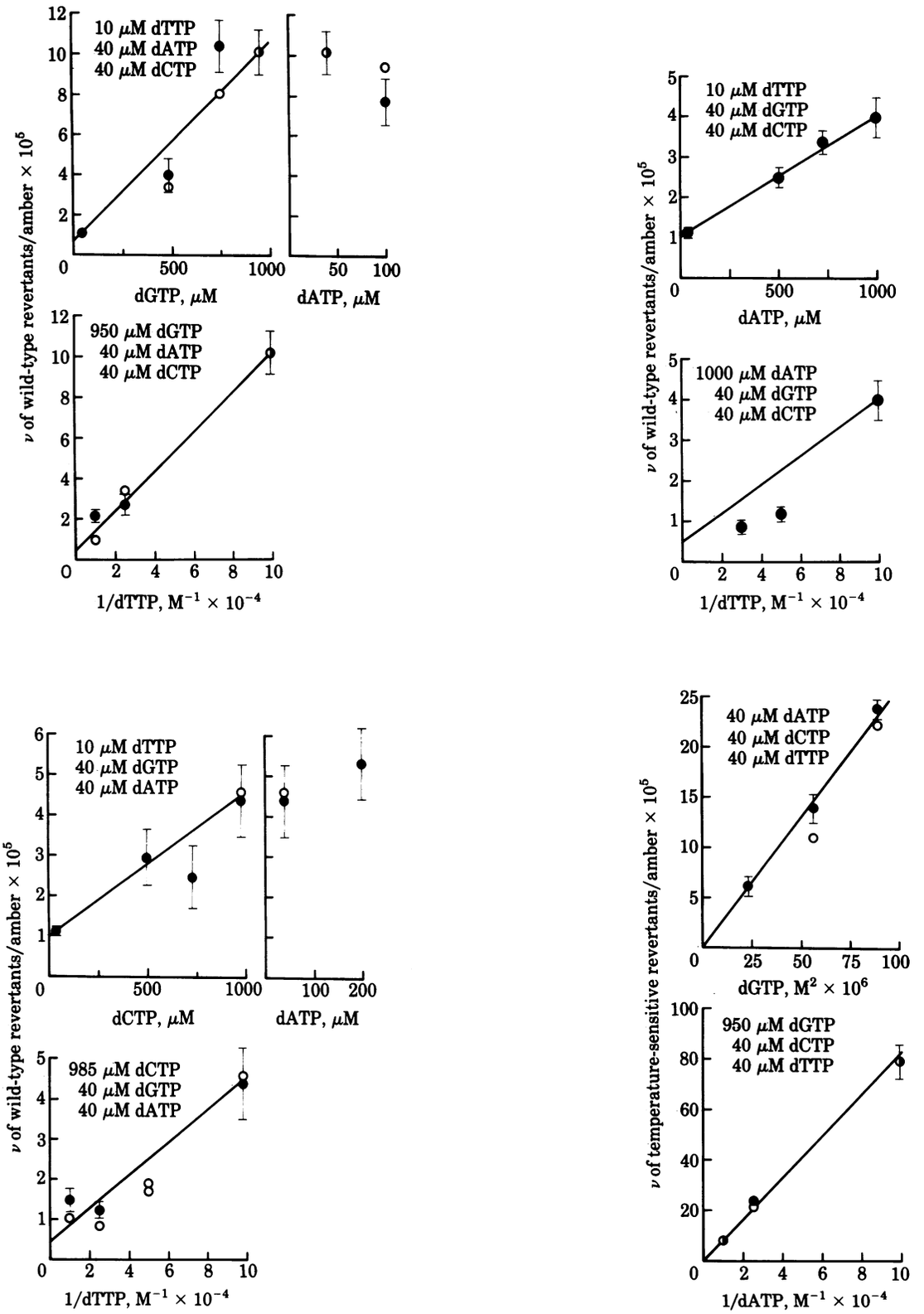

FIG. 1. Dependence of phage reversion frequencies (ν) on the bias of dNTPs during DNA replication ●, Infective center; ○, phage release assays. Wild type refers to all large revertant plaques at 30°C. The error bars are ± 1 SD, which equals $\pm \nu/\sqrt{n}$, where n is the number of revertant plaques scored (between 20 and 150 and typically about 80). (Error bars are not given for phage release assays because of the complications arising from the superposition of burst size.) (*Upper Left*) Production of wild-type revertants (TAG → GAG) indicated by dGTP competing with dTTP. The frequency is essentially independent of dATP concentration at 950 μM dGTP and 10 μM dTTP. (*Lower Left*) Mutation of TAG → CAG. Again, this is essentially independent of dATP concentration at 1000 μM dCTP and 10 μM dTTP. (*Upper Right*) Mutation of TAG → AAG. This is independent of the concentration of the following dNTP ($dNTP^{Fol}$ = dATP), being linear with dATP concentration. (*Lower Right*) Mutation of TAG → TGG producing temperature-sensitive (ts_{30}) revertants (because of the small burst size, the phage release assays have been multiplied by a factor of 2 to normalize to the infective-center assays, which are independent of burst size). The frequency is here dependent on the concentration of the following dNTP ($dNTP^{Fol}$ = dGTP), being linear with the square of the dGTP concentration.

which produce phage in proportion to the burst size and the number of infective centers formed, gave similar proportions of wild-type and ts_{37} revertants but only 5×10^{-7} ts_{30} revertants. The proportion of revertants could be increased dramatically by the appropriate biasing of the ratio of dNTPs. (It will be seen later that of the 2.3×10^{-6} wild-type revertants produced on transfection, 1.7×10^{-6} are calculated to be reverted during replication in 40 μM of each dNTP by replacing the T of the amber codon by A, C, or G.)

Classification of Mutants According to Kinetics of Production and Phenotype. Five different reversion pathways *in vitro* were identified from the rate laws of production of mutants (Eqs. 2 and 3). This classification was strengthened by the observation that the revertants fall into at least three and probably four classes according to their temperature sensitivity of growth (Table 2). Reversion to wild type (GAG) was clearly indicated by a series of experiments varying the concentrations of dGTP and dTTP (Fig. 1). At constant [dTTP], the production of mutants increased linearly with [dGTP], and at constant [dGTP], the production was inversely proportional to [dTTP]. dGTP was clearly competing with dTTP for the first position in the amber codon, forming revertants with a frequency of 1×10^{-6} [dGTP]/[dTTP]. The revertants formed large plaques up to about 42.5°C. Similarly, reversion to CAG, a codon known to give rise

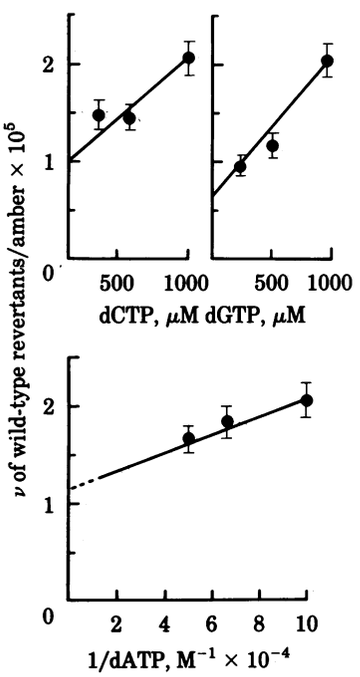

FIG. 2. Detection of pyrimidine·pyrimidine mispairing. The mutation of TAG → TCG was detected by infective-center assays of the DNA produced at high dCTP and dGTP and low dATP concentrations. (*Upper Left*) Increase in reversion frequency with dCTP concentration at 970 μM dGTP, 10 μM dATP, and 197 μM dTTP. (*Upper Right*) Increase in reversion frequency with dGTP concentration at 985 μM dCTP, 10 μM dATP and 197 μM dTTP. (*Lower*) Reversion frequency inversely proportional to dATP concentration at 985 μM dCTP, 970 μM dGTP, and 197 μM dTTP. It is calculated that, from the DNA produced at 985 μM dCTP, 970 μM dGTP, 197 μM dTTP, and 10 μM dATP, a frequency of 9.6×10^{-6} revertants results from TAG → TCG, 4.9×10^{-6} from TAG → GAG, and 1.8×10^{-6} from TAG → CAG. Also 4.1×10^{-6} are ts_{37} and 0.6×10^{-6} are wild-type revertants carried over from the parental DNA. Six out of 7 large plaques picked from a plate of 70 grown at 37°C were found to be phenotypically different from those previously isolated under conditions that produce GAG- and CAG-coding revertants (see Table 2). The shallow dependence of 1/dATP concentration is caused by the background of these latter mutants.

to viable phage (Table 1), was indicated by the kinetics of Fig. 1: revertants were formed with a frequency of 3.6×10^{-7} [dCTP]/[dTTP]. The revertants were as vigorous as wild type. Reversion by the third possible change in the first position, TAG → AAG, was also found (Fig. 1) with a frequency of 3×10^{-7} [dATP]/[dTTP]. These revertants were slightly more temperature sensitive than wild type or TAG → CAG, forming pinpoint plaques (<1-mm diameter) at 43°C, at which parallel studies with the other two gave small plaques (1–2 mm).

In all three cases, the rate of mutation followed the simpler Eq. 2 and was independent of the concentration of the "following" nucleotide, dATP, in the concentration ranges examined (apart from a small increase caused by the parallel pathway of TAG → AAG).

Reversion at the middle position was also readily observed at low dATP and high dGTP concentrations (Fig. 1). Under these conditions, *ts* mutants were formed that appeared to be the ts_{30} noted *in vivo*. These were readily distinguishable from the wild type produced by high dGTP and low dTTP concentrations because of their characteristic temperature sensitivity and small plaque size. They were produced with a frequency of 1×10^{-2} [dGTP]^2/[dATP] (Fig. 1 *Lower Right*). This is very similar to the rate law previously observed for the reversion of *am3*, which also involved TAG → TGG (6). Under the conditions of the present study, *am3* was found to revert with a similar frequency of 1.7×10^{-2} [dGTP]^2/[dATP]. The rate law of Eq. 3 is followed: the concentration of dGTP is involved twice as explained above.

The reversion TAG → TCG was also detected at high concentrations of both dCTP *and* the "following" nucleotide, dGTP, and low [dATP] (Fig. 2). The frequency followed 1×10^{-4} [dCTP][dGTP]/[dATP]. Although at the highest ratio of [dCTP] [dGTP]/[dATP] examined, there was only a two-fold increase above the background reversion frequency caused by the presence of spontaneous mutants and those induced by TAG → CAG + GAG at the high dCTP and dGTP concentrations, we are confident that there was the production of a new mutant because (*i*) the data obtained were the most precise of this study, the spheroplasts used being particularly active; and (*ii*), more importantly, the new mutants showed a further class of temperature sensitivity. The mutants produced at high dCTP, high dGTP, and low dATP concentrations were similar in temperature sensitivity to (and possibly slightly more than) those produced at high dATP and low dTTP concentrations but were markedly more temperature sensitive than those produced from high dCTP or high dGTP and low dTTP concentrations (Table 2). The distinction is fortunately unambiguous.

High concentrations of dTTP did not cause any increase in the number of revertants but, instead, gave a smaller proportion than produced at 40 μM each of dGTP, dTTP, dATP, and dCTP, presumably by decreasing the reversion at the first position in the amber codon.

No significant increase in the number of ts_{37} mutants was observed in any of the experiments *in vitro* despite ts_{37} being the major revertant *in vivo*. This revertant presumably arises *in vivo* at the ss → RF stage of replication. By the process of elimination, it must be one of three undetected transversions at the end of Table 2 that arise through either a T·T, C·C, or C·T mismatch at the RF → ss stage or through an A·A, G·G, or G·A mismatch at the ss → RF step.

Sinha and Haimes (8) have replicated both strands of ϕX174 *am16* RF DNA (RF → RF) using the T4 enzyme system. Wild-type revertants were noted at a frequency of 1.2×10^{-7} [dGTP]/[dTTP] and temperature-sensitive mutants at 1.6×10^{-7} [dGTP]/[dATP]. The latter are presumably the ts_{30} of this study.

DISCUSSION

ϕX174 *am16* has proven to be a most useful mutant for determining the nature of reversion pathways. Of the eight possible mismatch changes that can revert TAG to a sense codon, five have been observed *in vitro* and a sixth has been identified *in vivo* (Table 2). The classification has been greatly aided by the mutants falling into four or possibly five groups according to temperature sensitivity of growth. This has enabled us to measure the frequency of all three combinations of purine (Pur) and pyrimidine (Pyr) mismatches (Pur·Pur, Pur·Pyr, and Pyr·Pyr) and their dependence on the concentrations of dNTPs.

Concentration Dependence of Mispairing Depends on Sequence. The reversion frequencies induced during replication *in vitro* vary with the concentration of dNTPs according to the rate law (Eq. 1) that was previously derived (6) but fall into the simplified versions of Eqs. 2 and 3 (Table 2). For substitution of A, G, or C for the T in TAG, the misincorporation frequency follows the ratio of [dATP], [dGTP], or [dCTP] to [dTTP] and is independent of the concentration of dATP, the following nucleoside triphosphate, in the concentration range examined (>40 μM). However, when dGTP is the following nucleotide, as in the reversion to TGG or TCG, its concentration is critical. The factors governing this behavior are as yet unknown, but mutation frequencies are clearly dependent on the precise sequence of nucleotides.

Relative Frequencies of Mispairings Are Pur·Pur ≈ Pyr·Pur > Pyr·Pyr. Our data support qualitatively the theory of Topal and Fresco (16) on the nature of permitted mispairings. From model-building studies of mispairings that can form complementary hydrogen bonds and fit into the geometry of the double helix of DNA, they propose that transversions arise through Pur·Pur mismatches but not Pyr·Pyr. They also calculate that Pur·Pur mismatches are formed within an order of magnitude of Pur·Pyr mismatches. It is calculated from the rate laws in Table 2 that, for the substitution of another nucleotide for the T in TAG, the relative frequencies of A·G, A·A and A·C mispairings are 10:3:3.6. For the replacement of A in the second position, T·G pairing is 100 times more favored than T·C. The ts_{37} mutant, which can only arise through a Pyr·Pyr mismatch during the RF → SS stage, occurs at too low a frequency to be observed.

Thus, mispairings are catalyzed by DNA polymerase III holoenzyme with the relative frequencies Pur·Pur ≈ Pyr·Pur ≫ Pyr·Pyr.

The Error Rate of the Polymerase *in vitro* Is Similar to the Phage Mutation Frequency *in vivo*. The ratio of dATP to dCTP to dTTP to dGTP in exponentially growing *E. coli* is known with reasonable accuracy to be about 2.4:1.9:1.9:1 (17–19). There is uncertainty about the *absolute* concentrations, but dGTP appears to be in the range of 20–100 μM (6, 17–19). Substituting these values into the rate laws of Table 2 gives the following reversion frequencies for concentrations of dNTPs mimicking those *in vivo*: TAG → GAG, 5×10^{-7}; TAG → AAG, 4×10^{-7}; TAG → CAG, 3.6×10^{-7}; TAG → TGG, $8\text{–}40 \times 10^{-8}$; TAG → TCG, $1.6\text{–}8 \times 10^{-9}$. These figures are within an order of magnitude of those expected for reversion frequencies of ϕX174 *in vivo*, although there is some uncertainty in calculating the precise values *in vivo* because of the unusual mode of DNA replication. For example, in this study ts_{30} and ts_{37} are produced at frequencies of about $5\text{–}15 \times 10^{-7}$; Denhardt and Silver (2) report values of 1.1×10^{-6}, 1.7×10^{-6}, and 4.3×10^{-8} for the reversion of *am6*, *am8* and *am9*, respectively. Combined with our original observation on the accuracy (6), this strongly suggests that the error in base selection by the DNA polymerase is responsible for the spontaneous mutation rate of ϕX174 *in vivo*.

The chromosome of *E. coli* is replicated far more accurately than is the phage (the error rate is $1\text{–}100 \times 10^{10}$ (21)), although the same DNA polymerase is used for both. There is good evidence that the additional accuracy is achieved by a postreplicative mismatch repair system that patrols the double-stranded DNA, recognizes the daughter strand by its methylation pattern, and excises mismatches (5). Thus, the spontaneous mutation rate in *E. coli* is determined by a combination of the accuracy of the DNA polymerase and an attenuation factor due to mismatch repair. Bacterial mutator genes (22) may involve the DNA polymerization reaction *per se*, factors controlling the concentrations of dNTPs, the mismatch repair systems, and the DNA methylation systems.

We are deeply grateful to Dr. Arthur Kornberg for providing the enzymes necessary to continue the project initiated in his laboratory. This work was funded by Medical Research Council Programme Grant G977/985.

1. Goulian, M., Kornberg, A. & Sinsheimer, R. L. (1967) *Proc. Natl. Acad. Sci. USA* **58**, 2321–2328.
2. Weymouth, L. A. & Loeb, L. A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1924–1928.
3. Liu, C. C., Burke, R. L., Hibner, U., Barry, J. & Alberts, B. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 469–487.
4. Kornberg, A. (1980) *DNA Replication* (Freeman, San Francisco).
5. Glickman, B. W. & Radman, M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1063–1067.
6. Fersht, A. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4946–4050.
7. Hibner, U. & Alberts, B. M. (1980) *Nature (London)* **285**, 300–305.
8. Sinha, N. K. & Haimes, M. D. (1980) *Mechanistic Studies of DNA Replication and Genetic Recombination* ICN-UCLA Symposia on Molecular and Cellular Biology, ed. Alberts, B. (Academic, New York) Vol. 19, pp. 707–723.
9. Kunkel, T. A. & Loeb, L. A. (1980) *J. Biol. Chem.* **255**, 9961–9966.
10. Brown, N. L. & Smith, M. (1977) *J. Mol. Biol.* **116**, 1–30.
11. Sanger, F., Air, G. M., Barrell, B. G., Brown, N. L., Coulson, A. R., Fiddes, J. C., Hutchinson, C. A., III, Slocombe, P. M., Smith, M., Drouin, J., Friedman, T. & Smith, A. J. H. (1978) in *The Single Stranded DNA Phages*, eds. Denhardt, D. T., Dressler, D. & Ray, D. S. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 659–669.
12. Gillam, S. & Smith, M. (1979) *Gene* **8**, 81–97.
13. Benbow, R. M., Hutchinson, C. A., III, Fabricant, J. D. & Sinsheimer, R. L. (1971) *J. Virol.* **7**, 549–558.
14. Hirt, B. (1967) *J. Mol. Biol.* **26**, 365–369.
15. Eisenberg, S., Harbers, B., Hours, C. & Denhardt, D. T. (1975) *J. Mol. Biol.* **99**, 107–123.
16. Topal, M. D. & Fresco, J. R. (1976) *Nature (London)* **263**, 285–289.
17. Munch-Peterson, A. (1970) *Eur. J. Biochem.* **15**, 191–202.
18. Mathews, C. K. (1972) *J. Biol. Chem.* **247**, 7430–7438.
19. Neuhard, J. & Thomassen, E. (1976) *J. Bacteriol.* **126**, 999–1001.
20. Denhardt, D. T. & Silver, R. B. (1966) *Virology* **30**, 10–19.
21. Fowler, R. G., Degnen, G. E. & Cox, E. C. (1974) *Mol. Gen. Genet.* **133**, 179–191.
22. Cox, E. C. (1976) *Annu. Rev. Genet.* **10**, 135–56.