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Fidelity of a human cell DNA replication complex

(DNA polymerase α/DNA polymerase δ/mutagenesis/simian virus 40 replication)

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ABSTRACT **We have measured the fidelity of bidirectional, semiconservative DNA synthesis by a human DNA replication complex *in vitro*. Replication was performed by extracts of HeLa cells in the presence of simian virus 40 (SV40) large tumor antigen by using a double-stranded phage M13mp2 DNA template containing the SV40 origin of replication and either of two different target sequences for scoring mutations in the *lacZα*-complementation gene, which encodes the α region (specifying the amino-terminal portion) of β-galactosidase. Replicative synthesis was substantially more accurate than synthesis by the human DNA polymerase α–DNA primase complex purified from HeLa cell extracts by immunoaffinity chromatography, suggesting that additional factors or activities in the extract may increase fidelity during bidirectional replication. However, by using a sensitive opal codon reversion assay, single-base substitution errors were readily detected in the replication products at frequencies significantly higher than estimated spontaneous mutation rates *in vivo*. These data suggest that additional fidelity factors may be present during chromosomal replication *in vivo* and/or that the fidelity of replication alone does not account for the low spontaneous mutation rates in eukaryotes.**

Low spontaneous mutation rates in prokaryotic cells result from at least three processes: (*i*) the accuracy of the polymerization reaction, (*ii*) exonucleolytic proofreading of mistakes made during the polymerization reaction, and (*iii*) postreplication repair by specific error-correction systems. The first two steps are integral features of DNA synthesis carried out by prokaryotic DNA polymerases or their associated subunits (1) and have been studied extensively with purified DNA polymerases and with multisubunit protein complexes known to be involved in prokaryotic DNA replication (for review, see ref. 2).

In eukaryotes, spontaneous mutation rates have been estimated to be 10^{-10} to 10^{-12} errors per base pair per generation (e.g., ref. 3)—values equal to or lower than those found in prokaryotes (4). To elucidate the mechanisms by which this exceptional accuracy is achieved, our laboratory has been systematically investigating the fidelity of DNA synthesis carried out by the four classes of eukaryotic DNA polymerases (α, β, γ, and δ) by using M13mp2-based fidelity assays (5–7). From these and other studies (for a review, see ref. 8), it is clear that the accuracy of purified DNA polymerases is not sufficient to account for low spontaneous mutation rates *in vivo*. One explanation for such inaccuracy may be the loss, during purification, of subunits of the eukaryotic polymerases that function during replication to improve fidelity. Eukaryotic replicative complexes, as compared to purified polymerases, may contain additional components that reduce the number of errors produced during chromosomal replication *in vivo*. To address this issue, we have adapted the M13mp2-based fidelity assays to measure the error rate of a eukaryotic replication complex during bidirectional, semiconservative DNA replication *in vitro*.

The cell-free simian virus 40 (SV40) replication system (9–12) is a useful model for studying DNA replication in mammalian cells because synthesis initiates at a unique site (the SV40 origin), is bidirectional and semiconservative, and is carried out almost entirely by host cellular replication proteins. Only a single viral protein is required, the SV40 large tumor (T) antigen. This system contains all the factors necessary to convert double-stranded circular DNA templates containing the SV40 origin into double-stranded circular daughter molecules (9–12). We report here measurements, using an SV40 DNA replication system *in vitro*, of the fidelity of DNA synthesis catalyzed by this complex eukaryotic replication apparatus.

MATERIALS AND METHODS

Bacterial Strains and Bacteriophage. Bacteriophage M13mp2 and its derivatives and *Escherichia coli* strains CSH50, NR9099, MC1061, and S90C have been described (5, 13). *E. coli* NR9162 (constructed by R. M. Schaaper, National Institute of Environmental Health Sciences) is strain MC1061 that has been transduced by phage P1 to contain a *mutS* marker. Replicative form (RF) DNA was prepared from M13-infected *E. coli* (NR9099) as described (5), followed by ultracentrifugation in a CsCl/ethidium bromide density gradient.

Enzymes. DNA polymerase (pol) α–DNA primase complex from HeLa cells was provided by D. Weinberg and T. J. Kelly (Johns Hopkins University) and was purified by immunoaffinity chromatography essentially as described (14). This preparation contained the four major pol α subunits as described (15) and was assayed for fidelity within 3 weeks of its preparation. Topoisomerase I was the gift of L. F. Liu (Johns Hopkins University). All other enzymes were obtained from either New England Biolabs or Boehringer Mannheim and were used according to the manufacturer's instructions, except for *Dpn* I, which was used in the presence of 0.2 M NaCl.

Fidelity Assays with DNA pol α–Primase Complex. DNA synthesis on M13mp2 duplexes with single-strand gaps was carried out *in vitro* as described (6). Pol α gap-filling synthesis was performed in 50 μl of 20 mM Tris·HCl, pH 7.5/20% (vol/vol) glycerol/10 mM $MgCl_2$/10 mM dithiothreitol/200 μg of bovine serum albumin per ml/300 ng of "gapped" M13mp2 DNA per ml/either 100 μM of 500 μM deoxyribonucleoside triphosphates (dNTPs)/3.8 units of pol α per ml. (A unit of pol α activity is defined as that amount of enzyme that incorporates 1 nmol of dNTP into acid-insoluble material in 1 hr with activated calf thymus DNA as the template-primer.)

SV40 DNA Replication *in Vitro*. SV40 DNA replication reactions (25 μl) were carried out as described (10) in 30 mM

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Abbreviations: pol α and pol δ, DNA polymerases α and δ; RF, replicative form; SV40, simian virus 40; T antigen, SV40 large tumor antigen.

Hepes, pH 7.8/7 mM $MgCl_2$/4 mM ATP/ 200 μM each CTP, GTP, and UTP/100 μM each dATP, dGTP, dTTP, and [α-^{32}P]-dCTP (4000 cpm/pmol)/40 mM creatine phosphate/100 μg of creatine phosphokinase per ml/15 mM sodium phosphate, pH 7.5/40 ng of either M13mp2SV or M13mp2SVA89 RF DNA (123 pmol of nucleotide)/$\approx$1 μg of T antigen (purified either as described in refs. 16 or 17)/$\approx$75 μg of protein from HeLa cytoplasmic extract (18)/$\approx$250 units of topoisomerase I. After incubation at 37°C for 6 hr, the reaction was terminated, and the DNA was purified approximately as described (19). DNA replication products were treated with *Dpn* I prior to transfection of NR9162 cells, whereas DNA incubated with extract alone and untreated RF DNA were not. Transfection and plating were performed as described (13, 20). Because potential mutant replication products should be hemimethylated heteroduplexes and therefore subject to differential correction by the *E. coli* methyl-instructed mismatch repair system (21, 22), transfections were performed with host cells deficient in this repair system (NR9162, *mutS*).

RESULTS

This study utilized two fidelity assays based on complementation of β-galactosidase activity by the α-peptide portion of the enzyme encoded in the single-strand bacteriophage M13mp2. The first is a forward mutation assay for loss of α-complementation activity (5). Since the assay scores for the loss of a function that is not essential for plaque production, a wide variety of mutations can be scored and recovered for subsequent DNA sequence analysis to precisely define the error. The mutational target contains over 110 sites at which single-base substitution errors will produce a detectable phenotype (T.A.K., unpublished data; ref. 23) and 150 sites for frameshift errors (24). Similarly, deletions, additions, and more complex errors are easily scored (5).

The second assay (7, 13) focuses specifically on single-base substitution errors at a three-base mutational target, a TGA nonsense codon in the *lacZ*α sequence. This assay is highly sensitive, since the spontaneous background mutant frequency is much lower than the background frequency for the forward mutation assay. When combined with the ability to force base-substitution errors by intentionally imbalancing dNTP concentrations during synthesis, this reversion assay permits the fidelity of even highly accurate polymerization reactions to be monitored (e.g., refs. 25 and 26).

Construction of SV40 Origin-Containing M13mp2 Molecules. To extend our fidelity studies to a eukaryotic replication apparatus, we first constructed the necessary M13mp2 vectors for both fidelity assays. These contain both the *lacZ*α mutational target sequence and the SV40 origin of replication.

Wild-type (i.e., blue) M13mp2SV used in the forward assay was constructed by inserting the *Hin*dIII–*Sph* I fragment of SV40 (nucleotides 5171–128 on the SV40 map) [obtained from plasmid pJLO (10)] at the unique *Ava* II site in M13mp2. The SV40 origin-containing fragment and the *Ava* II-linearized M13mp2 molecule were treated separately with phage T4 DNA polymerase in the presence of all four dNTPs to generate blunt ends. The M13mp2 molecule was then dephosphorylated with calf intestinal alkaline phosphatase prior to ligation of the two fragments with T4 DNA ligase. Ligation products were used to transfect competent S90C cells as described (5). M13 phage that produced blue plaques were purified and examined for the presence of the SV40 origin by digestion of RF DNA by *Sty* I, which cleaves at a single site in the SV40 sequences. The SV40 origin and the flanking M13mp2 DNA, as well as the *lacZ*α region, were sequenced to confirm that no sequence changes had occurred during the construction. Note that the *Ava* II site has been recreated at the *Sph* I–*Ava* II junction in M13mp2SV.

The DNA substrate for the opal codon reversion assay, M13mp2SVA89, was constructed as follows. The single-stranded circular viral M13mp2SV DNA was hybridized with a denatured M13mp2A89 RF molecule previously linearized by *Ava* II digestion. The M13mp2A89 mutant molecule contains an opal codon (TGA) because of the presence of an adenosine in place of a guanosine at position 89 of the *lacZ*α sequence (position 1 is the first transcribed base of the *lacZ*α gene). Hybridization of these two molecules produces a double-stranded DNA molecule containing a G·T mismatch at position 89 and a single-strand gap across from the SV40 origin in M13mp2SV. The gapped heteroduplex hybridization products were used to transfect competent cells as described above, colorless (white) plaques were picked, single-stranded DNA was prepared, and the *lacZ*α region and the SV40 origin were sequenced to confirm the presence of the correct sequences. This procedure can be used to create a wide variety of reversion targets within the *lacZ*α gene in M13mp2SV.

Analysis of SV40 Replication *in Vitro*. We next examined the ability of the SV40 system to replicate the M13mp2SV molecule *in vitro*. Table 1 shows the dependence of this system on various components. As has been shown previously (9–12), replication occurred only in the presence of both the HeLa cell extract and the T antigen. No synthesis was observed with M13mp2 templates that lacked the SV40 origin (data not shown). The incorporation of >100% of the input DNA is most likely due in part to reinitiation of replication on previously replicated templates and in part to the presence of some rolling-circle synthesis (see below) as suggested (9–12).

To assess the quality of DNA synthesis, replication products were analyzed by electrophoresis in an agarose gel in the presence of ethidium bromide (Fig. 1). No radiolabeled DNA products were observed in reactions incubated in the absence of T antigen (lane 1). When the reaction was carried out in the presence of T antigen, a significant amount of synthesis occurred, and the majority of the M13mp2SV DNA products migrated coincident with RF I (supercoiled) or RF II (relaxed) monomer-length circular DNA (lane 2). Electrophoresis in the absence of ethidium bromide (data not shown) indicated that the RF I-type products were partially supertwisted monomer circles. The presence of some high molecular weight DNA products (e.g., lanes 2 and 3) is consistent with similar observations in other laboratories (9–12) and may represent replication intermediates (θ structures) or products of rolling-circle replication.

We have used the *Dpn* I assay (10–12) to specifically monitor semiconservative DNA synthesis in the SV40 system. As seen in Fig. 1, the ^{32}P-labeled product DNA was

Table 1. Dependence of DNA replication on components of the reaction mix

Reaction mixture	Incubation, hr	dNTP incorporated, pmol
Complete	2	111
	4	202
	6	245
Without T antigen	2	0.5
Without DNA	2	0.5
Without HeLa extract	2	0.5

DNA replication was carried out as described with M13mp2SV DNA (123 pmol of nucleotide) except that the reaction mixture was incubated at 37°C for the amount of time indicated. Acid-precipitable material was collected on glass fiber filters and assayed for radioactivity by liquid scintillation counting.

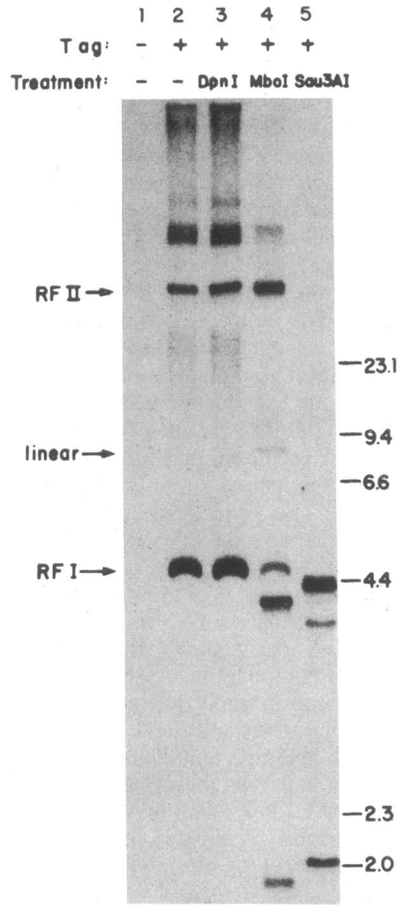

FIG. 1. Analysis of SV40 DNA replication products. Aliquots of replication reaction mixtures (6 hr) with M13mp2SV DNA were digested with the appropriate restriction enzyme and analyzed by agarose gel electrophoresis (2–3 V per cm) in 40 mM Tris acetate/1 mM EDTA containing 0.2 μg of ethidium bromide per ml of buffer, followed by autoradiography of the dried gel. RF DNA standards were run in parallel lanes. Numbers on the right of the figure represent the location of linear DNA size standards (a *Hin*dIII digest of bacteriophage λ DNA). The difference in mobility between the *Sau*3AI fragments and the *Mbo* I fragments was eliminated when the products of the restriction digestions were extracted with phenol prior to electrophoresis. We have noticed a reproducible difference in the sensitivity of the RF I- and RF II-type replication products to digestion with *Mbo* I, suggesting that a larger percentage of the RF I-type products have been through more than one round of replication; at present, we have no explanation for this observation. We have also noticed that high molecular weight material migrating slower than RF II DNA shows the greatest sensitivity to *Mbo* I, consistent with previous product analyses (11).

completely resistant to digestion by *Dpn* I (lane 3), an enzyme that cuts only fully methylated 5′-GATC-3′ sequences, whereas unreplicated M13mp2SV DNA was completely digested by *Dpn* I (data not shown). Thus, in the presence of T antigen, the DNA template underwent semiconservative replication. The product DNA was completely cut by the *Dpn* I isoschizomer *Sau*3AI (lane 5), which is insensitive to the state of adenine methylation at the five GATC sites present in this DNA, demonstrating that the products retain these sites in a cleavable form. Treatment of the product DNA with *Mbo* I (lane 4), which cuts only fully unmethylated GATC sequences, revealed that a substantial portion of the monomer circles are the products of a single round of replication—i.e., resistant to *Mbo* I digestion. The portion of the monomer circle products that were sensitive to *Mbo* I could reflect multiple rounds of replication in the extract. This would be consistent with the incorporation data (Table 1) and previous studies of SV40 replication *in vitro* (9, 11, 12). However, even if all the DNA products arose from two rounds of replication, our estimates of error rates would decrease by a factor of only 2. Alternatively, the *Mbo* I-sensitive DNA could result from a single round of replication of input template molecules that were not fully methylated (for example, see ref. 21).

Forward Mutagenesis Assay with the SV40 Replication Apparatus. Prior to initiating a study of the fidelity of DNA replication *in vitro*, we wanted to determine if replication errors, once made, would survive incubation in the crude HeLa extract. A complete M13mp2 heteroduplex containing a single A·C mismatch at position 89 was constructed (13). Each strand of the heteroduplex coded for a different and easily identified phenotype [(+)-strand, white; (−)-strand, dark blue]. This heteroduplex molecule was incubated with the HeLa cytoplasmic extract, and the DNA was then purified and used to transfect competent NR9162 cells. The frequency of recovery of the phenotypes of both strands and the frequency of mixed bursts were similar to that of the unincubated heteroduplex (Table 2). The data indicate that this mismatch was not efficiently repaired in the HeLa extract, at least not in the absence of T antigen-dependent replication. Thus, errors produced during replication *in vitro* should yield M13mp2SV plaques having altered LacZα phenotypes.

We then examined the forward mutation frequency in the wild-type *lacZα* sequence of M13mp2SV DNA, replicated at equal concentrations (100 μM) of all four dNTP substrates. The frequency of mutants in this DNA (Table 3) was similar to the mutant frequency in template DNA incubated with HeLa cytoplasmic extract but no T antigen. Both of these values were about 2-fold above the background mutant frequency in the starting preparation of untreated M13mp2-SV DNA. From these data, an initial estimate of the error rate of the replication complex can be made, as described in the legend to Table 3. This value, 6.7×10^{-6} or 1 error per 150,000 nucleotides incorporated, represents an average base substitution and frameshift error rate for a large number of different sites. This may be an underestimate of the fidelity of the replication complex for two reasons. First, there is only a small difference between the mutant frequency of the replicated samples and the unreplicated starting template DNA, a difference that is not convincingly different. Second, this error rate is calculated on the basis of the number of detectable sites for base-substitution errors obtained from polymerase gap-filling reactions using the regulatory sequences and the first 45 codons of the *lacZα* gene as a target. This number (110 sites) is a minimum estimate of the number of detectable sites, since the replication complex copies the entire *lacZα* sequence in M13mp2SV, potentially increasing the number of detectable sites in the target.

Table 2. Heteroduplex expression in M13mp2 DNA after incubation in HeLa extract

Treatment	Plaques scored			Frequency of expression	
	Blue	White	Mixed	(−)-Strand	(+)-Strand
None	839	392	102	0.71	0.37
HeLa extract	1066	498	135	0.71	0.37

An M13mp2 heteroduplex molecule containing a C [blue, (−)-strand]·A [white, (+)-strand] mismatch was used to transfect NR9162 cells either before or after incubation for 6 hr in a replication reaction containing HeLa cell extract but no T antigen. The frequency of expression of the (−)-strand is calculated by dividing the sum of blue and mixed plaques by the total plaque-forming units, whereas the frequency of expression of the (+)-strand is calculated by dividing the sum of white and mixed plaques by the total plaque-forming units. It is theoretically possible to have 100% expression of each strand, in which case all of the plaques would be mixed bursts.

Table 3. Forward mutant frequency of M13mp2SV DNA replicated by a human cell extract

Replication conditions	Plaques scored		Mutant frequency $\times 10^{-4}$
	Total	Mutant	
Experiment 1			
Untreated	64,975	21	3.2
Without T antigen	ND		
Complete	61,930	39	6.3
Experiment 2			
Untreated	11,983	4	3.3
Without T antigen	92,046	61	6.6
Complete	60,480	46	7.6

SV40 DNA replication products were prepared by incubation for 6 hr, purified as described in the legend to Fig. 1, and used to transfect *E. coli* NR9162 as described (5, 19). Because the frequency of mutants in the replicated DNA is close to the frequency in unreplicated DNA, the choice of which background value to subtract significantly affects the estimation of error rates. It is not clear that the difference between the untreated DNA and the DNA incubated with extract alone is real. The difference could be the result of the inherent fluctuation in dealing with small numbers or increased mutations by unknown mechanisms in DNA incubated with HeLa extract. The mean of the two "Untreated" control experiments yields a background mutant frequency of 3.2×10^{-4}. Subtracting this background from the mean of the two complete reaction values (6.9×10^{-4}), dividing by 0.5 (the approximate probability of expressing an error), and then dividing by the number of sites (110) yields an average error rate of 1 per 150,000 nucleotides incorporated. When the "Without T antigen" value is used as background, the error rate becomes 1 per 1,800,000. Based on the mutant frequencies in the reversion assay (see below), we conclude that incubation with the extract probably does not lead to additional mutations and that the difference between the controls in the forward assay reflects experimental variation. Thus, we favor the former error rate estimate of 1 per 150,000. ND, not determined.

Opal Codon Reversion Assay. Since errors were not observed at high frequency above background in the forward mutation assay, we next used the more sensitive opal codon reversion assay. We performed reactions with two different extracts, using an equimolar concentration (100 μM) of all four dNTP substrates. The replicated products yielded a reversion frequency greater than the unreplicated DNA (Table 4). However, as in the forward assay, the frequencies were only several fold above the spontaneous background. Therefore, replication reactions were carried out with dNTP substrate pool biases—with dGTP and dCTP in excess over dTTP and dATP. This experimental design is intended to increase base substitution errors at both the first and third positions of the opal codon because of the increased ratio of incorrect substrates to correct substrates.

The frequency of TGA revertants in DNA replicated under pool-bias conditions was consistently higher than the frequency of revertants in DNA replicated in the presence of equimolar dNTP pools (Table 4), indicating that base-substitution errors are readily detectable in this assay. The base-substitution fidelity could be estimated from the revertant frequencies as described in the legend to Table 4. When using the values from the 20-fold pool-bias reactions, the error rate per base pair incorporated at this TGA site was between 1 in 140,000 and 1 in 33,000. The observation that the error rate for base substitutions at this site may be slightly higher than the average base-substitution error rate throughout the target in the forward assay indicates that this site will be useful for future studies of high-fidelity replication systems.

A number of the revertants from the 20-fold pool-bias sample in experiment 1 (Table 4) were examined by DNA sequence analysis: all contained single-base transition and transversion errors at the first and third positions as would be expected from the pool bias used (Table 5). An estimate of the base-substitution fidelity for specific mispairs at the first and third positions of the opal codon could be made from the sequencing data. The error rates for individual base changes at this codon varied >10-fold during replication by human cell extracts (Table 5).

Table 4. Opal codon reversion frequency in M13mp2SVA89 DNA replicated by a human cell extract

Replication conditions	Plaques scored		Reversion frequency $\times 10^{-5}$
	Total	Revertant	
Experiment 1			
Untreated	900,000	8	0.9
Without T antigen	2,400,000	15	0.6
Complete			
Equal dNTP pools	2,400,000	38	1.6
G+C pool bias			
20-fold	870,000	127	15
Experiment 2			
Untreated	740,000	18	2.4
Without T antigen	460,000	6	1.3
Complete			
Equal dNTP pools	1,500,000	129	8.6
G+C pool bias			
5-fold	480,000	65	14
10-fold	220,000	42	19
20-fold	100,000	63	63
50-fold	16,000	13	81

DNA replication reactions were carried out for 6 hr as described except that the DNA used was M13mp2SVA89. The dGTP+dCTP (G+C) pool bias experiments were identical to the equal-pool experiments except that dNTP concentrations were as follows: 5-fold pool bias, 50 μM each dATP and dTTP and 250 μM each dGTP and dCTP; 10-fold, 50 μM dATP/dTTP and 500 μM dGTP/dCTP; 20-fold, 50 μM dATP/dTTP and 1000 μM dGTP/dCTP; 50-fold, 40 μM dATP/dTTP and 2000 μM dGTP/dCTP. Experiments 1 and 2 were performed with different extract preparations. We have noted some variation in mutant frequency between different extracts. The error rate per detectable base replicated is calculated as in the legend to Table 3 (by using the "Without T antigen" data as background), but in this case the frequency is divided by 3 (equal pool experiments) or 2 (pool bias experiments) to correct for the number of sites in the opal codon target.

Fidelity of HeLa Cell DNA Pol α–Primase Complex Purified by Immunoaffinity Chromatography. Since replication in the extract depends on pol α (10–12, 14), we examined the accuracy of gap-filling DNA synthesis by the pol α–primase

Table 5. Error rates at individual base pairs in the opal codon (TGA_{89}) of M13mp2SVA89

Mutation to	Potential mispairs involved	Revertants	Error rate
TG*G*	A:dCTP, T:dGTP	38	1/450,000
TG*C*	A:dGTP, T:dCTP	≤6	≤1/1,500,000
*C*GA	T:dGTP, A:dCTP	77	1/110,000
*G*GA	T:dCTP, A:dGTP	12	1/710,000

These data are derived from the 20-fold G+C pool bias in experiment 1 in Table 4. Only one codon (the original wild-type TGG) of the eight detectable codons derived from the TGA by single-base substitution yields a dark-blue phenotype. Thus, a direct color comparison of revertant to wild-type plaques permits this mutant to be quantitated without DNA sequencing. To confirm this, 10 dark blue revertants were sequenced; all contained the expected TGG codon. Fifteen of the light-blue mutants were chosen at random for DNA sequencing; 13 CGA and 2 GGA sequences were found. Of the 127 revertant plaques obtained under pool-bias conditions, 38 were dark blue and 89 were light blue. The total number of each of the light-blue revertants are estimates based on the fraction of those sequenced that contain the specific base change.

complex freshly purified from HeLa cell extracts by immunoaffinity chromatography. In the forward mutation assay, this enzyme complex generated mutants at a frequency of 130×10^{-4}, 20-fold above the spontaneous background. This value is similar to our previous measurements of the fidelity of pol α purified from several sources (including HeLa cells) by several different procedures (6). We conclude that, when considering a variety of errors at many positions, the intact pol α–primase complex is not highly accurate, having an error rate of 1.9×10^{-4} or 1 error per 5000 nucleotides incorporated.

DISCUSSION

The results presented here establish the fidelity of bidirectional, semiconservative DNA replication by an extract of human (HeLa) cells. Since SV40 DNA replication *in vitro* is known to require pol α (10–12, 14) and may also involve pol δ (27, 28), it is interesting to compare mutation rates in the SV40 replication system to the fidelity of these purified polymerases. Our data suggest that this human cell replication complex is substantially more accurate than is human pol α. The most direct comparison is in the forward assay, where the cell extract has an estimated fidelity of ≤ 1 error per 150,000 nucleotides incorporated, whereas the pol α–primase complex has an error rate of 1 per 5000. This difference suggests that pol α may operate during SV40 replication in a form that is much more accurate than the purified polymerase. Increased fidelity could result from additional protein subunits that alter processivity by increasing the affinity of the polymerase for template-primers. Fidelity could also be improved by exonucleolytic proofreading. Multisubunit forms of HeLa pol α have been reported, including one that contains a $3' \rightarrow 5'$ exonuclease that could provide a proofreading function (29). Similarly, pol α from other sources has been found to have an associated $3' \rightarrow 5'$ exonuclease (30, 31). A $3' \rightarrow 5'$ exonuclease activity also has been discovered in a highly purified preparation of pol α from *Drosophila melanogaster* (32).

The demonstration that pol δ has an intrinsic $3' \rightarrow 5'$ exonuclease (33) that is capable of proofreading base substitution errors (7) suggests the possibility that pol δ may play a major role in determining the accuracy of SV40 replication. Fidelity measurements for calf thymus pol δ (7) demonstrate that this enzyme can be even slightly more accurate than the replication apparatus in these extracts of human cells. It remains to be determined whether proofreading by either pol α or pol δ contributes to fidelity in the SV40 replication system *in vitro*.

The base substitution error rate per round of replication in the SV40 system ranges from 8.9×10^{-6} (for the TGA $\rightarrow$ CGA change) to $\leq 6.9 \times 10^{-7}$ (for the TGA $\rightarrow$ TGC change) (Table 5). Although further efforts with the SV40 system will be required to determine the fidelity for various mispairs at different sites, even the limited data shown here suggest that, just as with purified DNA polymerases (5, 6) and just as in chromosomal replication *in vivo* (K. R. Tindall and L. F. Stankowski, Jr., personal communication), the mutation frequency in this eukaryotic replication system varies widely depending on the site and mispair being examined.

It is clear that the SV40 replication system does commit errors *in vitro* at a rate that is much higher than the estimated spontaneous mutation rate for chromosomal loci *in vivo* (10^{-10} to 10^{-12}). It is possible that the HeLa cell extract used in this study is missing critical fidelity components. It is also possible that SV40 origin-dependent replication uses a form of a replication complex that is different from that used by the cell for chromosomal replication. However, it is intriguing to consider that DNA replication in human cells may not be highly accurate relative to the estimated spontaneous mutation rate in such cells, and that higher organisms may rely heavily on postreplication repair processes to ensure the integrity of genetic information.

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