INFIDELITY OF DNA SYNTHESIS BY REVERSE TRANSCRIPTASE

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SUMMARY: The fidelity of purified DNA polymerase from avian myeloblastosis virus in precisely copying polynucleotide templates was determined. With poly (dA-dT)·poly (dA-dT) as a template, one molecule of the incorrect base-paired nucleotide (dCTP) is incorporated for every 6000 nucleotides polymerized. When copying the ribo strand of poly (rA)·poly (dT) the error rate is approximately one in 600. It is suggested that the enzyme makes similar errors *in vivo* and thus could be mutagenic.

In a recent communication (1) from this laboratory we found evidence for a DNA polymerase in human leukemic lymphocytes that has an unusually high error rate when copying synthetic polynucleotide templates. Control extracts from phytohemagglutinin stimulated normal lymphocytes copied the same template with a 10-fold greater precision. The most direct explanations for the infidelity of DNA synthesis in the leukemic extracts were (a) the presence of a unique polymerase, possibly of viral origin, in the leukemic cells or (b) the presence of altered cellular polymerases that are faulty in base selection. We now report on studies which indicate that a DNA polymerase of an RNA tumor virus, avian myeloblastosis virus, permits many mistakes in base-pairing when copying polynucleotides.

Avian myeloblastosis virus was first isolated from plasma of infected chickens (gift of Dr. Beard) by velocity and equilibrium centrifugation. There after the purified virions were disrupted with Triton-X and the DNA polymerase (reverse transcriptase) was purified by the procedure of Hurwitz (2). The most purified fraction was tested for its precision in guaranteeing correct base-pairing when copying poly (dA-dT)·poly (dA-dT) and the ribo strand of poly (rA)·poly (dT) or poly (rA)·oligo (dT) (Table I). As may be seen the

TABLE I

Fidelity of DNA Replication with Several Homopolymers

Template	Correct Nucleotide Incorporation pmoles dTTP	Incorrect Nucleotide Incorporation pmoles dCTP	Level of Infidelity
poly (dA-dT)·poly (dA-dT)			
* Experiment I	467	0.070	1/6,670
* Experiment II	685	0.035	1/19,570
Experiment III	179	0.009	1/19,890
Experiment IV	114	0.010	1/11,400
Experiment V	281	0.055	1/5,100
poly (rA)·poly (dT)			
Experiment I	27	0.051	1/530
Experiment II	97	0.16	1/600
poly (rA)·oligo $(dT)_{12-18}$	78	0.078	1/1,000
poly (rC)·oligo $(dG)_{12-18}$	dGTP pmoles	dATP pmoles	
	140	0.048	1/2,920

* In preliminary experiments, a small amount of purified AMV DNA polymerase was generously provided by Dr. David Baltimore. Poly dT was a gift of Dr. Bollum, poly (rA)·oligo (dT) and poly (rC)·oligo (dG) were purchased from Collaborative Research. Poly (dA-dT)·poly (dA-dT) was made by the de novo reaction of E. coli DNA polymerase I. Poly (rA)·poly (dT) was synthesized by annealing poly (rA) to poly (dT). All labeled nucleoside triphosphates were purchased from New England Nuclear.

DNA Polymerase Activity: To measure the fidelity with which poly (dA-dT)·poly (dA-dT) is copied we measured the incorporation of correct nucleotides (dATP and dTTP) and incorrect nucleotide (dCTP) in separate assays. For determination of total synthesis a standard reaction mixture was employed containing in a total volume of 0.2 ml: 13.0 μM ^{3}H-dTTP (1500 dpm/pmole), 12.5 μM dATP, 20 μM dCTP, 10 mM $MgCl_2$, 5 mM KCl, 5 mM dithiothreitol, 0.05 M Tris-HCl, pH 8.0, and 92 μM phosphorus of poly (dA-dT)·poly (dA-dT). Assays were initiated with 5 λ of enzyme and incubated for 90 minutes at 37°C. For measurement of dCTP incorporation, identical reaction mixtures were used except that they contained unlabeled dATP and dTTP and 20 μM ^{3}H-dCTP which had 30,000 dpm/pmole. Incorporation was stopped by the addition of acid and washed as previously described (1). In experiments employing poly (rA)·poly (dT) as template reaction conditions were similar except for the presence of 70 μM phosphorus of poly (rA)·poly (dT). For measuring the fidelity of poly (rA)·oligo (dT) 50 μM phosphorus of this template was included in place of poly (rA)·poly (dT). In experiments employing poly (rC)·oligo (dG) as template the reaction mixtures measuring total synthesis contained 13 μM ^{3}H-dGTP (2,400 dpm/pmole), 20 μM dATP, and 100 μM phosphorus poly (rC)·oligo (dG). Reaction mixtures measuring incorrect nucleotide incorporation were the same as assays measuring correct synthesis except for the presence of 20 μM ^{3}H-dATP (25,000 dpm/pmole) and unlabelled 13.0 μM dGTP. In control experiments with poly (rA)·poly (dT) E. coli polymerase I made less than 1 error in 100,000 nucleotides polymerized.

Total incorporation with the different templates are not to be compared since different amounts of enzyme were used in different assays.

TABLE II

Reaction Requirements for Correct and Incorrect Nucleotide Incorporation

Template	poly (dA-dT)·poly (dA-dT) pmoles incorporation		poly (rA)·poly (dT) pmoles incorporation	
Reaction Mixtures	dTTP	dCTP	dTTP	dCTP
Complete	281	0.055	97	0.16
Minus Enzyme	0	0	0	0
Minus DNA	0	0.006	0	0.03
Minus Mg^{++}	0	0	0	0
Minus dATP & dTTP	-	0.006	-	0.013
Heated Enzyme*	0	0	0	0

* The enzyme was heated for 15 minutes at 70°C.

Reaction conditions are given in Table I.

Incorporation of incorrect bases occurs with a high frequency. The frequency of error in copying the ribo-strand of poly (rA)·poly (dT) or poly (rA)·oligo (dT) varied in different experiments from 1/500 to 1/1000. The frequency of errors when copying poly (dA-dT)·poly (dA-dT) was about an order of magnitude less than when copying poly (rA)·poly (dT). This infidelity does not simply result from chemical contamination of templates or radioactive precursor since the same reaction mixtures with homogeneous E. coli DNA polymerase I makes few mistakes (less than 1 in 100,000) (see legend to Table I).

The requirements for both correct and incorrect deoxynucleotide incorporation for copying poly (dA-dT)·poly (dA-dT) (a DNA template) and for copying the ribo-strand of poly (rA)·poly (dT) (an RNA template) are shown in Table II. The requirements for both correct and incorrect nucleotide incorporation were similar. Without the added divalent cation or polynucleotide template, incorporation of either correct or incorrect nucleotides was less than 20% of that obtained with the complete reaction mixtures. In assays without enzyme or with heated enzyme no incorporation was observed.

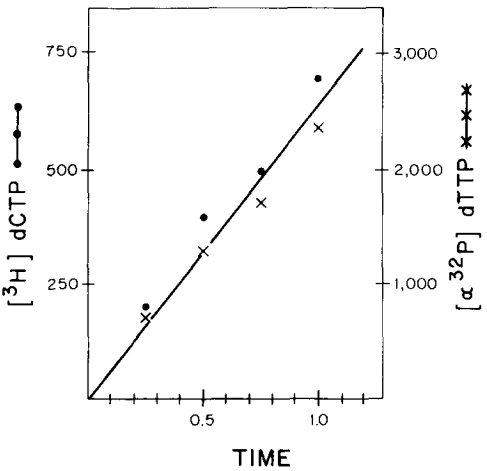

FIGURE 1: Time course of incorporation of correct (dTTP) and incorrect nucleotides (dCTP) with poly (rA)·poly (dT) as template. Reaction conditions are given in Table I except that 13.0 μM [α-^{32}P]dTTP (60 dpm/pmole) was employed. Time is in hours.

The omission of the correct nucleotides dATP and dTTP for copying poly (dA-dT)·poly (dA-dT) and for copying poly (rA)·poly (dT) reduced the incorporation of the incorrect nucleotide (dCTP) to less than 20% observed with the complete reaction mixture. This suggests that for faulty incorporation DNA polymerization is required. Further evidence that the incorrect nucleotide incorporation was not simply the addition of dCTP onto the end of polynucleotide chains is shown by the time course of the reaction (Fig. 1). In this experiment the incorporation of [α-^{32}P]dTTP (correct) and [^{3}H]dCTP (incorrect) was determined in the same reaction mixtures with poly (rA)·poly (dT) as template. The incorporation of the incorrect nucleotide paralleled that of the correct nucleotide suggesting that the incorrect base was not just added terminally but was inserted as DNA synthesis proceeded.

The product of the reaction using poly (rA)·poly (dT) as template-primer was analyzed in CsCl density gradients in order to determine if both correct and incorrect nucleotides were incorporated in an RNA-DNA hybrid product. Figure 2 illustrates that both [^{3}H]dCTP and [α-^{32}P]dTTP banded at a density expected of an RNA-DNA hybrid indicating that both were incorporated

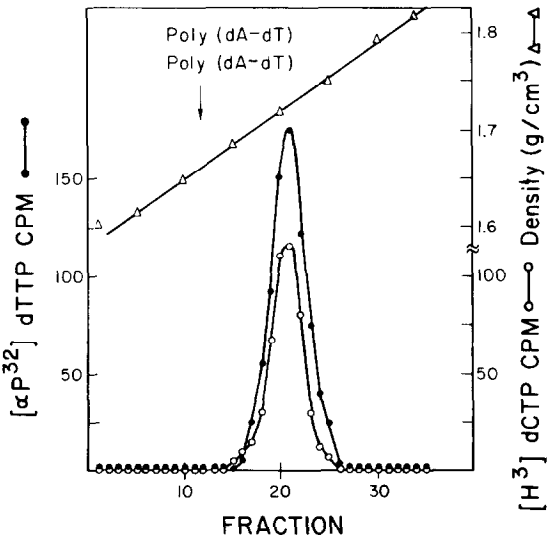

Figure 2: CsCl density gradient analysis of the product of the reaction using poly (rA)·poly (dT) as template. Five normal reaction mixtures containing 20 μM [^{3}H]dCTP (8,900 cpm/pmole) and 13.0 μM [α-^{32}P]dTTP (25 cpm/pmole) were employed. The polynucleotide product was dialyzed against 4 liters changed 3 times of 0.1 M Tris-HCl, pH 7.5, containing 1 M NaCl. After dialysis the product was suspended in CsCl (final density 1.75 gm cm^{-3}) in a final volume of 5 ml. The solution was centrifuged for 70 hours at 35,000 rpm (147,000 xg) at 20°. Eight drop fractions were collected from the top of the tube and the radioactivity determined. Separate gradients contained: Marker DNA consisting of poly [^{3}H] (dA-dT)·poly (dA-dT), and the reaction mixtures.

into hybrid molecules. The ratio of incorrect to correct nucleotides incorporated in material banding at the hybrid density agrees with the anticipated number of errors as determined from regular assays (1 in 750). The method for determining errors is the ratio of correct to incorrect incorporation and does not indicate the distribution of incorrectly incorporated nucleotides.

DISCUSSION:

The results presented here indicate that the purified DNA polymerase (reverse transcriptase) from avian myeloblastosis virus copies both poly (rA)·poly (dT) and poly (rA)·oligo (dT) with errors. The level of infidelity observed is similar to that reported using nucleic-acid free extracts from acute leukemic human lymphocytes (1) suggesting the infidelity in these malignant cells might indicate the presence of a polymerase similar to that found

in avian myeloblastosis virus. Evidence for a DNA polymerase present in human leukemic cells with properties similar to that of known polymerases from animal RNA tumor virus has been reported by Gallo et al. (3). It is now important to determine if a particular polymerase is present in leukemic cells with properties resembling a "reverse transcriptase" which is faulty in base-selection. If so, inexactness in copying polynucleotide templates might be an important marker for the presence of viral polymerases in human leukemic cells.

In general, DNA polymerases exhibit little if any specificity for homologous templates. It is conceivable that a viral enzyme is able to copy host cell DNA. If the infidelity we observe in vitro also occurs in vivo, copying of host cell DNA by a mutagenic viral enzyme may be an important mechanism for tumor progression (4, 5). A viral enzyme could be mutagenic leading to production of altered enzymes including cellular DNA polymerases.

It is of interest that AMV DNA polymerase is least able to copy without mistakes the ribo-strand of poly (rA)·oligo (dT) or poly (rA)·poly (dT). Additional studies will be needed to determine if this inexactness is prominent with other polyribonucleotide templates and if other RNA tumor viral polymerases make frequent errors when copying RNA and DNA templates. If so, RNA tumor viruses may exhibit an exceptionally high rate of mutations in vivo.

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