INFIDELITY OF DNA SYNTHESIS: A GENERAL PROPERTY OF RNA TUMOR VIRUSES

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SUMMARY. We have determined the frequency with which a non-complementary base-paired nucleotide is incorporated by the DNA polymerase of Rauscher leukemia virus using synthetic polynucleotides as templates. The observed error rates are very similar to those error rates previously reported with the DNA polymerase from avian myeloblastosis virus. This similarity suggests that a high level of infidelity may be a common characteristic of RNA tumor viruses. This high error rate may be relevant to the mode of action of the polymerase during carcinogenesis.

The fidelity of DNA synthesis has been determined with purified enzymes from prokaryotes (1), eukaryotes (2), and an RNA tumor virus (3). The unusually high error rate observed with the DNA polymerase of avian myeloblastosis virus indicated that this enzyme may have an intrinsic mutational capability. We now report that the DNA polymerase from Rauscher leukemia virus also has an extremely high error rate comparable to that of avian myeloblastosis virus.

Rauscher leukemia virus was obtained from Dr. E. Gruber. The virus was further isolated by sedimentation and equilibrium centrifugation. The virions were disrupted and the RNA directed DNA polymerase isolated through the phosphocellulose step as described by Hurwitz and Leis (4).

The ability of the purified DNA polymerase to copy synthetic polynucleotides accurately was then examined (Table 1). With poly(rA).oligo(dT) as template, this enzyme incorporates one molecule of non-complementary base-paired deoxyribonucleotide (dCTP) for every 525 molecules of complementary nucleotide (dTTP) polymerized. When a purine non-complementary nucleotide (dGTP) is utilized, the error rate using poly(rA).oligo(dT) as a template is reduced to less than 1/3600. With poly(rC).oligo(dG) as template, this enzyme incorporates one molecule of non-complementary base-paired deoxyribonucleotide (dATP) for every 307 molecules of complementary nucleotide (dGTP) polymerized.

TABLE I

Level of Infidelity by DNA Polymerase from
Rauscher Leukemia Virus

Template	Complementary Nucleotide	Non-Complementary Nucleotide	Complementary Nucleotide Incorporation (pmoles)	Non-Complementary Nucleotide Incorporation (pmoles)	Error Rate
poly rA.oligo dT	dTTP	dCTP	37.3	0.071	1/525
poly rA.oligo dT	dTTP	dGTP	32.0	< 0.009	<1/3600
poly rC.oligo dG	dGTP	dATP	101.6	0.33	1/307
poly dG.poly dC	dGTP	dATP	45.3	0.045	1/1010

RLV DNA Polymerase Activity: The incorporation of complementary (dTTP) and non-complementary (dCTP) nucleotides by RLV DNA polymerase using poly(rA).oligo(dT) as a template was measured at 37°C for 60 minutes. The standard reaction mixture (total volume 0.05 ml) contained 50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 20 mM KCl, 5 mM DTT, 2 ug BSA, 26 µM α-P^{32} dTTP (26.8 dpm/pmole), 30 µM ^{3}H-dCTP (55,000 dpm/pmole), or 30 µM ^{3}H-dGTP (30,000 dpm/pmole), and 1 µg of poly(rA).oligo(dT). For poly rC.oligo dG and for poly dG.poly dC, separate assays for each polynucleotide template were empolyed and measured complimentary (dGTP) and non-complimentary (dATP) nucleotide incorporation with ^{3}H-dGTP and ^{3}H-dATP respectively. The assay conditions were identical to those for poly (rA).oligo(dT) except that 30 µM dATP and 27 µM dGTP were added to the incubation mixture. All incubation contained 0.025 ml of purified RLV DNA polymerase. The wash procedure of Battula and Loeb was employed (5).

Poly(rA).oligo(dT) wwas hybridized by annealing poly(rA) to oligo(dT). The mass ratio of polymer to oligomer was 1:1. Poly(rC).oligo(dG) was purchased from Collaborative Research and poly(dG).poly(dC) was purchased from P-L Biochemicals. All labelled nucleoside triphosphates were purchased from New England Nuclear Corporation.

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TABLE II

Reaction Requirements for Complementary and Non-Complementary Nucleotide Incorporation

Reaction Mixture	Complementary Nucleotide(dTTP) Incorporation (pmoles)	Non-Complementary Nucleotide(dCTP) Incorporation (pmoles)
Complete	20.0	0.078
Minus Enzyme	< 1.0	<0.005
Minus Template	< 1.0	<0.007
Minus Mg^{2+}	< 1.0	0.017
Minus dTTP	< 1.0	0.021

The incubation conditions are identical to those described in Table 1.

These error rates of RLV DNA polymerase are of the same magnitude as those previously reported for AMV DNA polymerase (5), and are significantly higher than those reported for E. coli DNA polymerase I (1, 5). As shown in Table 1, the DNA polymerase from Rauscher leukemia virus also copies the deoxypolymer poly(dG).poly(dC) with a high level of infidelity. One molecule of non-complementary base-paired nucleotide (dATP) is incorporated for every 1011 molecules of complementary nucleotide (dGTP). This result is also comparable to those previously obtained with avian myeloblastosis virus (5).

The requirements for the incorporation of the complementary and non-complementary nucleotides are identical and are typical of a DNA polymerase (Table 2). With poly(rA).oligo(dT) as the template the incorporation of the non-complementary nucleotide is dependent upon complementary nucleotide, Mg^{++}, template, and enzyme. The removal of any of the required components of the reaction mixture results in a loss of greater than 80% of non-complementary nucleotide incorporation. Furthermore, with poly(rA).oligo(dT) the incorporation of the non-complementary nucleotide (dCTP) parallels the incorporation of the complementary nucleotide (dTTP) over the indicated time period (Fig. 1), suggesting that continued synthesis of the polymer is required for the continued incorporation of the incorrect nucleotide.

The distribution of the non-complementary nucleotides in the DNA product using poly(rA).oligo(dT) as a template was investigated by processive hydrolysis of the reaction product using snake venom phosphodiesterase (Figure 2). There is a coordinate release of [^{3}H] dCMP and [α-^{32}P] dTMP into acid soluble material.

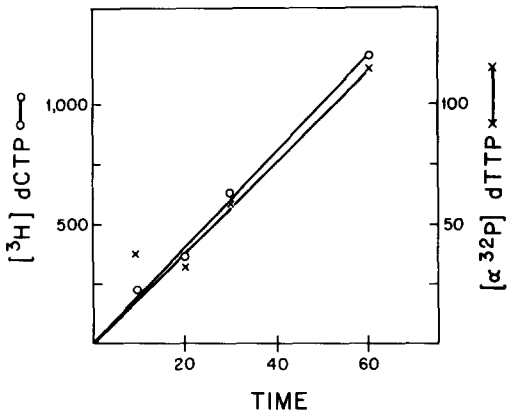

Figure 1 - The time dependency of complementary (dTTP) and non-complementary (dCTP) nucleotide incorporation employing poly(rA).oligo(dT) as the template, with Rauscher leukemia virus DNA polymerase. The assay conditions are identical to those in Table I. Incorporation is expressed in counts/min. and time in minutes.

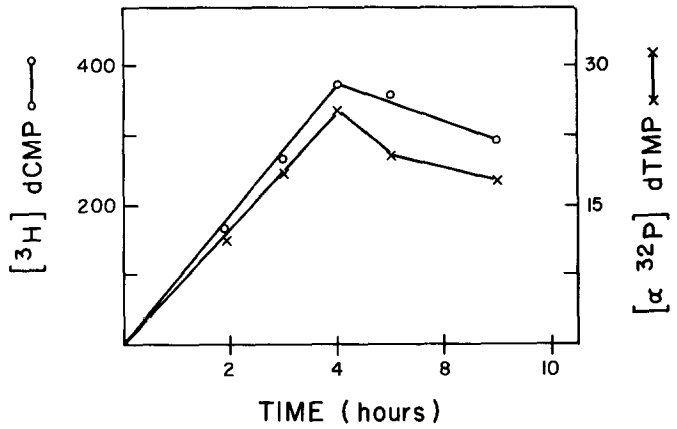

Figure 2 - Snake venom phosphodiesterase digestion of the DNA product using poly(rA).oligo(dT) as a template with Rauscher leukemia virus DNA polymerse. The product was formed using the assay conditions as described in Table 1. The wash procedure of Battula and Loeb (5) was employed to extract the product. The reaction mixture (total volume 2.5 ml) contained 100 mM Tris-HCl (pH 8.4), 5 mM $MgCl_2$, 50 µg BSA, 2.5 µg product, and 40 µg of snake venom phosphodiesterase. The assay was incubated at 37°C. Duplicate samples (100 µl) were taken at the indicated time periods and precipitated with 200 µl of 10% TCA and 100 µl of a heat denatured calf thymus DNA solution (1.5 mg/ml). Samples were centrifuged at 5,000 r.p.m. for 10 minutes and the acid soluble radioactivity determined in counts/min.

This suggests that the non-complementary nucleotide is evenly distributed throughout the DNA product and is not terminally added.

DISCUSSION. These results demonstrate that the DNA polymerase from Rauscher leukemia virus copies synthetic polynucleotide templates with a high level of infidelity which is of comparable magnitude to that previously observed with the DNA polymerase from avian myeloblastosis virus.

The ability of both of these tumor viruses to copy inaccurately both ribo-and deoxyribopolymers indicates that this high error rate may be a general characteristic of RNA tumor viruses. This capability to incorporate errors into DNA may be relevant to the molecular mechanism for tumor induction by the oncogenic RNA viruses.

It has also been observed (6) that the polymerases in extracts from human leukemic cells also have an extremely high error rates when copying synthetic homopolymers. In contrast, this is not the case with phytohemagglutinin-stimulated normal lymphocytes. Traces of an RNA tumor virus have been reported in human leukemic cells (7). In this regard, it would be interesting to correlate the error rates of purified enzymes from human leukemic cells with the error rates observed in RNA tumor viruses. The similarity of those error rates to those we have reported may indicate a possible molecular basis for human tumorogenesis.

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