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Partial Purification of Chromosomal DNA Polymerase from Rat Walker Tumor* (33346)

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Previous studies in this laboratory have shown that the nonhistone chromatin proteins from rat liver and calf thymus contain a replicative type DNA polymerase (1, 2). It was subsequently found that isolated cell nuclei from solid Walker tumor are especially rich in this enzymic activity. Investigation of the acidic chromosomal proteins from Walker 256 carcinosarcoma has resulted in the partial purification of a DNA polymerase. The method of the enzyme purification is described in the present report. It will be shown that the purified DNA polymerase requires only native DNA as template and a complete supplement of all four deoxyriboside triphosphates as substrates. The enzymic reaction also depends on the presence of Mg^{2+}, monovalent cations being inhibitory.

Materials and Methods. Walker 256 carcinosarcomas were developed for 7 days after tumor cells were injected intramuscularly into the hind legs of rats. The tumor cells had a mitotic cycle of 22–25 days and showed well-organized endoplasmic reticulum network. All the following operations to be described were carried out at 2–4°.

Cell nuclei were isolated from the tumor tissue by the method of Chauveau *et al.* (3). The tumor nuclei were extracted with 100 vol of 0.05 *M* Tris–HCl, pH 7.4, containing 5 m*M* $MgCl_2$, followed by repeating the buffer extraction three times. The nonhistone chromosomal proteins were prepared from the washed nuclei according to previously described procedure (4).

The isolated acidic chromosomal proteins were dialyzed overnight against 0.05 *M* Tris–HCl, pH 8.5, containing 1 m*M* 2-mercaptoethanol. To the dialyzed acidic protein solution, saturated ammonium sulfate (AS) solution (adjusted to pH 8.5 with NH_4OH) was added slowly with stirring to 40% saturation with respect to ammonium sulfate. After standing for 15 min, the mixture was centrifuged at 10,000*g* for 15 min and the precipitate was discarded. To the clear supernate enough ammonium sulfate was added to a final 60% saturation of ammonium sulfate. The precipitate (40–60% AS) thus formed

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TABLE I. Purification of DNA Polymerase from Rat Walker Tumor Nonhistone Acidic Proteins.

Fraction	Total protein (mg)	Total act. (units)[a]	Sp act. (units[a]/mg)
Total nonhistone acidic proteins	108	366	3.39
AS (40–60%)	19.8	113	5.76
DEAE-cellulose	4.4	51.9	14.3

[a] One unit of enzyme activity is defined as 1 mμmole of dTTP-^{3}H incorporated into acid-insoluble material per hour.

was collected by centrifugation, dialyzed against 0.02 *M* Tris–HCl, pH 8.5, containing 1 m*M* 2-mercaptoethanol, and clarified by centrifugation at 20,000*g* for 20 min. The 40–60% AS fraction was then adsorbed on a 1.2 × 12-cm DEAE-cellulose (DE-52, Reeve Angel) column and eluted successively with 0.025, 0.05, and 0.10 *M* NaCl in Tris–mercaptoethanol. The fraction eluted by 0.10 *M* NaCl represented the partially purified DNA polymerase which was used for the present study.

The DNA polymerase activity was assayed in a reaction mixture containing: glycine buffer, pH 8.0, 20 μmoles; 2-mercaptoethanol, 0.5 μmole; $MgCl_2$, 5 μmoles, thymidine-^{3}H-5′-triphosphate (Schwartz), and 5′-triphosphates of deoxyadenosine, deoxycytidine, and deoxyguanosine (PL labs), 50 μmoles each; calf thymus DNA, prepared according to Thomas *et al.* (5), 50 μg; and the purified enzyme, 10–30 μg. The reaction mixture, in a total volume of 0.50 ml, was incubated at 37° for 1 hr. The incorporation of radioactivity into acid-insoluble material was measured as described previously (1).

Chromatin was prepared from rat liver according to various methods (6–9). Reconstituted DNA-histone was prepared by extraction of isolated calf thymus nuclei with 1 *M* NaCl and precipitation of the reassociated DNA-histone from the salt extract by dilution with water to 0.14 *M* NaCl.

Results. Table I illustrates the purification steps of the chromosomal DNA polymerase from the rat Walker tumor. It can be seen that the DNA polymerase activity in the nonhistone acidic proteins is considerably higher than that reported in the normal (1) and regenerating (10) rat livers. Both ammonium sulfate precipitation and DEAE-cellulose chromatography steps increased the specific activity of the enzyme.

As shown in Table II, the tumor chromosomal DNA polymerase was completely dependent on the presence of all four deoxyriboside triphosphates for its activity. The enzymic reaction utilized only native DNA as template. Limited DNase digestion of the template doubled the rate of incorporation of dTTP-^{3}H, presumably due to increased 3′-hydroxyl termini. The product of

TABLE II. Requirements for Walker Tumor Chromosomal DNA Polymerase Reaction.

System	dTTP-^{3}H incorporated (μμmoles)
Complete system	403
Omit DNA, +heat-denatured DNA[a]	<2
Omit dCTP, dGTP, dATP	<2
Limited DNase treatment[b]	800
Postincubation with DNase[c]	<2
Omit enzyme	<2
Omit $MgCl_2$	<2
Omit $MgCl_2$, +$MnCl_2$ (5 μmoles)	55
Omit $MgCl_2$, +$CaCl_2$ (5 μmoles)	<2
+NaCl (20 μmoles)	241
+KCl (20 μmoles)	222

[a] Heat-denatured DNA was prepared by heating native DNA in boiling water bath for 10 min followed by rapid cooling.

[b] Native DNA was incubated with DNase (0.01 μg of DNase/0.5 mg of DNA per ml) at 37° for 5 min before it was used as the template.

[c] The complete reaction mixture, after incubation at 37° for 1 hr, was digested with 0.5 μg of DNase by incubation at 37° for 20 min.

TABLE III. Templating Activity of Chromatin in Walker Tumor Chromosomal DNA Polymerase System.

Template	dTTP-^{3}H incorporated ($\mu\mu$moles)	Incorporation (%)
DNA	162	100
DNA-histone, reconstituted	22.7	14
Chromatin:[a]		
Zubay and Doty (6)	61.8	38
Frenster *et al.* (7)	51.5	32
Huang and Bonner (8)	50.9	31
Dingman and Sporn (9)	57.4	35

[a] All in equivalent amount of 50 μg of DNA.

the enzymic reaction was completely susceptible to DNase treatment.

The tumor chromosomal DNA polymerase had an absolute requirement for Mg^{2+} for its optimal reaction. The Ca^{2+} was without effect, while Mn^{2+} gave only marginal activity. The Na^+ or K^+ was inhibitory to the enzymic reaction.

That the chromosomal DNA polymerase-catalyzed reaction utilized DNA as template was further shown by the result that isolated chromatin in which the DNA was not fully exposed exhibited only partial activity as compared with DNA (Table III). Templating activity of the reconstituted DNA-histone was the least, being 14% that of DNA.

Discussion. Results from the present study clearly show that the tumor chromosomal DNA polymerase is of the replicative type, requiring all four deoxyriboside triphosphates for its activity. The enzyme is present in high activity in the nonhistone acidic proteins which can be purified to relatively high specific activity. The Walker tumor chromosomal DNA polymerase was, however, extremely unstable upon storage. When kept in frozen state at $-20°$, the enzyme steadily lost its activity. Thus, some enzymic activity was undoubtedly being lost during the purification process.

Furlong and Williams (11) have purified DNA polymerase from the soluble fraction of Walker 256 carcinosarcoma by acidification and repeated ammonium sulfate precipitation. The soluble tumor enzyme can utilize both heat-denatured and, preferentially, native DNA as templates, a characteristic generally shown by DNA polymerase preparations from various mammalian sources (12–14), with the exception of calf thymus (15). However, the tumor DNA polymerase as described in the present report, differing from the soluble tumor enzyme, is of chromosomal origin, and is inactive with heat-denatured DNA as template. The requirement for Mg^{2+}, the inability of Ca^{2+} to substitute for Mg^{2+}, and the inhibition by monovalent cations for the chromosomal enzyme are in good agreement with those reported for the soluble Walker tumor DNA polymerase. Whether the template specificity underlines the difference between the soluble and chromosomal DNA polymerases from the Walker tumor remains to be elucidated.

The marked decrease in templating activity of reconstituted DNA-histone contrasts with that of the chromatin. One interpretation of this low templating activity is that when reconstituted with histones, the DNA molecule is probably masked more through binding with histones than DNA in the chromatin. This would suggest that without the participation of the nonhistone proteins, DNA and histones could not be associated into their native state. In view of the method by which the non-histone chromosomal proteins are prepared, this would suggest a more extensive interaction between histones and DNA in the absence of the nonhistone acidic proteins. It is thus conceivable that in its native configuration, perhaps part of DNA is in direct association with the acidic chromosomal proteins.

Summary. A replicative type DNA polymerase has been partially purified from the nonhistone chromosomal proteins of Walker 256 carcinosarcoma. The enzymic polymerization of deoxyribotides into DNA-like product depends on the presence of a complete supplement of all four deoxyriboside-5′-triphosphates and native DNA as template. The enzyme requires Mg^{2+} for its optimal reaction and is inhibited by Na^+ and K^+. The DNA in isolated chromatin exhibits 40% of its templating activity. The tumor chromosomal DNA polymerase shows only

marginal activity with reconstituted DNA-histone as template.

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Attempts to Infect Rhesus Monkeys with Human Type 4 Adenovirus (33347)

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Adenovirus infections constitute a major cause of acute respiratory disease in military recruits (1–3). For several years respiratory disease in this group was controlled by parenteral administration of polyvalent formalin-inactivated vaccines which incorporated adenovirus types 3, 4, and 7 (4,5). Increasing concern with potential oncogenicity of some adenoviruses and with the oncogenic properties of the contaminating SV40 virus led to the suspension of routine use of these vaccines (6–8).

In 1966 Chanock and associates (6) reported the development of a live adenovirus type 4 vaccine for oral administration as enteric-coated tablets. The unattenuated virus used in this vaccine was grown in the WI-38 human diploid fibroblast cell culture and has been shown to be free of known adventitious agents and oncogenic activity in the usual test systems. Volunteer studies have shown that virus administered in this manner bypasses the upper respiratory tract, selectively infects the lower intestine, and is readily excreted in the stools of vaccinees (9). Virus spread to nonimmune contacts did not occur under the usual conditions of life in the barracks (9).

Later studies suggested that in some families the vaccine virus spread from immunized to nonimmunized spouses (10). It seemed worthwhile to explore the use of the simian primate as a laboratory model in studying adenovirus infections. A review of the literature disclosed that others had failed to infect monkeys with human adenoviruses (11). This report describes our attempts to infect rhesus monkeys with human type 4 adenovirus and to determine communicability among uninoculated cagemates.

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