A DNA POLYMERASE IN LIVER NUCLEI WHOSE ACTIVITY RISES WITH DNA SYNTHESIS AFTER PARTIAL HEPATECTOMY

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SUMMARY

Rat liver nuclei contain at least two DNA polymerases that can be separated by extracting the nuclei with 5% Brij 58. The loosely-bound activity increases little or not at all after partial hepatectomy and is insensitive to cytosine arabinoside triphosphate (araCTP). The tightly-bound enzyme activity rises along with DNA replication and is inhibited by araCTP.

In addition to a distinct mitochondrial enzyme (1, 2), the mammalian cells that have been studied contain one or more nuclear DNA polymerases (3-11) and a cytosolic enzyme (3, 6, 7, 9, 10, 12-16). Exclusive of the mitochondrial enzyme, only two activities have been found in liver, one in the nucleus (4, 8, 10, 15), the other in the cytosol (10, 12-16). The level of the nuclear activity has been found to change little or not at all after partial hepatectomy of the rat (10, 15) and, for this reason, it has not been considered to be the replicative polymerase. The soluble activity does increase after removal of 70% of the liver (12, 15, 16) but it seems doubtful that it has a direct relationship with DNA replication since much of the increase takes place after the peak of DNA synthesis has been passed and the rise in activity is small as compared with the enhancement in liver DNA synthesis (15, 16).

The purpose of this report is to show that liver nuclei contain at least two DNA polymerases. The activity of one of them increases by more than ten-fold during the rise in liver DNA formation after partial hepatectomy.

EXPERIMENTAL PROCEDURE

Male Sprague-Dawley-derived rats (100 g) were obtained locally and were freely given food and water at all times. Partial hepatectomy refers to the removal of 70% of the liver. Injections were made in the tail vein. Unlabeled compounds were from Sigma, labeled compounds, from New England Nuclear. DNA polymerase activity was assayed in mixtures (0.25 ml) that contained 0.15 M Tris (pH 8.0), 3 mM $MgCl_2$, 20 mM 2-mercaptoethanol, 2% ethanol, dATP, dCTP, dGTP (each 8×10^{-5}M), 1.6×10^{-5}M ^{3}H-TTP (0.5 Ci/mmole), 150 μg of activated (17) calf thymus DNA, and the enzyme preparation. After incubation at 37° for 10 min, reactions were stopped with 1 ml of 1 M NaOH and 1 mg of calf serum protein was added to provide a coprecipitate. ^{3}H-TTP incorporation into DNA was measured as previously described (18). One unit of enzyme is defined as the amount that catalyzes the incorporation of 1 nmole of TTP in 10 min.

RESULTS

Levels of Loosely-and Tightly-Bound DNA Polymerase in Normal and Regenerating Liver Nuclei - The test mixture used to measure DNA polymerase provided optimal conditions for the tightly-bound activity. Under these conditions, regenerating nuclei had about four-times more activity than normal nuclei (Table 1) Brij extracts of the nuclei contained similar levels of polymerase but the tightly-bound activity of the regenerating nuclei was more than ten-fold greater than that of the normal nuclei. The tightly-bound activity could be extracted from the Brij pellets with 1 M NaCl (2) and the solubilized activity did not sediment during centrifugation at 105,000 x g for 2 h.

Nuclear DNA synthesis in the liver gradually rises beginning at 12 to 14 h

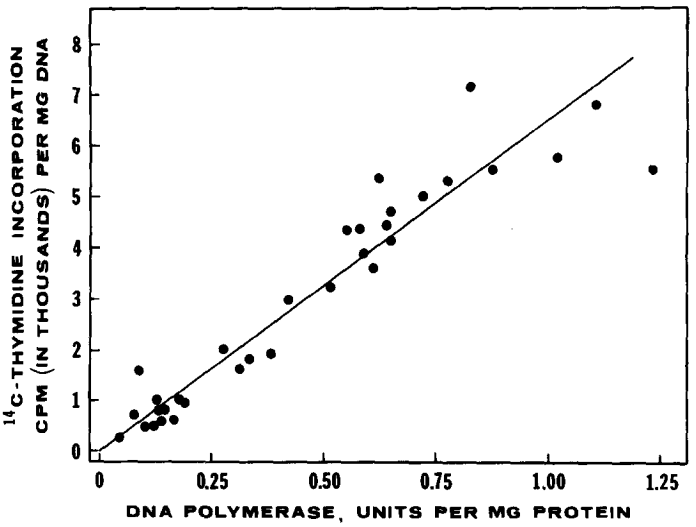

Fig. 1. Correlation between the level of liver DNA replication in vivo and the activity of the tightly-bound nuclear DNA polymerase after partial hepatectomy. Partially hepatectomized rats were given 0.5 μCi of ^{14}C-thymidine in the tail vein at 0 to 24 h after the operation and liver samples were taken 45 min later. DNA polymerase was measured in Brij residues prepared as for Table I. ^{14}C-Thymidine incorporation into DNA was estimated as previously described (18).

after partial hepatectomy of the rat as more nuclei enter the S period. To learn whether the increase in the activity of the tightly-bound DNA polymerase was correlated with DNA replication in vivo, the enzyme activity was measured in the Brij residue fraction of liver samples taken at various times after the operation (Fig. 1). As the figure shows, a good correlation was found.

Properties of the Loosely-Bound and Tightly-Bound DNA Polymerases of Regenerating Liver Nuclei - The loosely-bound and tightly-bound activities differed in two properties (Table II). First, the tightly-bound activity was more dependent upon the presence of the unlabeled deoxynucleoside triphosphates than was the loosely-bound enzyme. Second, only the tightly-bound activity was inhibited by araCTP. The table does not show that neither nuclear fraction was active when the activated DNA in the test mixture was replaced with untreated calf thymus DNA or with denatured DNA.

Table I

Levels of loosely- and tightly-bound DNA polymerase in normal and regenerating liver nuclei

Liver nuclei were isolated from an intact rat and from an animal at 20 h after partial hepatectomy. To the isolated nuclei (about 4 mg of DNA), suspended in 3 ml of 0.3 M sucrose, was added an equal volume of a solution of 10% Brij 58, 0.1 M Mg acetate, and 2.5 mM mercaptoethanol. The suspensions were shaken, kept at 0° for 10 min, and the nuclei were sedimented at 800 x g (5 min). The extracted nuclei, resuspended in 3 ml of 0.3 M sucrose, were extracted as before and the insoluble fraction (Brij residue) was washed once with 0.3 M sucrose. Finally, the Brij residue was dissolved in 1 M NaCl (2), the NaCl was removed by dialysis, and the NaCl extract and Chromatin fractions were separated by centrifugation. Assay of the DNA polymerase activities was as described under EXPERIMENTAL PROCEDURE. Protein was estimated colorimetrically (19) or by absorption at 280 nm (Brij extracts).

Fraction	Normal			Regenerating		
	Units	Protein	Specific activity	Units	Protein	Specific activity
		mg	units/mg protein		mg	units/mg protein
Nuclei*	5.2	17	0.31	22	18	1.2
Brij extract I	5.2	5.2	1.0	8.4	6.7	1.3
II	0.8	1.4	0.57	0.8	0.2	4.0
Brij residue	1.0	12	0.08	11	12	0.92
NaCl extract	0.36	2.0	0.18	6.0	2.1	2.9
Chromatin	0.46	8.2	0.06	3.0	8.0	0.38

* Under the assay conditions used (without ATP), "proper" DNA synthesis by the intact nuclei was negligible. Thus, without exogenous DNA, ^{3}H-TTP incorporation by the normal and regenerating nuclei was 0.15 and 0.51%, respectively of that with added calf thymus DNA.

Regenerating rat liver nuclei that are isolated and incubated under the conditions that have been described (18, 20) form "proper" DNA, that is, they elongate the DNA strands that were growing *in vivo*. AraCTP inhibited "proper" DNA synthesis by the intact nuclei and the reaction carried out by the tightly-bound nuclear activity to similar degrees at all the levels of the analogue tested (Table III).

Table II

Properties of the loosely-bound (Brij extract I) and tightly-bound (NaCl extract) DNA polymerases of regenerating liver nuclei

The Brij and NaCl extracts of regenerating nuclei were prepared as for Table I and the test mixture and assay procedure were described under EXPERIMENTAL PROCEDURE. Treatment with pancreatic DNase was for 5 min (37°) at the end of the 10 min period of incubation. In some cases, as indicated, a solution of Brij 58, Mg acetate, and mercaptoethanol, equal to the volume of the Brij extract tested, was added to the assay mixture with the NaCl extract.

Reactants	^{3}H-TTP incorporation	
	Brij extract I	NaCl extract
	cpm	
Complete system	3210	4670
-DNA	0	9
-dATP, dCTP, dGTP	2460	1050
-Mg^{++} + EDTA	12	2
+DNase (100 μg/ml)	5	148
+Brij solution		4840
+araCTP (200 μM)	3030	200
+Brij solution + araCTP (200 μM)		180
+Heated extract (55°, 10 min)	2	84

Table III

Inhibition by araCTP of "proper" DNA synthesis by intact nuclei and of the activity of the tightly-bound DNA polymerase

Nuclei were isolated from regenerating liver (18) and incubated in a reaction mixture (without exogenous DNA) that contained ^{3}H-TTP, the three unlabeled deoxynucleoside triphosphates, and ATP as previously described (20). The preparation of NaCl extract from regenerating nuclei and the assay conditions were as for Table I. The reaction mixtures for the nuclei and the NaCl extract contained identical levels of dCTP.

AraCTP	Intact nuclei		NaCl extract	
μM	cpm	% of control	cpm	% of control
0	8300	100	29,300	100
20	5120	62	18,100	62
40	3510	42	13,000	44
80	1630	20	5 240	18
120	630	8	3 160	11
200	20	0.2	510	2

DISCUSSION

When regenerating liver nuclei are isolated and assayed in the presence of ATP and without exogenous DNA (18, 20), only nuclei that were forming DNA *in vivo* are active *in vitro* and the incorporation of deoxynucleotides is for the elongation of DNA chains that were growing in the animal. It is reasonable to believe, therefore, that the isolated nuclei contain the replicative DNA polymerase. It remains to be established whether the tightly-bound polymerase is the replicative enzyme. The temporal relationship between DNA synthesis *in vivo* and the level of the tightly-bound activity and the almost identical effect of araCTP on the activities of the intact nuclei and the solubilized activity are consistent with this possibility.

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