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Multiple Forms of Mammalian Deoxyribonucleic Acid Polymerase

AN ATTEMPT TO RELATE THEIR INTERACTIONS WITH NUCLEI AND FREE DEOXYRIBONUCLEIC ACID *IN VITRO* WITH THEIR POSSIBLE FUNCTIONS *IN VIVO*

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The DNA polymerases of the following eukaryotic tissues were studied: regenerating rat liver, normal rat liver, rat thymus, normal mouse liver and Ehrlich ascites-tumour cells. In all cases two main polymerase forms are observed, one of mol.wt. 200000, preferring denatured DNA to native calf thymus DNA primer, designated type I, and the other, designated type II, of mol.wt. 100000, showing a variable and slight preference for native calf thymus DNA primer. Some catalytic properties of these polymerases are described. Nuclei have been isolated from some of these tissues by using two different buffer systems. The ionic composition of the isolation medium is found to affect greatly the amounts and types of polymerase that bind to the nuclei, and also affects the kinetic properties of the polymerases. The way the polymerases and nuclei change properties as the ionic composition of the buffers is changed suggests that ionic effects may be a significant factor in the control of DNA synthesis *in vivo*. These ionic effects also explain much of the previous confusion over the localization of specific DNA polymerases.

The enzyme responsible for DNA synthesis by isolated nuclei is, depending on the preparation procedure, usually a member of that class of mammalian DNA polymerases that prefers native DNA (Mantsavinos & Munson, 1966; Bellair, 1968; Iwamura, Ono & Morris, 1968; Meyer & Simpson, 1968; Ove, Lazlo, Jenkins & Morris, 1969*a*; Greene & Korn, 1970). This enzyme(s) is a constituent of chromatin in various cells (Patel, Howk & Wang, 1967; Howk & Wang, 1970).

However, mammalian cells contain a further class of DNA polymerase, which is distinguished from the above class by its molecular weight and its primer requirements (Bollum; 1960; Keir, 1962; Smellie, 1963; Gold & Helleiner, 1964; Furlong & Williams, 1965; Calvin, Kosto & Williams-Ashman, 1967; Iwamura *et al.* 1968; Ove *et al.* 1969*a*; Ove, Brown & Lazlo, 1969*b*; Roychoudhury & Bloch, 1969*a*,*b*; Chiu & Sung, 1970; Ove, Jenkins & Lazlo, 1970). This enzyme has a higher molecular weight (determined by Sephadex chromatography) than the DNA polymerase that prefers native DNA (Iwamura *et al.* 1968; Ove *et al.* 1969*a*,*b*), and usually shows a strong preference for denatured DNA. The denatured-DNA-preferring DNA polymerase(s) is generally found in highest concentration in tissues undergoing rapid cell division, such as tumours and developing tissues (Iwamura *et al.* 1968; O'Neill & Strohman, 1969; Chiu & Sung, 1970), and in rat liver the enzyme activity has been reported to increase during liver regeneration (Iwamura *et al.* 1968). Our results agree with these findings.

The correlation between the elevation of DNA polymerase activity and the metabolic state of the cell is not a simple one, and in fact the peak activities of denatured-DNA-preferring polymerase in regenerating rat liver occur well after the period of maximum DNA synthesis (Ove *et al.* 1970). Moreover, this DNA polymerase has generally been observed in the soluble fraction rather than in the nuclei. Bazill & Philpot (1963) and O'Neill & Strohman (1970) have noted that only a small percentage of the total cell DNA polymerase remains associated with isolated nuclei, although Main & Cole (1964) found that the presence of Ca^{2+} in the isolation medium increases the amount of enzyme bound to rat thymus nuclei. However, there is some evidence that DNA polymerase becomes particulate and migrates from the cytoplasm to the nucleus (Loeb & Fansler, 1970) during active DNA synthesis in L-cells (Littlefield, McGovern & Margeson, 1963), and in the developing sea urchin embryo.

In the present paper we examine some of the factors that influence the partition of the DNA polymerases between the nuclear phase and free solution.

EXPERIMENTAL

Chemicals. [α-^{32}P]dTTP (1 Ci/mmol at date of synthesis) was prepared as described by Symons (1969). ATP, dATP, dCTP, dGTP, dTTP, Ficoll and phosphoenolpyruvate were from Sigma Chemical Co. (St Louis, Mo., U.S.A.).

Buffers. Buffer A contained 60 mM-KCl, 15 mM-NaCl, 0.15 mM-spermine, 0.5 mM-spermidine, 15 mM-2-mercaptoethanol and 15 mM-tris adjusted with HCl to pH 7.4. Buffer D contained 35.5 mM-KCl, 2 mM-NaCl, 0.05 mM-spermine, 10 mM-glycine, 15 mM-2-mercaptoethanol, 10 mM-Hepes [2-(*N*-2-hydroxyethylpiperazin-*N'*-yl)-ethanesulphonic acid], adjusted to pH 7.4 with KOH.

Isolation of nuclei. All operations were carried out at 0–4°C. Nuclei were isolated in buffer A in the presence or absence of EDTA and EGTA* as described by Burgoyne, Waqar & Atkinson (1970). For nuclei in buffer D, the tissue was homogenized in buffer D containing 15% Ficoll, 2 mM-EDTA and 0.5 mM-EGTA, filtered through muslin and centrifuged for 20 min at 12000*g*. The pellet was resuspended in buffer D containing 15% Ficoll, 1.32 M-sucrose and 0.1 mM-EGTA, then layered over a step gradient of buffer D containing 15% Ficoll, 1.4 M-sucrose, 0.1 mM-EGTA (bottom) and buffer D containing 15% Ficoll, 1.32 M-sucrose, 0.1 mM-EGTA (top). The gradient was centrifuged for 45 min at 75000*g*. The top layers were carefully removed, contamination of the nuclear pellet being avoided. After one wash by centrifuging for 15 min at 1000*g* in buffer D containing 15% Ficoll, 0.1 mM-EGTA and 0.1 mM-EDTA, the nuclei were dispersed in buffer D before assaying for DNA polymerase.

Separation and purification of DNA polymerases. (*a*) Sephadex G-200 chromatography. The procedures described by Ove *et al.* (1969*b*) were followed. (*b*) Ammonium sulphate fractionation. The tissue was homogenized in 0.34 M-sucrose–buffer A and a 1 h × 100000*g* supernatant fraction was obtained. The pellet from a pH 5 precipitation of the supernatant fraction was dissolved in buffer A, and used for the ammonium sulphate fractionation.

Calcium phosphate gel. To 100 ml of 0.1 M-potassium phosphate buffer, pH 7.4, 15 ml of 0.1 M-$CaCl_2$ was added slowly with vigorous stirring, the pH being kept at 7.4 by the addition of KOH. After being stirred for 1 h the gel was separated by centrifuging for 10 min at 2000*g*, then dispersed in 50 ml of 0.02 M-potassium phosphate, pH 7.4, and equilibrated overnight at room temperature. The 30–40%-satd. ammonium sulphate fraction from regenerating rat liver cytosol was treated with calcium phosphate gel in 0.16 M-potassium phosphate buffer, pH 7.4, to remove unwanted protein. The polymerase was then adsorbed to another batch of calcium phosphate gel in 0.08 M-potassium phosphate buffer, pH 7.4, and eluted with 0.3 M-potassium phosphate buffer, pH 7.4.

DNA polymerase assays. Assays were performed as described by Waqar, Burgoyne & Atkinson (1971) except that acid-soluble material was removed by washing the paper discs seven times with cold 0.12 M-H_2SO_4–0.05 M-H_3PO_4–0.25 M-Na_2SO_4 and twice with ethanol.

* Abbreviation: EGTA, ethanedioxybis(ethylamine)-*NNN'N'*-tetra-acetic acid.

RESULTS

Rat liver

Two forms of DNA polymerase. Separation of DNA polymerase activity on Sephadex G-200 columns and by ammonium sulphate fractionation indicated that two polymerases were present in both normal and regenerating rat liver, although in different proportions (Figs. 1*a* and 1*b*). The two polymerases could be distinguished by their molecular weights and by their preference for primer DNA. The denatured-DNA-preferring polymerase (designated type I) was eluted with the protein front on Sephadex G-200, indicating a molecular weight at or above 200000. From calibration of the Sephadex G-200 column with marker proteins, the molecular weights of the native-DNA-preferring polymerase (designated type II) was estimated to be approx. 100000.

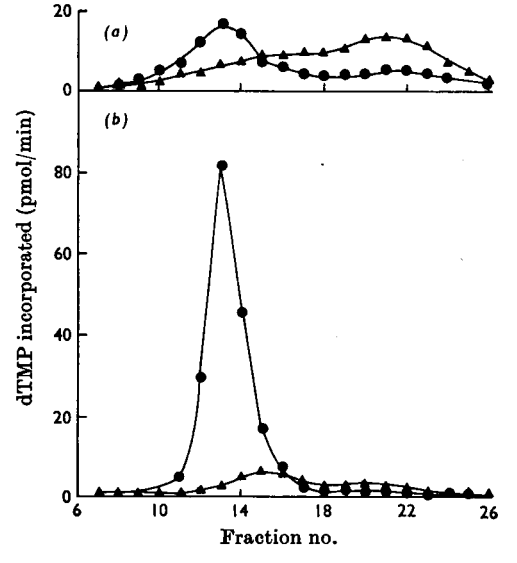

Fig. 1. Sephadex G-200 chromatography of extracts of (*a*) normal rat liver and (*b*) 40 h-regenerating rat liver. The extraction procedures of Ove *et al.* (1969*b*) were followed. Fractions (5 ml) were collected, and 50 μl samples were assayed at 37°C for 30 min, with either native or denatured DNA primer. Over 90% of the protein loaded was recovered from the column and most was eluted soon after the void volume. ▲, Activity with native DNA primer; ●, activity with denatured DNA primer. When tissues were extracted with buffer A instead of by the above procedure virtually the same results were obtained.

A similar separation of the two types of polymerase could be achieved by ammonium sulphate fractionation of extracts from normal and regenerating rat livers (Figs. 2*a* and 2*b*). The type I polymerase activity appeared in the 30–40%-satd. ammonium sulphate fraction and the type II polymerase was in the 60–70%-satd. ammonium sulphate fraction. This procedure gave an approximately 200-fold purification of type II polymerase (Table 1). The polymerase activity in the 30–40%-satd. ammonium sulphate fraction of regenerating liver could be further purified by treatment with calcium phosphate gel to give an approximately 300-fold purification of type I polymerase (Table 1). Sucrose-density-gradient centrifugation of the purified type I and type II polymerases with molecular-weight marker proteins confirmed that their molecular weights were approx. 200000 and 100000 respectively.

Influence of the isolation medium on the association of polymerases with nuclei in vitro. Nuclei were isolated by two main procedures with variations as stated in the text. In the first procedure buffer A was used (Burgoyne *et al.* 1970) and in the second buffer D was used (see the Experimental section). Buffer D was chosen to give an isolation medium of lower ionic strength and the lowest concentrations of polyamine stabilizers that would still permit preparation and handling of liver

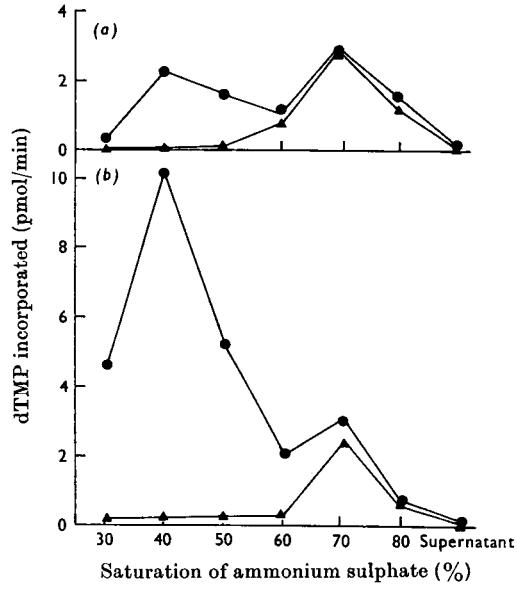

Fig. 2. Ammonium sulphate fractionation of extracts of (*a*) normal rat liver and (*b*) 40h-regenerating rat liver. The ammonium sulphate precipitates were resuspended and dialysed against buffer A. DNA polymerase assays were carried out at 37°C for 20min with either native or denatured DNA primer. ▲, Activity with native DNA primer; ●, activity with denatured DNA primer.

Table 1. *Purification of polymerase I from 40h-regenerating rat liver and polymerase II from normal rat liver*

Tissue homogenization and fractionation was carried out in the buffer A system as described in the Experimental section. DNA polymerase assays were at 37°C for 30min. Native calf thymus DNA was dissolved in buffer A. Denatured and activated primer were prepared as described by Aposhian & Kornberg (1962).

	Specific activity of polymerase with different primers (nmol of dTMP incorporated/s per μg of DNA per mg of protein)					
	Polymerase I			Polymerase II		
Fraction Primer ...	Native	Denatured	Activated	Native	Denatured	Activated
Crude homogenate	0.018	0.032	0.062			
18000*g* supernatant	0.034	0.084	0.298	0.0015	0.0016	0.0063
100000*g* supernatant	0.130	0.745	1.96	0.0022	0.0024	0.0105
pH5 fraction	0.245	0.584	2.37	0.015	0.0114	0.082
30–40%-satd. ammonium sulphate fraction	0.147	2.50	5.10			
Second 30–40%-satd. ammonium sulphate fraction	0.719	4.05	9.73			
Calcium phosphate gel purification		9.85				
60–70%-satd. ammonium sulphate fraction				0.30	0.24	0.90

nuclei. The 15% Ficoll in the buffer D system increased the mechanical stability of the nuclei, and was particularly important when buffer D had potassium chloride added to it. In both procedures chelating agents were used to bind the Ca^{2+} and Mg^{2+}, which are required for endonuclease cleavage and thus activation of the nuclear DNA (Burgoyne *et al.* 1970).

The nuclei were assayed for the amount of unstimulated DNA synthesis, the activities of polymerases I and II, and the amount of the Ca^{2+}-dependent activation.

Freshly isolated nuclei from resting or regenerating liver were extremely primer-limited, as their net potential polymerase activity, measured with added primer, was always much higher than their unstimulated activity (Table 2). Although nuclei prepared by the buffer A procedure were markedly stimulated by Ca^{2+}, as previously reported (Burgoyne *et al.* 1970; Waqar *et al.* 1971), nuclei prepared by the buffer D procedure were only weakly stimulated by Ca^{2+}, but stimulation could be restored by the addition of a mouse nuclear endonuclease (D. R. Hewish & L. A. Burgoyne, unpublished work).

Nuclei prepared by the buffer A procedure from resting or regenerating liver reacted with added primer like the free polymerase II, i.e. they showed a slight preference for native rather than denatured DNA, or were indifferent. Moreover, when the polymerase was extracted from these nuclei, and chromatographed on Sephadex G-200, or when these nuclei were centrifuged through a saline gradient (Fig. 3), only one peak of polymerase activity was detected, and this had the molecular weight and catalytic properties of polymerase II. The study of the affinity of polymerase II for the nuclear material posed a slight technical problem as extraction of nuclei with strong salt solutions results in an intractable gel. Consequently the extraction was carried out by centrifuging the lysing nuclei through saline gradients as shown in Fig. 3.

Significant amounts of polymerase I were found in regenerating liver tissue, but distribution studies of the two polymerases in the buffer A system indicated that under these conditions, although some polymerase II stayed associated with the nucleus, no polymerase I stayed associated with the nucleus (Table 3).

However, in nuclei prepared from regenerating liver by the buffer D procedure, an appreciable proportion of the polymerase I was also bound to the nuclei (Table 2). It could be assayed *in situ* with added primers or detached by washing the nuclei with buffer A (Table 2). Thus it appeared that the major factor determining whether or not appreciable amounts of polymerase I would stay associated with the nucleus was the ionic composition of the buffer. This was further illustrated by preparing nuclei in buffer D and washing separate samples of the nuclear suspension in buffer D containing additional potassium chloride, and then measuring the amount of polymerase I that was detached. Buffer D alone did not give maximum affinity between nucleus and polymerase I, as maximum affinity appeared to occur at a net potassium chloride concentration of 0.08M. A net concentration of potassium chloride that gave negligible affinity was 0.22M (Fig. 4).

The differences between buffer A and buffer D are not simply due to ionic strength but rather to a multifactorial interaction between all the buffer components. However, many of the important differences between buffer A and buffer D can be reproduced by varying the ionic strength.

Different affinities of the DNA polymerases for DNA. Studies were carried out with the two types of purified polymerase to determine their activity and affinity for native, denatured and activated calf thymus DNA, and also to determine whether this was influenced by the ionic composition of the medium. The apparent K_m and $V_{max.}$ values are shown in Table 4. Although polymerases I and II have been classified by their preference for de-

Table 2. *Activities of polymerases I and II in nuclei prepared in buffer D*

A and *B* refer to nuclei prepared in Ficoll–buffer D as described in the Experimental section. *C* refers to nuclei prepared by washing nuclei '*B*' once with buffer A. DNA polymerase assays were at 37°C for 20min. To eliminate any differences caused by buffer at the level of the assays, all assays were carried out in a 1:1 mixture of buffers A and D.

	DNA polymerase activity (pmol/min per mg of nuclear DNA)		
Additions to assay mixture	*A* (Normal liver nuclei)	*B* (40h-regenerating liver nuclei)	*C* [Nuclei (*B*) washed with buffer A]
—	1.3	1.0	1.1
Ca^{2+} (1mM)	5.0	6.0	4.0
Native DNA	14.0	16.0	15.0
Denatured DNA	17.0	116.0	18.0
Activated DNA (i.e. nicked)	64.0	278.0	62.0

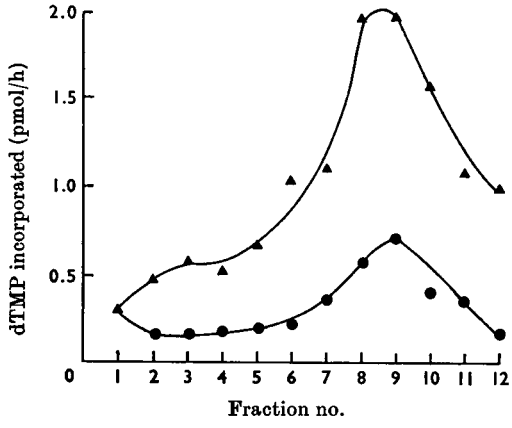

Fig. 3. Saline gradient dissociation and extraction of nuclei. A 5 ml linear gradient was set up between buffer A–0.2 mM-EGTA–0.2 mM-EDTA (the light solution) and 1.2 M-KCl–0.1 M-potassium phosphate (pH 7.4)–0.2 mM-EGTA–0.2 mM - EDTA–15 mM - 2 - mercaptoethanol (the heavy solution). A portion (0.25 ml) of nuclei (1.55 mg of DNA) from a normal rat liver was loaded and then the MSE 50 SW 40 centrifuge head was accelerated in three 15 min stages to 200*g*, 1000*g* and 3000*g*, then run for 7 h at 75 000 *g*. Under these conditions the nuclei move slowly down the gradient and are progressively dissociated by the increasing salt concentration. At the end of the run the gradient was collected in 13 fractions from above the gelatinous pellet, then dialysed against buffer A before 0.025 ml samples were assayed for 60 min with 34.5 μg of DNA. ▲, Activity with native DNA primer; ●, activity with denatured DNA primer.

natured and native DNA respectively as primer, it was apparent that the optimum primer for both enzymes was nicked double-stranded DNA, and polymerase I even preferred nicked DNA to denatured-nicked DNA. Nicked or activated DNA is DNA that has been subjected to slight DNAase I digestion to produce single-strand breaks that act as priming sites for DNA polymerase. Although the values for K_m and $V_{max.}$ were not absolute values because of the difficulty of specifying the nicks, the essential conclusion from these results was that the change in ionic composition from buffer D to buffer A greatly altered the affinity of polymerase I for nicked DNA but had no effect on the affinity of polymerase II. This change in the affinity of polymerase I for activated DNA could also be brought about by increasing the concentration of potassium chloride in buffer D (Table 4).

With respect to the $V_{max.}$ values, polymerase I was more active in buffer D, whereas polymerase II had higher activity in buffer A. Similar changes in activity were also obtained by changing the salt concentration of buffer D (Table 4).

Thus, under ionic conditions similar to those in buffer D, polymerase I could be expected to compete with polymerase II for nicks in the nuclear DNA, and would have a tenfold higher activity than polymerase II under these conditions. However, if the ionic conditions were changed and became similar to those of buffer A, polymerase I would no longer be able to compete with polymerase II successfully. The studies with nuclei would suggest

Table 3. *DNA polymerase activity in nuclear and soluble fractions from normal and 24 h-regenerating rat liver*

Livers were homogenized in 0.34 M-sucrose–buffer A and filtered through muslin. The homogenate was layered over 1.72 M-sucrose–buffer A and centrifuged for 30 min at 50000*g*. There fractions could be distinguished: an upper soluble fraction, a 'mitochondrial layer' on top of the dense sucrose layer (this would also contain any unbroken cells and a few trapped nuclei), and a nuclear pellet at the bottom of the tube. The nuclei were dispersed in 0.34 M-sucrose–buffer A. DNA polymerase assays were carried out at 37°C for 10 min. DNA primer was added at a final concentration of 1 mg/ml. Results are expressed as total polymerase activity per fraction derived from 1 g wet wt. of original tissue.

	DNA polymerase activity (pmol of TMP incorporated/min per fraction)			
	Normal liver		24 h-regenerating liver	
Primer	Nuclear fraction	Soluble fraction	Nuclear fraction	Soluble fraction
None added	0.35*	2.9*	2.4*	5.8*
Native	1.2	25.8	3.1	30.4
Denatured	0.9	20.3	2.3	111.0
Activated	5.3	92.5	4.0	350.0

* The activity in these fractions is above the 'blank' values and is presumably a complex function of the concentrations of DNA and accompanying nucleases which may activate and/or destroy both endogenous primer and product.

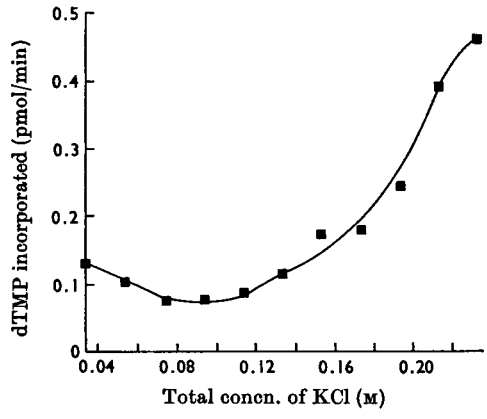

Fig. 4. KCl extraction of nuclei from 40 h-regenerating rat liver. Nuclei were prepared by the buffer D procedure. Nuclei equivalent to 51 μg of DNA were added to each of 11 separate 0.1 ml lots of 15% Ficoll–buffer D–0.1 mM-EGTA containing a sequence of KCl concentrations (see horizontal axis in the figure). Each suspension was left at 4°C for 15 min and then was centrifuged at 20000g for 15 min. Each supernatant was then assayed for DNA polymerase as described in the Experimental section. The assay contained 0.025 ml of the supernatant to be tested, 0.025 ml of buffer D containing 20 μg of nicked DNA and a calculated amount of KCl so that during the assay there was a net KCl concentration of 0.1355 M in all assays. This KCl correction in the assays was necessary as KCl has a large effect on the activity of polymerase I. The extracted nuclei were assayed after each extraction and found to have lost significant amounts of polymerase I only. This was virtually all removed at a KCl concentration of 0.22 M. The polymerase II was not extracted significantly within this range of KCl concentrations.

that, in parallel with these events, polymerase I might be expected to be released from the nuclei.

Some comparative studies with other tissues

Rat thymus. Similar studies to those described above were carried out with rat thymus. However, the rat thymus nuclei tended to lyse in buffer D, so they could not be studied quite as readily. The results obtained with rat thymus were essentially similar to those obtained with regenerating liver. Sephadex G-200 chromatography of the whole tissue indicated high polymerase I activity and low polymerase II activity, but only polymerase II was found in nuclei prepared in buffer A (Table 5). The amount of polymerase II in the nuclear fraction was increased in the presence of Ca^{2+}, but polymerase I remained in the soluble fraction (Table 6). Nucleoprotein prepared in buffer D systems with the net concentration of potassium chloride raised to 0.08 M contained more polymerase I than polymerase II and gave less Ca^{2+} stimulation than nuclei prepared in buffer A (Table 5).

Resting mouse liver and ascites-tumour cells. Young and adult mouse liver were shown by Sephadex G-200 chromatography to contain both type I and type II polymerases, with a preponderance of type I, although nuclei prepared in a Ca^{2+}-free buffer A system contained virtually no DNA polymerase of either type. The nuclei did, however, contain the Ca^{2+}-dependent endonuclease (D. R. Hewish & L. A. Burgoyne, unpublished work) and in the presence of Ca^{2+} the nuclei would act as primer for polymerase I that had been obtained from ascites-tumour cells. With mouse nuclei the presence of Ca^{2+} and Mg^{2+} in the buffer A isolation medium resulted in some DNA polymerase activity being weakly bound, possibly to the nicks in the primary DNA structure produced by the Ca^{2+}-dependent endonuclease, but it was bound in variable amounts and was progressively lost from the nuclei during preparation and washing.

Ehrlich ascites cells also contained both type I and type II polymerases, with the type I polymerase being the major species observed in the Sephadex G-200 fractionation, the cells resembling regenerating rat liver in this respect. Despite repeated attempts we have not been able to obtain satisfactory nuclear preparations from Ehrlich ascites cells without extensively modifying these procedures.

DISCUSSION

The aim of these studies has been to understand some of the factors controlling DNA synthesis in eukaryotic cells *in vivo* and the problem has been studied from a number of aspects. Two types of DNA polymerase exist in eukaryotic cells, and although these can be distinguished most easily by their preference for denatured or native DNA, the optimum substrate for both enzymes is nicked DNA. Polymerase I activity increases markedly during liver regeneration and is generally found to be high in cells with the ability to replicate, such as Ehrlich ascites cells or thymocytes, and this has led to the speculation that it may be the true replicative polymerase. Polymerase II seems more likely to be involved in some process characteristic of resting cells, because it is present in resting liver and regenerating liver in similar amounts. It also remains tightly bound to the nuclei under a variety of ionic conditions. Thus we tentatively suggest that the major function of polymerase II is in DNA repair.

If the initiation sites for DNA synthesis are regions with nicks, then polymerases I and II might be expected to compete with each other. The

Table 4. *Apparent K_m and $V_{max.}$ values of purified polymerases I and II with different primers*

Polymerases I and II were purified by ammonium sulphate fractionation and dialysed against the appropriate buffer. Polymerase assays were at 37°C for 20min. The final concentration of DNA in the appropriate buffer was varied over the range 0.02–1.0mg/ml. The apparent K_m and $V_{max.}$ values were calculated from Lineweaver–Burk reciprocal plots. It should be noted that the absolute values of the apparent K_m and $V_{max.}$ are only valid with the particular batches of DNA we used. This is because of the great difficulty in accurately describing the amounts and intramolecular distributions of nicks (active priming sites) in both activated DNA and unactivated DNA (i.e. not deliberately activated). However, in this experiment, all our native and denatured DNA in all buffers was from one batch of stock solution and similarly all our activated DNA was from another single batch of solution. Thus within these two groups meaningful comparisons can be made. The differences between the groups reflect the effects of the activation by deoxyribonuclease I as both activated and non-activated DNA were derived from one original batch.

K_m (μg of DNA/ml)

	Native DNA		Denatured DNA		Activated DNA		Activated DNA	
Buffer ...	A	D	A	D	A	D	D+ 0.22M-KCl	D+ 0.08M-KCl
Polymerase I	37	8	40	71	185	31	251	86
Polymerase II	15	26	29	11	8	12	83	4

$V_{max.}$ (pmol of dTMP incorporated/min)

	Native DNA		Denatured DNA		Activated DNA		Activated DNA	
Buffer ...	A	D	A	D	A	D	D+ 0.22M-KCl	D+ 0.08M-KCl
Polymerase I	0.024	0.039	0.05	0.08	1.0	3.1	0.67	2.7
Polymerase II	0.43	0.57	0.34	0.17	0.59	0.26	0.28	0.15

Table 5. *DNA polymerase activity in rat thymus nuclei*

Nuclei were prepared in buffer A or D as described in the Experimental section. Polymerase assays were at 37°C for 20min. DNA primer was added to a final concentration of 1mg/ml.

	DNA polymerase activity (pmol of TMP incorporated/min per mg of nuclear DNA)	
Buffer ...	A	D
Additions to assay		
$-Ca^{2+}$	0.9	2.1
$+Ca^{2+}$	2.1	3.3
+Native DNA	1.6	5.7
+Denatured DNA	0.9	21.7
+Activated DNA	6.7	121.8

apparent K_m and $V_{max.}$ values presented above indicate that the concentrations and types of ions present might be a major factor in determining whether polymerase I or II will operate. This theory would suggest that DNA repair might be less active during DNA replication than in non-replicative phases and it would suggest that ion pumps are involved in the programming of DNA replication and repair.

The concepts presented here are probably gross oversimplifications because the apparent K_m values almost certainly do not simply measure the affinity of the enzyme for the nicks. Moreover, the enzymes used are not pure, and the presence of other proteins with affinity for DNA would be expected to affect both the $V_{max.}$ and K_m values. In addition, the natural substrate of these enzymes is nucleoprotein, not free nicked DNA. Despite these reservations, the general agreement between the kinetic studies and the nuclei–polymerase association studies makes us believe that these ionic effects may be important in nucleus–cytoplasm interactions.

Although these postulated ionic control mechanisms may explain some aspects of the control of DNA synthesis, a number of aspects of DNA synthesis are largely unexplained. One important question is posed by the almost complete lack of priming activity in the nuclei we obtained from regenerating liver. When prepared in buffer D systems these nuclei possess both polymerases and are very readily activated by added primer, so it would appear that the properties of the nuclear DNA are the limiting factor. Lynch, Brown, Umeda, Langreth & Lieberman (1970) and Hyodo

Table 6. *DNA polymerase activity in nuclear and soluble fractions from rat thymus*

Nuclear and soluble fractions were prepared in buffer A, or buffer A–1 mM-$CaCl_2$–5 mM-$MgCl_2$ as described in Table 3. DNA polymerase assays were at 37°C for 10 min. DNA primer was added to a final concentration of 1 mg/ml. Results are expressed as total polymerase activity per fraction derived from 1 g wet wt. of tissue.

	DNA polymerase activity (pmol of TMP incorporated/min per fraction)			
Buffer ...	A	A	A+Ca^{2+}	A+Ca^{2+}
DNA primer	Nuclear fraction	Soluble fraction	Nuclear fraction	Soluble fraction
—	1.8	8.4	8.2	5.2
Native	2.0	28.4	9.9	13.1
Denatured	4.5	271.5	13.0	98.0
Activated	14.1	535.0	30.3	228.0

& Ono (1970) appear to have obtained nuclei containing some of the natural priming sites intact but in our preparations it would appear that the natural priming sites are becoming virtually completely blocked.

Although there seem to be broad similarities between the nuclei from rat thymus and rat liver there are definite differences in detail, e.g. rat thymus nuclei are much more fragile in buffer D than rat liver nuclei. Even larger differences may appear between species, as resting mouse liver nuclei, prepared in buffer A, contain no polymerase, whereas similarly prepared resting rat liver nuclei contain polymerase II. Consequently it seems likely that, even at this level, details of nuclear behaviour vary from species to species and even between tissues.

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REFERENCES

Aposhian, H. V. & Kornberg, A. (1962). *J. biol. Chem.* **237**, 519.
Bazill, G. W. & Philpot, J. St L. (1963). *Biochim. biophys. Acta*, **76**, 223.
Bellair, J. T. (1968). *Biochim. biophys. Acta*, **161**, 119.
Bollum, F. J. (1960). *J. biol. Chem.* **235**, 2399.
Burgoyne, L. A., Waqar, M. A. & Atkinson, M. R. (1970). *Biochem. biophys. Res. Commun.* **39**, 254.
Calvin, H. I., Kosto, B. & Williams-Ashman, H. G. (1967). *Archs Biochem. Biophys.* **118**, 670.
Chiu, J.-F. & Sung, S. C. (1970). *Biochim. biophys. Acta*, **209**, 34.
Furlong, N. B. & Williams, N. L. (1965). *Biochim. biophys. Acta*, **108**, 489.
Gold, M. & Helleiner, C. W. (1964). *Biochim. biophys. Acta*, **80**, 193.
Greene, R. & Korn, D. (1970). *J. biol. Chem.* **245**, 254.
Howk, R. & Wang, T. Y. (1970). *Archs Biochem. Biophys.* **136**, 422.
Hyodo, M. & Ono, T. (1970). *Expl Cell Res.* **60**, 401.
Iwamura, Y., Ono, T. & Morris, H. P. (1968). *Cancer Res.* **28**, 2466.
Keir, H. M. (1962). *Biochem. J.* **85**, 265.
Littlefield, J. W., McGovern, A. P. & Margeson, K. B. (1963). *Proc. natn. Acad. Sci. U.S.A.* **49**, 102.
Loeb, L. A. & Fansler, B. (1970). *Biochim. biophys. Acta*, **217**, 50.
Lynch, W. E., Brown, R. F., Umeda, T., Langreth, S. G. & Lieberman, I. (1970). *J. biol. Chem.* **245**, 3911.
Main, R. K. & Cole, L. J. (1964). *Nature, Lond.*, **203**, 646.
Mantsavinos, R. & Munson, B. (1966). *J. biol. Chem.* **241**, 2840.
Meyer, R. R. & Simpson, M. V. (1968). *Proc. natn. Acad. Sci. U.S.A.* **61**, 130.
O'Neill, M. & Strohman, R. C. (1969). *J. cell. Physiol.* **73**, 61.
O'Neill, M. & Strohman, R. C. (1970). *Biochemistry, Easton*, **9**, 2832.
Ove, P., Brown, O. E. & Lazlo, J. (1969*b*). *Cancer Res.* **29**, 1562.
Ove, P., Jenkins, M. D. & Lazlo, J. (1970). *Cancer Res.* **30**, 535.
Ove, P., Lazlo, J., Jenkins, M. D. & Morris, H. P. (1969*a*). *Cancer Res.* **29**, 1557.
Patel, G., Howk, R. & Wang, T. Y. (1967). *Nature, Lond.*, **215**, 1488.
Roychoudhury, R. & Bloch, D. P. (1969*a*). *J. biol. Chem.* **244**, 3359.
Roychoudhury, R. & Bloch, D. P. (1969*b*). *J. biol. Chem.* **244**, 3369.
Smellie, R. M. S. (1963). *Expl Cell Res.* Suppl. 9, p. 245.
Symons, R. H. (1969). *Biochim. biophys. Acta*, **190**, 548.
Waqar, M. A., Burgoyne, L. A. & Atkinson, M. R. (1971). *Biochem. J.* **121**, 803.