Mitochondrial DNA polymerase, deoxyribonuclease and ribonuclease
H activities from brain of chick embryo

L. Soriano, J. Smith, Y. Croisille, and B. Dastugue*

Institut d'Embryologie et Tératologie expérimentales du C.N.R.S.,
49bis, Avenue de la Belle-Gabrielle, 94130 Nogent-sur-Marne, France

Received 10 June 1974

ABSTRACT

R-DNA polymerase, D-DNA polymerase, DNase and RNase H activities in mitochondria from chick embryonic brain were studied by ion-exchange chromatography. Two main fractions were separated according to their chromatographic behaviour: a fraction M Ib which is eluted with the washing buffer from two successive DEAE-cellulose columns and a fraction M IV which is eluted at 400 mM KCl from a phosphocellulose column. Although the two fractions contain both the DNA polymerase and the degrading activities, all the specific activities are higher in fraction M IV than in fraction M Ib. Heat inactivation experiments have shown that R-DNA polymerase is inactivated in both fractions, whereas RNase H and DNase are not affected. Thus, degrading activities and R-DNA polymerase activity seem to be catalyzed by different molecular entities. However the fact that in most cases these activities co-chromatograph suggests that the corresponding molecules form rather stable complexes.

INTRODUCTION

Avian myeloblastosis viruses contain a DNA polymerase which catalyzes dGMP polymerization by transcription of polyribocytidylate sequences of either $(C)_n \cdot (dI)_n$ or $(C)_n \cdot (I)_n$ duplex structures¹. This activity has also been observed in other oncogenic RNA viruses by using $(dG)_{12-18} \cdot (C)_n$ as primer-template^{2,3}.

One of us⁴ has shown for the first time in eukaryotes the presence of a DNA polymerase which polymerizes dGMP when $(dG)_n \cdot (C)_n$ is provided as primer-template. This activity was probably due to a contamination of chick material with virus C-type particles, since a similar activity was observed by using $(dG)_{12-18} \cdot (C)_n$ as primer-template with cultured mouse fibroblast L/929 cells⁵ which were in fact probably contaminated with an endogenous murine leukaemia virus^{6,7}. On the other hand, three recent reports mention the presence

of $(dG)_{12-18} \cdot (C)_n$ -dependent DNA polymerase activity, in leukocytes from a child-hood acute lymphoblastic leukaemia³, in a human breast tumour cell line⁸, and in murine plasmocytomas⁹. These cellular enzymes are considered to be identical to reverse transcriptase from oncogenic RNA viruses.

Ribonuclease H, an enzyme which specifically degrades the RNA strand of DNA.RNA hybrids to acid-soluble products, was originally discovered in calf thymus^{10,11}. Subsequently, its presence was recorded in KB cells and chick embryo¹². Special attention has also been given to the association of DNA polymerase and ribonuclease H activities in several oncogenic RNA viruses^{13,14}.

It has been shown that neither exonuclease nor pyrophosphorolysis activity is present in purified calf thymus 3.4 S DNA polymerase¹⁵. It is interesting to compare these results with those obtained with E. coli DNA polymerase which contains two associated exonuclease activities and also catalyzes pyrophosphorolysis and a pyrophosphate exchange reaction¹⁶. However, the existence of degrading activities associated with DNA polymerase in other eukaryotic systems cannot be excluded since the presence of an endonuclease associated with a mitochondrial DNA polymerase has recently been shown¹⁷.

In the present paper we have analyzed the DNA polymerase, deoxyribonuclease and ribonuclease H activities present in mitochondria isolated from 11-day-old chick embryonic brain. We have distinguished two separate DNA polymerase activities, a D-DNA polymerase and an R-DNA polymerase (nomenclature according to FRY and WEISSBACH⁵). The former is characterized by its ability to catalyze either dGMP or dAMP polymerization when provided with $(dG)_n \cdot (dC)_n$ and $(dT)_n \cdot (dA)_{12-18}$ respectively, the latter by the polymerization of dTMP when using $(dT)_n \cdot (A)_n$ or $(dT)_{12-18} \cdot (A)_n$ as primer-template. Deoxyribonuclease and ribonuclease H activities (hereafter DNase and RNase H) are tested by using as substrates $[^3H] (dT)_n \cdot (A)_n$ and $(dT)_n \cdot (dA)_{12-18} - [^3H] (A)_n$ respectively.

MATERIAL AND METHODS

A) Preparation of enzymes

About 250-300 brains from eleven-day-old chick embryos are removed as previously described⁴. Mitochondria are isolated according to a slight modification of the method of CHAPPELL and HANSFORD¹⁸. Brains are homogenized in buffer A (50 mM Tris-HCl pH 7.6, 25 mM KCl, 5 mM $\text{Mg}(\text{CH}_3\text{COO})_2$, 1 mM dithiothreitol (DTT), 0.88 M sucrose). After filtration on gauze, the homogenate is centrifuged at 600 x g for 15 min, then the supernatant is centrifuged at 10,000 x g for 15 min. The pellet, which contains the mitochondria, is washed three times with buffer A and is then suspended in buffer B (50 mM Tris-HCl pH 7.8, 500 mM KCl, 1 mM EDTA, 1 mM DTT) and Nonidet P 40 (Shell) is added to a final concentration of 0.4 % (v/v). The extract is then incubated 15 min at 37° and after centrifugation at 10,000 x g for 30 min the supernatant is recovered and $(\text{NH}_4)_2 \text{SO}_4$ is added in two steps : up to 25 % saturation and between 25 and 80 % saturation. In the second precipitation step most of the DNA polymerase and degrading activities are recovered. This precipitate is dissolved in a small volume of buffer B and dialyzed against 400 volumes of buffer C (50 mM Tris-HCl pH 8.3, 50 mM KCl, 0.5 mM EDTA, 5 mM MgCl_2 , 1 mM DTT). After concentration of the sample by ultrafiltration (Amicon membrane UM 20 E), it is adsorbed on a DEAE-cellulose column. Protein concentration is determined by the method of LOWRY et al.¹⁹, and correction for the colour contribution of Tris-containing buffers is made.

B) Ion-exchange chromatography.

6 ml of crude extract (11.5 mg protein/ml) are loaded on to a column (10 mm x 260 mm, volume 20 ml) of DEAE-cellulose (exchange capacity 0.82 meq/g) equilibrated with buffer C. After washing with 6 column-volumes of the equilibrating buffer, 200 ml of 175 mM KCl are applied. Mitochondrial fraction M Ia (Fig. 1), eluted with the washing buffer, is concentrated by ultrafiltra-

tion and loaded on to a second DEAE-cellulose column, which is equilibrated with buffer C. After washing with 100 ml of equilibrating buffer, 200 ml of 350 mM KCl are applied. 10 ml-fractions are collected from both DEAE-cellulose columns at a flow-rate of 15-20 ml/hr. Mitochondrial fraction M II (Fig. 1), eluted with 175 mM KCl, is concentrated and then loaded on to a phosphocellulose column (the same dimensions as the DEAE-cellulose column; exchange capacity 7.4 meq/g) which is equilibrated with buffer D (the same as buffer C except that it contains 175 mM KCl). After washing with 100 ml of the equilibrating buffer, 100 ml of 400 mM KCl are applied and 5-ml fractions are collected at the above-mentioned flow-rate.

Fractions eluted with the washing buffer from the second DEAE-cellulose column (mitochondrial fraction M Ib, Fig. 3), with the washing buffer from the phosphocellulose column (mitochondrial fraction M III) and at 400 mM KCl (mitochondrial fraction M IV, Fig. 2) are concentrated and stored at -20° in glycerol (50 % v/v).

C) Templates and substrates.

$(dG)_n \cdot (dC)_n$ ($S_{w,20}=16.3$), $(dT)_n \cdot (A)_n$ ($S_{w,20}=8.7$, $E_{(p)}^{\max} = 6.7$, $n=300$ nucleotides) are obtained from Miles Laboratories, $(dT)_{12-18} \cdot (A)_n$, $(dT)_n \cdot (dA)_{12-18}$ are from Collaborative Research. The extinction coefficient of $(dT)_n \cdot (A)_n$ is that communicated by the manufacturer. For $(dG)_n \cdot (dC)_n$, $(dT)_{12-18} \cdot (A)_n$ and $(dT)_n \cdot (dA)_{12-18}$ the extinction coefficients are calculated on the basis of published values (RILEY et al.²⁰, BOLLUM²¹) and the hypochromicity indicated for each one by the supplier. [3H] deoxyguanosine 5'-triphosphate (11 Ci/mmol), methyl [3H] thymidine 5'-triphosphate (28 Ci/mmol) and adenosine [3H] 5'-triphosphate (20.5 Ci/mmol) are supplied by the Radiochemical Centre, Amersham).

Ribonuclease H substrate.

$(dT)_n \cdot (dA)_{12-18} - [^3H] (A)_n$ is synthesized in a 1500- μ l reaction mixture

containing : 100 mM Tris-HCl pH 7.6, 2.5 mM MnCl_2 , 1 mM DTT, 0.4 μM adenosine 5'-triphosphate, 48.3 nmoles of $(\text{dT})_n \cdot (\text{dA})_{12-18}$, 45 μg of purified yeast RNA polymerase (a gift from Dr. A. SENTENAC) 10 μCi adenosine $[\text{}^3\text{H}]$ 5'-triphosphate. After 15 min incubation at 37° , sodium dodecyl sulfate (SDS) is added to a final concentration of 0.2 % (v/v). The sample is dialyzed against 50 mM Tris-HCl pH 7.6 containing 1 mM EDTA. The specific activity of the complex is 70 cpm/pmole. Ribonuclease A (Worthington) has no action on this substrate.

Deoxyribonuclease substrate.

$[\text{}^3\text{H}](\text{dT})_n \cdot (\text{A})_n$ is prepared in a reaction mixture of 1500 μl containing : 50 mM Tris-HCl pH 8.3, 60 mM KCl, 1mM DTT, 1 mM MnCl_2 , 280 nmoles of $(\text{dT})_n \cdot (\text{A})_n$, 100 μg of mitochondrial fraction M Ib (See Fig. 3), 15 μCi methyl $[\text{}^3\text{H}]$ thymidine 5'-triphosphate. After 60 min incubation at 37° , SDS is added to a final concentration of 0.2 % (v/v) and the sample is dialyzed against 50 mM Tris-HCl pH 8.3, 60 mM KCl, 1 mM EDTA. After recovery of the complex by ethanol precipitation, it is dried in vacuo and dissolved in 150 mM NaCl. The specific activity of the duplex is 14 cpm/pmole.

1 unit of either RNase H or DNase is the amount of enzyme which transforms 1 nmole of the respective $[\text{}^3\text{H}]$ substrate into acid-soluble form in 20 min. This time is chosen to define the unit since under our experimental conditions the rates of degradation are linear up to 20 min and can be taken as initial velocities.

D) Standard DNA polymerase assays.

In a final volume of 100-150 μl the reaction mixture contains 40 mM Tris-HCl pH 8.3, 60 mM KCl, 1 mM DTT, either 1 mM MgCl_2 (when $(\text{dG})_n \cdot (\text{dC})_n$ or $(\text{dT})_n \cdot (\text{dA})_{12-18}$ is provided) or 1 mM MnCl_2 (when $(\text{dT})_n \cdot (\text{A})_n$ or $(\text{dT})_{12-18} \cdot (\text{A})_n$ is used), one of the synthetic primer-templates and the enzyme fractions in the amounts indicated for each experiment, one of the three $[\text{}^3\text{H}]$ deoxynucleoside 5'-triphosphates : 7.2 nmoles $[\text{}^3\text{H}]$ dGTP (specific activity 62 cpm/pmole),

Fig. 1. DEAE-cellulose column chromatography of mitochondrial extract. Fig. 2. Fraction M II from the first DEAE-cellulose column is concentrated and applied to a phosphocellulose column. Fig. 3. Fraction M Ia from the first DEAE-cellulose column is concentrated and applied to a second DEAE-cellulose column. For details see Material and Methods. 100 μ l aliquot of each fraction is used to test R-DNA polymerase (62.1 nmoles of $(dT)_n.(A)_n$) ($\circ$), DNase (193 pmoles of $[^3H]-(dT)_n.(A)_n$) ($\blacktriangle$) and RNase H (45 pmoles of $(dT)_n.(dA)_{12-18}-[^3H]-(A)_n$) ($\triangle$). Time of incubation for R-DNA polymerase 60 min. Time of incubation for DNase and RNase H 210 min.

3.2 nmoles $[^3H]dTTP$ (specific activity 147 cpm/pmole), 3.2 nmoles $[^3H]dATP$ (specific activity 165 cpm/pmole). All incubations are carried out at 37° . The time of incubation is 60 min. 20-50 μ l are withdrawn and processed for determination of acid-precipitable radioactivity as described by SPIEGELMAN et al²².

1 unit of R-DNA or D-DNA polymerase is defined as the quantity of enzyme which catalyzes the incorporation of 1 nmole of $[^3H]dNMP$ into acid-insoluble form in 60 min.

RESULTS

A) DEAE-cellulose and phosphocellulose chromatography.

The elution profiles of R-DNA polymerase, DNase and RNase H of the mitochondrial extract on DEAE-cellulose and phosphocellulose are shown in Fig. 1, 2 and 3. Fig. 1 shows that R-DNA polymerase (checked by using $(dT)_n.(A)_n$) is resolved into two peaks: the first one (fraction M Ia) is eluted with the washing buffer and the second one (fraction M II) at 175 mM KCl. In spite of a widespread distribution of DNase and RNase H activities, one can see that

M Ia and M II fractions contain both the degrading activities. Fig. 3 shows that R-DNA, DNase and RNase H activities co-chromatograph when fraction M Ia is concentrated and applied to a second DEAE-cellulose column. All three activities are eluted with the washing buffer. This indicates that no overloading of the first DEAE-cellulose column has occurred.

When fraction M II is concentrated and applied to a phosphocellulose column (Fig. 2), one can see that R-DNA polymerase, DNase and RNase H are eluted as a single peak at 400 mM KCl (fraction M IV). However, a part of R-DNA polymerase and RNase H activities is also eluted with the washing buffer (fraction M III).

Fig. 4 and 5. Kinetics of DNase (Fig. 4) and RNase H (Fig. 5) activities with fractions M Ib (4 μ g) ($\bullet$), M III (12 μ g) ($\square$) and M IV (0.5 μ g) ($\circ$). In a final volume of 200 μ l the reaction mixture contains 50 mM Tris-HCl pH 7.6, 50 mM KCl, 1 mM DTT, 2 mM MgCl_2 either 3.2 nmoles of $[\text{H}^3]$ -(dT) $_n$ -(A) $_n$ of 1.5 nmoles of (dT) $_n$ -(dA) $_{12-18}$ $[\text{H}^3]$ -(A) $_n$. At the end of the indicated times 20 μ l aliquots are withdrawn and processed for determination of the remaining acid-precipitable radioactivity.

Fig. 6. Heat-inactivation experiment. Fractions M Ib (full symbols) and M IV (empty symbols) are incubated at 50° for the indicated times in the presence of 50 μ g/ml of bovine serum albumin. 0.5 μ g of each heated fraction M Ib and M IV is used to check (dT) $_n$ -(A) $_n$ -dependent DNA polymerase ($\bullet$, $\circ$), DNase ($\blacktriangle$, $\triangle$) and RNase H ($\blacksquare$, $\square$) activities. 100 % of R-DNA polymerase is 82 and 195 pmoles of dTMP incorporated per 60 min by non-heated fractions M Ib and M IV respectively. 100 % of either DNase or RNase H activities is 260 and 78 pmoles released from the respective $[\text{H}^3]$ -substrates in 120 min by non-heated fractions M Ib and M IV.

B) R-DNA, D-DNA polymerase, DNase and RNase H activities in purified mitochondrial fractions.

Table I shows that the highest levels of enzyme activities are found in fraction M IV, where specific activities of R-DNA polymerase, DNase and RNase H indicate 60-70-fold purification. Fraction M Ib, which represents another molecular species, since it has a different chromatographic behaviour, also shows the three described activities but with lower specific activities.

Fig. 4 and 5 show the kinetics of DNase and RNase H activities when fraction M Ib, M III and M IV are used. It can be seen that fractions M Ib and M IV contain both DNase and RNase H activities but fraction M III shows no DNase and only traces of RNase H.

Table II shows the D-DNA and R-DNA polymerase activities of fractions M Ib, M III and M IV, when different synthetic primer-templates are provided.

Two remarks can be made :

1) $(dG)_n \cdot (dC)_n$ -dependent and $(dT)_n \cdot (dA)_{12-18}$ -dependent DNA polymerase activities are higher in fraction M IV than in fraction M Ib (12.4 and 5.9 times respectively).

2) $(dT)_{12-18} \cdot (A)_n$ -dependent DNA polymerase activity is considerably higher in fraction M IV than in fraction M Ib (ratio : 76.3). In contrast, $(dT)_n \cdot (A)_n$ -dependent DNA polymerase activity is 2.3-fold higher in fraction M IV than in fraction M Ib.

C) Heat-inactivation.

Fig. 6 shows the inactivation pattern of R-DNA polymerase in both fractions M Ib and M IV when samples are heated at 50° at the indicated times in the presence of 50 µg/ml of bovine serum albumin. In contrast to the polymerase neither RNase nor DNase is affected by heating.

TABLE I
Purification of mitochondrial R-DNA polymerase, DNase and RNase H

Step	Procedure	Total protein mg	R-DNA polymerase			DNase			RNase H		
			Total activity units	Specific activity $\frac{\text{Units}}{\text{mg}}$	Recovery %	Total activity units	Specific activity $\frac{\text{Units}}{\text{mg}}$	Recovery %	Total activity units	Specific activity $\frac{\text{Units}}{\text{mg}}$	Recovery %
1	Crude extract Amm. Sulfate Fraction C.E	69.300	443	6.4	100	838	12.1	100	1078	15.5	100
2 ^a	1st DEAE-cell.										
	Fraction M Ia	1.400	156	112.0	35.3	34	24.2	4	233	167.0	21.6
	Fraction M II	5.540	40	7.3	9.1	302	54.6	36	37	6.8	3.4
2 ^b	2d. DEAE-cell.										
	Fraction M I b	0.100	17	176.8	3.1	4	44.0	0.5	28	285.0	2.6
3	Phospho cell.										
	Fraction M III	2.900	3	1.3	0.7	—	—	—	—	—	—
	Fraction M IV	0.013	5	403.3	1.2	9	748.8	1.0	14	1120.0	1.3

For details see Material and methods. R-DNA polymerase is detected by using 62.1 nmoles of $(dT)_n \cdot (A)_n$ and $(^3H) - dTTP$. DNase and RNase H activities are tested as indicated in Fig. 4.

TABLE II

Primer-template utilization by purified mitochondrial-fractions

Template	Substrate	Fraction MIb	Fraction MIII	Fraction MIV	Ratio $\frac{MIV}{MIb}$
$(dG)_n \cdot (dC)_n$	$(^3H)dGTP$	362 ± 10	2 ± 0.05	4486 ± 187	12.4
$(dT)_n \cdot (dA)_{12-18}$	$(^3H)dATP$	14 ± 0.01	0.1 ± 0.01	83 ± 2.2	5.9
$(dT)_n \cdot (A)_n$	$(^3H)dTTP$	176 ± 6	1.3 ± 0.05	403 ± 12.2	2.3
$(dT)_{12-18} \cdot (A)_n$	$(^3H)dTTP$	3 ± 0.4	0.1 ± 0.01	229 ± 5.5	76.3

D-DNA and R-DNA polymerase assays are performed according to the standard DNA polymerase assay described in Material and methods. Volume of the reaction mixture 100 μ l. The following amounts of the primer-templates are used: 11.3 nmoles of $(dG)_n \cdot (dC)_n$, 5.8 nmoles of $(dT)_n \cdot (dA)_{12-18}$, 62.1 nmoles of $(dT)_n \cdot (A)_n$, 5.6 nmoles of $(dT)_{12-18} \cdot (A)_n$. The results are expressed in units/mg of the corresponding fractions. Standard error is calculated on the basis of 18 determinations.

DISCUSSION

In a previous report²³, we have shown that there are at least two D-DNA polymerases in the mitochondrial preparation from chick embryonic brain, since on DEAE-cellulose chromatography, although the bulk of the enzyme is eluted at 150-175 mM KCl, a small but significant amount is eluted with the washing buffer. The study of an R-DNA polymerase (by using $(dT)_{12-18} \cdot (A)_n$) revealed that all of this activity is eluted together with the bulk of D-DNA polymerase, and that no $(dT)_{12-18} \cdot (A)_n$ -dependent activity could be detected

in the washing buffer fractions. A similar result has recently been obtained¹⁷ with HeLa mitochondrial DNA polymerase. In this case, the bulk of D-DNA polymerase activity was eluted with the washing buffer and no $(dT)_{12} \cdot (A)_n$ -dependent activity could be detected in this fraction. From these and our previous results, one could be tempted to think that the DEAE-cellulose washing buffer fraction of chick embryonic brain and HeLa mitochondrial DNA polymerase contains exclusively D-DNA polymerase activity. In fact, according to the results presented in this paper, mitochondrial fraction M Ib (eluted with the washing buffer of the second DEAE-cellulose column, Table II) shows a high D-DNA polymerase activity ($(dG)_n \cdot (dC)_n$ -dependent activity) but also a low R-DNA polymerase activity when $(dT)_{12-18} \cdot (A)_n$ is used as primer-template. In contrast, when $(dT)_n \cdot (A)_n$ is provided, fraction M Ib shows a considerable R-DNA polymerase activity. Thus, it appears that in chick embryonic brain mitochondria D-DNA and R-DNA polymerase activities cannot be separated. This feature of the partially purified mitochondrial enzyme is not unexpected, since it is observed in other highly purified cellular DNA polymerases^{24,25,26}.

We have seen that DNase and RNase H are found together with DNA polymerase in fractions M Ib and M IV. Recent reports^{15,27} show that exonuclease activity is not essential for DNA polymerization in eukaryote systems, and the presence of degrading activities in partially purified mitochondrial fractions could be interpreted as contamination. However, the association of a nuclease activity with mitochondrial DNA polymerase cannot be excluded since an endonucleolytic activity has been found in HeLa mitochondrial DNA polymerase¹⁷. On the other hand, the presence of an RNase H activity associated with DNA polymerase I of *E. coli* and DNA polymerase II from KB cells has been described¹².

The differential thermal lability of R-DNA polymerase and both degrading activities could suggest that RNase H and DNase activities are catalyzed by molecular species different from R-DNA polymerase and for this reason

are to be considered as contaminants. However, the fact that R-DNA polymerase and both degrading activities co-chromatograph under various conditions suggests that the corresponding enzymes might form rather stable complexes and that they present a high affinity for each other. On the other hand, the possibility of association of degrading and polymerizing activities to the same molecule cannot be completely excluded merely on the basis of their different thermal inactivation profiles.

A fact which deserves a final comment is the comparison of the kinetic patterns of DNase activity (Fig. 4) in fractions M Ib (sigmoid curve) and M IV (hyperbolic curve). The apparent differences observed could suggest the existence of different DNase activities contaminating or associated with R-DNA polymerase. We are presently trying to analyze these DNase activities in more detail.

ACKNOWLEDGEMENTS

We are grateful to Professor Et. WOLFF for his help and encouragement, to Professor F. CHAPEVILLE and Professor J. KRUEH for their critical reading of the manuscript and their helpful suggestions.

The technical assistance of P. CHAUDOUET, L. ADDADE, P. COLTEY and E. BOURSON is deeply acknowledged.

*Institut de Pathologie Moléculaire,
24, Rue du Faubourg Saint-Jacques, 75014 Paris, France

BIBLIOGRAPHY

1. SPIEGELMAN, S., BURNY, A., DAS, M.R., KEYDAR, J., SCHOLM, J., TRAVNICEK, M. and WATSON, K. (1970). *Nature*, **228**, 430-432.
2. BALTIMORE, D. and SMOLER, D. (1971). *Proc. Nat. Acad. Sci. U.S.A.*, **68**, 1507-1511.
3. Mc CAFFREY, R., SMOLER, D. and BALTIMORE, D. (1973). *Proc. Nat. Acad. Sci. U.S.A.* **70**, 521-525.
4. SORIANO, L. (1972). *FEBS Letters*, **22**, 310-314.
5. FRY, M. and WEISSBACH, A. (1973). *J. Biol. Chem.*, **248**, 2678-2683.
6. KINDIG, D.A. and KIRSTEN, W.H. (1967). *Science*, **155**, 1543-1545.
7. LOWY, D.R., ROWE, W.P., TEICH, N. and HARTLEY, J.W. (1971). *Science*, **174**, 155-156.
8. GERWIN, B.I. and BASSIN, R.H. (1973). *Proc. Nat. Acad. Sci. U.S.A.*, **70**, 2453-2456.
9. PENIT, C., PARAF, A. and CHAPEVILLE, F. (1974). *Nature* (in the press).
10. STEIN, H. and HAUSEN, P. (1969). *Science*, **166**, 393-395.
11. HAUSEN, P. and STEIN, H. (1970). *Eur. J. Bioch.*, **14**, 278-283.
12. KELLER, W. and CROUCH, R. (1972). *Proc. Nat. Acad. Sci. U.S.A.*, **69**, 3360-3364.

-
13. GRANDGENETT, D.P., GERARD, G. and GREEN, M. (1973). Proc. Nat. Acad. Sci. U.S.A., 70, 230-234.
 14. LEIS, J.P., BERKOWER, I. and HURWITZ, J. (1973). Proc. Nat. Acad. Sci. U.S.A., 70, 466-470.
 15. CHANG, L.M.S. and BOLLUM, F.J. (1973). J. Biol. Chem., 248, 3398-3404.
 16. KORNBERG, A. (1969). Science, 163, 1410-1418.
 17. FRY, M. and WEISSBACH, A. (1973). Biochemistry, 12, 3602-3608.
 18. CHAPPELL, J.B. and HANSFORD, R.G. (1969). In Subcellular Components, pp. 43-56, Butterworths, London.
 19. LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. and RANDALL, R.S. (1951). J. Biol. Chem., 193, 265-275.
 20. RILEY, M., MALING, B. and CHAMBERLIN, M.J. (1966). J. Mol. Biol., 20, 359-389.
 21. BOLLUM, F.J. (1968). In Methods of Enzymology, vol. XII, Part. B, pp. 591-611, Academic Press, New-York and London.
 22. SPIEGELMAN, S., BURNY, A., DAS, M.R., KEYDAR, J., SCHLOM, J., TRAVNICEK, M. and WATSON, K. (1970). Nature, 227, 563-567.
 23. SORIANO, L. and CROISILLE, Y. (1973). FERS, Letters, 31, 143-148.
 24. CHANG, L.M.S. and BOLLUM, F.J. (1972). Biochemistry, 11, 1264-1272.
 25. STAVRIANOPOULOS, J.G., KARKAS, J.D. and CHARGAFF, E. (1972). Proc. Nat. Acad. Sci. U.S.A., 69, 2609-2613.
 26. SMITH, R.G. and GALLO, R.C. (1972). Proc. Nat. Acad. Sci. U.S.A., 69, 2879-2884.
 27. WANG, T.S.F., SEDWICK, W.D., and KORN, D. (1974). J. Biol. Chem., 249, 841-850.