Eukaryotic ternary transcription complexes. I. The release of ternary transcription complexes of RNA polymerases I and II by the endogenous nucleases of rat liver nuclei

David R.Sargan and Peter H.W.Butterworth

Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, UK

Received 12 March 1982; Revised 7 June 1982; Accepted 5 July 1982

ABSTRACT

Autodigestion of rat liver nuclei in magnesium-containing buffers leads to the release of about 80% of DNA-dependent RNA polymerases I and II, together with 4 to 8% of the DNA. The RNA polymerases are at least partially DNA bound as judged by the effect on *in vitro* transcription of (1) Actinomycin D, (2) preirradiation of the enzymes in the presence of 8-methoxypsoralen and (3) heparin. The released DNA migrates as ladders of nicked nucleosome-size fragments on electrophoresis. On sucrose gradients, most RNA polymerase activity sediments as two peaks: one slightly smaller than the 11S mononucleosome and the other with the dinucleosome. The released material can act as a source of ternary transcription complexes for further structural studies.

INTRODUCTION

Much interest is currently focussed on the structure and organisation of chromatin in regions of transcriptional activity. Some evidence (reviewed in 1) suggests that all four nucleosome core histones are present in active chromatin, but the sensitivity to nucleases of actively transcribed sequences (as opposed to bulk chromatin) indicates differences in chromatin organisation. Actively transcribed DNA sequences must by definition contain ternary complexes of RNA polymerase, DNA and RNA. Differential release of active regions of the chromatin by nucleases will therefore give rise to the simultaneous release of transcription complexes for subsequent structural analysis. Mild micrococcal nuclease digestion has been shown to release ternary complexes containing RNA polymerase II from rat liver nuclei (2,3).

Mammalian nuclei, isolated from tissues using an initial homogenisation in isotonic sucrose, contain RNA polymerases I and II predominantly in a chromatin-bound form (4). Incubation of such nuclei in magnesium-containing buffers at 37°C and low ionic strength results in the release of both classes of RNA polymerase into the incubation medium (5,6). Release of the polymerase activities is probably due to the action of non-sequence specific deoxyribo-

nuclease(s) which are known to exist in mammalian nuclei (7). Many of these are magnesium or magnesium and calcium activated.

Here we show that autodigestion results in the release of a small proportion of the nuclear DNA in parallel with the release of both RNA polymerases I and II and that a significant proportion of the polymerase is in a DNA-bound form. We have analysed the pattern of DNA fragments released by the unknown nuclear nuclease(s) and the sedimentation characteristics of released RNA polymerase activity.

MATERIALS AND METHODS

Preparation of rat liver nuclei

Rat liver nuclei were prepared by a modification of the procedure of Widnell and Tata (8). Briefly, livers from 200g female Sprague-Dawley rats were homogenised in 0.32M sucrose containing 25mM KCl, 3mM MgCl₂ and 0.1mM dithiothreitol (DTT). The homogenate was adjusted to 0.25M sucrose by the addition of deionised water and a crude nuclear pellet was obtained by centrifugation at 700 x g for 15 min. Pellets were resuspended in 2.4M sucrose containing 25mM KCl, 1mM MgCl₂ and 0.1mM DTT and nuclei were collected by centrifugation at 25000 x g for 90 min. All procedures were carried out at 0° to 4°C and nuclei were either used immediately or stored at -70°C for up to 6 weeks.

Endogenous nuclease digests

Nuclei were washed by centrifugation through Buffer A (25% glycerol, 5mM MgCl₂, 0.5mM DTT and 20mM Tris/HCl, pH 7.9) containing 70mM KCl for 10 min at 3000 x g and 2°C, and were resuspended in the same buffer at a concentration of 2.5×10^8 nuclei ml⁻¹. Thereafter, the method follows that of Coupar and Chesterton (6); nuclei were allowed to autodigest at 37°C for 30 min and were pelleted as above. The supernatant was collected and the pellets were resuspended in the same volume of Buffer A containing 120mM KCl. Autodigestion was repeated for a further 30 min at 37°C and the supernatant was collected as before. For most purposes, the two supernatants were combined and were either used immediately or were made up to 50% glycerol and stored at -70°C.

RNA polymerase assays

Assays for RNA polymerase activity were carried out and processed as described previously (4). RNA polymerase I activity was measured in the presence of 100µg ml⁻¹ α-amanitin (Boehringer), 80mM KCl, 1mM MnCl₂ and 5mM MgCl₂; RNA polymerase II activity was the activity sensitive to 1µg ml⁻¹ α-amanitin at 130mM (NH₄)₂SO₄ at the same divalent cation concentrations. The specific radioactivity of the labelled ribonucleoside triphosphate used (³H-UTP) was 120 - 600 counts min⁻¹ pmole⁻¹. In some cases, 40µg ml⁻¹ calf thymus DNA was included as an exogenous template. Incubation times and temperatures are given in the legends to figures and tables.

Modification to the assay conditions to define DNA-bound polymerase activity

Three types of modified assays were used:

1. In order to prevent de novo initiation of RNA chains, heparin at 1mg ml⁻¹ was added to assays which were brought to 25°C 1 min prior to the addition of ribonucleoside triphosphates.

2. The protocol of Yu and Fiegelson (9) as modified by Kellas et al. (4) was used: samples were assayed using poly d(A-T) at $20\mu\text{g ml}^{-1}$ as template in the presence and absence of $200\mu\text{g ml}^{-1}$ Actinomycin D (Sigma). All other assay conditions were as above, incubations being at 25°C for 15 min.

3. 'Bound'-RNA polymerases were inhibited by preirradiation of the nuclear extract (in 50% glycerol) with long-wave UV light (366nm) in the presence of $100\mu\text{g ml}^{-1}$ 8-methoxypsoralen (Sigma) and 25mM β -mercaptoethanol for 30 min at 25°C . Following irradiation, RNA polymerase activity was measured in the normal way using calf thymus DNA as a template. A detailed description of this reaction and its applications is included in the accompanying paper (10).

Electrophoretic analysis of DNA fragments

DNA extractions were as described by Butterworth (11). Gel electrophoresis was in agarose LE (Miles) gels buffered with 90mM Tris, 90mM boric acid, 2.5mM EDTA at pH 8.3, or in polyacrylamide gels by the method of Maniatis et al. (12).

Sucrose gradient centrifugation

After vacuum dialysis against 25mM KCl, 0.1mM EDTA, 0.1mM DTT and 20mM Tris/HCl (pH 8.0) for at least 3 hours (resulting in about a four-fold decrease in volume), 1ml samples of the extract were layered onto 55ml linear gradients of sucrose (7-30%) in the same buffer. After centrifugation for 18 hours at $1000000 \times g$ and 40°C , the gradients were fractionated by upward displacement with the optical density at 260nm being monitored.

DNA assays

DNA assays were by the method of Giles and Myers (13).

Micrococcal nuclease and DNase I digestion

The conditions of Pauletti (14) were used for digestions with micrococcal nuclease (Sigma).

Other Materials

Except where stated otherwise, all materials were from Fisons UK Ltd or BDH Chemicals Ltd and were analytical reagent grade.

RESULTS

RNA-polymerases I and II are progressively released from nuclei during 'autodigestion'

Figure 1 shows a typical example of the progressive release of RNA polymerases I and II and DNA during the low ionic strength, magnesium-activated 'autodigestion' of rat liver nuclei, according to the protocol described in Methods. We have used a number of different protocols for the digestion, varying the ionic strength and the digestion time. The standard method shown here gives the maximum release of polymerase activity with relatively low DNA release. The shift in ionic strength is necessary to maximise the release of RNA polymerase II. The release of polymerase II is also highly dependent on the presence of magnesium ions in the extraction buffers, but that of polymerase I is less so. Included in the data is an

Fig. 1. Release of DNA and RNA polymerases I and II during 'autodigestion' of rat liver nuclei. Autodigestion was as described in Methods. At the time points shown, aliquots were removed from the digests and separated into a supernatant fraction containing released RNA polymerases and DNA, and a nuclear pellet by centrifugation at $8000 \times g$ for 2 min. (a) DNA in supernatant expressed as a percentage of the total nuclear DNA (data from 5 determinations); (b) RNA polymerase assays were for 15 min at 25°C . Released enzymes were assayed at a single (suboptimal) salt concentration of 250mM KCl (at which both RNA polymerases I and II activities are at about 75% of maximum) in the presence and absence of α -amanitin; nuclear polymerase activity was assayed at 100mM $(\text{NH}_4)_2\text{SO}_4$ in the presence of 1 mg ml^{-1} heparin. $\circ$, released RNA polymerase I; $\bullet$, released RNA polymerase II; $\square$, nuclear polymerase activity.

analysis of the polymerase activity remaining in the nuclear pellets, to provide an indication of the extent of enzyme release. This analysis was carried out in the presence of heparin to maximise the nuclear polymerase activity by removal of nuclear 'blocking' proteins (see 15,16). The data on released polymerase on the one hand and the remaining nuclear activity on the other are not strictly comparable because of the inhibition of initiation and the increased rate of chain elongation in the presence of heparin.

However, as the nuclear preparation used here contains RNA polymerases primarily in a DNA-bound form (4), it is reasonable to conclude that about 80% of the RNA polymerase activity of the nuclei is released during auto-digestion in these conditions, together with a small proportion of the nuclear DNA ($6.2 \pm 2.5\%$ from 5 determinations).

The presence of DNA-bound RNA polymerases in the nuclear extract

1. Heparin resistant polymerase activity. If the nuclear extract contains any polymerases in the form of ternary complexes, these complexes will synthesise a limited amount of RNA *in vitro* in the presence of heparin (i.e. 'run-off' from the bound DNA fragment only), as heparin is a potent inhibitor of initiation but not of the elongation of RNA chains (15). There was heparin-resistant polymerase activity in the nuclear extract (Fig. 2), 66% of which was polymerase I, the remainder being polymerase II according to α -amanitin sensitivity.

2. Actinomycin D-sensitivity of poly d(A-T) transcription. An alternative approach to identifying DNA-bound RNA polymerases is to trap the enzymes in the transcription complex so that they cannot transcribe an added (exogenous) template. The classical method for this is to preincubate putative polymerase complexes with Actinomycin D. This intercalates into the DNA of the complex, restricting transcription and the release of the polymerase. This prevents access to the poly d(A-T) template, the transcription of which is not affected by Actinomycin D. Part of Table 1 summarises such an anal-

Fig. 2. Heparin-resistant RNA polymerase activity in the nuclear extract. 100 μ l aliquots of the nuclear extract were preincubated in the presence or absence (control) of heparin at 1 mg ml⁻¹ for 5 min at 25°C; after the addition of ribonucleoside triphosphates, aliquots were removed at the time points shown and processed as described in Methods. ●, control; □, preincubated with heparin.

TABLE 1

Sensitivity of RNA polymerases in the nuclear extract to inhibition by Actinomycin D and 8-methoxypsoralen (8-MOP)

Enzyme	Inhibitor	pmole ^3H -UMP incorp in RNA		Inhibition %
		-	+	
RNAp I	Actinomycin D	24.29	12.64	47.96
RNAp II	Actinomycin D	8.86	3.98	55.13
RNAp I	8-MOP	3.29	2.64	19.93
RNAp II	8-MOP	6.75	5.08	24.76

Assays were incubated at 25°C for 15 min. In the case of 8-MOP, the nuclear extract was preirradiated separately (-) or together with (+) the psoralen derivative prior to assay as described in Methods.

ysis of the nuclear extract, showing that transcription by both polymerases is about 50% inhibited by Actinomycin D with poly d(A-T) as template.

3. Photochemical crosslinking of the DNA in the transcription complexes.

Another technique to trap the polymerase in the transcription complex is to use the photochemical crosslinking of pyrimidine residues in adjacent polynucleotide chains by psoralen derivatives (17,18). Irradiation of the nuclear extract at 366nm in the presence of 8-methoxypsoralen reduced polymerase activity on calf thymus DNA by approximately 25% for both polymerases (see Table 1).

Gel analysis of DNA fragments in the nuclear extracts

DNA fragments released from the nuclei during autodigestion were purified and analysed by electrophoresis on agarose gels (Fig. 3) and under denaturing conditions on polyacrylamide gels (Fig. 4). The DNA released was nucleosomal, showing a predominance of mono- and di-nucleosome sizes, especially at early times in the digestion. This contrasts with the behaviour of these nuclei under micrococcal nuclease digestion in which polynucleosome fragments predominate early in the digestion and are reduced in average length as the digestion proceeds (19). It is noticeable that the average size of the 'mononucleosomal'-DNA in these extracts was slightly higher than that of this DNA arising from micrococcal nuclease digests of

Fig. 3. Analysis of DNA fragments released from nuclei as a function of time during 'autodigestion' by endogenous nuclear nucleases. 5 - 8 μ g of DNA purified from supernatants prepared at various times during autodigestion (see Legend to Fig. 1) was analysed by electrophoresis on a 2.4% agarose gel. Lanes 1 + 13: Hpa II digest of plasmid pAT 153 as markers; lane 2: DNA in the supernatant of nuclei washed with Buffer A; lanes 3, 4 and 5: DNA in the supernatant from nuclei autodigested for 10, 20 and 30 min; lane 6: DNA in the supernatant of nuclei washed with Buffer B after autodigestion in Buffer A for 30 min; lanes 7, 8 and 9: DNA in the supernatant from nuclei subsequently autodigested in Buffer B for 10, 20 and 30 min respectively; lane 10: DNA from combined supernatants (total extract) from autodigestion; lanes 11 + 12: DNA remaining in the nuclear pellets (8 and 16 μ g respectively) at the completion of autodigestion. Gels were stained with ethidium bromide.

similar extent (173 ± 15 compared with 165 ± 8 base-pairs). The dimer and trimer members of the nucleosome series were also larger in the autodigestion, but thereafter the nucleosomal repeat interval was very similar (approx. 196-198 base pairs). When analysed on polyacrylamide gels under denaturing conditions, the DNA fragments exhibited a pattern of single-standed nicking at 10 base intervals with the highest discrete member running at 160 bases (Fig. 4). This digestion pattern is rather similar to that found in chromatin after DNase I digestion (20) and the extent of nicking has an important affect on the interpretation of the DNA-bound polymerase data in

Fig. 4. Denaturing gel electrophoresis of DNA released from nuclei during autodigestion (see Fig. 3). DNA released from nuclei was purified and 4µg samples were denatured by boiling in formamide and were analysed by electrophoresis on a 10% polyacrylamide gel (19:1 acrylamide/methylene bis-acrylamide) containing 7M urea. Lane 1: DNA in the supernatant of nuclei washed with Buffer A; lanes 2, 3 and 4: DNA in the supernatant from nuclei autodigested in Buffer A for 10, 20 and 30 min respectively; lane 5: DNA in the supernatant from nuclei washed with Buffer B after autodigestion in Buffer A; lanes 6, 7 and 8: DNA in the supernatant from nuclei autodigested in Buffer B for 10, 20 and 30 min respectively; lane 9: DNA in the total endogenous nuclease extract; lane 10: DNA remaining in the nuclear pellet (8µg) at the completion of autodigestion; lanes 11 and 12: DNA from the supernatant of a 5 min and a 30 min digest of nuclei (2.5×10^8 nuclei ml^{-1}) at 37°C with 10µg ml^{-1} micrococcal nuclease.

Table I and Fig. 2 (see Discussion below).

Sucrose gradient analysis of the nuclear extracts

Sucrose gradients were used to examine the extracts for chromatin subunits containing RNA polymerases (Fig. 5). The DNA associated with each optical density peak was analysed by gel electrophoresis (Fig. 6). This showed that the material in peak 4 was nucleosomal, whilst peak 5 contained some 140 base-pair DNA and possibly some smaller material. Material sedimenting right through the gradient appeared to consist of aggregates of mono-

Fig. 5. Sucrose density gradient analysis of the total endogenous nuclear nuclease extract. Samples were prepared as described in Methods and were sedimented through 7-30% linear sucrose gradients for 23 hr at 100,000 x g. RNA polymerase activity was assayed in gradient fractions at 250mM KCl (●) and OD₂₆₀ peaks were bulked as shown.

Fig. 6. Analysis of DNA from pooled sucrose gradient fractions. 10 μ g samples of DNA from each pool (see Fig. 8) were analysed by electrophoresis on 1.2% agarose gels. Lanes 1 - 5: DNA from pools 1 - 5 of sucrose gradient (see Fig. 8); lane 6: total DNA from a 4 min digest of rat liver nuclei with micrococcal nuclease (conditions as for Fig. 5, lane 11).

and small oligo-nucleosome-sized DNA's, and not of very large DNA's. Peak 2 contained DNA fragments of the size of nucleosome 4-6 mers, but may well be caused by 40S HnRNA particles. The other inflections of optical density correspond to the various nucleosome multimers.

The main peaks of RNA polymerase activity at high ionic strength (mainly polymerase II) in the gradient travel in two areas, one around optical density peaks 3 and 4, the other in front of peak 5 (Fig. 5). The former is 32.1% suppressed by Actinomycin D in the presence of poly d(A-T) and the latter only 15.6% suppressed. RNA polymerase I behaved rather similarly, but the first peak spreads further down the gradient to optical density peak 2 (data not shown).

DISCUSSION

The data presented above show that in the 'autodigestion' method for the extraction of RNA polymerases from eukaryotic nuclei, DNA is released concurrently with the release of the enzymes (Fig. 1). It appears that a proportion of both RNA polymerases I and II are bound to chromatin in the nuclear extract as judged by Actinomycin D sensitivity, 8-methoxypsoralen inhibition and heparin-resistance of RNA synthesis by the extracted enzymes (Table 1 and Fig. 2). Because the nuclei as prepared here contain very little RNA polymerase III (less than 5% of the total polymerase activity) no data has been collected on this enzyme.

None of the three assay techniques we have used suggested that all the RNA polymerase molecules released were in a DNA-bound form. To establish DNA-binding, each of the techniques depends on a minimum length of DNA being available to the polymerase for transcription in the complex. If the DNA in the complex is very short, it may contain no sequences suitable for 8-methoxypsoralen crosslinking or Actinomycin D intercalation: in each case, a specific dinucleotide is preferred (21,22). Similarly, polymerases which fall off the DNA very rapidly will hardly be seen in any of the assays: since the DNA is highly nicked (Fig. 4) and since RNA polymerases can terminate transcription at nicks, it is likely that each of these techniques will underestimate the proportion of DNA-bound polymerases in the nuclear extract.

The RNA polymerases are bound to a very small proportion of DNA. Each nucleus contains about 30,000 molecules of RNA polymerases (23) together with about 2.9×10^9 base-pairs of DNA (24) so that the polymerases are unlikely to be in contact with more than 0.1% of the DNA. Therefore, to investigate the structure of the ternary transcription complexes released by autodigest-

ion, it is necessary either to devise a further purification for these complexes (see 3) or to find a means of identifying the complexes within the released chromatin mixture. An attempt to purify the transcription complexes by fractionation of released chromatin by solubility in 100mM NaCl (25) was not successful (data not shown). Therefore we have adopted an alternative approach which is described in the accompanying paper (10).

The finding that the bulk of the DNA-bound RNA polymerase sediments only slightly faster than a mono-nucleosome particle in sucrose gradients suggests that it is not released in this system in the same state as the hexanucleosome particle reported by Tata and Baker (2). Since the polymerases in these smaller chromatin units are active in RNA synthesis on their associated DNA fragments, the hexanucleosome particle is not absolutely necessary for RNA polymerase function. It should be emphasised that the rate of sedimentation on these gradients cannot give an absolute indication of the accompanying DNA size as the folding of the particle is not known: unfolded nucleoproteins are known to display very aberrant sedimentation values on sucrose gradients (26).

We have performed studies on the sizes of the DNA fragments released during autodigestion, a detailed description of which will be published elsewhere. The cutting pattern is similar to that seen by Duerksen and Connors (27) and by Simpson and Whitlock (28) for calcium-dependent hepatic endogenous nucleases. There are one or two intriguing differences however. The core particle size is slightly smaller than that seen at the start of the Simpson and Whitlock digestions and does not change during their digestion. Nucleosome multimers *increase* in the extract as the digest proceeds, perhaps because friable areas of chromatin are very rapidly degraded before a less selective attack occurs.

A variety of nucleases appear to fractionate chromatin into transcriptionally active and inactive components (e.g. DNase II (29); micrococcal nuclease (30,31) and DNase I (32,33)). In the light of these findings, the associated release of RNA polymerases and part of the nuclear DNA in the autodigestion technique invites speculation that the released DNA may be enriched for transcribed sequences. A preliminary report confirming this has already been published (34) and indirect evidence from other groups has suggested that hepatic endogenous nucleases may preferentially digest transcriptionally active chromatin (27,35). A more detailed analysis of the DNA sequences released in our system is in preparation.

ACKNOWLEDGEMENTS

We would like to thank Dr. Stephen Gregory, Chris Konialis and Niall Dillon for reviewing this manuscript and Stephen Thomas for technical assistance. D.R.S. was in receipt of a Science Research Council Studentship and the work is supported by the Medical Research Council (Project Grant No. G80/0300/2CB and the Wellcome Trust.

REFERENCES

1. Mathis, D., Oudet, P. and Chambon, P. (1980) *Prog. Nuc. Acid Res. Mol. Biol.* **24**, 1 - 55.
2. Tata, J.R. and Baker, B. (1978) *J. Mol. Biol.* **118**, 249 - 272.
3. Hatayama, T., Omori, K., Inoue, A. and Yukioka, M. (1981) *Biochim. Biophys. Acta* **652**, 245 - 255.
4. Kellas, B.L., Austoker, J.L., Beebe, T.J.C. and Butterworth, P.H.W. (1977) *Eur. J. Biochem.* **72**, 583 - 594.
5. Chesterton, C.J. and Butterworth, P.H.W. (1971) *Eur. J. Biochem.* **19**, 232 - 241.
6. Coupar, B.E.H. and Chesterton, C.J. (1975) *Eur. J. Biochem.* **59**, 25 - 34.
7. Sierakowska, H. and Shugar, D. (1977) *Prog. Nuc. Acid Res. Mol. Biol.* **20**, 59 - 130.
8. Widnell, C.C. and Tata, J.R. (1966) *Biochim. Biophys. Acta*, **123**, 478 - 492.
9. Yu, F.-L. and Fiegelson, P. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2177 - 2180.
10. Sargan, D.R. and Butterworth, P.H.W. (1982) accompanying paper.
11. Butterworth, P.H.W. (1977) in "Subnuclear Components" ed G.D. Birnie, Butterworths Press, London, pp 307 - 314.
12. Maniatis, T., Jeffreys, A. and Van de Sande, H. (1975) *Biochemistry* **14**, 3787 - 3794.
13. Giles, K.V. and Myers, A. (1965) *Nature* **206**, 93.
14. Pauletti, J. (1978) *Biochem. Biophys. Res. Commun.* **81**, 193 - 198.
15. Coupar, B.E.H. and Chesterton, C.J. (1977) *Eur. J. Biochem.* **79**, 525 - 533.
16. de Pomerai, D.I., Chesterton, C.J. and Butterworth, P.H.W. (1974) *FEBS Letters* **42**, 149 - 153.
17. Isaacs, S.T., Shen, C.-K.J., Hearst, J.E. and Rapaport, H. (1977) *Biochemistry* **16**, 1058 - 1064.
18. Ou, C.-N., Isai, C.-H., Tapley, K.J. and Song, P.-S. (1978) *Biochemistry* **17**, 1047 - 1053.
19. Sargan, D.R. (1981) Ph.D. Thesis, University of London, UK.
20. Lutter, L.C. (1978) *J. Mol. Biol.* **124**, 391 - 420.
21. Reich, E. and Goldberg, I.H. (1964) *Prog. Nuc. Acid Res. Mol. Biol.* **3**, 183 - 234.
22. Scott, B.R., Pathak, M.A. and Mohn, G.R. (1976) *Mut. Res.* **39**, 29 - 74.
23. Cochet-Meilhac, M., Nuret, P., Courvalin, J.C. and Chambon, P. (1974) *Biochim. Biophys. Acta* **353**, 185 - 192.
24. Sober, H.A. (1968) in *Handbook of Biochemistry* (Canadian Rubber Co. Cleveland, USA), pp H-58.
25. Levy-Wilson, B. and Dixon, G.H. (1978) *Nuc. Acids Res.* **5**, 4155 - 4163.
26. Gordon, V.C., Knobler, C.M., Olins, D.E. and Schmaker, V.N. (1978) *Proc. Nat. Acad. Sci. USA* **75**, 660 - 663.
27. Duerksen, J.D. and Connor, K.W. (1978) *Mol. Cell. Biochem.* **19**, 93-112.

28. Simpson, R.T. and Whitlock, J.P. (1976) Nuc. Acid Res. 3, 117 - 127.
29. Gottesfeld, J.M. and Partington, G.H. (1977) Cell, 12, 953 - 962.
30. Bloom, K.S. and Anderson, J.M. (1978) Cell 15, 141 - 150.
31. Bellard, J.D., Gannon, F. and Chambon, P. (1977) Cold Spring Harbor Symp. Quant. Biol. 42, 779 - 791.
32. Weintraub, H. and Groudine, M. (1976) Science 193, 848 - 856.
33. Garel, A. and Axel, R. (1977) Cold Spring Harbor Symp. Quant. Biol. 42, 701 - 708.
34. Sargan, D.R. and Butterworth, P.H.W. (1981) Cell Biol. Int. Reports 5, 59 - 64.
35. Smith, R.J. and Duerksen, J.D. (1977) Can. J. Biochem. 55, 227 - 238.