Biochemical Pharmacology, Vol. 31, No. 6, pp. 1063–1067, 1982.
Printed in Great Britain

0006-2952/82/061063–05 $03 00/0

INFLUENCE OF 3-METHYLCHOLANTHRENE ON LIVER NUCLEOLAR AND NUCLEOPLASMIC ACTIVITIES OF PROTEIN KINASES AND RNA POLYMERASES

U. KLEEBERG, J. SZEBERÉNYI,* P. JUHASZ,* A. TIGYI* and W. KLINGER

Institute of Pharmacology and Toxicology, Friedrich-Schiller University, Jena, G.D.R., and *Department of Biology, University Medical School, Pecs, Hungary

(*Received* 31 *March* 1981; *accepted* 9 *October* 1981)

Abstract—The experiments were designed to investigate some details of the action of 3-methylcholanthrene (3-MC) on the regulation of transcription. After a single intraperitoneal dose of 3-MC a significant increase in the activities of both nucleolar and nucleoplasmic protein kinases in hepatic cells of young rats was found. The maximal stimulation took place 24 hr after the administration of 3-MC and the extent of activation was much greater in the nucleolar fraction. There is a significant elevation of the activities of both functional forms, free and template-engaged, of RNA polymerase A 24 hr after a single injection of 3-MC. Free and engaged forms of extranucleolar RNA polymerase B show a different behaviour: after 24 hr of 3-MC administration the engaged form is markedly enhanced while the activity of the free enzyme shows a significant decrease. The more moderate increase in total RNA polymerase B activity is obviously preceded by a transfer of the enzyme from 'free' to 'engaged' form. Since the enhancement of protein kinase activities was accompanied by the stimulation of nuclear RNA polymerases we suggest that both kinds of enzymes are involved in an epigenetic mechanism of the inducing action of 3-MC on cytochrome P_1-450.

The enzyme induction phenomenon in eukaryotic cells mediated by the polycyclic aromatic hydrocarbon (PAH) 3-MC involves the enhancement of the activity of a special part of the microsomal monooxygenase system, the aryl hydrocarbon hydroxylase (AHH) activity [1–3].

The biological significance of nuclear protein phosphorylating enzymes for important cellular processes such as differentiation, proliferation, enzyme induction, response to hormonal actions etc. was emphasized in several review articles [4–8]. In these processes the increased phosphorylation of nuclear proteins is followed by alterations in the transcriptional activity of eukaryotic cells. 3-MC produces early changes in the RNA synthesis in rat liver cells and thus an enhancement of transcription is directly involved in the enzyme induction process caused by 3-MC. Enhanced RNA synthesis after administration of inducing compounds have been described by investigating the *in vivo* uptake of orotic acid [9], by *in vitro* measurement of DNA-dependent RNA synthesis [10], and by estimation of chromatin template activity [11] (cf. [12]). It was indicated by Gelboin [12] that the elevation in microsomal protein synthesis caused by 3-MC treatment could be prevented by actinomycin D. This suggests an alteration at the transcriptional level produced by 3-MC. Therefore the behaviour of both the protein kinase and the RNA polymerase activities in rat liver nucleolar and nucleoplasmic fractions after 3-MC administration seemed to be of high interest. In previous experimental series the relative selectivity of the 3-MC induction stimulus in young rats on the hepatic AHH activity could be confirmed, the magnitude was dependent on the dose of inducer and the time of administration [13].

Now we differentiated between the free and engaged forms of DNA-dependent RNA polymerases in nucleolar and nucleoplasmic fractions. The existence of two functionally different forms of eukaryotic RNA polymerases was first supported by the studies of Yu [14, 15]. It could be demonstrated that the free and engaged forms of RNA polymerases show a characteristic alteration during processes leading to changes in gene expression. Liver regeneration [16, 17], differentiation [18], tryptophan treatment [19], and thioacetamide administration [20] were found to be accompanied by characteristic changes in the activities of the free and template-engaged forms of nuclear RNA polymerases.

The experimental results presented in this paper indicate an early increase in the activities of nucleoplasmic and mainly of nucleolar protein kinases in the livers of young rats following administration of the inducer 3-MC. The stimulation of protein kinases is accompanied by remarkable changes in both functional forms, i.e., free and engaged, of nucleolar and nucleoplasmic RNA polymerases.

The significance of changes in template activity, of interconversion processes between the free and engaged RNA polymerase molecules, and the possible role of phosphorylation of chromatin proteins during the inducing effect of 3-MC have to be discussed.

MATERIALS AND METHODS

Chemicals. [γ-^{32}P]ATP (0.5–2 Ci/mmole) was prepared as described by Glynn and Chappell [21]. Unlabelled ATP was purchased from Reanal (Budapest). Cyclic AMP, casein and EGTA were obtained from CalBiochem (Los Angeles, CA),

3-MC and EDTA from Serva (Heidelberg, FDR). [^{3}H]UTP (12 Ci/mmole) was obtained from the Radiochemical Centre (Amersham, U.K.). Unlabelled nucleoside triphosphates and β-mercaptoethanol were purchased from Reanal (Budapest), creatine phosphate, creatine phosphokinase, dithiothreitol, α-amanitin and calf thymus DNA from CalBiochem (Los Angeles, CA). All other chemicals were of reagent grade.

Animals. Groups of 6 to 8 ten-day-old Wistar rats weighing 20–30 g received a single intraperitoneal dose of 3-MC (100 mg/kg body wt) at different times before sacrifice. The control animals received the solvent only (10 ml of corn oil/kg body wt).

Isolation of nuclear fractions. Rat liver nuclei were isolated by the method of Chauveau *et al.* [22]. The purified nuclear pellet was prepared for the sonication procedure of Higashinakagawa *et al.* [23] in order to separate the nucleoli from the nucleoplasmic fraction. The nucleolar sediment was suspended in a buffer containing 50 mM Tris–HCl, pH 7.9, 0.25 mM EDTA, 5 mM β-mercaptoethanol, 20% (v/v) glycerol and its purity was verified by phase contrast microscopic examination.

Protein kinase assay. The standard incubation medium contained in a total volume of 0.2 ml (pH 6.0): 50 mM glycerolphosphate, 20 mM NaF, 0.3 mM EGTA, 0.2 mM EDTA, 2 mM theophylline, 6 mg/ml of casein, 10 mM $MgCl_2$, 0.5 mM unlabelled ATP, 0.1 mM ATP with γ-^{32}P label and—if necessary—5 μM cyclic AMP. The reactions were initiated by the addition of 0.1 ml of nucleolar and nucleoplasmic fractions corresponding to about 50 and 100 μg protein, respectively. The incubations were performed for 30 min at 37°. Reactions were terminated by pipetting 0.15 ml of reaction mixture onto squares (4 × 4 cm) of Whatman 3MM filter paper. The papers were washed four times in cold 5% trichloroacetic acid, two times in methanol, rinsed with ether and dried. The radioactivity was measured in vials filled with a PPO–POPOP–toluene cocktail by means of a Beckman LS 230 counter.

One unit of enzyme activity is defined as the amount of enzyme which transfers 1 pmole of ^{32}P phosphate from [γ-^{32}P]-ATP to protein during 1 min in the standard assay mixture.

RNA polymerase assay. The activity of the engaged form of the nucleolar RNA polymerase (A) was determined *in vitro* with a standard reaction mixture of 0.15 ml total volume containing 50 mM Tris–HCl, pH 7.9, 6 mM $MgCl_2$, 40 mM $(NH_4)_2SO_4$, 5 mM dithiothreitol, 0.67 mM each of ATP, GTP and CTP, 6.7 μM unlabelled UTP, 0.5 μCi [^{3}H]-UTP, 10 mM creatine phosphate, 5 μg creatine phosphokinase. The engaged form of the extranucleolar RNA polymerase (B) was assayed in a total volume of 0.15 ml containing 50 mM Tris–HCl, pH 7.9, 2 mM $MnCl_2$, 100 mM $(NH_4)_2SO_4$, 5 mM dithiothreitol, 0.67 mM each of ATP, GTP and CTP, 6.7 μM unlabelled UTP, 10 mM creatine phosphate, 5 μg creatine phosphokinase. The reactions were initiated by the addition of 0.1 ml of nucleolar and nucleoplasmic fractions corresponding to about 50 and 100 μg of protein, respectively, and incubated at 37° for 15–30 min. At the end of the incubation, 0.12 ml of reaction mixture was pipetted onto squares (4 × 4 cm) of Whatman 3MM paper, which were washed and measured in the same way as described above for protein kinase assay.

Aliquots of nucleolar as well as nucleoplasmic suspension were incubated under identical conditions, but 20 μg of calf thymus DNA were added to the reaction mixtures. Under these conditions the activities of the free and engaged RNA polymerase molecules together can be determined. The activity of the free form was calculated by subtracting the radioactivity incorporated in the absence of DNA from the value of incorporation obtained in the presence of the exogenous DNA template.

One unit of activity is defined as the amount of enzyme which catalyzes the incorporation of 1 pmole of UMP into RNA per minute.

Determination of protein. Protein concentration of nucleolar and nucleoplasmic fractions was assayed with the technique of Lowry *et al.* [24] using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

In order to investigate the action of 3-MC on rat liver nuclear protein kinases and different forms of RNA polymerases we prepared nucleolar and nucleoplasmic fractions from control animals as well as from animals injected with a single dose of 3-MC at different times before being killed. All these fractions were tested for phosphotransferase and RNA polymerase activities. The results of the protein kinase measurement are illustrated in Fig. 1. It can be seen that the administration of 3-MC is followed by an increase in the activities of both the nucleolar and nucleoplasmic protein kinases. In the case of *nucleolar* fractions the maximal stimulation occurs between 24 and 48 hr after 3-MC treatment (190%, $P < 0.01$, and 170%, $P < 0.02$, of the control activity, respectively). After 48 hr exposure to 3-MC there is a decrease of protein kinase activity, but control values are not reached until 96 hr.

The elevation of the *nucleoplasmic* protein kinase activity also takes place after a lag period, reaching the first maximum at 24 hr of exposure to 3-MC, but

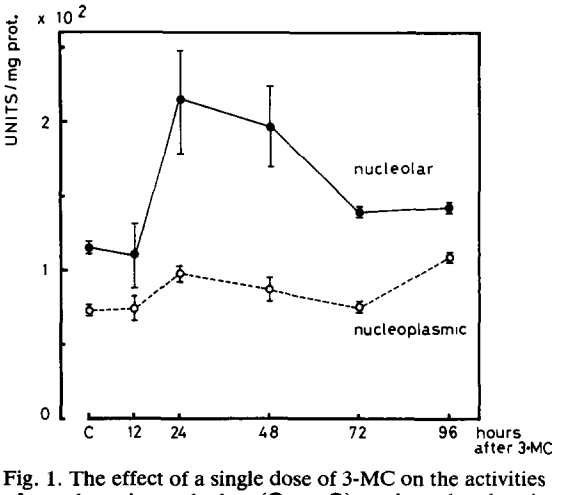

Fig. 1. The effect of a single dose of 3-MC on the activities of rat hepatic nucleolar (●——●) and nucleoplasmic (○---○) protein kinases. The results represent the means of three independent experiments ± S.E. (C = Controls).

the extent of stimulation, while significant, is not as marked as that of the nucleolar fraction (133% of the control value, $P < 0.01$). After a transient decrease up to control activity there is a second elevation of the activities of protein kinases in the extranucleolar fractions 96 hr after the injection of 3-MC.

The action of cyclic AMP upon nuclear protein kinase was thoroughly investigated according to the same time schedule of 3-MC pretreatment. But cyclic AMP had no noticeable effect on protein kinases, neither in the nucleolar, nor in the nucleoplasmic fractions of control and 3-MC-pretreated animals. The lack of the stimulating effect of cyclic AMP on nuclear protein kinase activities appears somewhat surprising. But there could be simple explanations for that: Firstly, crude nuclear subfractions used in our experiments contain multiple protein kinases, some of them being cyclic AMP-dependent, but others are cyclic AMP-independent or even inhibited by this cyclic nucleotide [4]. Secondly, the stimulatory effect of cyclic AMP on protein kinases seems to be considerable only with substrates of polycationic character (protamine, histone). Casein used as phosphate acceptor in our experiments has no such effect [25, 26].

Concerning the significance of RNA synthesis we tested in some experimental series the action of α-amanitin on the RNA polymerase activity of nucleoplasmic and nucleolar fractions (data not shown), as it was found by Seifart *et al.* [27] that RNA polymerase B is nearly completely inhibited *in vitro* by 3 μg/ml of α-amanitin while the activity of RNA polymerase A is not affected by this concentration. In our experiments the RNA polymerase activity of nucleoplasmic fractions was inhibited in a range of nearly to 90%, while the enzyme activity of nucleolar fractions in the presence of the same concentration of the toxin was always higher than 90% of the control values. These results indicate that the separation of the nucleolar and nucleoplasmic parts of nuclei was successful and that cross-contamination between the two fractions is negligible. The similar inhibition of the free and engaged form of RNA polymerases by α-amanitin supports the idea of Yu [14, 15] about the structural identity of the different functional states of these enzymes.

The results on RNA polymerase activity in liver *nucleolar* fractions of control and 3-MC treated animals are illustrated in Fig. 2. After a lag period both the free and the engaged form show a great stimulation following 3-MC treatment. The maximal activity level of the free form is obtained 24 hr after the administration of 3-MC (235% of the control value, $P < 0.001$). After this time only a slow decrease in the activity of the free enzyme can be observed, but the activity remains higher in comparison to that of the controls at least up to 96 hr (180%, $P < 0.01$). The changes observed in the activity of the engaged fraction of RNA polymerase A after 3-MC injection are quite similar except that there is a constant, slowly increasing tendency of enzyme activity after 24 hr reaching the maximum between 72 and 96 hr after 3-MC treatment (240%, $P < 0.001$). As a consequence of these changes the total activity of RNA polymerase A shows an elevation 24 hr after the administration of 3-MC (220%, $P < 0.001$) and remains at this high level at least in the first four days after 3-MC administration.

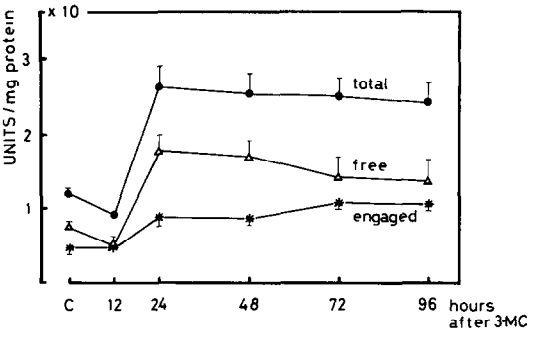

Fig. 2. The effect of a single dose of 3-MC on the activity of rat hepatic nucleolar RNA polymerase. ★——★ engaged enzyme activity; △——△ free enzyme activity; ●——● total (free + engaged) enzyme activity. All values are means of four independent experiments ± S.E.

The behaviour of the *extranucleolar* RNA polymerase (B) under the influence of 3-MC is different from that of the nucleolar RNA polymerase (Fig. 3). 24 Hours after 3-MC-administration the activity of the engaged form shows a significant enhancement (160% of the control values, $P < 0.01$), whereas the free form is strongly depressed at this time (20% of the control activity, $P < 0.001$). Beginning two days after 3-MC administration the activity of the engaged form of RNA polymerase B is gradually decreasing, reaching the control value at 96 hr. In the meantime the activity of the free enzyme increases rapidly and the activity is significantly higher than that of the control fraction on the fourth day (240%, $P < 0.001$). In consequence of this different behaviour of the free and engaged forms, the total activity of extranucleolar RNA polymerase shows only a small elevation during the first four days after 3-MC injection (at 96 hr the activity is 55% higher than that of the control sample, $P < 0.001$).

In the total activity of RNA polymerases the stimulating effect of 3-MC was apparently more pronounced in the nucleolar RNA synthesis than in the nucleoplasmic one. This finding is in good agreement with the results of Bresnick *et al.* [9]. We observed similar differences between the activation of nucleolar and nucleoplasmic protein kinases by 3-MC.

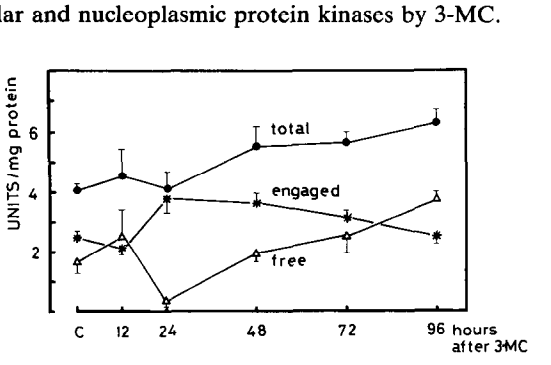

Fig. 3. The effect of a single dose of 3-MC on the activity of rat hepatic extranucleolar RNA polymerase. ★——★ engaged enzyme activity; △——△ free enzyme activity; ●——● total (free + engaged) enzyme activity. Results are expressed as means of four independent experiments ± S.E.

Phosphorylation of nuclear proteins plays a paramount role in a wide variety of processes which lead to alterations in the regulation of gene expression in eukaryotic cells. Cell differentiation, tissue proliferation, the action of steroid as well as peptide hormones are accompanied—and probably mediated—by the stimulation of certain nuclear protein kinases and by the phosphorylation of chromatin proteins (for review see [4, 5, 28]). Even cancer is considered by several authors to be a disease of cellular gene regulation [28, 29] connected with marked alterations in protein kinase activities and phosphorylation patterns of nuclear proteins.

There exists also convincing evidence of the involvement of nuclear protein kinases in the regulation of enzyme induction. In different kinds of cells the induction of various specific enzymes was mediated by cyclic AMP-dependent protein kinases [30], (for review see [7]). Our results do not allow the differentiation between cyclic AMP-dependent and cyclic AMP-independent protein kinase actions connected with the inducing effect of 3-MC. However, we suggest that the stimulation of nuclear protein kinases may be involved in the induction process caused by 3-MC.

It appears to be highly informative to investigate cells that had been induced *in vivo* by a chemical inducer such as 3-MC. As a sign of marked response to chemical stimulation by PAHs we applied the very sensitive model of AHH induction in young rats. The administered dose of 100 mg 3-MC per kg of body weight comes up to a saturation level of the inducing compound and after 3 to 4 days the maximum of hepatic AHH acitivities is reached [13]. At least a connection in time between the activation of nuclear protein kinases and the induction of AHH activity is confirmed in our experiments. According to the present knowledge phosphorylation of histones and especially of non-histone chromatin proteins mediated by protein kinases may act primarily on the template activity and on RNA polymerases, too, initiating the synthesis of new RNA chains (reviewed in [7]).

Bresnick [10] and Madix and Bresnick [11] concluded that the most important event in rat liver cell nuclei evoked by 3-MC is an alteration in the template capacity of chromatin. The enhancement of the activity of the engaged forms of RNA polymerases A and B appears to be in good agreement with this concept. However, the elevation of the template activity of chromatin cannot account for the marked stimulation of the free forms of RNA polymerases in our experiments. It is highly probable that 3-MC stimulates the synthesis of RNA polymerase molecules. Another possibility is that the enzyme molecules are activated by phosphorylation. It was found by several investigators that the phosphorylation of certain subunits of eukaryotic RNA polymerases led to an increased catalytic activity of these enzymes [31–36]. Our findings support the view that phosphorylation of certain nuclear proteins may be involved in the stimulation of RNA polymerase activity and perhaps in the redistribution process of free and engaged RNA polymerase molecules in 3-MC induction. New synthesis or activation of free enzyme molecules occurs early after 3-MC administration followed by a conversion into the template-engaged enzyme form.

We suggest that this interconversion process between free and engaged enzyme forms represents an important finding of these experiments. Especially within the nucleoplasmic RNA polymerase species this interconversion process, favouring the actual chromatin-engaged form, seems to be the decisive phenomenon in 3-MC-induction. On the contrary the marked increase both in the free and engaged nucleolar RNA polymerases A leads to a distinct higher total level of enzyme activity.

Furthermore, the earliest nuclear ultrastructural alteration in liver cells induced by a single 3-MC injection seems to be the hypertrophy of nucleoli [37].

All these facts strongly suggest that the primary nuclear target of 3-MC is the nucleolus and the synthesis of preribosomal RNA. Several observations about the binding of PAHs to cellular macromolecules raise some possibilities for suggestions. PAHs bind not only to nucleic acids, but also to nuclear proteins [38, 39]. The binding proteins in the cytosol appear to be functionally similar to steroid receptor proteins [40–44]. The binding of PAHs to nuclear histone and non-histone proteins is well documented [29]. Recent studies of Moses *et al.* [45] and Spelsberg *et al.* [46] indicated that a large part of labelled 3-MC administered *in vivo* is bound to a transcriptionally active subfraction of chromatin. In experiments using the fractionation method of Webster *et al.* [47] for rat liver nuclei, we* found a significant stimulation of protein kinase and RNA polymerase activities in the same chromatin fraction which is most capable to bind labelled 3-MC. We assume that 3-MC molecules or 3-MC-receptor protein complexes may bind to transcriptionally active parts of chromatin and thus enhance the activity and/or amount of protein kinases and RNA polymerases.

We tried to receive experimental data on the early stages of epigenetic activation in liver cells after administration of 3-MC, which is characterized by changes of protein kinases and RNA polymerases and finally by the induction of hepatic AHH activity. Our results support the hypothesis that nuclear protein kinases and RNA polymerases are directly involved in the inducing action of 3-MC, consequently emphasizing the role of epigenetic regulation.

Acknowledgements—We would like to thank Dr. Judit Gaàl for cooperation in some experiments on protein kinase, and Dr. L. Komáromy for valuable advice and criticism. The skilled technical assistance of Mrs. Melinda Kiss is greatly appreciated.

* J. Szeberényi *et al.* (unpublished results).

REFERENCES

1. A. H. Conney, E. C. Miller and J. A. Miller, *J. biol. Chem.* **228**, 753 (1957).
2. D. W. Nebert and H. V. Gelboin, *J. biol. Chem.* **243**, 6242 (1968).
3. D. W. Nebert and H. V. Gelboin, *Archs Biochem. Biophys.* **134**, 76 (1969).
4. L. J. Kleinsmith, *J. Cell Physiol.* **85**, 459 (1975).

5. G. S. Stein, T. C. Spelsberg and L. J. Kleinsmith, *Science* **183**, 817 (1974).
6. R. A. Jungmann and D. H. Russell, *Life Sci.* **20**, 1787 (1977).
7. R. A. Jungmann and E. G. Kranias, *Int. J. Biochem.* **8**, 819 (1977).
8. P. Greengard, *Science* **199**, 146 (1978).
9. E. Bresnick, M. E. Synerkohn and G. T. Tizard, *Molec. Pharmac.* **4**, 218 (1968).
10. E. Bresnick, *Molec. Pharmac.* **2**, 406 (1966).
11. J. C. Madix and E. Bresnick, *Biochem. biophys. Res. Commun.* **28**, 445 (1967).
12. H. V. Gelboin and J. P. Whitlock, Jr., in *Symposia Med. Hoechst* 14. *The Induction of Drug Metabolism* (Eds. R. W. Estabrook and E. Lindenlaub), p. 67. F. K. Schattauer, Stuttgart (1979).
13. U. Kleeberg, A. Barth, J. Roth and W. Klinger, *Acta biol. Med. Germ.* **34**, 1701 (1975).
14. F.-L. Yu, *Nature* **251**, 344 (1974).
15. F.-L. Yu, *Biochim. biophys. Acta* **395**, 329 (1975).
16. F.-L. Yu, *Biochem. biophys. Res. Commun.* **64**, 1107 (1975).
17. T. Matsui, T. Onishi and M. Muramatsu, *Eur. J. Biochem.* **71**, 361 (1976).
18. C. C. Hentschel and J. R. Tata, *Devl Biol.* **57**, 293 (1977).
19. A. P. N. Majumdar, *Experientia* **34**, 1258 (1978).
20. T. B. Leonard and S. T. Jacob, *Biochemistry* **16**, 4538 (1977).
21. I. M. Glynn and J. B. Chappell, *Biochem. J.* **90**, 147 (1964).
22. J. Chauveau, Y. Moule and C. Roiller, *Expl Cell Res.* **11**, 317 (1956).
23. I. Higashinakagawa, M. Muramatsu and H. Sugano, *Expl Cell Res.* **71**, 65 (1972).
24. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
25. M. Shimoyama, M. Kawai, S. Yamamoto, H. Imai, A. Kitamura, S. Nasu, K. Dohi and I. Ueda, *Life Sci.* **17**, 1445 (1975).
26. A. D. Deana, F. Meggio and L. A. Pinna, *Biochem. J.* **179**, 693 (1979).
27. K. H. Seifart, B. J. Benecke and P. P. Juhasz, *Archs Biochem. Biophys.* **151**, 519 (1972).
28. G. S. Stein, J. L. Stein and J. A. Thomson, *Cancer Res.* **38**, 1181 (1978).
29. T. H. Zytkovicz, H. L. Moses and T. C. Spelsberg, in *The Cell Nucleus*, Vol. VII (Ed. H. Busch), p. 479. Academic Press, New York (1979).
30. J. Wimalasena and W. D. Wicks, *Molec. Pharmac.* **16**, 449 (1979).
31. C. J. Martelo and J. Hirsch, *Biochem. biophys. Res. Commun.* **58**, 1008 (1974).
32. G. I. Bell, P. Valenzuela and W. J. Rutter, *Nature* **261**, 429 (1976).
33. M. E. Dahmus, *Biochemistry* **15**, 1821 (1976).
34. R. A. Jungmann, P. C. Hiestand and J. S. Schweppe, *J. biol. Chem.* **249**, 5444 (1974).
35. E. G. Kranias, J. S. Schweppe and R. A. Jungmann, *J. biol. Chem.* **252**, 6750 (1977).
36. E. G. Kranias and R. A. Jungmann, *Biochim. biophys. Acta* **517**, 439 (1978).
37. A. Tigiy, J. Szeberényi, L. Komáromy, U. Kleeberg and J. Gaál, *Acta Biol. Acad. Sci. Hung.* **31**, 329 (1980).
38. J. M. Pezzuto, M. A. Lea and C. S. Yang, *Cancer Res.* **36**, 3647 (1976).
39. K. Hemminki, I. Blaszek and M. Vanhkonen, *Sci. Proc. Am. Ass. Cancer Res.*, Abstr. 170 (1978).
40. M. S. Dickens and S. Sorof, *Biochem. biophys. Res. Commun.* **79**, 713 (1977).
41. A. M. Sariff, K. L. McCarthy, S. Nesnow and C. Heidelberger, *Cancer Res.* **38**, 1438 (1978).
42. T. M. Guenthner and D. W. Nebert, *J. biol. Chem.* **252**, 8981 (1977).
43. J. M. B. Carlstedt-Duke, G. Elfström, B. Högberg and J.-A. Gustafsson, *Cancer Res.* **39**, 4653 (1979).
44. D. W. Nebert, *Pharmac. Ther.* **6**, 395 (1979).
45. H. L. Moses, R. A. Webster, G. D. Martin and T. C. Spelsberg, *Cancer Res.* **36**, 2905 (1976).
46. T. C. Spelsberg, T. H. Zytkovicz and H. L. Moses, *Cancer Res.* **37**, 1490 (1977).
47. R. A. Webster, H. L. Moses and T. C. Spelsberg, *Cancer Res.* **36**, 2896 (1976).