Activation of Genetic Transcription in Rat Liver Chromatin by 3-Methylcholanthrene

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SUMMARY

Previous studies had indicated that the administration of 3-methylcholanthrene to rats is attended by an increase in the "aggregate" RNA polymerase activity in rat liver. Chromatin has been isolated from the livers of both control and 3-methylcholanthrene-treated rats. The experimental chromatin did not differ from control material in RNA:DNA and histone to DNA ratios, ultraviolet absorption, or transition temperature. The efficacy of the chromatin as a template was determined in the *Micrococcus lysodeikticus* RNA polymerase system. The liver chromatin from 3-methylcholanthrene-treated rats proved to be a better template. Adrenalectomy did not affect this finding. The nearest neighbor frequency of the RNA elaborated on the chromatin template was also determined. The RNA product made in the presence of chromatin isolated from 3-methylcholanthrene-treated rats differed substantially from that made in the presence of control chromatin or rat liver DNA itself. These studies provide further evidence that the administration of the polycyclic hydrocarbon leads to an activation of a region(s) on the genome.

INTRODUCTION

In multicellular organisms, a large portion of the genome is not present in a readily transcribable form but exists in a "masked" condition. For example, Paul and Gilmour (1) have reported that only 5–10% of the chromatin from mammalian organs is available for transcription. Presumably, the restriction of template activity is mediated through the association of proteins (histones and non-histone proteins) with the DNA in the deoxyribonucleoprotein structure. The exact means by which specific loci are made available for the transcription process forms a part of a sophisticated control mechanism about which little is known. Results obtained recently in this laboratory bear upon this mechanism of control of genetic transcription.

Several years ago, we reported (2) the "aggregate" RNA polymerase activity of liver nuclei to be significantly elevated after the administration of 3-methylcholanthrene to rats. This observation has been confirmed by Gelboin *et al.* (3). For a number of reasons, the enhancement in "aggregate" enzyme activity was viewed as the end effect of an activation of the genome rather than an elaboration of additional enzyme molecules (2). Preliminary studies (4) were in concert with this hypothesis, since chromatin isolated from the livers of 3-methylcholanthrene-treated rats proved to be a better template in the synthesis of RNA by purified bacterial RNA polymerase with ^{14}C-uridine triphosphate as precursor.

The activational effect has now been

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studied in greater detail. The results presented here provide further evidence of the increased template efficacy for the synthesis of RNA of chromatin isolated from the livers of 3-methylcholanthrene-treated rats. Furthermore, the data establish conclusively that the RNA product elaborated in the presence of this chromatin differs substantially in composition from that made in the presence of either control liver chromatin or liver DNA. These results suggest that 3-methylcholanthrene directly or indirectly produces an activation of the genome.

METHODS

Male rats, 50–60 g, purchased from Cheek-Jones Company (Houston), were treated by intraperitoneal injection of either 0.5 ml of corn oil (controls) or 0.5 ml of corn oil containing 1 mg of 3-methylcholanthrene, 20 mg/kg of body weight. In several experiments, rats were employed that had been adrenalectomized 3–5 days previously. The latter received 1% NaCl in their drinking water.

Isolation of liver chromatin and DNA. The control and experimental rats were lightly anesthetized with ether, and the livers were perfused *in situ* via the portal system with cold 0.25 M sucrose. The livers were removed and washed in cold 0.25 M sucrose. The remaining procedure was conducted in a cold room at 4°. The livers from three rats were passed through a cooled Harvard mincer, and the minced tissue was homogenized in 12 volumes of 2.3 M sucrose–3 mM $CaCl_2$. The liver nuclei were isolated and purified by the Chauveau procedure (5). Chromatin was isolated from the purified nuclei by the method of either Marushige and Bonner (6) or Dingman and Sporn (7). The final chromatin preparation was dialyzed overnight at 4° against 0.01 M Tris buffer, pH 8.0.

TABLE 1

Composition of liver chromatin

Chromatin was extracted from liver nuclei of animals that had received either corn oil (control) or 3-methylcholanthrene in corn oil 12 hr previously. The chromatin was isolated by the method of either Dingman and Sporn (7) (Experiment I) or Marushige and Bonner (6) (Experiment II). The values represent the averages of 6–10 determinations with individual groups of rats. A single determination was performed on the chromatin extracted from the nuclei obtained from three livers.

Source	RNA: DNA	Histone: DNA
I. Control	0.01	0.8
3-Methylcholanthrene, 12 hr	0.02	0.9
II. Control	0.01	0.9
3-Methylcholanthrene, 12 hr	0.01	1.0

The compositions of the chromatin prepared by these two methods are compared in Table 1. The RNA:DNA and histone to DNA ratios of the chromatin isolated by both methods were similar.[2]

The ultraviolet absorption measurements on the chromatins from the livers of corn oil- and 3-methylcholanthrene-treated rats showed $A_{250}:A_{260}$ and $A_{280}:A_{260}$ ratios of 0.81 and 0.61, respectively.

DNA was prepared from rat liver by the procedure of Savitsky and Stand (8).

Assay of template activity. The DNA and chromatin samples were assayed for template activity with an RNA polymerase purified from *Micrococcus lysodeikticus*, which was either obtained from Miles Laboratories or purified from the bacterial organism by the method of Nakamoto *et al.* (9). Unless otherwise stated, the assay mixture contained Tris buffer, pH 7.5, 10 μmoles, $MnCl_2$; 1 μmole; spermidine phosphate, 0.05 μmole; ATP, GTP, CTP, and UTP, 0.1 μmole each, one of which was present as nucleoside triphosphate-α-^{32}P (Schwarz BioResearch), 2.5 μC; RNA polymerase, 5–10 units (9); and DNA or chromatin, 0.1–10 μg of DNA, in a total volume of 0.25 ml. The amount of incorporation of nucleoside monophosphate-^{32}P

[2] Although the values given in Table 1 were obtained from rats treated with 3-methylcholanthrene 12 hr prior to death, similar results were obtained at times up to 36 hr postinjection. It is important to emphasize that the similarity in the RNA:DNA and histone to DNA ratios of the control and experimental chromatin does not preclude any subtle changes in these values that might have resulted after administration of the polycyclic hydrocarbon.

into RNA after a 10–15-min incubation at 37° was determined by a disc method described previously (4).

Nearest neighbor frequency analysis. After incubation at 37° for 10 min, the tubes containing the constituents of the RNA polymerase system were immersed in an ice-bath and 3 mg of yeast RNA were added as carrier. The RNA was precipitated upon the addition of 2.5 ml of cold 5% trichloracetic acid–1% sodium pyrophosphate, and the precipitate was collected by centrifugation. The RNA precipitate was washed four times with 2 ml of cold 5% trichloracetic acid to remove traces of the nucleotide precursor and was finally dissolved in 0.5 ml of 0.3 N KOH. The alkaline hydrolysis was conducted at 37° overnight. To the hydrolysate, phenol red was added as an internal indicator, and the nucleotides were neutralized with 4 N $HClO_4$. The neutralized material was maintained at 4° for several hours to ensure the complete precipitation of $KClO_4$. To 0.3 ml of neutralized extract, 0.2 ml of distilled water and 0.5 ml of 0.1 N HCl were added. The nucleotide composition was determined on Dowex 50 according to the method of Katz and Comb (10); the ^{32}P content of the respective nucleotides was measured in a liquid scintillation counter using Bray's phosphor mixture (11).

Analytical methods. The DNA was determined by the diphenylamine method (12); RNA, by the orcinol procedure (13); and protein, by the method of Lowry *et al.* (14). The "histone" composition of the chromatin was estimated after a 30-min extraction at 4° with 0.4 N HCl and precipitation of the basic proteins from the HCl extract upon the addition of trichloracetic acid to a concentration of 20%. The final precipitate was dissolved in 0.1 N NaOH, and the protein content was determined according to Lowry *et al.* (14) using purified rat liver histones as standard. The latter was a gift from Dr. W. C. Starbuck. Temperature transition studies were performed on the DNA and chromatin samples in 0.015 M NaCl–0.0015 M sodium citrate, pH 7.0, using a Gilford spectrophotometer with recorder and temperature probe.

RESULTS

The hyperchromic effect as a function of increasing temperature is shown in Fig. 1 for rat liver DNA, for control and experimental chromatins. The transition temperature (T_m) for rat liver DNA in 0.015 M NaCl–0.0015 M sodium citrate, pH 7.0, was 69.2°; the T_m for chromatin was 81.5°. The slopes of the curves were less with the chromatin than with DNA. Furthermore, in every case, a small inflection was noted in the hyperchromicity curves with chromatin. However, no alteration either in the shape of the curve or in the magnitude of the T_m was apparent when control and experimental chromatins were compared. These experiments indicated that the administration of 3-methylcholanthrene was not attended by any gross alteration in the extent of hyperchromicity of the liver chromatin.

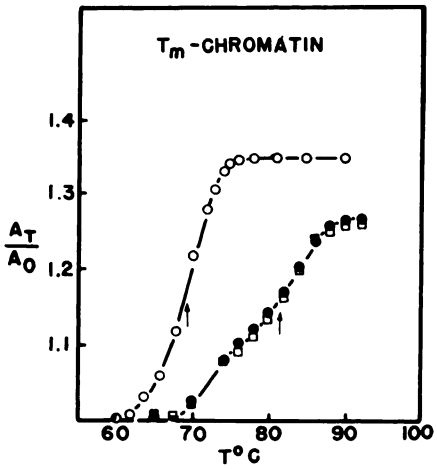

FIG. 1. *Transition temperature of rat liver chromatin*

A_T, absorbance at specific temperature ($T°C$); A_0, absorbance at room temperature, i.e., 25°; T_m, transition temperature (indicated by the arrows); O——O, rat liver DNA; ●——●, chromatin of control liver; □- - -□, chromatin of liver of 3-methylcholanthrene-treated rats, 12 hr after injection.

The efficacy of control and experimental chromatin as templates in a bacterial RNA polymerase system is shown in Fig. 2. These results indicate the enhanced ability of chromatin isolated from the livers of 3-

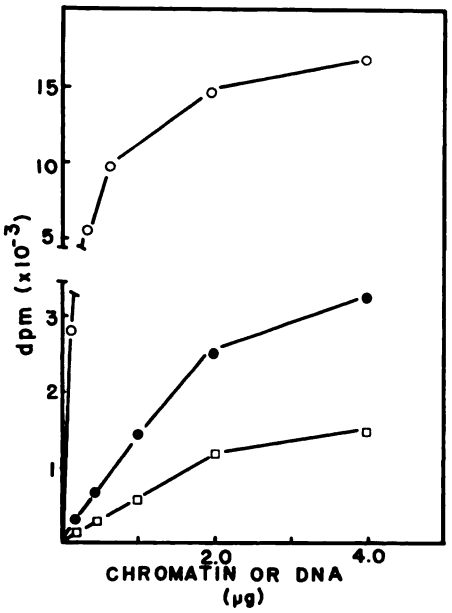

FIG. 2. *Efficacy of chromatin as template in the RNA polymerase assay*

Rat liver DNA (○——○), control liver chromatin (□——□), and liver chromatin from 3-methylcholanthrene-treated rats, 15 hr after administration (●——●), were employed as templates in the *Micrococcus* RNA polymerase system (10 units of enzyme); the chromatin was isolated by the method of Dingman and Sporn (7). ATP-α-^{32}P (280 μC/μmole, 0.5 μC/assay) was the precursor. The time of incubation at 37° was 10 min. The ordinate represents the disintegrations per minute (× 10^{-3}) of AMP incorporated into RNA, while the abscissa represents the amount of DNA or chromatin per assay system as micrograms of DNA.

methylcholanthrene-treated rats to serve as template for RNA synthesis. The results of Fig. 2 also show the relative effectiveness as templates of chromatin as compared to rat liver DNA. The comparative template abilities of chromatin and rat liver DNA are indicated in Table 2. Omission of either Mn^{2+} or the three nucleoside triphosphates abolished enzyme activity. Control liver chromatin exhibited one-twelfth the activity of purified rat liver DNA in the synthesis of RNA catalyzed by RNA polymerase. Chromatin from 3-methylcholanthrene-treated rats was one-fifth as active as rat liver DNA; this chromatin was at least twice as active in the synthesis of RNA as control chromatin. Previous data (4) had indicated that the increased template efficacy was apparent as early as 6 hr.

To eliminate any contribution of corticosteroids to the activation phenomenon, adrenalectomized animals were chosen for the preparation of liver chromatin. The results presented in Fig. 3 show that the observed activation was not due to enhanced elaboration of corticosteroids.

The enhanced template activity of the chromatin from the livers of 3-methylcholanthrene-treated rats could be the result of generalized increase in the rate of transcription of the loci which are already being "read," the exposure of additional genetic sites, or the transcription of select loci. If the first of these possibilities is true,

TABLE 2

Template activity of chromatin in the RNA polymerase system

Chromatin was extracted from liver nuclei, by the method of Dingman and Sporn (7), from rats that had received either corn oil (control) or 3-methylcholanthrene 12 hr previously. Rat liver DNA was isolated as described in the text. The template efficacy was determined using the assay system presented in METHODS, with ATP-α-^{32}P (280 μC/μmole, 0.5 μC) as the precursor.

Assay conditions	AMP incorporated with the following templates:		
	3-Methylcholanthrene chromatin	Control chromatin	DNA
		μμmoles/μg DNA	
Complete	14.8	5.6	65.6
GTP, CTP, and UTP omitted	<0.4	<0.4	<0.4
Mn^{2+} omitted	<0.4	<0.4	<0.4

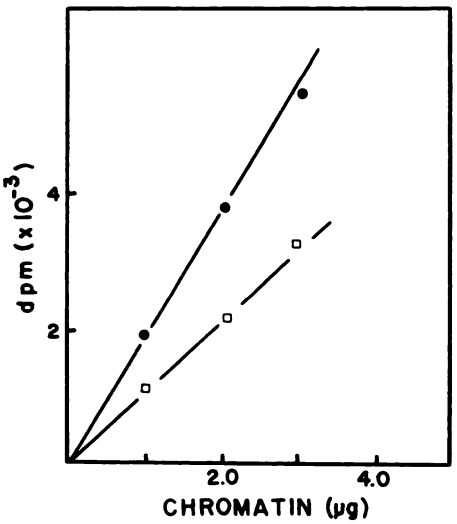

FIG. 3. *Template efficacy of liver chromatin from adrenalectomized rats*

The labeled precursor was GTP-α-^{32}P (40 μC/μmole, 4.0 μC). The chromatin was isolated from the livers of adrenalectomized rats (3–5 days previously) (□——□) and from the livers of 3-methylcholanthrene-treated, adrenalectomized rats, 15 hr after administration (●——●), by the method of Dingman and Sporn (7). The time of incubation at 37° was 10 min. The chromatin is represented in the abscissa in terms of micrograms of DNA.

the RNA product should possess the identical base composition when either control or experimental chromatin acts as template. If 3-methylcholanthrene either directly or indirectly affects the appearance of additional loci for transcription or influences the reading of only certain loci, the gene product should possess an altered base composition. The hypothesis was tested by determining the nearest neighbor frequency of the RNA product using α-^{32}P-labeled nucleoside triphosphates as precursors. These results are presented in Tables 3–5.

With UTP-α-^{32}P as precursor (Table 3) and experimental chromatin as template, increases in the nearest neighbor analysis were apparent with GpU and CpU, while decreases occurred with UpU and ApU. The nearest neighbor frequency with the chromatins as templates differed markedly from that obtained with rat liver DNA as template.

With GTP-α-^{32}P as the precursor (Table 4) and experimental chromatin as template, small increases occurred in UpG and in ApG, as in GpG. The composition of the product made in the presence of DNA was similar whether the concentration of the labeled nucleotide was 2.8×10^{-5} or 4.0×10^{-4} M. The composition of the RNA product elaborated on the chromatin template was similar when the assay was conducted for either 10 or 20 min.

The nearest neighbor analysis with CTP-α-^{32}P as precursor is presented in Table 5. An increase in UpC was noted with the ex-

TABLE 3

Nearest neighbor analysis of RNA elaborated on chromatin template

RNA polymerase (10 units) was incubated for 10 min under the assay conditions described in METHODS. The labeled precursor was UTP-α-^{32}P (80–90 μC/μmole), 0.1 μmole, and the templates were either rat liver DNA, 4 μg; control liver chromatin, 2–4 μg of DNA; or chromatin obtained from the livers of 3-methylcholanthrene-treated rats 15 hr previously, 2–4 μg of DNA. The radioactivity (counts per minute) was corrected for activity in the absence of template. The nearest neighbor analysis is reported as a percentage of the total activity recovered in the form of the specific nucleotide.

Template	UpU	GpU	CpU	ApU
	% cpm	% cpm	% cpm	% cpm
DNA (2)[a]	32.6[b]	21.0[b]	26.8[b]	18.6[b]
Control chromatin (5)	42.4 ± 0.7[c,d]	19.7 ± 0.9[b]	18.9 ± 0.6[e]	19.0 ± 0.6[d]
3-Methylcholanthrene chromatin (5)	38.8 ± 0.7[d]	24.8 ± 0.6[b]	22.9 ± 0.9[e]	14.1 ± 0.7[d]

[a] Numbers in parentheses denote number of determinations.
[b] $p = 0.01$ (Student's t-test).
[c] Mean ± standard error.
[d] $p = 0.05$ (Student's t-test).
[e] $p = 0.02$ (Student's t-test).

TABLE 4

Nearest neighbor analysis of RNA elaborated on chromatin template

The assay conditions were similar to those described in the legend to Table 3, but the radioactive precursor was GTP-α-^{32}P (50 μC/μmole), 5.0 μC, except as noted below.

Template	UpG	GpG	CpG	ApG
	% cpm	*% cpm*	*% cpm*	*% cpm*
DNA	32.5[a]	26.9[a]	4.0[a]	36.6[a]
DNA (3)[b]	32.4 ± 0.9[c]	29.2 ± 0.5	4.3 ± 0.1	37.3 ± 1.0
Chromatin				
Control (3)	33.9 ± 1.0	33.2 ± 1.0	10.0 ± 0.4	22.2 ± 0.4
3-Methylcholanthrene (3)	35.2 ± 0.9	29.9 ± 0.8	11.0 ± 0.5	24.0 ± 0.6
3-Methylcholanthrene, 20-min incubation	36.0	29.1	10.5	24.4

[a] In these experiments, the specific activity of the GTP-α-^{32}P added was 690 μC/μmole.

[b] Numbers in parentheses denote number of determinations.

[c] Mean ± standard error.

perimental chromatin as template; decreases in CpC and ApC were also apparent.

These data clearly indicate that the DNA transcribed from the chromatin isolated from the livers of 3-methylcholanthrene-treated rats has a different composition from that manufactured in the presence of control chromatin or rat liver DNA.

TABLE 5

Nearest neighbor analysis of RNA elaborated on chromatin template

The assay conditions were similar to those described in the legend to Table 3, but the radioactive precursor was CTP-α-^{32}P (90 μC/μmole), 0.1 μmole.

Template	UpC	GpC	CpC	ApC
	% cpm	*% cpm*	*% cpm*	*% cpm*
DNA (3)[a]	34.0 ± 0.6[b]	23.6 ± 0.5	21.8 ± 0.5	20.6 ± 0.4
Control chromatin (3)	35.5 ± 0.8	21.5 ± 0.5	22.2 ± 0.5	22.8 ± 0.5
3-Methylcholanthrene chromatin (3)	40.5 ± 0.9	21.7 ± 0.6	18.4 ± 0.4	19.4 ± 0.4

[a] Numbers in parentheses denote number of determinations.

[b] Mean ± standard error.

DISCUSSION

The results presented here suggest that the increase in the activity of the "aggregate" RNA polymerase of liver nuclei which occurs following the administration of 3-methylcholanthrene is largely the outcome of an activation of the genome. This conclusion is founded on the following evidence. (a) Chromatin isolated from the livers of rats treated with 3-methylcholanthrene exhibits greater efficacy as template for the *in vitro* synthesis of RNA using a purified *Micrococcus* RNA polymerase. (b) The enhanced template property of the experimental chromatin is not secondary to a stimulation in the secretion of adrenal corticosteroids by 3-methylcholanthrene. (c) The RNA product formed in the presence of the liver chromatin isolated from 3-methylcholanthrene-treated rats differed substantially in its nearest neighbor analysis from the RNA formed with either rat liver DNA or control chromatin as template.[3]

[3] We have also observed that the RNA formed in the presence of chromatin obtained from the livers of 3-methylcholanthrene-treated rats differed substantially from that manufactured in the presence of chromatin from phenobarbital-treated rats as template. This experiment further accentuates the difference between the actions of 3-methylcholanthrene and phenobarbital as "inducers" of the drug-metabolizing enzymes.

The magnitude of the genomic activation is not sufficient to alter such properties of chromatin as chemical composition, ultraviolet absorption, or the transition temperature. The detection of subtle differences in these parameters, however, would be beyond the sensitivity of the measurements.

What the relation is between the observed genomic activation and the function of the gene product is not established in these studies. However, we have recently demonstrated that the administration of the polycyclic hydrocarbon to rats is attended by a profound elevation in the incorporation of labeled orotic acid into the 45 S cytoplasmic particle of liver (15). The maximum increase occurred by 15 hr after injection, although a substantial elevation as noted as early as 3 hr. This effect was also evident in adrenalectomized rats. Concomitantly with the increased incorporation into the 45 S particle there occurred an augmentation in the synthesis of 18 S and 28 S ribosomal RNA fractions in liver cytoplasm. These data suggest an increase in the formation of ribosomes subsequent to the administration of 3-methylcholanthrene. The ribosomes may be required to augment the rate of translation of microsomal enzymes.

These events are reminiscent of the sequence of biochemical alterations which occur after the administration of hormones to rats and draw attention to the "pseudo"-hormonal action of the polycyclic hydrocarbon. One of the earliest effects noted after injection of gonadal hormones (16–19) or glucocorticoids (20–22) is the increased activity of the "aggregate" RNA polymerase system in the nuclei of the target cell. Lukács and Sekeris (23) reported a significant increase in RNA synthesis when cortisol was incubated with rat liver nuclei and the isolated aggregate enzyme was subsequently assayed for RNA polymerase activity. That the enhanced aggregate enzyme activity was the result of an increased capacity of the chromatin as a template in the polymerase reaction has been demonstrated by Barker and Warren (24) in the uterus after estradiol administration, and by Stackhouse *et al.* (25) in liver after cortisol injections.

A stimulation of the synthesis of ribosomal RNA follows the administration of a variety of hormones, including thyroxine, cortisol, and growth hormone (26–29). After the hormonally induced stimulation in ribosomal RNA synthesis, an increased rate of hepatic protein synthesis was observed.

The mechanism by which 3-methylcholanthrene administration is attended by a genomic activation is not presently understood. We had previously reported (30) that labeled 3-methylcholanthrene (or a derivative) forms a complex with protein in rat liver after administration *in vivo*. Subsequently, this complex formation has been accomplished *in vitro* using a high-speed rat liver supernatant fraction as a source of protein.[4] The labeled polycyclic hydrocarbon is also bound to kidney supernatant fractions, although spleen is not active in this regard. It is interesting to speculate that the effect of 3-methylcholanthrene (or a derivative) on template efficacy is preceded by its combination with a receptor molecule. The latter would be responsible for the transport into the nucleus and the interaction with the genomic complement. Studies based on this hypothesis are under way.

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