INCREASED EFFICACY OF LIVER CHROMATIN AS A TEMPLATE FOR RNA SYNTHESIS AFTER ADMINISTRATION OF 3-METHYLCHOLANTHRENE

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A single intraperitoneal injection of 3-methylcholanthrene to rats effects a marked increase in the activities of several drug metabolizing enzymes located within the microsomal fraction of liver (1-7). The increased activity appears to be the result of new enzyme synthesis (6-11). Previous work in this and other laboratories has shown that aggregate RNA polymerase activity in liver nuclei is enhanced after the intraperitoneal administration of 3-methylcholanthrene (12,13). The latter effect was observable 12 hours before the drug metabolizing enzymes of the liver responded to the polycyclic hydrocarbon. The mechanisms underlying the drug-induced elevation of the 'aggregate' RNA polymerase activity could include: an increased synthesis of the enzyme; the presence of activators or diminished production of inhibitors; allosteric conformational change of RNA polymerase leading to increased activity; the presence within the chromatin-RNA polymerase complex of a template with improved transcriptive

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ability. The first three possibilities were eliminated in the previous study (12). This paper demonstrates that the enhanced RNA polymerase activity seen in rat liver after the administration of 3-methylcholanthrene is caused by an activation of the transcriptive ability of liver chromatin.

Experimental

Male 50-60 g Cheek-Jones (Houston) rats were given a single intraperitoneal injection of 3-methylcholanthrene, 1 mg/50 g body wt, in corn oil. Control animals received a corn oil injection. The animals were then sacrificed by cervical dislocation at varying times after injection, their livers removed, perfused with cold 0.25 M sucrose and processed for isolation of DNA or chromatin according to the procedures detailed below.

Animals from which the liver DNA was to be isolated were fasted 24 hr before sacrifice in order to reduce the level of liver glycogen. DNA was then isolated by the procedure of Savitsky and Stand (14).

Chromatin was isolated by a modified Dingman and Sporn (15) procedure from nuclei prepared by the method of Kim and Cohen (16). The modification involved an overnight dialysis of the final chromatin solution at 4°C against 0.01 M Tris, pH 8.0, to remove EDTA[3] .

DNA and chromatin samples were assayed for template activity in an RNA polymerase system at 37°C for 10 min. In earlier

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[3] The following abbreviations are employed: EDTA, ethylene diamine tetraacetate; ATP, GTP, CTP, UTP, 5'-triphosphates of adenosine, guanosine, cytidine and uridine, respectively; TCA, trichloroacetic acid; UMP, uridine-5'-monophosphate; 3-MC, 3-methylcholanthrene; SSC, 0.15 M NaCl and 0.015 M Na citrate, pH 7.0; G, guanosine; C, cytidine.

studies, RNA polymerase was isolated from E. coli B by both the procedure of Furth et al (17) and of Chamberlain and Berg (18). In the chromatin experiments, the enzyme from Micrococcus lysodeiticus, purchased from Miles Laboratories, was employed. The assay mixture contained 10 μmoles tris buffer, pH 8.0; 1 μmole $MgCl_2$; 0.25 μmole $MnCl_2$; 3 μmole β-mercaptoethanol; 0.05 μmole spermidine phosphate; 0.1 μmole each of ATP, GTP, CTP; UTP-2-^{14}C (25 μc/μmole), 0.1 μc; RNA polymerase (5 units); DNA or chromatin, 0.1-10 μg, in a total volume of 0.25 ml. The reaction was stopped by plunging the tubes in ice and 1 mg cold UTP and 10 mg bovine serum albumin were added to each reaction mixture. An aliquot of 0.025 ml was removed and placed on 2.5 cm discs of Whatman #1 paper. The discs were washed 5 times in cold 5% TCA, twice in 95% ethanol and were dried. They were then placed in a toluene scintillation fluid and the radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer. Efficiencies were determined with toluene-^{14}C and the μμmoles UMP-^{14}C incorporated into RNA were calculated.

DNA was determined by the diphenylamine method (19); RNA by the orcinol procedure (20); proteins by the Lowry method (21). Temperature transition studies were performed on the DNA and chromatin samples. We express our appreciation to Dr. M. Mandel of the M. D. Anderson Hospital and Tumor Institute for instructing us in this technique.

Results

The protein/DNA and RNA/DNA of the DNA preparations from control liver were 0.01 and 0.06, respectively; the corresponding values of the liver DNA from the 3-MC-treated animals were 0.02 and 0.10, respectively. The composition of the chromatins is presented in Table I. The RNA/DNA of control liver chromatin

Table I

Composition of Isolated Chromatin

Treatment	RNA/DNA	Protein/DNA	Non-Basic Protein/DNA*
None	0.05	1.2	0.6
3-MC**	0.06	1.1	0.5

*The non-basic proteins included the material which was soluble in 4 N HCl and precipitable by 20% TCA.

**The values at 3, 7, 10, 12 and 16 hr after administration of 3-MC were combined since they did not significantly differ.

(average of at least 5 determinations) was 0.05. The values after administration of 3-MC were not significantly different. The protein/DNA of control liver chromatin was 1.2, while the corresponding value after 3-MC administration was 1.1. The non-basic protein/DNA did not differ significantly after 3-MC treatment.

Representative melting temperature curves of normal and 3-MC-treated chromatins are presented in Figure 1. The T_m for control chromatin varied between 83-85°C, while the T_m for 3-MC chromatin varied from 82-85°C (average of 3, 10, 16 hr pretreatment with 3-MC), a range not significantly different from control values.

The increased template efficacy of chromatin isolated from animals treated with 3-MC can be seen in Figures 2 and 3. Increased activity was observed as early as 3 hr after treatment, and was still present 16 hr after injection. Studies with adrenalectomized animals indicate that adrenal hormones are in no way responsible for the observed increase in template activity. These studies will be reported at another time.

DNA isolated from the livers of control and 3-MC-treated

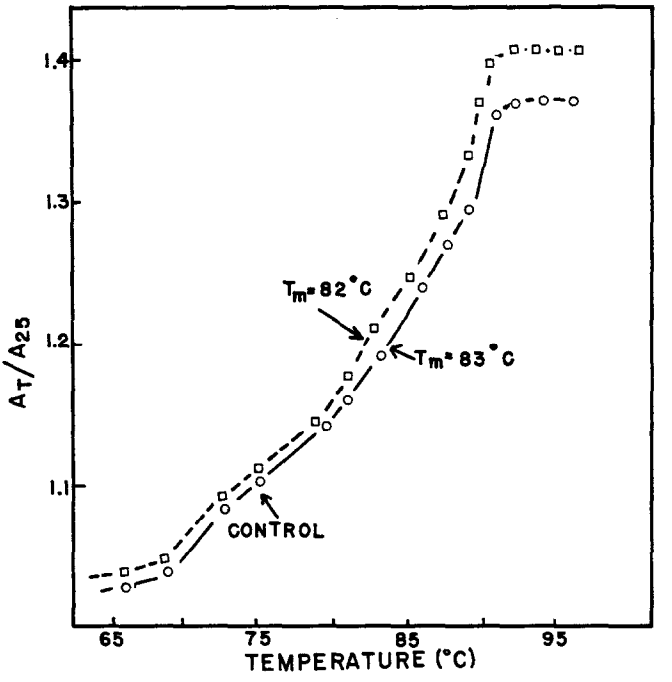

Figure 1. Transition Temperature of Control and 3-MC-Treated Chromatin.

Chromatin was diluted in SSC and the absorbance at 260 and 320 mμ was determined. The A_T represents the absorbance at 260 mμ - absorbance at 320 mμ at the respective temperature. The A_{25} was the value obtained at room temperature. Hyperchromicity is expressed as A_T/A_{25}. o——o, control chromatin; □- - - -□ , chromatin at 16 hr after administration of 3-MC.

The transition temperatures (T_m) are given on the graph.

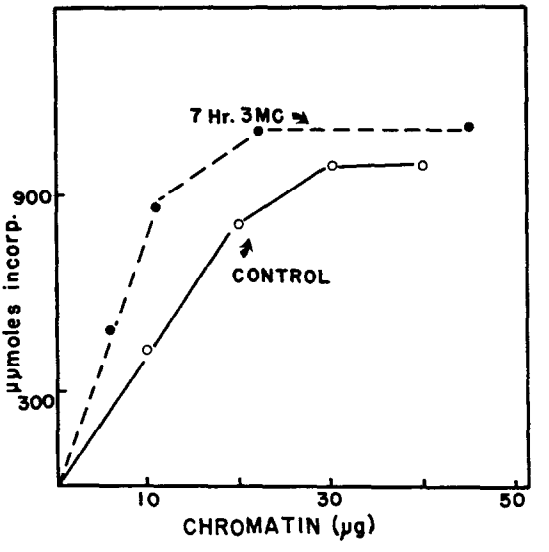

Figure 2. Template Activity of Chromatin from the Livers of Rats Pretreated with 3-MC for 7 hr.

See the Experimental Section for details.

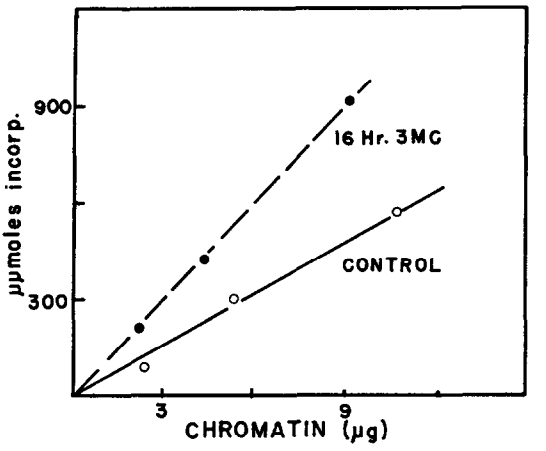

Figure 3. Template Activity of Chromatin from the Livers of Rats Pretreated with 3-MC for 16 hr.
See the Experimental Section for details.

rats (at 6, 12 or 24 hr) were equally effective in promoting RNA synthesis in the bacterial RNA polymerase system. The enhanced template activity was therefore a property of the chromatin per se and not of the DNA.

Studies are presently underway to determine the base composition of the RNA synthesized in the RNA polymerase system in the presence of the chromatin preparations. Preliminary data indicate an altered base composition of RNA transcribed from 3-MC treated chromatin, with a shift towards a higher G-C content.

Discussion

Previous work in this and other laboratories (12,13) has shown that 3-MC effects an enhanced activity of the rat liver aggregate RNA polymerase system, and suggests that this enhanced activity is the result of genomic activation. The present work clearly supports this suggestion. It is shown that administration of 3-MC results in a two-fold increase in template efficacy in an in vitro bacterial RNA polymerase system. The increase was ob-

servable as early as 3 hr following administration of the polycyclic hydrocarbon and remained at this elevated rate as long as 16 hr.

An enhanced template activity of chromatin has been demonstrated in the liver by Dahmus and Bonner (22) after administration of hydrocortisone to adrenalectomized rats; in the liver by Tata and Widnell (23) after administration of thyroid hormones; in the prostate by Liao and Lin (24) after administration of testosterone to the castrate animal; and in the muscle after injection of testosterone or growth hormone (25). It is becoming apparent that this type of activation may be a common mechanism of action of hormones.

3-Methylcholanthrene may act similarly to produce a genomic activation. In view of previous work which demonstrated considerable binding of 3-MC to liver protein (26), it is believed that the genomic activation is indirect and is accomplished by the 3-MC protein complex. Studies are presently underway in two directions: the determination of the exact nature of the proteins to which 3-MC binds; the determination of the nature of the RNA being transcribed from the activated template.

In summary, the experiments presented in this report suggest that 3-MC either directly or indirectly causes an activation of liver chromatin such that an increased number of sites are now available for the transcription of RNA. This (or these) RNA (or RNA's) would presumably be responsible for the elaboration of the drug-metabolizing enzymes.

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