STUDIES ON IN VITRO RNA SYNTHESIS BY CHROMATIN FRACTIONS FROM RAT LIVER AND NOVIKOFF HEPATOMA

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Summary

Eu- and heterochromatin fractions prepared from normal liver and Novikoff hepatoma were tested for their capacity to direct the synthesis of RNA. Using purified RNA polymerase, template activity was measured by the incorporation of C^{14} GTP into RNA. Unlike DNA, chromatin fractions had increased template activity at increased salt concentrations. No difference was observed in the priming capacity of either eu- or heterochromatin fractions derived from normal liver or Novikoff hepatoma.

It is widely believed that genetic information is actively transcribed from extended chromatin, i.e. euchromatin, and that latent genetic information is present but remains unexpressed in the condensed, i.e. heterochromatin fraction (1,2). This hypothesis proposes that, as a cell differentiates, those genes not functioning during a particular stage of differentiation are maintained in an inactive state by an unknown repression mechanism, and are in the form recognizable as heterochromatin. Furthermore, as differentiation proceeds, there may be simultaneous activation and repression of different genes to accommodate the changing needs of the cell. Both morphological and biochemical lines of evidence support this hypothesis. It is well known that, as cells become more highly differentiated and lose their ability to synthesize RNA, heterochromatinization, clumping of the nuclear contents, can be observed in histological sections. This is perhaps most evident during the maturation of the nucleus during differentiation of erythroid cell precursors.

In this instance the nucleus of the late orthochromic normoblast, a cell virtually inactive in DNA and RNA synthesis (3), is markedly heterochromatic (4). Frenster, Allfrey, and Mirsky have presented biochemical evidence to support this hypothesis (2). They fractionated the nuclear chromatin and showed that RNA synthesized in isolated nuclei was primarily associated with the euchromatin. The heterochromatin contained little newly-synthesized RNA.

A preliminary report from this laboratory demonstrated that Novikoff hepatoma contained a greater percentage of heterochromatin when compared to normal liver (5). Based on the above hypothesis, this finding would agree with the observed loss in the capacity to synthesize a number of enzymes, a loss which accompanies the process of malignant transformation (6). The present study was designed to test this hypothesis by the direct measurement of the biological activity of eu-, and heterochromatin in normal liver and Novikoff hepatoma. Chromatin was prepared from the nuclei of these two tissues by minor modifications of techniques detailed elsewhere (7). The chromatin was then fractionated into eu- and heterochromatin according to the method of Frenster et al. (2). RNA polymerase was isolated from E. coli B, according to the method of Chamberlin and Berg (8). The enzyme was purified to the fraction III stage. The template activity of the chromatin in fractions was evaluated in the following system: RNA polymerase, 0.15 units; Tris HCl, 40 mM, pH 8.0; $MgCl_2$, 4 mM; $MnCl_2$, 1 mM; βmercaptoethanol, 0.12M; spermidine HCl, 0.2 mM; ATP, 0.4 mM; CTP, 0.4 mM; C^{14} GTP, 0.4 mM; 25 μc/mM; and UTP, 0.4 mM. Duplicate 0.5 ml. assays were incubated at 37° for 20'. The reaction was stopped by the addition of trichloracetic acid to a final concentration of 7% at 0° C. The precipitates were collected by filtration on Millipore filters. Radioactivity was measured in a Wide Beta II low background gas flow counter Salmon testes DNA (Worthington Biochem.) was used for standardization of the assay and for comparison with chromatin. DNA was measured by the diphenyl-

amine reaction (9).

Initial studies were performed to measure the proportion of heterochromatin in the total chromatin (Table 1). The results revealed that Novikoff hepatoma cells contained 167% as much heterochromatin as did normal liver cells. If the postulate that heterochromatin represents inactive genetic material is correct, this finding suggests that more genetic information functioning in the normal mature liver cell may be suppressed in the dedifferentiated tumor cell than is induced in the process of dedifferentiation.

TABLE 1

Percent of total chromatin existing as heterochromatin

Normal liver	Novikoff hepatoma	
	Solid form	Ascitic form
8.7±1.6	14.9±2.0	14.4±2.3

TABLE 2

RNA polymerase activity expressed as CPM incorporated/ugm DNA

Primer	0.25M - NaCl	
	Absent	Present
DNA (Salmon testes)	5,800	5,000
Liver euchromatin	14	73
Liver heterochromatin	22	85
Hepatoma euchromatin	15	77
Hepatoma heterochromatin	22	75

When normal liver or hepatoma chromatin was used as a primer for RNA polymerase, it was relatively inactive in comparison with purified salmon

testes DNA (Table 2). Little difference was found in the priming capacity of hetero- and euchromatin regardless of its origin. The chromatin fractions as initially isolated were relatively insoluble. During attempts to solubilize heterochromatin, which is considerably less soluble than euchromatin, it was found that increased ionic strength solubilized the chromatin. The results of a systematic study on the solubility of both eu- and heterochromatin in NaCl is depicted in Fig. 1. Eu- and heterochromatin of both normal liver and tumor origin were suspended in increasing concentrations of NaCl. The insoluble portions were removed by centrifugation and optical density of the supernatant fraction was measured at 260 and 280 mμ. The results presented in Fig. 1 thus represent the relative solubility of eu- and heterochromatin as a function of NaCl concentration. As the salt concentration was increased to 0.25 M, euchromatin became considerably less

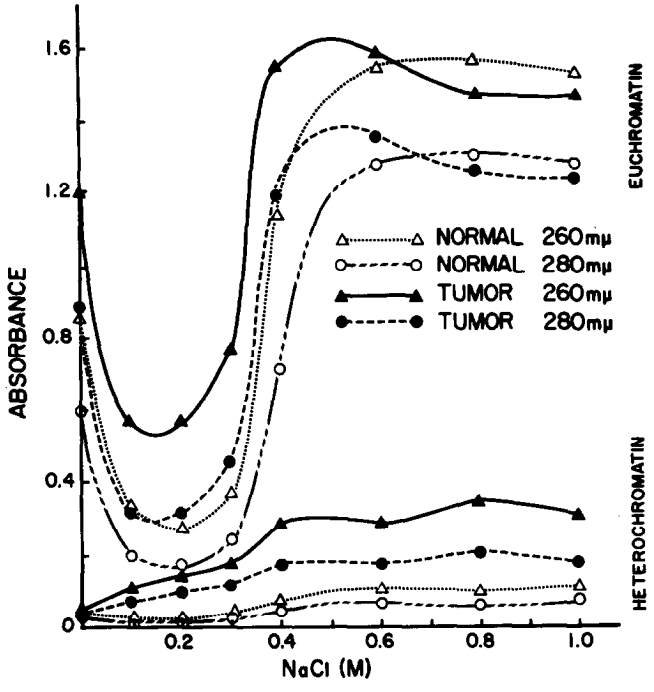

Fig. 1 - Solubility of eu- and heterochromatin of normal liver (Normal) and Novikoff hepatoma (Tumor) origin (measured as optical density at 260 and 280 mμ). See text for preparation of samples.

soluble. Above this concentration there was resolubilization of the eu-chromatin to a maximal level at 0.6 M NaCl. In contrast, heterochromatin was least soluble in water and became more soluble as the salt concentration was increased. The 260/280 ratio remained relatively constant for all chromatin preparations at all salt concentrations tested.

When sub saturated suspensions of eu- and heterochromatin were assayed for template activity in increasing concentrations of NaCl, there was marked enhancement of priming capacity to a maximum level at 0.25M NaCl (Fig. 2a,b). No significant difference was found between eu- and heterochromatin nor between normal and tumor chromatin (Table 2, Fig. 2c,d). At optimal NaCl concentrations priming activity was linear up to 4-7 micrograms of chromatin DNA (Fig. 2c,d). These findings were in direct contrast with results ob-

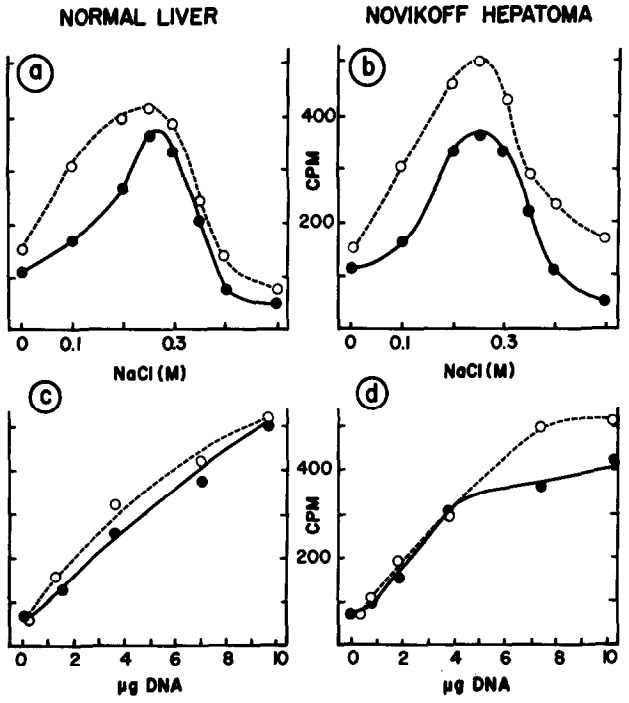

Fig. 2 - Priming capacity of euchromatin (●——●), and heterochromatin (O- - -O). a,b. - Effect of the addition of NaCl to the assay system. Equal amounts of chromatin were added to each assay (7γ chromatin DNA/assay). c,d. - Priming capacity of increasing amounts of chromatin (assayed in the presence of 0.25M NaCl).

tained with purified salmon testes DNA, where priming capacity was maximal in the absence of NaCl (Fig. 3a), and where the RNA polymerase was saturated with 0.3 micrograms of DNA (Fig. 3b). With increasing concentrations of NaCl there was a linear decrease in activity of 13% up to 0.25M NaCl. Higher concentrations led to a precipitous drop in priming activity paralleling the loss of activity seen with chromatin. This loss of activity is thought to be related to the dissociation of RNA polymerase and template DNA

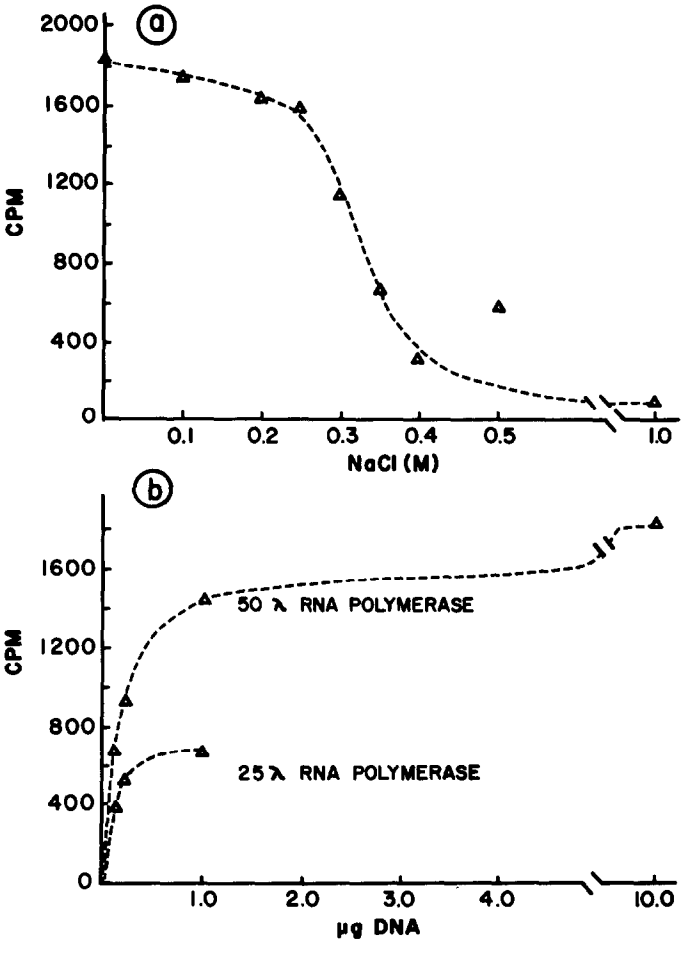

Fig. 3 - Priming capacity of salmon testes DNA. a. - Effects of the addition of NaCl to the assay system (10γ DNA added per assay). b. - Priming capacity of increasing amounts of DNA. The upper curve was obtained using 50λ RNA polymerase (the amount normally used in the assay) and the lower curve using 25λ RNA polymerase.

seen with increasing salt concentration (10). It is obvious from the present study that the ionic strength of the assay system is vital for the measurement of the priming capacity of chromatin. That this is not merely a reflection of the solubility of chromatin, is evident from the observation that euchromatin is maximally active at an ionic strength at which it is least soluble.

The finding that eu- and heterochromatin from tumor or liver were equally effective as template for the RNA polymerase reaction was unexpected. This contrasts with the observations of Frenster et al. (2) and needs further clarification. A possible explanation concerns the relative solubility of eu- and heterochromatin. If chromatin must be in the soluble state to have priming capacity, then the increased solubility of euchromatin relative to heterochromatin at all ionic strengths may allow for a greater biological expression of euchromatin provided that nuclear chromatin exists in a saturated state in vivo, i.e. heterochromatinization renders chromatin insoluble and consequently it loses its priming capacity. An alternative explanation may involve the quantity of endogenous RNA polymerase associated with eu- and heterochromatin. Since exogenous RNA polymerase was added to the system in the present study, we cannot rule out this possibility. Until the differences are resolved, however, acceptance of the hypothesis that condensed chromatin, heterochromatin, is inactive in the RNA synthetic reaction must be reserved.

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