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Nature of hydrocortisone-elicited amplification of template activity of liver chromatin

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Summary

The template efficiency of euchromatin region and number of RNA polymerase binding sites on this region of rat liver chromatin were significantly elevated at 3.5 h after administration of hydrocortisone to adrenalectomised rats. The euchromatin from the liver chromatin of horomone-treated rats was also found to have significantly increased levels of nonhistone proteins as compared to those in euchromatin fraction derived from adrenalectomised rats.

Introduction

Hydrocortisone mediated stimulation in synthesis of hepatic RNA has been ascribed mainly to activation of template efficiency of chromatin in the liver (1,2). Based on the evidence that the eukaryotic interphase nuclei contain varying amounts of dispersed (euchromatin) and condensed (heterochromatin) chromatin regions (3) it was felt of interest to investigate relative influence of hydrocortisone administration on the transcription efficiency of the two regions of the liver chromatin.

The results presented in this communication indicate that the hormone specifically elevates the template activity of the euchromatin region probably by increasing the rate of RNA chain initiation.

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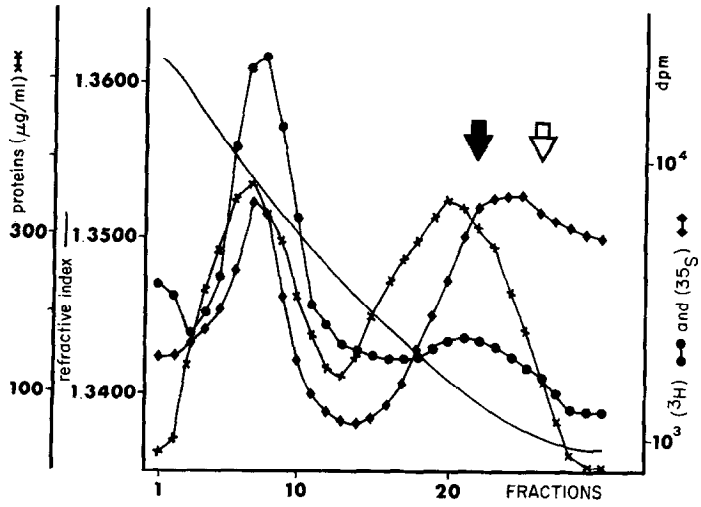

Fig. 3 Labelling of the plasma membrane. T.brucei cells were incubated 15 min at 22° C in the presence of (●——●) [3H]concanavalin A or (◆——◆) [35S]p-diazobenzenesulfonate before disruption. The homogenate was separated on a CsCl density gradient. (———) refractive index; (x——x)proteins. Equilibrium density of free concanavalin A and p-diazobenzenesulfonate are indicated by filled and open arrows respectively.

incorporated increases with temperature (Fig. 2B) indicating fusion or endocytosis (7). Endocytosis can be ruled out since no inhibitory effect at 4° C and 37° C could be detected in the presence of 10 mM KCN, NaF or NaN_3, reported to be inhibitors of endocytosis in T.brucei (13), or of 10 µM colchicine.

It has to be pointed out that at 37° C, lipid transfer is of minor importance (7), although at 2° C it may predominate (14).

Subcellular fractionation.

In order to investigate membrane-liposome interaction, chemical labelling of the plasma membrane and subcellular fractionation has been undertaken. When parasites, incubated in the presence of [3H]concanavalin A or [35S]diazobenzenesulfonate and subsequently disrupted, were layered on the top of a continuous CsCl density gradient, both [3H]- and [35S]-labelled material were recovered in the same peak, at the density of 1.20 g/cm^3 (Fig. 3). Under the same conditions free concanavalin A and diazobenzenesulfonate were recovered at lower densities, (1.06 and 1.02 g/cm^3 respectively). Since these reagents are non-permeant, it was concluded that the labelled fraction was the plasma membrane of the trypanosome; further characterisation would need marker enzymes which are not yet realiable for such organisms. If the cells are treated

method (11) and estimated for radioactivity. To rule out the possibility of non-specific incorporation of (γ- ^{32}P) ATP due to protein kinase and polyphosphate kinase associated with chromatin fractions (12), suitable controls were used.

Incorporation of ^{3}H-leucine into histone and nonhistone proteins in rat liver :

Adrenalectomised and hydrocortisone-treated rats were injected with DL (^{3}H) leucine (600/ /uCi/100 g body wt.) 1 h prior to sacrifice. Histones and nonhistone proteins were isolated from liver nuclei by the method of Teng et al. (13). The histones were dissolved in water while nonhistones were dissolved in 0.01 M phosphate buffer (pH 7.2) for protein estimation and radiactivity determinations. Protein was estimated by the method of Lowry et al. (14) using bovine serum albumin as standard. DNA was estimated by Burton's diphenylamine reaction using calf thymus DNA as standard (15). RNA was estimated by the orcinol reaction using yeast RNA as standard (16).

Results and Discussion

The method adopted for fractionation of the liver chromatin into euchromatin and heterochromatin regions was based on their differential sedimentation properties during ultracentrifugation through sucrose density gradients. The sedimentation pattern of liver chromatin is depicted in Fig. 1. Based on the method of Leake et al. (17), it was found that an aliquot of the euchromatin fraction stayed in solution containing 5 mM $MgCl_2$ after centrifugation at 2000 x g for 20 min at 4°C, whereas an aliquot of the heterochromatin fraction was pelleted under these conditions. The DNA content of euchromatin region comprising about 11-16% of total chromosomal DNA was in the range of those reported by others using different fractionation methods (7).

Results on the template efficiency of the two regions of liver chromatin derived from adrenalectomised and hydrocortisone-treated rats are incorporated in Table 1. The template efficiency was determined using exogenous RNA polymerase isolated from normal rat liver. As seen from the Table hydrocortisone had considerable

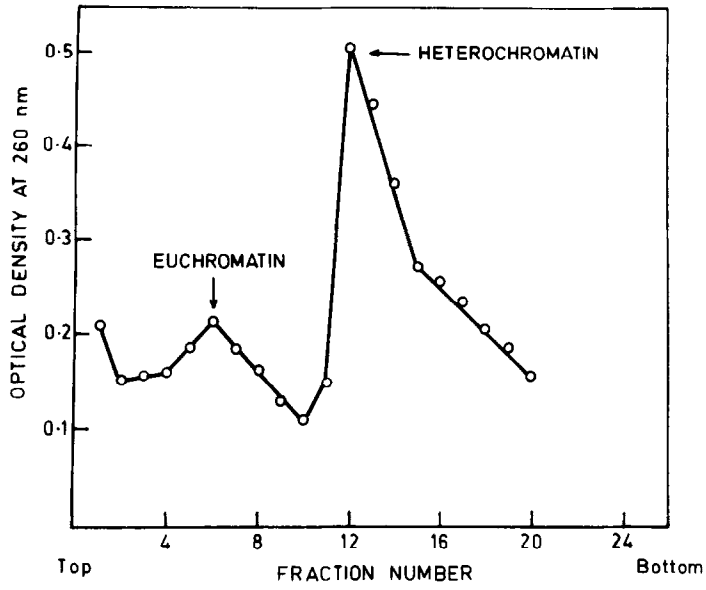

Fig. 1. : Fractionation of liver chromatin. The sheared chromatin corresponding to 8 A_{260} units in 2 ml buffer (0.01 M Tris-HCl, pH 8.0) was layered on 21 ml 5-30% sucrose density gradients in 0.01 M Tris-HCl (pH 8.0) and centrifuged at 80,000 x g for 3.5 h. One ml fractions were collected for the determination of absorbance at 260 nm.

Table 1

Effect of hydrocortisone on template activity of rat liver chromatin and chromatin fractions

		ΔMP incorporated* pmoles/10 min/20 µg DNA		
	DNA	Chromatin	Euchromatin	Heterochromatin
Adrenalectomised	349 ± 12.70	64 ± 3.46	89 ± 6.35	54 ± 2.30
Hydrocortisone administered (3.5 h)	342 ± 10.40	96 ± 2.88	148 ± 9.23	63 ± 4.04

*RNA synthesis by enzyme alone (19.6 pmoles) and chromatin alone (2.0 pmole) is subtracted. Each value is an average of three independent determinations ± S.E.M.

stimulatory effect on the template activity of euchromatin region but has not on that of heterochromatin region.

Table 2

Macromolecular composition of rat liver chromatin fractions : action of hydrocortisone

	DNA	RNA (*)	Histone	Non-histone
Adrenalectomised				
Euchromatin	1.0	0.02	0.94	0.91
Heterochromatin	1.0	0.01	1.02	0.28
Hydrocortisone-treated (3.5 h)				
Euchromatin	1.0	0.034	0.92	1.38
Heterochromatin	1.0	0.004	1.10	0.32

(*) The values are expressed as mg based on 1 mg DNA. Each value is average of three independent experiments.

The data on macromolecular composition of euchromatin and heterochromatin regions are given in Table 2. The levels of non-histone chromosomal proteins in euchromatin and heterochromatin regions were, respectively, 50% and 15% higher in hydrocortisone-administered rats as compared to adrenalectomised rats. On the other hand, no significant differences could be noticeable between hormone-deficient and - administered animals in respect of histone contents of euchromatin and heterochromatin regions of liver chromatin.

Results on the rate of incorporation of ^{3}H-leucine into chromosomal proteins are shown in Table 3. The incorporation of the radioactive precursors into nonhistone proteins of euchromatin region was considerably increased after hormone administration. This is indicative of increase in the rate of nonhistone proteins synthesis (or increased accumulation of nonhistone proteins due to decreased rate of degradation) in the euchromatin region under hormone influence.

Table 3

Action of hydrocortisone on labelling of histone and nonhistone proteins

	Histone (Specific activity cpm/mg protein)	Nonhistone (Specific activity cpm/mg protein)
Adrenalectomised		
Euchromatin	620 ± 10.39	990 ± 8.66
Heterochromatin	846 ± 12.12	385 ± 13.85
Hydrocortisone-treated (3.5 h)		
Euchromatin	642 ± 9.24	1507 ± 8.08
Heterochromatin	872 ± 19.63	402 ± 6.35

Values shown are means ± standard error for three separate experiments.

There was no significant change in the labelling of histones of euchromatin region in response to hormone administration. Rates of incorporation of (^{3}H) leucine into the two classes of chromosomal proteins of the heterochromatin region did not show any significant alterations as a result of hydrocortisone administration. These results are in consonance with the results shown in Table 2.

There have been reports implicating stimulation in syntheses or turnovers of nonhistone proteins in various gene activation phenomena (18). The present findings with hydrocortisone also suggest a possible link between stimulation of syntheses or turnovers of some nonhistone proteins associated with euchromatin regions and increased template activity of this region.

The data on the number of RNA chain initiation sites on the two chromatin fractions and the influence of hydrocortisone

Table 4

RNA chain initiation on rat liver euchromatin and heterochromatin : action of hydrocortisone

	Number of RNA chain* initiation sites/ µg DNA	
	Euchromatin	Heterochromatin
Adrenalectomised	0.024 ± 0.0011	0.0044 ± 0.0002
Hydrocortisone administered (3.5 h)	0.050 ± 0.0034	0.0060 ± 0.0001

*Number of RNA chain initiation sites are expressed as pmole of (γ- ^{32}p) ATP incorporated into RNA under the standard conditions. Each value represents the average of three independent experiments ± S.E.M.

administration are shown in Table 4. There was significant enhancement in the number of available initiation sites on the euchromatin region whereas those on the heterochromatin region were only marginally increased.

These studies strongly suggest that action of hydrocortisone on rat liver chromatin *in vivo* is selective at the euchromatin region. Both template activity and the number of available initiation sites of euchromatin are considerably increased at 3.5 h following hormone administration. On the other hand, heterochromatin region remains unchanged in terms of these two functional attributes.

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