GENETICS

Effects of Thyroid Hormones on the Transcription and Structure of the Malic Enzyme Gene in Rat Liver

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Functionally different chromatin fractions have been isolated whose DNAs are used in hybridization with ^{32}P-labeled fragments of the malic enzyme gene. In thyroidectomized animals, homologs of the malic enzyme gene are found predominantly in the transcriptionally inactive chromatin fraction. In triiodothyronine-treated rats, the most intense hybridization signals are obtained from the chromatin fraction DNA hypersensitive to micrococcal nuclease and from DNA associated with the nuclear matrix. Thus, induction of the malic enzyme mRNA with triiodothyronine is attended by rearrangements at the higher levels of DNA packing.

Key Words: *thyroid hormones; malic enzyme gene; structural organization; transcription*

The higher levels of DNA organization in chromatin may play an important role in the regulation of gene activity [2]. Transcription is believed to be preceded by the transfer of the expressed chromatin site from transcriptionally inactive state into active. The possible manifestations of such a transfer are depletion of the transcriptionally active chromatin for H1 histone and enrichment with a highly mobile group of proteins (HMG) [2,3,15], changed pattern of histone phosphorylation and acetylation [4,10], DNA hypomethylation [7], hypersensitivity of the active chromatin DNA to nucleases [15], and association of active chromatin with the nuclear matrix [6,9].

Detection of the thyroid hormone receptors in the nuclear matrix [1] and in chromatin sites hypersensitive to nucleases [12] as well as stimulation of various modifications of nuclear proteins with thyroid hormones, which changes their affinity for DNA [10], implies that thyroid hormones may contribute to the creation of "active" chromatin conformation, i.e., affect the higher levels of DNA packing.

The present study is an attempt to test this hypothesis. A fragment of the malic enzyme gene was used as a probe. The presence of the gene homologs in functionally different chromatin fractions was tested by DNA-DNA hybridization.

MATERIALS AND METHODS

Hypothyrosis in rats was induced by removal of the thyroid. The animals were sacrificed 4-5 weeks after the operation. Triiodothyronine (T_3) was injected intraperitoneally in a dose of 30 μg/100 g body weight 48 h prior to sacrifice. The functionally different chromatin fractions were isolated as previously [9,13]. The first fraction is an extract obtained after brief hydrolysis of the nuclei with micrococcal nuclease, the second is an extract of the nuclei eluted with 2 M NaCl, and the third is the nuclear sediment obtained after treatment with 2 M NaCl (nuclear matrix).

All chromatin fractions and the total nuclear fraction were treated with RNAse 1, proteinase K,

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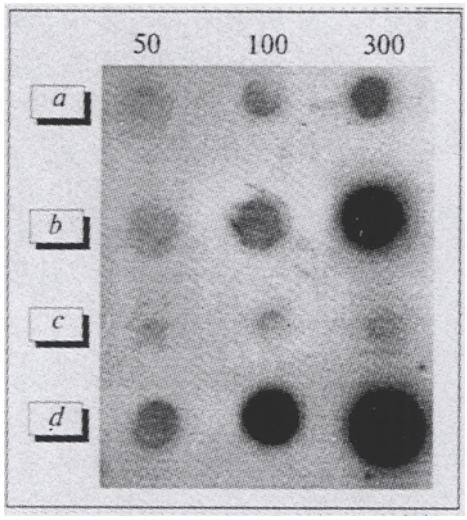

Fig. 1. Spot hybridization between immobilized DNA from various fractions of nuclei with the EcoRI fragment of the malic enzyme gene. Here and on Fig. 2: *a*) total nuclear fraction; *b*) fraction of nuclei hydrolyzed with micrococcal nuclease; *c*) 2 M NaCl extract of nuclear sediment obtained after nuclease hydrolysis; *d*) nuclear matrix. The figures show the content of filter-bound, ng.

and deproteinized with phenol-chloroform, after which DNA was precipitated with ethanol [5]. The EcoRI fragment (1.3 kb) of the malic enzyme gene was kindly provided Dr. J. H. Oppenheimer (USA). Nick translation of DNA was carried out using an Amersham Nick translation kit according to the manufacturer's instructions. DNA was labeled with [^{32}P]dCTP for DNA-DNA hybridization or with [^{3}H]dCTP for DNA-RNA hybridization. DNA-DNA hybridization was performed after DNA immobilization on nylon filters with UV exposure [11]. RNA was isolated using guanidine thiocyanate as described elsewhere [8]. Polyadenylated RNA was separated by affinity chromatography on a poly(U)-Sepharose column. RNA-DNA hybridization was performed after immobilization of poly(A$^+$)RNA on nitrocellulose filters [14].

RESULTS

Figure 1 shows the spots resulting from hybridization between ^{32}P-labeled fragment of the malic enzyme gene and total DNA or DNA of each of the three fractions isolated from intact rats. The intensity of signals from the same fraction on the radioautography image correlates with the content of DNA immobilized on the filter. At the same time, the content of sequences hybridizing with the labeled probe varies considerably for a unit of DNA of each fraction: with the same amounts of immobilized DNA, the most intense hybridization signals were obtained from DNA of fraction *d* and the least intensive signals from DNA of fraction *c*. This pattern of hybridization is not constant and seems to depend on the thyroid status of animals. Such a conclusion may be drawn from the data shown in Fig. 2. In contrast to normal rats, in thyroidectomized rats the greatest amount of malic enzyme homologs is detected in fraction *c*. Compensatory injection of T_3 to these rats alters the hybridization pattern, approximating it to that typical of intact rats.

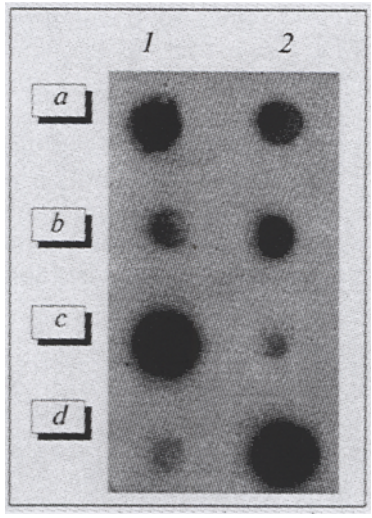

Fig. 2. Distribution of the malic enzyme gene in functionally different DNA fractions from rats with different thyroid status. *1*) thyroidectomy; *2*) injection of triiodothyronine.

Quantitation of the malic enzyme mRNA by DNA-RNA hybridization showed that injection of T_3 to intact animals results in a 3-4-fold increase in the production of the malic enzyme mRNA in comparison with that in thyroidectomized rats (Fig. 3). Thus, the T_3-induced redistribution of the malic enzyme gene from chromatin fraction *c* to fractions *b* and *d* is associated with an increase in the transcriptase activity of the sequences coding for this enzyme.

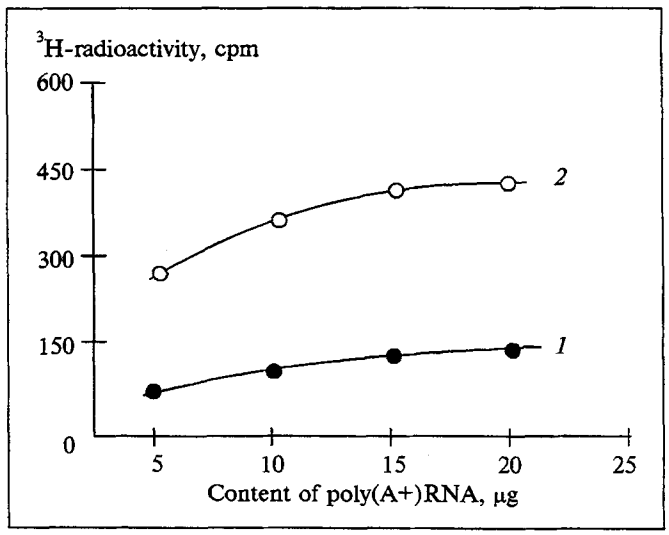

Fig. 3. Hybridization of ^{3}H-labeled fragment of the malic enzyme gene with poly(A$^+$)RNA from the liver of thyroidectomized rats (*1*) and triiodothyronine-treated rats (*2*).

The T_3-induced enrichment of fractions *b* and *d* in the malic enzyme gene was not surprising; moreover, it was in line with the results obtained in similar systems with other genes [6,9,13]. It was reported that fraction *b* is characterized by a high content of nonhistone proteins, low content of H1 histone, and is rich in HMG proteins, i.e., has the parameters of the transcriptionally active chromatin [9,13]. Fraction *c* is rich in histone H1, contains all histones of the cortex, and corresponds to transcriptionally inactive chromatin; fraction *d* contains the binding sites for RNA polymerase, hormone-receptor complexes, and various transcription factors [6,9], i.e., it is also functionally active.

Hence, the redictribution of T_3-reactive gene in functionally different chromatin fractions after administration of the hormone to animals indicates that thyroid hormones affect not only transcription, but also structural organization of DNA in chromatin.

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