ROLE OF THYROID HORMONES IN REGULATION OF GENETIC ACTIVITY OF NORMAL AND TRANSFORMED HUMAN CELLS

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Thyroid hormones labeled with ^{125}I are localized on structures of the interphase nucleus and metaphase chromosomes of fibroblasts from 8-10-week human embryos in culture. Meanwhile, although labeled thyroid hormones are present in interphase nuclei of HeLa cells, by contrast with normal cells they are not accepted by their metaphase chromosomes. It is suggested on the basis of the results that the acceptor region of the genome of HeLa cells during transformation have lost their ability to bind their own receptor complexes with thyroid hormones.

KEY WORDS: thyroid hormones; metaphase chromosomes; interphase nucleus; site-specific region; receptor; triiodothyronine.

Thyroid hormones participate in the regulation of genetic activity by stimulating the synthesis of various classes of RNA, activating the protein-synthesizing apparatus of the cells [15], inducing the synthesis of qualitatively new populations of high-polymer nuclear RNA [1], and inducing the synthesis of nonhistone proteins of chromatin [4] and various functionally important protein molecules [13]. However, the mechanisms of participation of thyroid hormones in the regulation of genetic activity have received little study.

Thyroid hormones, like steroids, specifically interact with intracellular receptor molecules [3, 11, 17]. The hormone–receptor complex *in vitro* activates RNA-polymerase I and II of purified nuclei, increases the template activity of chromatin, and increases the number of transcription initiation sites in chromatin [3].

According to existing information [5, 6, 8, 14, 16], steroid receptors are a protein product of the regulator gene. After specific interaction with hormone, the receptor molecules become capable of "recognizing" the operative part of the inducing operon.

If this concept of the molecular mechanism of action of steroids is applicable to thyroid hormones, which like steroids can penetrate into hormone-sensitive cells [11], where they bind specifically with receptor molecules of the cytoplasm [3] and chromatin [17], it can be expected that during incubation of hormone-sensitive cultured cells in the presence of labeled thyroid hormones the latter must penetrate into the cytoplasm and nucleus, and as a hormone–receptor complex, must be accepted by the regulatory structures of the genome. During packing of the chromatin into the chromosomal structures the labeled hormone, bound with chromatin, is transferred into the structures of the chromosomes and can be detected there by autoradiography [2].

The object of the present investigation was to test this hypothesis experimentally on a normal culture of human fibroblasts and a culture of transformed HeLa cells.

EXPERIMENTAL METHOD

To test this hypothesis, a primary culture of fibroblasts from 8-10-week human embryos and a continuous line of HeLa cells growing in medium No. 199 containing 10% calf serum were treated with the following radioactive compounds, each in a dose of 10 μCi/ml medium: $Na^{125}I$,

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TABLE 1. Number of Labeled Nuclei and Metaphase Chromosomes and Mean Number of [^{125}I]Triiodothyronine Tracks above Interphase Nuclei and Metaphase Chromosomes of Human Embryonic Fibroblasts and HeLa Cells

Cell structure	Percent of labeled nuclei	Mean No. of tracks above nucleus	% labeled metaphase chromosomes	Mean No. of tracks above metaphase chromosomes
Primary culture of human embryonic fibroblasts	77,6±3,6	40,8±1,9	39,1±1,7	16,2±2
HeLa	68,3±3,2	35,0±1,6	4,4±0,8	1,2±0,5

[^{125}I]diiodotyrosine, [^{125}I]triiodothyronine, or [^{125}I]thyroxine. After incubation with the labeled hormones for 1 h at 37°C the growth medium was replaced by fresh medium. To remove traces of radioactive hormone, a container with the cell culture was rinsed several times with the same solution, after which incubation continued in the presence of colchicine (0.04 µg/ml) at 37°C for 3 h. In additional experiments HeLa cells were incubated in the presence of the radioactive hormones throughout the period of interphase, i.e., in the G_1, S, and G_2 periods of the cell cycle. The cells were removed from the surface of the glass by energetic shaking in 0.25% trypsin solution and harvested by centrifugation. The cell residue was suspended in 0.075 M KCl and fixed in a mixture of methyl alcohol and acetic acid (3:1). Preparations of chromosomes and interphase nuclei were obtained by burning off the fixative. Type M emulsion was used. The exposure for autoradiography was 8 days.

EXPERIMENTAL RESULTS

The results are given in Table 1.

The background activity of the preparations per mean area of nuclei and metaphase chromosomes was 0.8±0.21 and 1.1±0.5 respectively.

The experiments showed that [^{125}I]triiodothyronine, unlike $Na^{125}I$ and [^{125}I]diiodotyrosine (data not shown), penetrates into the cell, is localized in the interphase nucleus, and is accepted by metaphase chromosomes of fibroblasts of 8-10-week-old human embryos in culture (Fig. 1). This points to definite specificity of interaction, for correlation is observed between the hormonal activity of compounds and their acceptance by interphase nuclei and metaphase chromosomes. According to data in the literature [17], $Na^{125}I$ and [^{125}I]diiodotyrosine do not possess hormonal activity.

Not all the hormonal label which penetrates into the nucleus is later accepted by metaphase chromosomes of fibroblasts.

As a result of experiments carried out under identical conditions, in which a culture of malignantly transformed HeLa cells was incubated with [^{125}I]triiodothyronine, the labeled hormone was found to penetrate into the cell and bind with the nucleus, but none of it was accepted by the metaphase chromosomes of these cells (Table 1; Fig. 2).

As Table 1 shows, a high proportion of the interphase nuclei and metaphase chromosomes of fibroblasts did not carry the hormonal label. Control experiments with incubation of labeled hormone with isolated dividing cells showed that [^{125}I]triiodothyronine does not interact with metaphase chromosomes of normal cells which are already formed.

The absence of acceptance of labeled hormones by metaphase chromosomes of HeLa cells appears to be an interesting phenomenon (Fig. 2). This phenomenon is evidently unconnected with the state of the interphase nuclei at the moment of addition of the labeled hormone, for in control experiments in which the duration of contact between these cells and the labeled hormone throughout interphase was increased to 12 h, i.e., with involvement of the G_1, S, and G_2 phases of the cycle in this process, the same negative result was obtained.

The localization of labeled triiodothyronine in the interphase nuclei of normal fibroblasts and transformed HeLa cells shows that in the cells of both lines the intracellular receptors can interact normally with the hormone and that this complex is perhaps translocated into the nucleus. Evidence in support of this view is given by investigations which showed a decrease in the affinity of the hormone for the receptor of cells carrying mutations in the

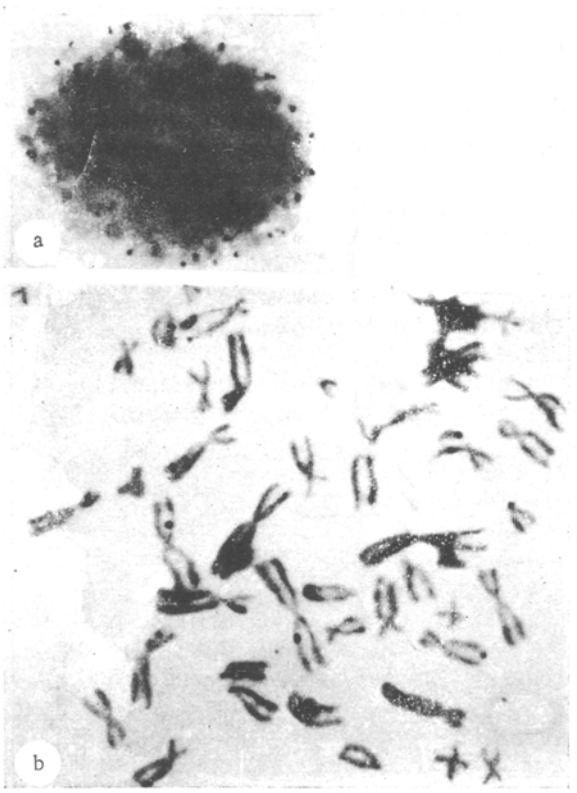

Fig. 1

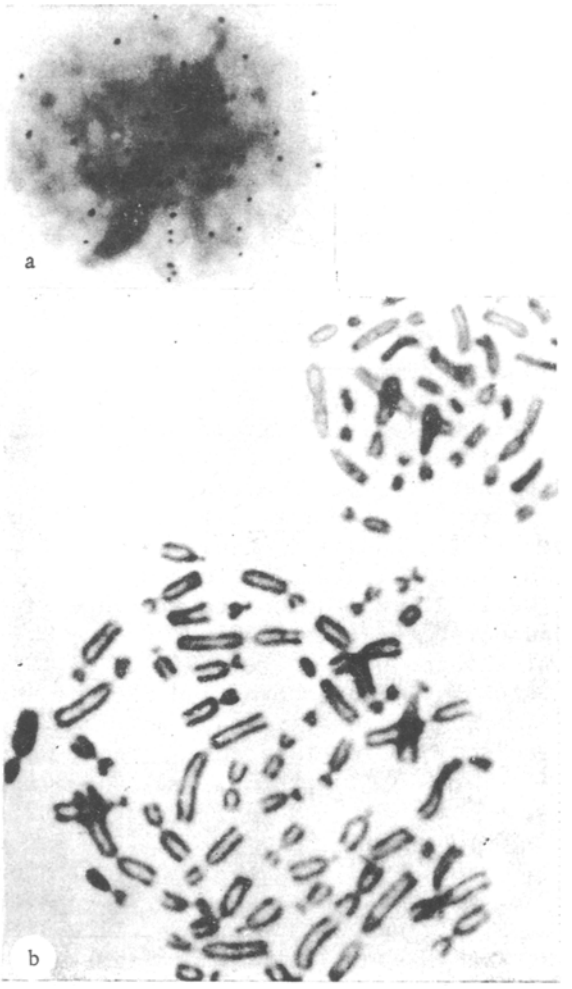

Fig. 2

Fig. 1. Interphase nucleus (a) and metaphase chromosomes (b) of fibroblasts from 8-10-week human embryos in culture. Cells incubated for 60 min in growth medium containing 10 μCi/ml [^{125}I]triiodothyronine. Crystal violet, 1350×.

Fig. 2. Interphase nucleus (a) and metaphase chromosomes (b) of HeLa cells. Cells incubated for 60 min in growth medium containing 10 μCi/ml [^{125}I]triiodothyronine. Crystal violet, 1350×.

gene coding the receptor molecule for androgens. Androgens cannot penetrate into such cells [10, 12].

The absence of acceptance of labeled hormone by chromosomes of HeLa cells, even though they are present at this time in the interphase nucleus, points to the existence of a hitherto unknown linke between penetration of the hormone into the nucleus, i.e., between the processes of translocation and acceptance by genetic material.

In the light of data in the literature on the molecular basis of the phenomenon of androgen insensitivity in men [10, 12], insensitivity to androgens of mice of the Tfm/Y line [5-8, 16], and the steroid-resistance of various normal and transformed cells [9, 14], in which close correlation has been established between mutations of the structural gene of receptor synthesis and the acceptor site of the genome, the writers suggest two alternative explanations of this phenomenon of absence of localization of labeled triiodothyronine on chromosomes of HeLa cells.

1. Intracellular receptors of HeLa cells normally interact with thyroid hormones and are translocated into the nucleus, but the receptor–hormone complex cannot interact with the acceptor site of chromatin as a result of changes in the structure of the site-specific region of the receptor.

2. The receptor-combining sites in the structure of the chromatin of interphase HeLa cells are modified.

However, other explanations of the absence of chromosomal acceptance of thyroid hormones by transformed cells are also possible.

Further investigations of interaction between the chromatin of normal and transformed cells and thyroid hormones, their specific receptors, and the hormone–receptor complex will probably provide a complete answer to this question.

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