Increased Phosphorylation of Liver Histone F_1 Following the Administration of 3,5,3'-Triiodo-L-Thyronine (T_3)

Although the regulation of transcription in eucaryotic cells is still largely unclear, there is growing evidence that phosphorylation of lysine-rich histone F_1 may be involved in this process. In fact, it has repeatedly been suggested that enhanced phosphorylation of histone F_1 could cause derepression of DNA template activity and stimulation of RNA and protein biosynthesis[1-4]. The present study was designed to investigate whether the increased RNA and protein biosynthesis induced in rat liver by T_3 administration[5] may be related to enhanced histone F_1 phosphorylation.

Materials and methods. Normal and thyroidectomized male Wistar rats, 140 to 180 g, were employed. Thyroidectomized rats were used 4 weeks postoperatively. T_3 (Merck A.G., Darmstadt, Germany) was injected i.p. with single daily doses of 30 µg/100 g body wt. for 1 or 4 consecutive days. The rats were killed 16 h after the last injection of the hormone. ^{32}P-phosphate (The Radiochemical Centre, Amersham, England) in 0.14 *M* NaCl containing 10^{-4} *M* carrier inorganic phosphate was injected i.p. at a dosage of 1 mCi/100 g body wt. 2 h before death. All animals were fasted overnight prior to sacrifice. Liver histone F_1 was prepared according to LANGAN[6], and contaminating nonhistone phosphoproteins were removed by chromatography on DEAE-cellulose[7]. Liver inorganic phosphate and alkali-labile histone phosphate were determined by the method of MARTIN and DOTY[8]; ^{32}P radioactivity was measured in a Beckman liquid scintillation counter according to ELLIS et al.[9]. The observed counts were corrected to account for individual variations in the labelling of liver inorganic phosphate pool and expressed as nmoles ^{32}Pi according to TAKEDA and OHGA[10]. Proteins were determined by the LOWRY technique[11].

Results. The data reported in Table I demonstrate that T_3 administered to intact rats significantly stimulated incorporation of ^{32}Pi into liver lysine-rich histone F_1. The rate of histone phosphorylation was increased to about 60% 16 h after a single injection of T_3 and to 224% when the hormone was injected for 4 consecutive days.

As can be seen from Table II, marked increases in histone phosphorylation were also detected in livers from thyroidectomized rats treated with T_3.

Assuming that histone phosphorylation is implicated in the stimulation of transcription, the results reported could suggest that the well-known increase induced by T_3 administration in rat liver RNA and protein biosynthesis is supported, at least in part, by the enhanced phosphorylation of lysine-rich histone F_1.

Table I. In vivo phosphorylation of liver histone F_1 from intact rats treated with T_3

No. of rats	Days of treatment	Rate of histone phosphorylation	Change (%)
10	—	2.35 ± 0.30	
6	1	3.78 ± 0.48	+ 60.8
4	4	7.62 ± 0.64	+ 224.2

The rate of histone phosphorylation is expressed as nmoles ^{32}Pi incorporated/2 h per mg histone. The values are means ± S.D.

Table II. In vivo phosphorylation of liver histone F_1 from thyroidectomized rats treated with T_3

No. of rats	Days of treatment	Rate of histone phosphorylation	Change (%)
10	—	1.80 ± 0.25	
6	1	2.42 ± 0.34	+ 34.4
4	4	5.48 ± 0.65	+ 204.4

The rate of histone phosphorylation is expressed as nmoles ^{32}Pi incorporated/2 h per mg histone. The values are means ± S.D.

Riassunto. Si è dimostrato che la somministrazione di 3, 5, 3'-Triiodo-L-Tironina stimola nettamente la fosforilazione in vivo dell'istone F_1 nel fegato del ratto. Si avanza l'ipotesi che l'aumentata fosforilazione dell'istone F_1 possa favorire, nel fegato del ratto trattato con l'ormone tiroideo, il processo di trascrizione e la biosintesi proteica.

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[1] L. J. KLEINSMITH, V. G. ALLFREY and A. E. MIRSKY, Proc. natn. Acad. Sci., USA *55*, 1182 (1966).
[2] M. G. ORD and L. A. STOCKEN, Biochem. J. *107*, 403 (1968).
[3] T. A. LANGAN, J. biol. Chem. *244*, 5763 (1969).
[4] K. LETNANSKY and L. REISINGER, Biochem. biophys. Res. Commun. *49*, 312 (1972).
[5] J. R. TATA, Biochem. J. *104*, 1 (1967).
[6] T. A. LANGAN, Proc. natn. Acad. Sci. USA *64*, 1276 (1969).
[7] R. H. BUCKINGHAM and L. A. STOCKEN, Biochem. J. *117*, 509 (1970).
[8] J. B. MARTIN and D. M. DOTY, Analyt. Chem. *21*, 965 (1949).
[9] M. K. ELLIS, S. N. WAMPLER and R. H. YAGER, Analyt. chim. Acta *34*, 169 (1966).
[10] M. TAKEDA and Y. OHGA, J. Biochem. *73*, 621 (1973).
[11] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).

The Effect of Isoniazid on Human Chromosomes Studied by a New Method

Isoniazid (IN, isonicotinic acid hydrazide), a drug originally used in the treatment of tuberculosis and more recently as a psychic energizer, was reported to cause chromosomal abnormalities (gaps, breaks, deletions, fragmentations) in bone marrow cells of a rat after a single weekly injection of 65 µg IN/g body weight[1]. When we cultured human peripheral lymphocytes in the presence of IN at several concentrations and for different times of exposure to IN, we did not observe any structural differences under a light microscope between the treated

[1] L. CIRNU-GEORGIAN and V. LENGHEL, Lancet *2*, 93 (1971).