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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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

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Binding of tritiated poly-L-lysine to human metaphase chromosomes

Chromosome	$G_e - G_c$ per chromatid (top)[a] or $\Sigma \Delta G$ per chromatid $\times 10$ (bottom)[b]									Control
	4 hours			24 hours			48 hours			
	20 μg/ml	40 μg/ml	80 μg/ml	20 μg/ml	40 μg/ml	80 μg/ml	20 μg/ml	40 μg/ml	80 μg/ml	
A_1	−2.40	1.10	4.13	−0.53	1.37	0.37	− 1.27	−2.17	−1.73	—
	0.88	−5.46	4.36	−2.94	3.62	− 1.16	1.40	0.16	0.46	−1.40
A_2	−8.68	−6.72	−4.64	−5.90	−8.86	−18.74	6.22	2.66	−8.40	—
	3.50	4.96	5.10	−0.36	0.44	0.12	−12.94	−1.18	−1.14	−8.05
A_3	3.03	−0.97	1.20	−0.93	−0.70	− 1.40	2.30	−2.37	0.07	—
	−2.76	0.26	2.38	0.20	0.68	1.28	− 7.87	1.10	−1.74	0.94

[a] $G_e - G_c$, difference in average number of grains per unit weight ($g \times 10^{-15}$) of chromatid between experimental (IN-treated) and control chromosomes. [b] $\Sigma \Delta G$, summation of the differences in grain density (G) between long, medium and short IN-treated chromosomes $[(G_L - G_M) + (G_L - G_S) + (G_M - G_S)]$.

and the control chromosomes. However, we did observe differences between them in binding of tritiated poly-L-lysine[2] (^{3}H-PL).

Materials and methods. Tissue culture of human peripheral lymphocytes and the preparation of metaphase spreads was done according to the published procedure[3]. IN, at the final concentration of 20 μg/ml, 40 μg/ml and 80 μg/ml was added to cultures at 4, 24 or 48 h before termination of cultures (72 h). Slides containing IN-treated or control metaphase spreads were treated with ^{3}H-PL (mol. wt. 50,000–100,000, specific activity 3.32×10^3 cpm/mg) as follows: 0.1 ml of 0.1% ^{3}H-PL solution in 0.1 *N* acetic acid was applied over chromosomes, and the slide was covered with a cover slip. After 20 min at room temperature the slips were removed, the slides washed with water and dried. After exposure to NTB-2 emulsion for 7 days they were examined under a microscope. Grouping of chromosomal complements according to the degree of contraction was based on the measurement of A_1 chromosome (short, 8.3 μm; medium, 10.0 μm; long, 12.5 μm). Average number of grains per unit weight ($g \times 10^{-15}$) of chromatid for the IN-treated (G_e) and the control (G_c) chromosomes was determined on at least 3 karyotypes for each condition of treatment (over 100 in total). Differences in the average grain density for each chromatid between the three classes of chromosomes (long ,medium, short) were also calculated. A summation of these differences ($\Sigma \Delta G$) was used for comparison of different chromatids. Identification of individual chromosomes in a spread was aided by the application of a Giemsa banding procedure after treatment of chromosomes with trypsin[4,5].

Results and discussion. Over 95% of the total grains was located over the chromosomes and the remainder over the background. Typical karyotypes of ^{3}H-PL-treated chromosomes are shown in the Figure. Density and distribution of grains is different for the IN-treated and the control karyotype. Analysis of a large number of such karyotypes (at least 3 for each condition of treatment, over 100 in total) revealed reproducible differences (G_e–G_c)

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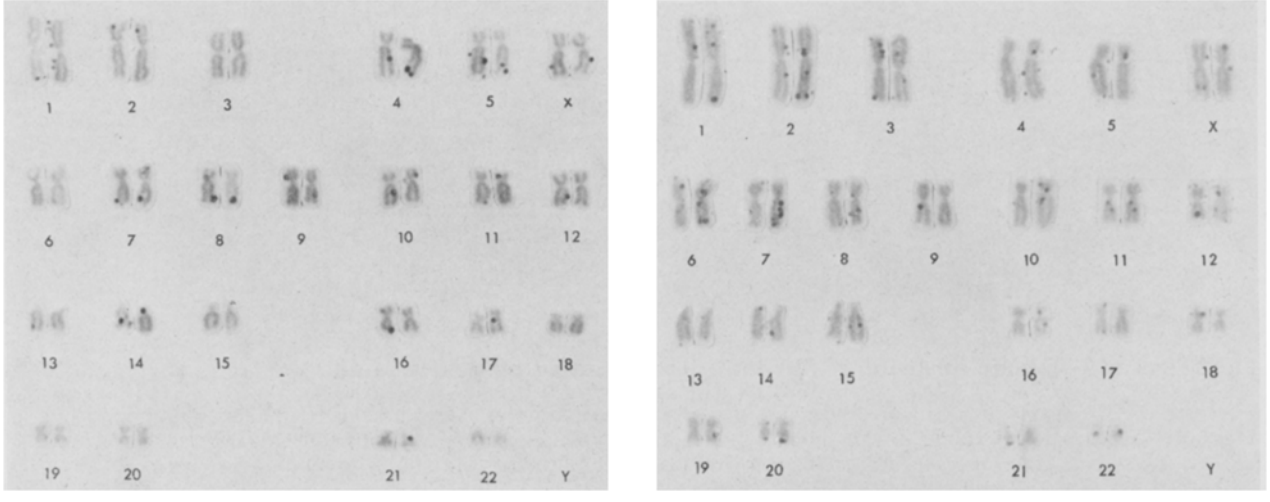

Typical karyotypes of human metaphase chromosomes with bound tritiated poly-L-lysine. Left, chromosomes isolated from isoniazid-treated culture (80 μg/ml, added at 48 h before termination of culture); right, control chromosomes.

in ^{3}H-PL binding between the IN-treated and the control karyotypes. The probable error computed for several groups of karyotypes corresponding to different conditions of treatment was between 15 to 30% of the mean G_e–G_c values.

The G_e–G_c values for A group chromosomes are given in the Table. The most striking effect of IN-treatment was on A_2 chromosome. It did bind less ^{3}H-PL than the control A_2 chromosome under nearly all conditions of IN-treatment. It was also observed that there was a dependence of ^{3}H-PL binding on the degree of chromosomal contraction (Σ ΔG values). A long A_2 chromosome exposed to IN for 4 h did bind more ^{3}H-PL than a short A_2 chromosome. The opposite result was obtained for the 48 h exposure time. In addition, there was observed for A_2 chromosome a gradual decrease of Σ ΔG with the exposure time for each IN dose employed.

The results obtained for A group chromosomes, particularly for A_2 chromosome, suggest that the organization of chromosomal nucleic acids and proteins might be different in IN-treated chromosomes than in controls. The ^{3}H-PL binding would be expected to occur most readily in those regions of metaphase chromosomes that contain exposed segments of nucleic acids. The binding occurs through ionic interactions between positively charged ε-amino groups of ^{3}H-PL and negatively charged phosphodiester linkages of nucleic acids. At least 10 to 20 such interactions would be required per one ^{3}H-PL molecule to maintain a stable complex, since the interaction energy for one mole of ion pairs is of the order of 1 Kcal.

Zusammenfassung. Nachweis, dass die Bindung von tritiummarkiertem Poly-L-Lysin (^{3}H-PL), mit den Chromosomen der A-Gruppe (speziell mit dem A_2-Chromosom) aus isoniazidhaltigen Kulturen der menschlichen Lymphocyten isoliert, verschieden von der Bindung desselben mit nicht behandelten Chromosomen war. Das Bindungsvermögen des behandelten A_2-Chromosoms ist überdies von der Chromosomenlänge abhängig.

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The Chromosomes of Italian Sturgeons

The cytogenetics of the Acipenseridae is interesting for two main reasons: 1. they are rather primitive fishes; 2. their taxonomy is complicated; many interspecific and intergeneric hybrids are described[1] and artificially obtained[2].

The only careful description of the karyotype so far published, to our knowledge, is that by Ohno et al.[3] of the shovelnose sturgeon, *Scaphirhynchus platorhynchus*, which has about 112 chromosomes (diploid number) with about 48 dot-like microchromosomes. The karyotypes of *Huso huso* L., *Acipenser ruthenus* L., and their hybrid were studied by Serebryakova[4]; but the microchromosomes were not noticed and complements of only 60 chromosomes were described for both species and their hybrids. Also for the *A. stellatus* Pall. and *A. nudiventris* Lov. karyotypes of 60 chromosomes were described, whilst *A. güldenstädti* Br. should have more than 130 chromosomes[2].

In Northern Italy, 3 species are present[5]: *Huso huso* L., *Acipenser sturio* L. and *Acipenser Naccarii* Bonaparte; this last species is found only in the North Adriatic. We studied the karyotypes of some specimens of these 3 species, collected from the Po river during spring and autumn 1973.

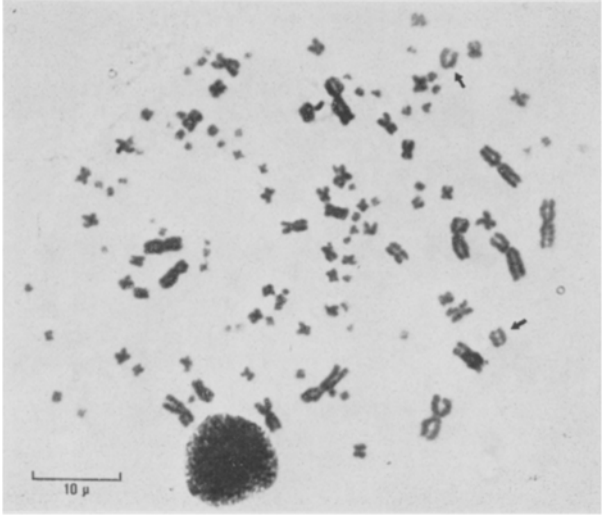

Fig. 1. Metaphase plate of *Huso huso*, arrows indicate the pair of large acrocentric chromosomes.

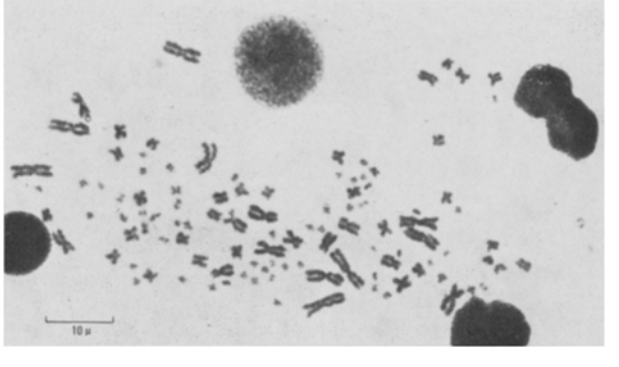

Fig. 2. Metaphase plate of *Acipenser sturio*.

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