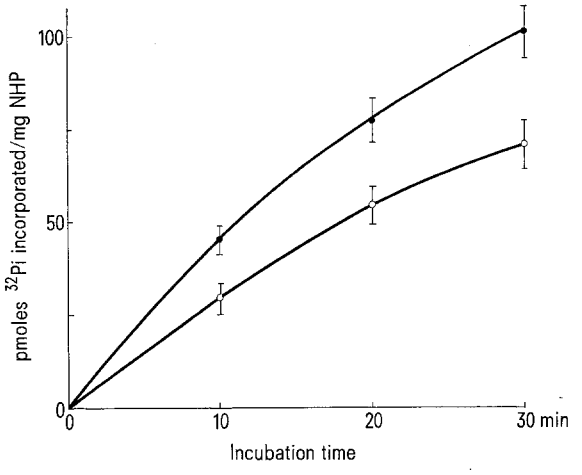
Stimulation of Nuclear Protein Kinase Activity in Rat Liver Induced by the Administration of 3,5,3'-Triiodo-L-Thyronine (T_3)[1]

Considerable evidence has accumulated recently which suggests that the chromatin non-histone phosphoproteins are involved in the regulation of gene activity[2,3]. The phosphate groups bound to these proteins have been shown to turn over rapidly, and the phosphorylation and dephosphorylation reactions are thought to induce an alteration in the conformation of these non-histone proteins, an alteration which, in turn, leads to changes in the structure and template activity of the chromatin[4,5].

If the non-histone phosphoproteins do indeed play a role in the control of gene expression, a study of the protein kinase activity responsible for their phosphorylation is important in order to understand this process in mammalian cells.

The present study was designed to investigate whether the stimulated RNA biosynthesis, induced in rat liver by T_3 administration[6], may be related to an increase of the protein kinase activity associated to non-histone proteins. In fact, it has been demonstrated that the chromatin non-histone protein preparations are endowed with kinase activity and are actively phosphorylated without addition of exogenous enzyme[7,8].



Effect of T_3 on the rate of phosphorylation of liver non-histone chromatin protein (NHP) by the associated kinase activity. For the enzyme assays, the standard reaction mixture (1.0 ml), prepared according to TAKEDA et al.[10], contained 500 µg of NHP obtained from the liver of control rats (○) or of rats treated with T_3 for 2 consecutive days (●). The points represent the mean ± SD of 12 experiments. In each experiment liver NHP pooled from 5 rats was used.

Effect of T_3 administration on the phosphorylation of casein by NHP-associated protein kinase activity

Days of treatment	No. of experiments	Rate of casein phosphorylation	Increase (%)	Student's t-test (p)
–	10	262 ± 38		
1	5	330 ± 54	+25.9	< 0.01
2	8	372 ± 83	+42.0	< 0.01

The rate of phosphorylation is expressed as pmoles ^{32}Pi incorporated into casein/20 min per mg of NHP isolated from the livers of control or T_3 treated rats and 800 µg of casein as substrate. The values are corrected for the incorporation of ^{32}Pi into NHP and represent the mean ± SD of the experiments. In each experiment, non-histone proteins pooled from 4 to 6 rats were used.

Materials and methods. Thyroidectomized male Wistar rats, 140 to 180 g, 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 2 consecutive days. The rats were killed 18 h after the last injection of the hormone. All animals were fasted overnight prior to sacrifice.

Liver non-histone proteins were isolated by the method of WANG[9]. This consisted of dialyzing the 1 *M* NaCl nuclear extract against water to 0.15 *M* with respect to NaCl. By this method, DNA- histone precipitated while the non-histone proteins remained in the supernatant fraction. The dilute non-histone protein solutions were concentrated in the cold by dehydrating in dialysis tubing covered with polyethylene glycol. Aliquots of this fraction were used for protein kinase determination according to TAKEDA et al.[10]. ATP-γ-^{32}P (250,000 cpm/nmole; the Radiochemical Centre, Amersham, England) as phosphoryl donor, and casein (Sigma Chemical Co. Ltd., St. Louis, MO, USA) as substrate, were utilized. The reaction was terminated and the protein-bound ^{32}P was estimated by the method B of REIMANN et al.[11]. Proteins were determined by the technique of LOWRY et al.[12].

Results and discussion. The Figure illustrates the incorporation of ^{32}P from ATP-γ-^{32}P into non-histone protein fractions obtained from control and T_3 treated rat livers without addition of exogenous enzyme. As can be seen, this protein kinase activity is significantly stimulated in liver preparations from thyroidectomized rats treated with T_3 for 2 consecutive days.

Additional evidence for the increase in protein kinase activity induced by T_3 administration was obtained in the assays with added casein which was routinely employed as a model substrate. The results presented in the Table demonstrate that the phosphorylation of casein is stimulated by 25.9% and 42.0% in liver preparations from animals treated with T_3 for 1 and 2 consecutive days, respectively.

In another set of experiments not reported here, it was shown that the protein kinase activity associated with chromatin non-histone proteins is able to phosphorylate casein or phosvitin but does not utilize at all calf thymus histone or protamine as substrate. Furthermore, in agreement with the observations of TAKEDA et al.[10], this kinase activity was not stimulated by 3',5'-cyclic AMP.

In conclusion, our data demonstrate that T_3 administration induces an increase in the kinase activity associated to non-histone proteins of chromatin. Such an increase

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probably depends on higher enzyme level. As far as we know, no earlier investigations are available on the stimulatory effect of thyroid hormones on this nuclear protein kinase activity.

Although the physiological roles of the nuclear protein kinases are yet to be clearly understood, it is tempting to speculate that the stimulated protein kinase activity, induced by T_3 administration, may result in an increase in non-histone protein phosphorylation, which could lead in turn to enhanced template activity of chromatin and hence to the well-known increase in liver RNA biosynthesis. The possibility of an enhanced template activity of rat liver chromatin following thyroid hormone administration warrants further investigation.

Riassunto. Si è dimostrato che la somministrazione di 3, 5, 3′-Triiodo-L-Tironina esalta nettamente l'attività protein cinasica associata alle proteine non istoniche della cromatina epatica del ratto tiroidectomizzato. Tale cinasi, che fosforila le proteine non istoniche della cromatina, la caseine e la fosvitina, non agisce sugli istoni o sulla protamina e non viene stimolata dal 3′, 5′-AMP ciclico. Si prospetta l'ipotesi che nel fegato del ratto trattato con l'ormone tiroideo la maggiore attività della cinasi associata alla cromatina possa aumentare il contenuto in fosfato delle proteine non istoniche ed esaltare il processo della trascrizione.

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Fibrillation of α-Elastin Induced by Proteoglycan

α-Elastin, a high-molecular weight polypeptide derived from insoluble elastin[1], undergoes reversible coacervation at high temperatures. On prolonged coacervation, an insoluble product is formed which shows elasticity typical for native elastin[2]. It has been shwon recently that coacervation of α-elastin results in fibril formation[3].

Formation of an insoluble elastic product from α-elastin has been observed to take place also due to interaction of α-elastin with a number of sulphated polysaccharides[4], some of which are contained in connective tissue in the form of proteoglycans. It has been well established that these proteoglycans interact with collagen[5,6] and are able to influence the course of its fibrillation[7].

The present paper reports on fibrillation of α-elastin as a result of interaction of α-elastin with connective tissue proteoglycan. This observation suggests a possible involvement of proteoglycans in elastogenesis.

Materials and methods. Insoluble elastin was prepared from bovine ligamentum nuchae by hot alkali treatment[8]. The insoluble elastin was hydrolyzed with oxalic acid[1] and α-elastin was isolated from the hydrolysate and purified by repeated coacervation at 60–80 °C and adsorption of coacervated α-elastin on Super Hyphlo Cell. No contaminating material was detected in the purified product.

Proteoglycan was prepared from bovine nasal cartilage by the dissociative procedure[9]. Isoelectric focusing revealed typical proteoglycan bands with isoelectric points of 3.6–3.9. No contaminating material was present.

The occurrence of interaction between α-elastin and proteoglycan was established by optical density measurements at 440 nm of α-elastin – proteoglycan mixtures in water solutions at varying ratios of both components and constant total concentration.

Electron microscopic observation was carried out on a Tesla-BS 613 electron microscope.

α-Elastin – proteoglycan interaction product (complex coacervate) was prepared from 108 μg of α-elastin plus 24 μg of proteoglycan per 1 ml of water (maximum interaction) at pH 3.0. The samples were negatively stained with a 12 m*M* uranyl acetate – 19.5 m*M* oxalic acid solution[3], pH 3.0.

Results. The plot of optical density at 440 nm vs. α-elastin/proteoglycan ratio at 20 °C demonstrated the occurrence of interaction at pH values 2.5 (the lowest pH followed) to 4.0–4.5. As an example, the dependence is shown of the complex coacervate formation on α-elastin/proteoglycan ratio at pH 3.0 (Figure 1).

Electron microscopic observation revealed in the complex coacervate the presence of a certain amount of amorphous material together with a significant proportion of typical fibrillar structures (Figure 2). In the fibrils, fine filaments were clearly discernable. The maximum observed width of fibre was 5000 Å.

Discussion. Interaction between α-elastin and proteoglycan under suitable ionic conditions results in the formation of complex coacervate. The first stage of the complex coacervate formation is the electrostatic interaction between oppositely charged macroions through which

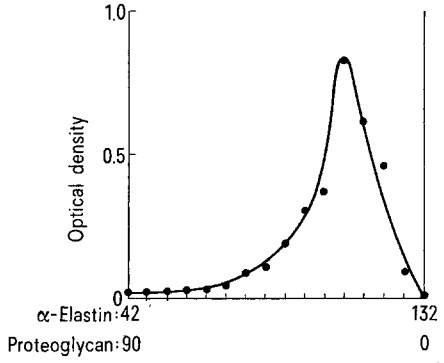

Fig. 1. Optical density at 440 nm vs. α-elastin/proteoglycan ratio. The amounts given are in μg/ml.

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