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REGULATION BY THYROID HORMONE OF THE SYNTHESIS OF A CYTOSOLIC THYROID HORMONE BINDING PROTEIN DURING LIVER REGENERATION

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Abstract - To understand the regulation by thyroid hormone, 3,3',5-triiodo-L-thyronine (T_3), of the synthesis of a cytosolic thyroid hormone binding protein (p58-M_2) during liver regeneration, the synthesis of p58-M_2 was evaluated. The synthesis of p58-M_2 was measured by metabolic labeling of primary cultures derived from the regenerating liver of euthyroid, hypo- or hyperthyroid rats. During regeneration, the increase in the liver/body weight ratio is ~25% higher in hyper- than in hypothyroid rats. However, T_3 has no effect on the rate of overall liver regeneration observed in four days. In mature liver, T_3 increased the synthesis of p58-M_2 by ~2.5-fold. During regeneration, however, the change in the synthesis of p58-M_2 varied with the thyroid status. In euthyroid rats, the synthesis of p58-M_2 continued to increase up to 2-fold during liver regeneration. In hyperthyroid rats, after an initial increase by 1.5-fold on day 1, the synthesis of p58-M_2 subsequently declined during regeneration. In hypothyroid rats, the synthesis of p58-M_2 remained virtually unchanged during regeneration. These results indicate that T_3 regulates the synthesis of p58-M_2 in mature and regenerating liver.

Introduction - We have purified a cytosolic thyroid hormone binding protein from human epidermoid carcinoma A431 cells (1). It has a molecular weight of 58,000 (p58-M_2). Its cDNA was isolated and characterized (2). Sequence analysis indicates that it is a subunit of pyruvate kinase, subtype PKM_2. p58-M_2 binds to T_3 and exhibits analog specificity. Tetrameric PKM_2 does not bind T_3 (2,3). *In vitro* fructose

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Abbreviations: T_3, 3,3',5-triiodo-L-thyronine; p58-M_2, pyruvate kinase, subtype M_2; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; mAb, monoclonal antibody; TCA, trichloroacetic acid.

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1,6-bisphosphate, a metabolite of the glycolytic pathway, stimulates the association of the monomeric p58-M_2 to the tetrameric PKM_2 (2,3). *In vivo*, at the physiological concentration of glucose, 30-35% of PKM_2 exist as a monomer. However, PKM_2 dissociates into monomer upon deprivation of glucose due to a reduction of intracellular fructose 1,6-bisphosphate (4). By manipulating the glucose concentration in the medium to change the intracellular concentration of p58-M_2, we found that the T_3-dependent transcriptional activity of T_3 nuclear receptor is modulated by p58-M_2. Thus, p58-M_2 functions as a regulator for cytosolic T_3 (5).

The above findings were derived from studies using cultured cells. The function(s) of p58-M_2 in target tissues is currently unknown. To assess further the functional role of p58-M_2 in thyroid hormone action, the present study evaluated the effect of T_3 on the synthesis of p58-M_2 in mature and regenerating liver.

Materials and Methods

Materials: L-[^{35}S]methionine (1050 Ci/mmole) were from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Methionine-free minimum essential medium was prepared in the National Institutes of Health media unit. Methimazol was obtained from Nacala Tesque (Kyoto, Japan). 3,3',5-triiodo-L-thyronine, collagenase, phenylmethanesulfonyl fluoride, leupeptin, aprotinin and 3-[(3-cholamidopropyl)dimethylammoni)-1-propanesulfonate (CHAPS) were obtained from Sigma Co. (St. Louis, MO). Monoclonal antibody J11 was prepared and purified as described by Obata et al. (6).

Preparation of animals: Male Sprague-Dawley rats, 200-250g (Nippon Rat Co, Ltd) were used for all studies. Hypothyroidism was induced as described by Freake and Oppenheimer (7). The rats were fed methimazole (0.1% w/v) in the drinking water for three weeks. Serum T_3 was not detectable and no apparent weight gain was apparent. Hyperthyroidism was induced by injecting T_3 (10 μg/100g body weight/day) for one week before and after hepatectomy. Euthyroid rats were used as a control. All rats underwent surgery to remove ~70% of liver (medium and left lateral lobes) by the methods of Higgins and Anderson (8).

Metabolically labeling of p58-M_2 in hepatocyte cultures: After hepatectomy, the liver was perfused with collagenase (0.05%, w/v) in Leibovitz's L-15 salt solution (L15 buffer, 136.9 mM NaCl, 5.36 mM KCl, 1.4 mM KH_2 PO4/pH 7.4) for ~10 min at 37°C. A portion of liver was removed and digested with collagenase for an additional 5 min. The released cells were filtered through platinum metal mesh (80-gauge) and collected by centrifugation (500 xg, 1 min). The parenchymal cells were washed with L15 buffer once and cultured in minimal essential medium containing 5% (v/v) of fetal bovine serum. Hepatocytes (3 x 10^8 cells/35 mm dish) in 3 ml of medium were labeled with [^{35}S]methionine (0.5 mCi/dish) for 5 hrs at 37°C.

Immunoprecipitation of p58-M_2: After labeling the cells with [^{35}S]methionine for 5 hrs, the hepatocytes were washed with Ca^{++} and Mg^{++}-free phosphate-buffered saline. Cells were extracted by shaking gently in 2 ml of buffer containing 3 mM CHAPS, 50 mM NaCl, 3 mM PO4/pH 7.4, 1 mM EDTA, leupeptin (1 μg/ml), aprotinin (20 μg/ml) and phenylmethanesulfonyl fluoride (0.5 mM) for 30 min at 4°C. The cellular extracts were clarified by centrifugation (105,000 xg) for 30 min. One ml of cellular extract was used for reacting with the monoclonal antibody J11 (5 μg/ml). Analysis of the immunoprecipitates by SDS-PAGE was

carried out as described previously (6). In some experiments, the secreted albumin in culture medium was analyzed by SDS-PAGE. The radioactive bands were quantified by a computerized densitomer (Ambis System, San Diego, CA).

The newly synthesized proteins were estimated by the TCA precipitation method. Ten μl of the [^{35}S]methionine-labeled cellular extracts or medium was spotted onto GF/C glass filter. The glass filter was boiled in 5% (w/v) TCA for 3 min followed by washing with 5% (w/v) TCA twice. The filter was incubated with 1 ml of solubilizer for 24 hrs. After addition of scintillation cocktail, the radioactivity was determined in a β-counter.

Results

Effect of thyroid hormone on liver regeneration: To understand the effect of thyroid hormone on the synthesis of p58-M_2 during liver regeneration, we first examined the regeneration response. We evaluated the rate of the restoration of liver mass under euthyroid, hyper- and hypothyroid conditions. As shown in Fig. 1, the liver/body weight ratio continued to increase up to four days under all three conditions. In euthyroid rat, the rate of restoration of liver mass is 0.0063 ± 0.001/day (mean ± S.D. n=3). On day 4, the ratio is 0.038 which is similar to that reported by Higgins and Anderson in which a ratio of 0.03 was reported (8). Figure 1 also shows that the increase in the liver/body weight ratio is ~25% higher in hyper- than in hypothyroid rats. However, the rates of regeneration for hyper- and hypothyroid rats are 0.0075 ± 0.0005 and 0.00625 ± 0.001/day, respectively. These results indicate that thyroidal status does not affect the rate of regeneration.

T_3 increases the synthesis of p58-M_2: Primary cultures of liver from euthyroid, hyper- and hypothyroid rats were prepared each day during regeneration. Cells were metabolically labeled with [^{35}S]methionine and p58-M_2 was immunoprecipitated with a specific antibody against p58-M_2 (6). Figure 2 shows the synthesis of [^{35}S]methionine-labeled p58-M_2 during regeneration under three

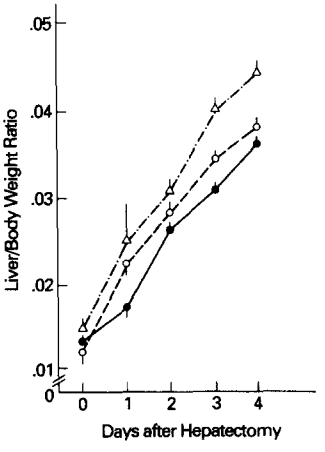

Figure 1. Rate of liver regeneration from rats of different thyroidal state. Hepatectomy was carried out as described in Methods. The weights of wet liver and rat was determined each day for four days.

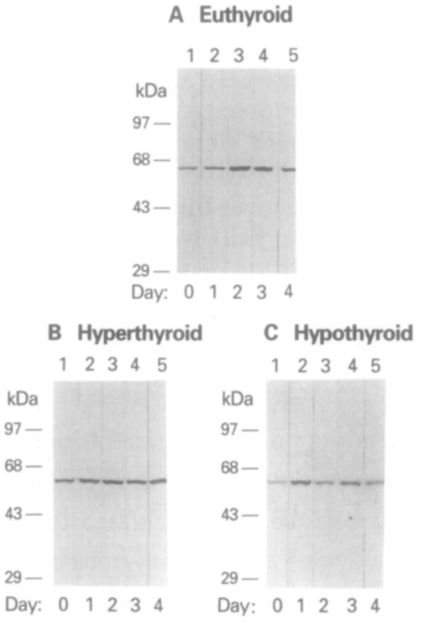

Figure 2. Autoradiograms of the p58-M_2 after immunoprecipitation with mAb J11.

Isolated hepatocytes ($3x10^8$ cells/35 mm dish) were incubated with 0.5 mCi of [^{35}S]methionine in 3 ml methionine-free medium for 5 hrs at 37°C. Cellular extracts were prepared and immunoprecipitated with mAb J11 (5 μg/ml) as described in Methods. The immunoprecipitates were analyzed by SDS-PAGE. The gel was dried and autoradiographed. Lane 1, before hepatectomy, lanes 2, 3, 4, and 5 are 0,1,2,3 and 4 days after hepatectomy, respectively.

conditions. The intensity of the bands was quantified by a computerized densitomer and the data were compared as shown in Fig. 3 and Fig. 4.

The data on day 0 represent the cellular level of p58-M_2 before hepatectomy under these conditions. Comparism of the p58-M_2 levels in the liver of hyper- (lane 1 in Fig. 2B) and hypothyroid rats (lane 1 in Fig. 2C) indicates that T_3 stimulated the synthesis of p58-M_2 by ~2.5 fold.

Effect of T_3 on the synthesis of p58-M_2 during liver regeneration: The effect of T_3 on the synthesis of p58-M_2 and albumin during regeneration is compared in Fig. 3. Since the rate of the synthesis of albumin in the regenerating liver has been reported by several laboratories (9,10), we also measured the synthesis of albumin as a positive control. Fig. 3 indicates that in euthyroid rat, synthesis of albumin was slightly increased (~25%) after 24 hr., but the level of albumin remained virtually constant during subsequent days of liver regeneration. This observation is consistent with the earlier reports in which only a slight change in the net rate of albumin synthesis was reported in the regeneration of liver of euthyroid rats (9,10).

The increase in the synthesis of p58-M_2 during regeneration, however, is more pronounced than albumin. Initially, a small increase (~25%) was observed after 24 hrs. After 48 hrs, a 2-fold increase was seen. This increase was sustained for

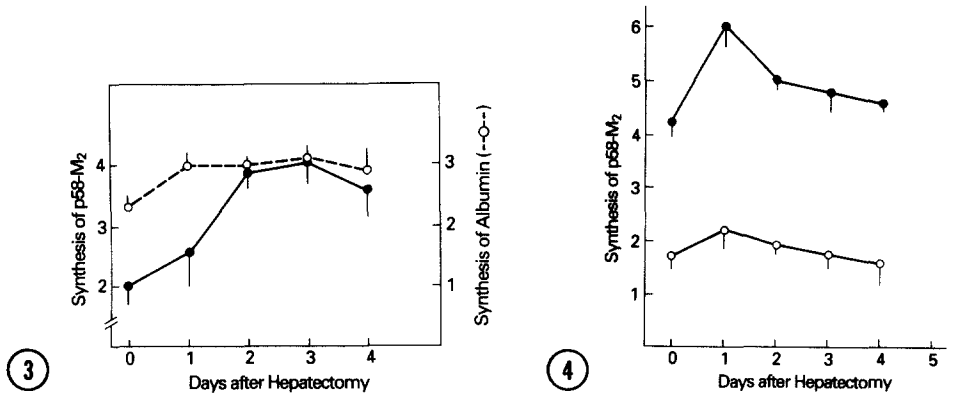

Figure 3. Synthesis of p58-M_2 in liver of euthyroid rat during liver regeneration.

The radioactive bands of p58-M_2 (-●-) bands from Fig. 2A were quantified by a computerized densitometer. Albumin (--O--) from the medium was determined similarly. The data are expressed as the ratio of the newly synthesized protein versus total synthesized proteins. Data were obtained in duplicate and the average of three experiments is shown. The bars represent standard deviations.

Figure 4. Synthesis of p58-M_2 in liver of hyper- and hypothyroid rats.

p58-M_2 from the liver of hyperthyroid (-●-) and hypothyroid (-O-) rats shown in Fig. 2B and 2C were quantified. Data were obtained in duplicate and the average of three experiments is shown. The bars represent standard deviation.

an additional 24 hrs. However, on day four, the synthesis of p58-M_2 began to decline (Fig. 3).

The change in the synthesis of p58-M_2 in the liver of hyperthyroid rats is different from that of euthyroid rats. Figure 4 shows that an initial 1.5-fold increase of p58-M_2 was seen 24 hrs after hepatectomy. However, it was followed by a steady decline. On day 4, the level was restored to the same level as on day 0 which is 2-fold higher than that in euthyroid rats (see also Fig. 3).

The synthesis of p58-M_2 in the liver of hypothyroid rat was not affected significantly by regeneration. As shown in Fig. 4, the level of p58-M_2 is lower than those in the liver of normal and hyperthyroid rats. Initially, a small increase was seen after 24 hrs., but it was followed by a slow and steady decline.

Discussion

The present study evaluated the effect of T_3 on the synthesis of p58-M_2 before and during liver regeneration. Our results clearly indicate that the synthesis of p58-M_2 is regulated by T_3. T_3 up-regulated the cellular level of p58-M_2 by ~2.5-fold. These results are similar to p58-M_1 which was found to be up-regulated by T_3 in GH_3 cells (11). p58-M_1 is a subunit of pyruvate kinase, subtype M_1. It also binds thyroid hormone with affinity and specificity in GH_3 cells (12). The T_3-induced stimulatory effect on the expression of p58-M_2 has important implications. p58-M_2 is a subunit of PKM_2. PKM_2 is a key enzyme for the generation of ATP in the glycolytic pathway.

The increase in the expression of the enzyme responding to the increase of T_3 is entirely consistent with the increase of metabolic rate observed in hyperthyroidism.

p58-M_2 is a multifunctional protein. In addition to being a subunit for PKM_2, it is a T_3 binding protein. Recently, we have demonstrated that p58-M_2 functions as a regulator for cytosolic T_3 (5). By regulating the availability of the free cytosolic T_3 to the T_3 nuclear receptors, p58-M_2 indirectly modulates the gene regulatory activity of T_3 nuclear receptors. The findings that the level of p58-M_2 correlates with the change in thyroidal status is consistent with its functional role as a cytoplasmic regulator for T_3.

The present study indicates that livers of hyper-, hypo- and euthyroid rats regenerate with the same rate after partial hepatectomy. Previously, the effects of thyroid hormone on liver regeneration had been evaluated using rats which were treated with thyroxine or thiouracil and no significant effect was detected (13,14). These data suggest that the initiation and/or sustaining of liver regeneration is not dependent on thyroidal status. Therefore, T_3 probably is not essential for the interplay of many factors in the transcriptional machinery during liver regeneration. Our data do indicate, however, that the increase in the synthesis of p58-M_2 is coupled to regeneration only in the liver of euthyroid and hyperthyroid rats. In euthyroid rat, the increase in the synthesis of p58-M_2 was detected on day 1 and was maximal on days 2 and 3. In hyperthyroid rat, the p58-M_2 levels are ~2-fold higher than that in euthyroid rat. A thrust of an additional 1.5-fold increase was seen in the synthesis of p58-M_2 in the liver of hyperthyroid rats 24 hrs. after hepatectomy. Therefore, T_3 controls the synthesis of p58-M_2 not only in the differentiated adult liver but also in the regenerating liver.

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