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Thyroid hormone regulates tubulin expression in mammalian liver. Effects of deleting thyroid hormone receptor-α or -β

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Vallejo, Carmen G., Ana M. Seguido, Pilar S. Testillano, and María-Carmen Risueño. Thyroid hormone regulates tubulin expression in mammalian liver. Effects of deleting thyroid hormone receptor-α or -β. *Am J Physiol Endocrinol Metab* 289: E87–E94, 2005. First published February 15, 2005; doi:10.1152/ajpendo.00436.2004.— Microtubules are made from polymers of α/β dimers. We have observed in rat liver that, on the first day after birth, α-subunit is relatively high and β-subunit low with respect to adult values. In the hypothyroid neonate, both subunits were found to be low, therefore indicating that thyroid hormone (TH) regulates these developmental changes. TH was also found to activate tubulin expression in adult liver, especially β-subunit. To investigate the role of TH receptors (TRs) in tubulin expression, we analyzed mice lacking TRα or TRβ compared with the wild type in both normal and TH-deprived adult animals. The results suggest that, in vivo, β-tubulin protein expression in the liver is primarily under TRβ positive control. In euthyroid mice lacking TRβ, β-tubulin expression was low. However, in the corresponding hypothyroid animals, it was found increased, therefore suggesting that the unliganded TRα might also upregulate β-tubulin expression. Accordingly, TH administration to hypothyroid TRβ-deprived mice reduced their high β-tubulin expression. In parallel, the relatively high messenger level observed with these hypothyroid animals was reduced to the euthyroid level after T_3 treatment. The microtubular network of the mutant livers appeared, by immunofluorescence confocal microscopy, generally disorganized and drastically reduced in β-tubulin in mice lacking TRβ. In conclusion, our results indicate that β-tubulin is critically controlled by TRβ in the liver and that both TRs are probably needed to maintain the microtubular network organization of the liver.

rat; knockout mice; Northern analysis; Western analysis; histological examination; immunofluorescence confocal microscopy

MICROTUBULES ARE MADE FROM POLYMERS of α/β dimers and characterized by high dynamic instability, turning over every few minutes in interphase cells or even seconds in mitotic spindles. These characteristic dynamics endow microtubules with the capacity of exploring the cell and intervening in multiple processes involving traffic of molecules or organelles, cell division, or cell shape. These functions are accomplished by periods of microtubules growing and shrinking, which are processes of high-energy cost. Different cellular factors probably contribute to microtubule turnover in cells, a subject of present intense research (8). In this paper, we examine the influence of thyroid hormone (TH) on tubulin protein expression in both rat and mouse liver and how the deletion of TH receptor (TR)α or -β affects this expression in the liver. TH exerts the physiological actions of increasing or decreasing expression of target genes by binding specific transcription factors, the nuclear TRs that recognize TH-responsive elements on DNA (17). There are two TR genes, α and β, that generate each TR form by alternative splicing and several truncated forms of which some do not bind triiodothyronine (T_3) (9, 25). The unliganded TRs may have a different effect on expression than those bound by TH (6–8). How the two TR gene products contribute differentially to TH-mediated responses is only beginning to be understood. The recent generation of knockout mice that lack a particular TR isoform is allowing studies that permit ascribing specific functions to individual TR isoforms (6, 7, 16, 18, 24). In the present work, we present data indicating that tubulin expression, especially β-tubulin, is under TH regulation, at both the protein and the RNA levels. Analysis in mice with TRα or TRβ deficiency suggests that, in the liver, tubulin expression is under TRβ control and that the unliganded TRα may also upregulate. The microtubular network of the mutant livers appeared disorganized and decreased to a different extent depending on the deleted TR isoform, thus suggesting that TH regulates both tubulin expression and organization.

EXPERIMENTAL PROCEDURES

Animals and treatments. Maintenance and handling of animals were in the IIB animal facility (no. 28079-37-A), according to BOE RD 223/88 and EEC Directive 86/609. The animal procedures were revised and approved by the Consejo Superior de Investigaciones Científicas Bioethics Committee. For rat studies, adult male Wistar rats produced in our animal facilities were used. Hypothyroidism was induced by surgical thyroidectomy and the supply of 0.05% 2-mercapto-1-methylimidazole in the drinking water for ≥1 mo before the start of the experiment. In the TH-treated rats, T_3 (20 μg/100 g body wt) was administrated daily by intraperitoneal injections for 3 days. The normal (euthyroid) and hypothyroid animals received only vehicle injections. In the experiments with postnatal rats, to induce hypothyroidism, 0.02% 2-mercapto-1-methylimidazole and 1% sodium perchlorate were administered in the drinking water to the pregnant rats from *day 9* of pregnancy, treatment that was continued until the animals were killed.

For mouse experiments, mice completely deficient in TRα ($TR\alpha^{0/0}$) or TRβ ($TR\beta^{-/-}$), generated respectively as described (6, 16) and independently bred, were used together with wild-type mice of the same strain. $TR\alpha^{0/0}$ mice lack TRα1, TRα2, TRΔα1, and TRΔα2. $TR\beta^{-/-}$ mice lack TRβ1 and TRβ2. Hypothyroidism was induced by administration of 0.02% 2-mercapto-1-methylimidazole and 1% sodium perchlorate in the drinking water to adult male mice for 8 wk. Hormonal treatment was accomplished by daily intraperitoneal injections of T_3 (2 μg/100 g body wt) for 4 days, as described (16), and experiments were terminated 4–5

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h after the last injection. The control groups of animals received only vehicle injections.

Protein extraction and Western blot analysis. Total liver extracts were obtained either by homogenizing in medium [210 mM mannitol, 70 mM sucrose, 1 mM EDTA, 5 mM Tris·HCl, pH 7.5, plus protease inhibitors (PMSF, E-64, pepstatin A)] or in boiling medium (65 mM Tris·HCl, pH 6.8, 10% glycerol, 0.14 M mercaptoethanol, 2% SDS), followed by centrifuging at 17,400 *g* for 20 min (22). Proteins (25–60 mg) were separated in 10% SDS-polyacrylamide gels and transferred to Immobilon-P membranes (Millipore, Bedford, MA). The Western blots were probed with monoclonal α- (Sigma, T9026; St Louis, MO) and β-tubulin (isotypes I and II, Sigma T8535; NeoMarkers MS 582-P; Fremont, CA) and polyclonal actin (Sigma, A2026) antibodies. Protein disulfide-isomerase polyclonal antibody (19) was used to control load. As secondary antibodies, anti-mouse and anti-rabbit peroxidase conjugates (Bio-Rad, Hercules, CA) were employed. The immunoreactive proteins were visualized by chemiluminescence (ECL detection system; Amersham, Buckinghamshire, UK).

RNA extraction and Northern analysis. Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA) from frozen mouse liver and brain samples. Twenty micrograms of RNA were separated on 1.5% (wt/vol) agarose-formaldehyde gels and transferred onto Zeta-Probe blotting membranes (Bio-Rad) with 10× SSC (1× SSC = 150 mM NaCl and 15 mM sodium citrate, pH 7.0). Hybridization was accomplished in 7% SDS and 0.25 M sodium phosphate, pH 7.4 or alternately, when β-tubulin probes were used, in Ultrahyb hybridization buffer (Ambion, Austin, TX) that effectively increases hybridization signal. Probes for β-tubulin isotypes 2 (II) and 5 (I) (21), 5′-deiodinase type 1 (3), and 7S (1) were labeled with ^{32}P by use of a random priming system (Amersham).

Histological methods and immunofluorescence studies. Mice under anesthesia were perfused either transcardially or by cannulation of the portal vein with 4% (wt/vol) paraformaldehyde in phosphate-buffered saline (PBS) buffer, pH 7.4, maintaining the perfusion fluids at 37°C. The livers were dissected out, immersed in buffered 4% paraformaldehyde for 9 h at 37°C, washed in PBS (5 min at 37°C, three times) and transferred to buffered 0.1% paraformaldehyde for storage at 4°C.

For examination of liver structure, fixed samples were dehydrated in an ethanol series and embedded in Araldite resin. Semithin sections (1 μm) were obtained from resin blocks, stained with toluidine blue, and observed under phase contrast on a Zeiss photomicroscope.

For immunofluorescence assays, sections (40 μm) were obtained with a vibratome directly from the fixed samples. Microtubular structure was analyzed by immunofluorescence and confocal microscopy observation. Sections were deposited on a drop of buffered 4% paraformaldehyde in slides with excavated wells covered with (3-aminopropyl)triethoxysilane (APTES, Sigma) to improve section adhesion, dried, and stored at 4°C. The sections were permeabilized by incubation in 0.5% Triton X-100 for 20 min, washed in PBS, and incubated with 5% bovine serum albumin (BSA) in PBS for 10 min. That was followed by an incubation with monoclonal antibodies, either anti-α-tubulin (NeoMarkers) or anti-β-tubulin (Sigma), diluted 1:25 and 1:500, respectively in 1% BSA for 60 min at room temperature. After washes in 1% BSA, sections were incubated with anti-mouse secondary antibody conjugated with Alexa 488 or 546 fluorophores (Molecular Probes, Eugene, OR) at 1:25 dilution in 1% BSA for 1 h, followed by washes in PBS. Sections were finally mounted with Mowiol (Sigma).

Examination of the immunofluorescence signal in the diverse mouse liver samples was carried out with a laser scanning confocal microscope (Bio-Rad). Controls were done in all cases by avoiding the first antibody.

Statistical analysis. Data are expressed as means ± SD. The significance of differences between groups of genetically different mice was analyzed by one-way ANOVA and Dunnett's post hoc tests, and the significance of differences between vehicle-treated and T_3-treated animals was analyzed by the same tests or Student's *t*-test. Data were reported as significant if $P < 0.05$.

RESULTS

Tubulin expression is tissue specific in mammals. Although tubulin expression is generally considered constitutive and either α- or β-tubulin subunits are frequently used as load controls, their expression is not homogeneous among mammalian tissues. Tubulin expression is highly tissue specific in the mouse, with α- and β-proteins being most abundant in brain and testis (Fig. 1). The same relative results were obtained from rat tissues (not shown).

Expression patterns of tubulin subunits do not vary in parallel during liver postnatal development: regulation by TH. The analysis of tubulin levels during postnatal development in the rat liver indicated that the two subunits' ratio changes in the postnatal days. The β-tubulin subunit level increased steadily from the first day after birth to obtain the adult value (Fig. 2). On the contrary, the α-subunit was abundant after birth, decreasing afterward to reach a level similar to that of the adult value from *day 14* onward. The results indicate that the steady-state expression of the two tubulin subunits can be unbalanced in physiological conditions.

We also analyzed the expression patterns of both subunits in rat neonates born from hypothyroid mothers (Fig. 2). The patterns of α- and β-subunits were similar during the postnatal period but different from the corresponding one observed with the normal animals. Protein disulfide-isomerase, a protein whose expression is not affected by thyroid status, was determined in order to allow for load control. These results indicated that TH regulates tubulin expression in the postnatal development of rat liver.

TH administration increases tubulin expression in adult rat liver. We also investigated whether tubulin expression in the adult rat liver was sensitive to TH administration. The analysis of tubulin subunits expression in the liver of normal, hypothyroid and T_3-treated hypothyroid rats indicated that T_3 increased significantly the β-subunit protein level (Fig. 3*A*). Protein disulfide-isomerase determination was used to control protein load. The T_3 status of the three groups of animals was confirmed by determining the mRNA levels of 5′DI (deiodinase), a T_3-responsive gene (Fig. 3*B*). The results thus indicate that

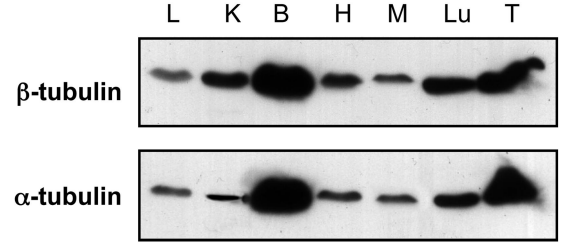

Fig. 1. Tubulin protein expression in adult mouse tissues. Total tissue extracts were obtained, and Western analysis was performed as described in EXPERIMENTAL PROCEDURES. β- and α-Tubulin proteins in different mouse adult tissues are expressed at relative similar levels. Protein disulfide-isomerase (PDI) is not included, as in other Western blot figures, to control protein load, because its expression is tissue specific. L, K, B, H, M, Lu, and T stand, respectively, for liver, kidney, brain, heart, skeletal muscle, lung, and testis. Very similar Western blots were obtained with rat tissues, although the signal was lower (not shown). Two independent experiments, for each mouse and rat tissue, were carried out with the same results.

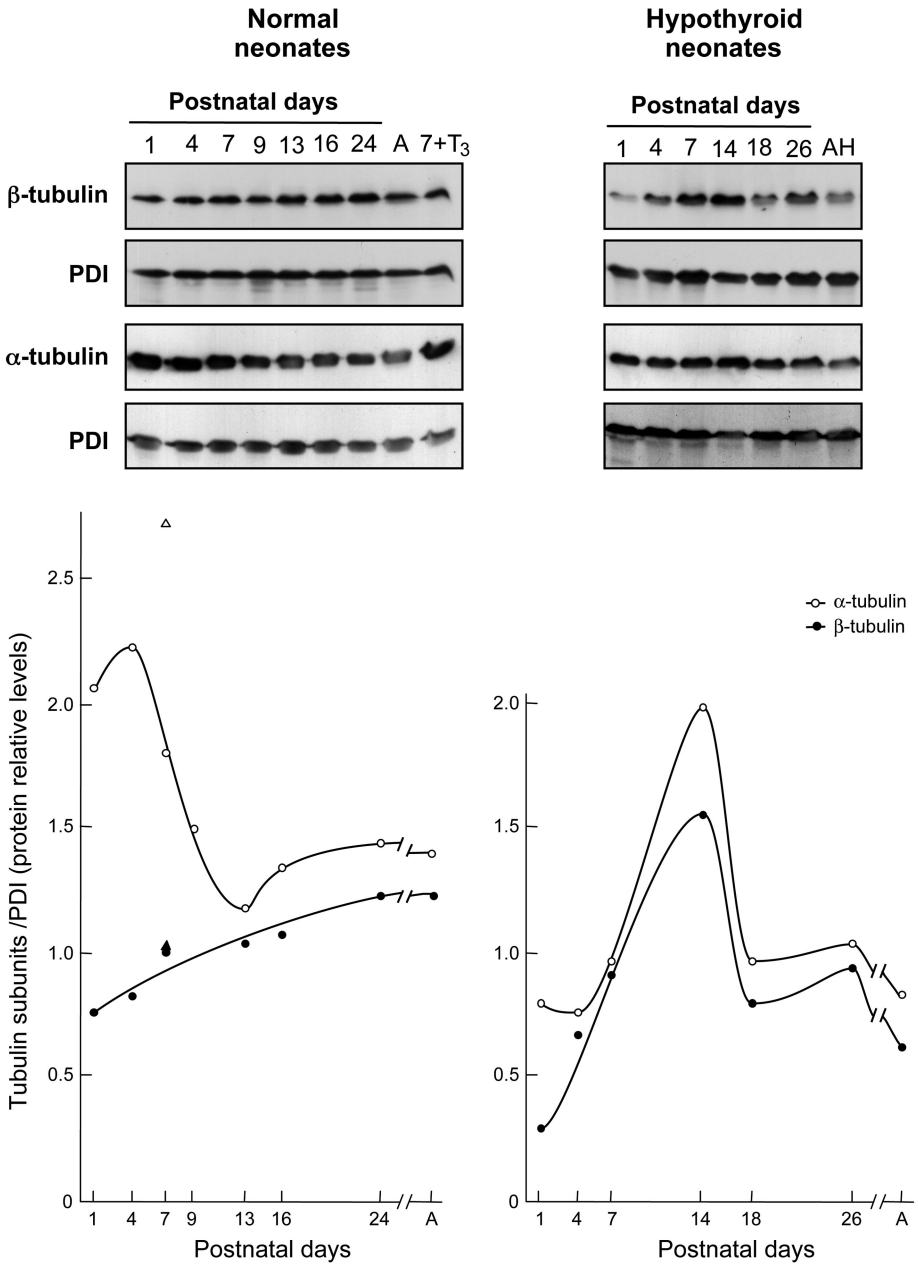

Fig. 2. Different expression patterns of α- and β-tubulin subunits during rat liver postnatal development, and their thyroid hormone (TH) regulation. Normal (euthyroid) and hypothyroid rats belonging to the same litter were killed at the different times indicated after birth. A, adult; AH, adult hypothyroid; T_3, triiodothyronine; triangle, 7-day-old hyperthyroid animals. Animal treatments and Western blot analysis were performed as described in EXPERIMENTAL PROCEDURES. Anti-PDI antibody was used to control protein load. Two independent experiments were carried out with the same results.

β-tubulin expression in the adult rat liver is sensitive to TH administration.

Tubulin expression in wild-type mice and mice lacking TRα or TRβ: effect of TH deprivation and T_3 administration. To investigate the role of TRs in the expression of tubulin, we measured the amounts of both subunits in wild-type mice and in mice of the same strain lacking TRα or TRβ (Fig. 4). The results indicated that β-tubulin protein, compared with the wild type, appears to be significantly lower in mice with TRβ deletion. The three types of mice were made hypothyroid and the levels of tubulin subunits determined. TH deprivation resulted in significant decrease of β-tubulin expression in wild-type and in TRα-lacking mice, but surprisingly, a significant increase existed in the mice without TRβ. The effects observed appeared to be specific for β-tubulin subunit, as little effect was seen with α-tubulin subunit or with actin, another cytoskeletal protein. Protein load was controlled with protein disulfide-isomerase. T_3 injected into hypothyroid mice lacking TRβ (Fig. 5) decreased the β-tubulin level down to about that of normal mice. Therefore, the results indicate that the effects of TH deprivation on the liver β-tubulin expression can be reversed by T_3 administration.

The results suggest that liver β-tubulin expression in vivo is primarily under TRβ control. However, the fact that hypothyroidsm and T_3 treatment increased or decreased, respectively, β-tubulin levels in TRβ$^{-/-}$ animals suggests that β-tubulin might also be upregulated by unliganded TRα.

TH regulates β-tubulin expression at the mRNA level. To investigate whether TH regulates β-tubulin expression at the mRNA level, RNA was isolated from the livers of wild-type mice and normal, hypothyroid, and hypothyroid T_3-treated wild-type animals and animals lacking either TRα or TRβ. A

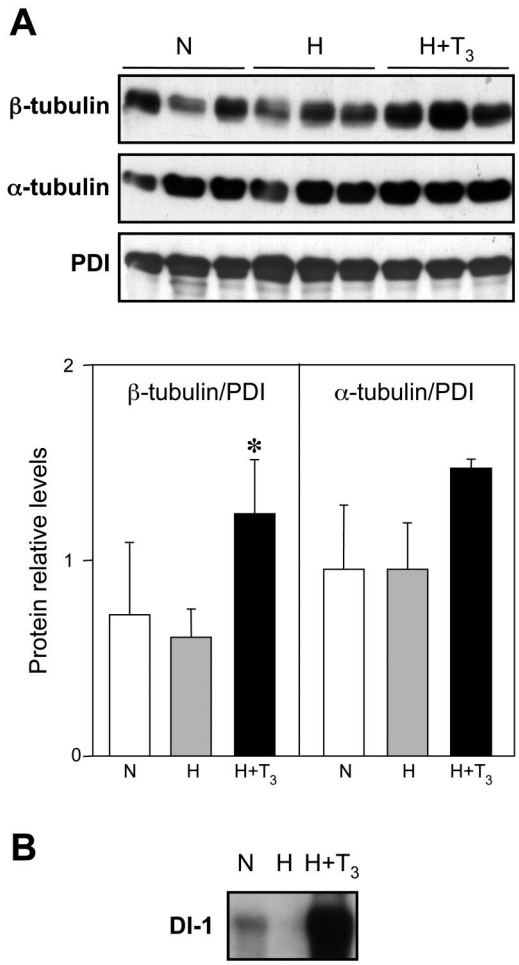

Fig. 3. Increase in tubulin protein expression in hypothyroid adult rat liver after T_3 treatment. *A*: total protein extracts obtained from the liver of normal (N), hypothyroid (H), and hypothyroid T_3-treated adult ($H+T_3$) rats were subjected to Western blot analysis, performed as indicated in EXPERIMENTAL PROCEDURES. Each lane corresponds to a different animal. Anti-PDI antibody was used to control protein load. Significant differences of β-tubulin in T_3-treated rats are marked, $*P < 0.05$. *B*: total RNA was isolated from livers of the 3 groups of animals, and Northern blot analysis was performed as indicated in EXPERIMENTAL PROCEDURES, using a rat 5′-deiodinase (5′DI-1) probe.

Northern blot containing RNA from the livers of normal and hypothyroid wild-type mice, TRα- or TRβ-deleted mice (Fig. 6*A*) was hybridized with the labeled cDNA probe of 5′DI, a control TH-responsive gene. The abundance of the specific mRNA observed in each sample reproduced previous results from the laboratory where the knockout animals were generated (16). The signal of the TRα-deleted mice was higher than that of the wild type and the signal of the TRβ-deleted mice much lower than the other two, whereas the hypothyroid animals of any type gave no detectable signal. This allowed confirming the status, both genetic and hormonal, of the animals used. The 5′DI probe used was the liver-specific isotype 1, and for that reason, no signal was observed in the lane containing RNA from wild-type brain. Brain RNA was included in the Northern blots as a sensitivity test of the assay because the liver β-tubulin message appeared to be rare and could be detected only with the aid of a commercial enhancer of hybridization (see EXPERIMENTAL PREOCEDURES). β-Tubulin mRNA in the liver comprises two mRNA isotypes (2 and 5, or II and I) which are also present in the brain (13). In Fig. 6*A*, brain RNA showed a strong signal, whereas in liver RNA the signal was clearly detected only in the liver of the hypothyroid TRβ-deleted mice. These results roughly parallel those obtained with the Western blots (Fig. 4), in that the strongest signal was observed with the hypothyroid TRβ-deleted mice. However, the modulation in the protein signal of the other samples seen in Western blots was not detected at the mRNA level, probably because the liver β-tubulin message was too scarce except in the case of the hypothyroid TRβ-deleted mice. Identical results were obtained with probes for mRNA isotypes 2 or 5, although the signal with the isotype 5 probe was always lower. The results indicating that the increase in β-tubulin protein expression produced by TH deprivation in TRβ-deleted mice can be reversed by T_3 administration (Fig. 5) were also reproduced at the mRNA level. As shown in Fig. 6*B*, the relatively high level of β-tubulin message observed in the TRβ-deleted hypothyroid mice was significantly decreased to

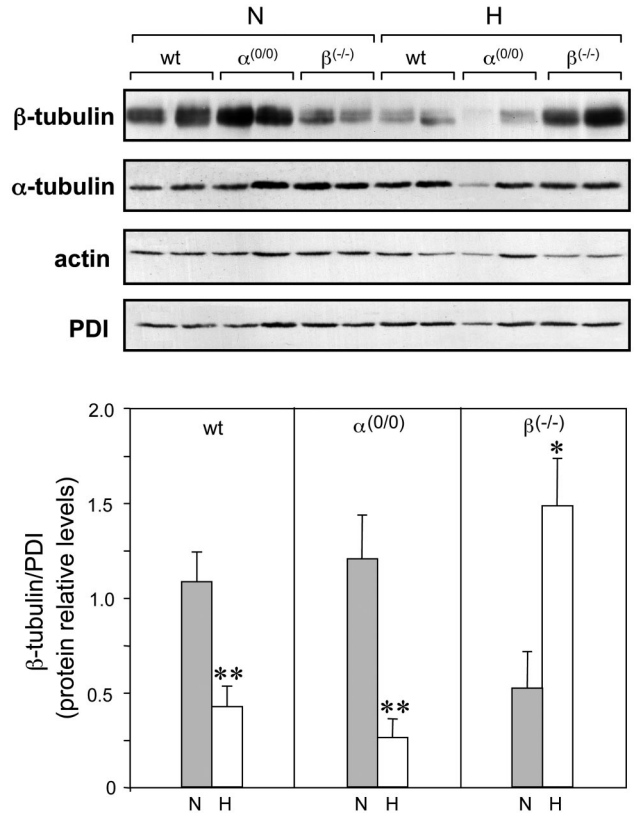

Fig. 4. Tubulin β-subunit protein is differently expressed in livers of wild-type (wt) mice and mice lacking TRα ($\alpha^{0/0}$) or TRβ ($\beta^{-/-}$) and differently affected by TH deprivation. Mice were treated, liver whole extracts were obtained, and Western blot analysis was performed as indicated in EXPERIMENTAL PROCEDURES. Western blots were probed with anti-tubulin β- and α-subunits and actin to check for other cytoskeletal protein and with PDI to check for protein load. Each lane corresponds to a different animal. Five animals were analyzed for each group, of which data from 2 animals are shown. The estimated marginal means of the data from each group are represented with SD. Statistical analysis indicated that wt mice were significantly different ($*P < 0.05$) from mice lacking TRβ, and mice lacking TRα significantly different ($*P < 0.05$) from mice lacking TRβ. For each genetic group, normal and hypothyroid animals were compared: wt mice ($**P < 0.01$), $TR\alpha^{-}$ mice ($**P < 0.01$), $TR\beta^{-}$ mice ($*P < 0.05$).

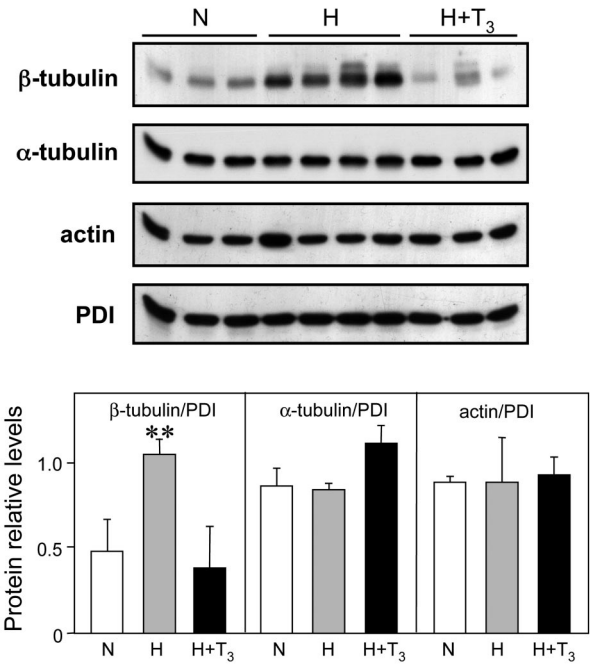

Fig. 5. Increased protein expression of tubulin β-subunit in the livers of TRβ-deleted hypothyroid mice is reduced upon T_3 administration. Animals were treated as indicated in EXPERIMENTAL PROCEDURES. Total protein extracts obtained from liver of N, H, and H+T_3 mice were subjected to analysis performed as indicated in the same section. Western blots were probed with anti-tubulin β- and α-subunits and actin to check for other cytoskeletal protein and with PDI to check for protein load. Each lane corresponds to a different animal. Three to four animals were analyzed for each group. The significant differences of β-tubulin in the hypothyroid TRβ$^-$ mice are marked, $^{**}P < 0.01$.

about the same levels of the euthyroid animals upon T_3 treatment.

Our mRNA results indicate that, at least in the TRβ-deleted mice, TH regulates expression of liver β-tubulin at the mRNA level.

Analysis of liver structure by section staining and of microtubular structure by immunofluorescence confocal microscopy, in wild-type mice and TRα- or TRβ-deleted mice. The differences in the amounts of β-tubulin (Fig. 4) found in wild-type mice and in mice lacking TRs, either TRα or TRβ, prompted us to analyze whether the liver structure of these animals was affected. The toluidine blue staining of liver sections (Fig. 7) indicated that, by this criterion, the livers of the three types of animals were apparently normal. We also analyzed the organization of the microtubular cytoskeleton by immunofluorescence confocal microscopy using anti α- and β-tubulin antibodies (Fig. 8). In the wild-type mice, the characteristic microfibrillar arrays of the tubulin cytoskeleton were observed with both antibodies. In agreement with Western blot data (Fig. 4), in mice lacking either TRα or TRβ, the amount of signal with α-tubulin antibody was similar to that observed in the wild-type mice. However, the cytoskeleton appeared somewhat disorganized, punctuated, and with less fibers. The β-tubulin antibody also gave rise to a punctuated pattern in the mice lacking TRα. In the mice without TRβ, the signal obtained with the β-tubulin antibody was in addition very low, in coincidence with the Fig. 4 data. This indicated that the microtubular structure was not only disorganized but clearly decreased in the mice lacking TRβ. The results suggest that both TRs are required for the microtubular organization observed in the wild-type liver and that β-tubulin expression, as also indicated from Western experiments data (Fig. 4), is critically controlled by TRβ.

DISCUSSION

Tubulin protein expression in rat and mouse tissues is tissue specific, with both subunits coordinately expressed in the adult tissues (Fig. 1). However, we have observed that the two subunits' ratio changes in the liver during the postnatal devel-

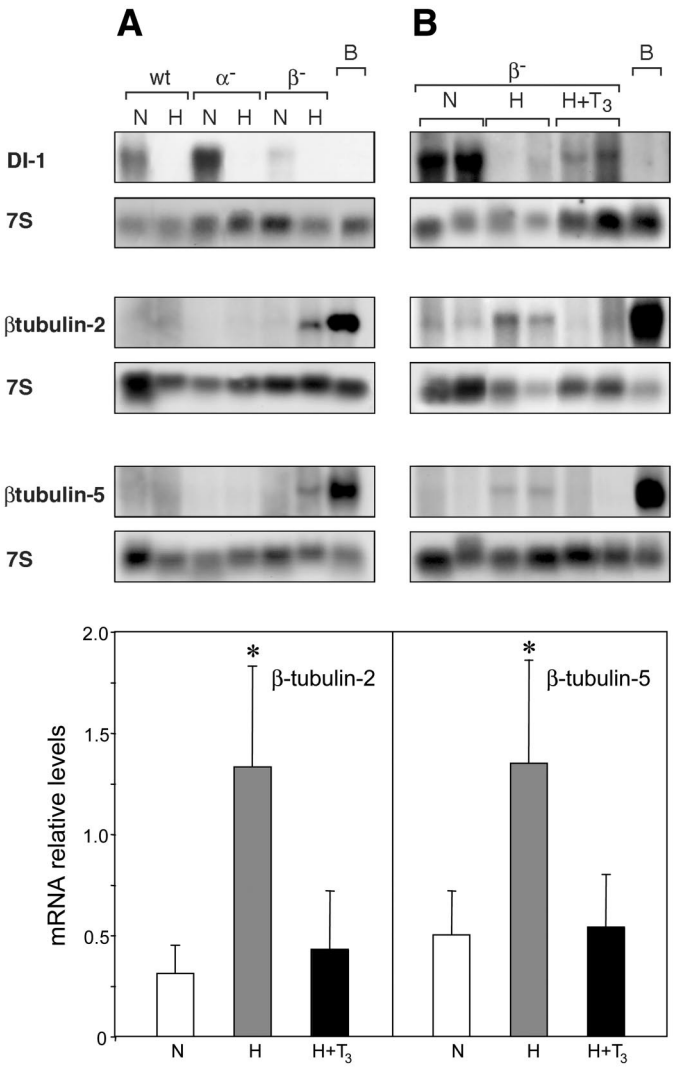

Fig. 6. TH regulates liver tubulin β-subunit at the mRNA level. Total RNA was isolated from the brain of wt mice, and livers of wt mice and mice lacking TRα or TRβ in the different N, H, and H+T_3 hormonal states, and Northern analysis was performed as indicated in EXPERIMENTAL PROCEDURES. Brain RNA (B) was included to compare levels of expression in the 2 tissues. Mouse 5′DI-1 probe was used to identify mice genetically and hormonally (16), and 7S to control RNA load. *A*: results obtained with RNA from wt mice and mice lacking TRα or TRβ, both N and H, are presented. *B*: results are from mice lacking TRβ, from N, H, and H+T_3 animals; 2 different animals per group are shown. Two independent experiments were carried out with the same results; 4 animals per group were analyzed in total. Statistical analysis indicated that the hypothyroid TRβ$^-$ mice were significantly different from both N and H+T_3 TRβ$^-$ mice ($^*P < 0.05$).

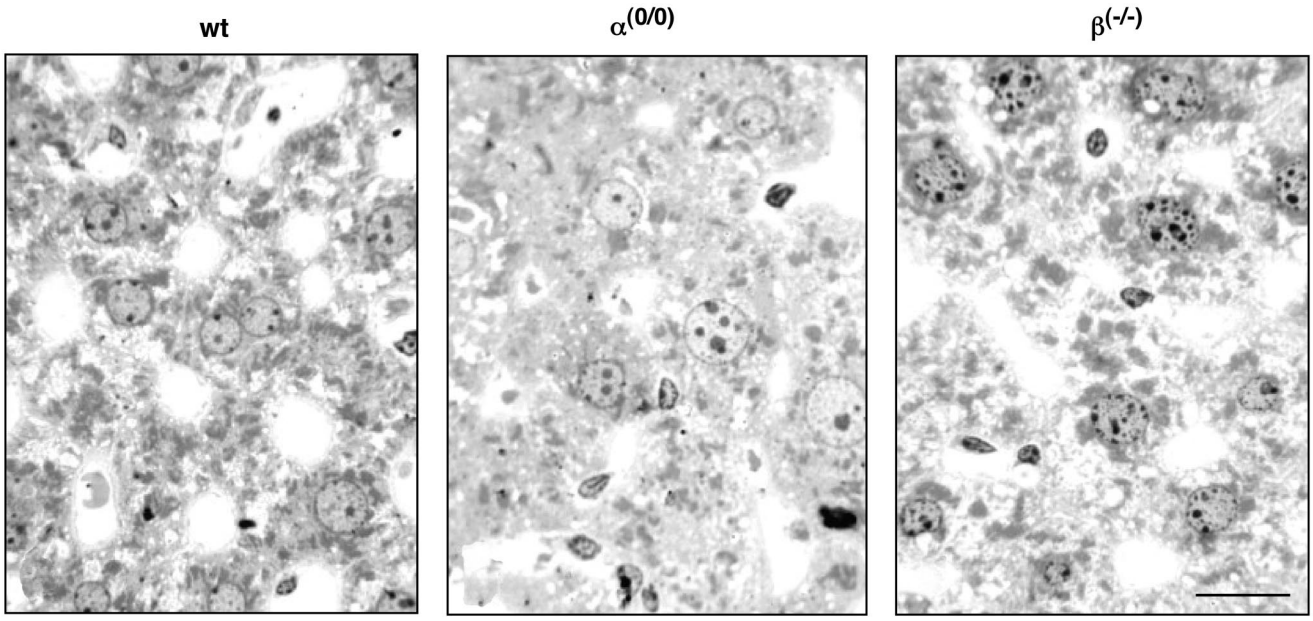

Fig. 7. Liver structure of wt mice and mice lacking TRα or TRβ. Liver sections obtained as indicated in EXPERIMENTAL PROCEDURES were stained with toluidine blue. Several sections from ≥2 animals of each type were examined with the same results. No significant difference among mice types was noticed. Bar, 50 μm.

opment (Fig. 2), with the α-subunit being relatively high with respect to the adult value and the β-subunit low, on the first day after birth. Although it is known that tubulin expression is regulated by TH in the brain (15), regulation in the liver has not been investigated. The analysis of α- and β-subunit protein levels in the livers of hypothyroid rat neonates indicated that T_3 affects the developmental patterns of both subunits during postnatal development. The β-subunit shows a sustained increase from birth to the adult stage, a pattern that is altered in hypothyroidism. Regarding α-tubulin, it is interesting to observe that, from 10 days after birth, changes in α-tubulin expression in hypothyroid newborns are quite similar to those described just after birth for normal animals. This result agrees well with a delayed development of hypothyroid fetuses and newborns, an observation found with other genes regulated during the perinatal period (2). The different patterns of tubulin subunit expression may be related to the developmental regulation of TRs. T_3 binding capacity in the fetal liver is solely accounted for by TRα (22). TRβ starts being expressed after birth and, by 15 days of age, it represents one-third of the total T_3 capacity, which increases to 80% in the adult liver (20). We also investigated whether tubulin expression in the adult rat liver was sensitive to TH administration by analyzing tubulin subunits expression in normal, hypothyroid, and T_3-treated hypothyroid rats. The results indicated that TH mainly activated β-tubulin expression. The observation that the hypothyroid rats showed normal levels of β-tubulin and that the T_3 effect was only evident after T_3 supplementation of these animals resembles the behavior of other T_3-responsive genes that respond only to high T_3 doses (5). The reductions in protein and mRNA levels of β-tubulin 2 and 5 isotypes were reported in the brain of congenitally hypothyroid *hyt/hyt* mice (4). β-Tubulin 2 and 5 isotypes are also present in the liver. To analyze the role of both TRs in tubulin expression, we measured tubulin levels in mice lacking either all the TRα isoforms (16) or TRβ1 and TRβ2 (6). The relative contributions of TRα1 and TRβ in the liver of mice are similar to those in the rat (15, 16). The results suggest that T_3 regulation of β-tubulin is mediated mainly through TRβ. However, the fact that hypothyroidism and T_3 treatment increased or decreased, respectively, β-tubulin levels in TRβ$^{-/-}$ animals suggests that β-tubulin might also be upregulated by unliganded TRα. The results on tubulin expression during rat liver postnatal development can be better interpreted in light of the results obtained with the knockout mice. The weak signal observed with β-tubulin in the neonates and the pattern of expression afterward (Fig. 2) paralleled the expression of TRβ (20). α-Tubulin is probably under TRα control in the fetus, as suggested by the repressed expression in hypothyroid neonates (Fig. 2) and by the fact that fetal liver contains only TRα1 (15). Our results support previous data in mutant mice (16) indicating that deficiency in one of the TR genes is not compensated for by the other. The mRNA analysis of these mice indicated that TH regulates β-tubulin at this level. The relatively high mRNA level observed with hypothyroid TRβ-deprived mice was reduced to the euthyroid level after T_3 treatment (Fig. 6). However, the undetectable levels of the β-tubulin messages in the other animal types precluded the comparative analysis with the wild-type and TRα-deleted mice.

Although the histological examination of liver structure in wild-type and mutant mice indicated no obvious differences, the microtubular organization showed alterations. The immunofluorescence analysis of liver sections from animals of each type using anti-tubulin α- and β-subunit antibodies (Fig. 8) indicated that, compared with the wild type, and in agreement with the results of Western analysis (Fig. 4), the signal given by the anti-β-subunit was very low in the animal without TRβ. In the same animal, the α-subunit signal was high, as observed in Western blots (Fig. 4), but appeared punctuated, indicating low polymerization, likely due to the lack of the β-subunit required to form the heterodimers. Similar images were observed with β-tubulin mutants expressed in tissue culture cells

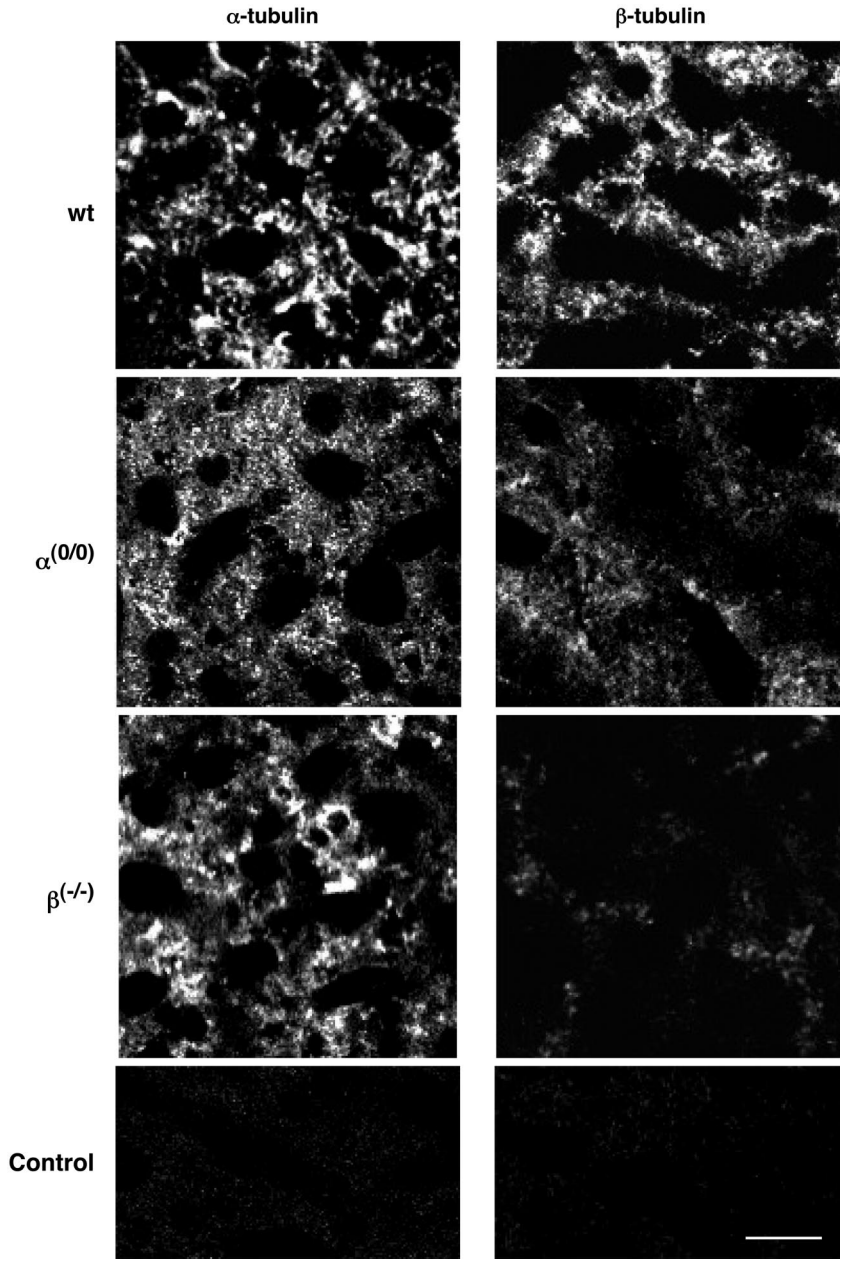

Fig. 8. Microtubular liver structure of wt mice and mice lacking TRα or TRβ was analyzed by immunofluorescence confocal microscopy. Liver sections from the 3 mouse types were incubated with anti-tubulin β- or α-subunit antibody, as indicated in EXPERIMENTAL PROCEDURES, and microtubular structure was analyzed in a confocal microscope. Several sections from ≥2 animals of each type were examined with the same results. Controls were carried out with wt mice without α- or β-tubulin antibody, respectively. Bar, 100 μm.

where they failed to polymerize with endogenous α-tubulin (26). Unexpectedly, despite the high expression of both subunits in the liver of TRα-depleted mice (Fig. 4), the microtubular network showed decreased polymerization with both subunit antibodies, suggesting that TRα may be involved at a certain level in achieving polymerization or in modulating microtubule dynamics. Multiple factors seem to be involved in the synthesis, transport, and storage of α- and β-tubulin in the folding of the heterodimers and the polymerization, stabilization, or dynamics of microtubules (14). In the TRβ-deleted mice, the distinctive punctuated α-tubulin immunofluorescence pattern may indicate that folding is affected. A similar punctuated pattern was described for free α-hemoglobin precipitated when expressed in cell experiments in the absence of the specific chaperone (11). The accumulation of unfolded protein and protein aggregates is detrimental and may lead to cell death and, to limit this accumulation, the cell activates a specific stress-signaling pathway (10).

To summarize, our results indicate that TH regulates tubulin expression in the mammalian liver. In studies with knockout mice, specifically, β-tubulin appears to be critically controlled by TRβ, with unliganded TRα probably also upregulating expression. It is suggested that both TRs may be required for the microtubular organization observed in the wild-type liver.

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