β-Trace Gene Expression Is Regulated by a Core Promoter and a Distal Thyroid Hormone Response Element*

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David M. White‡‡, Teiji Takeda‡‡, Leslie J. DeGroot‡, Kari Stefansson‡‡, and Barry G. W. Arnason‡

From the ‡Department of Neurology and the Brain Research Institute, and the §Thyroid Study Unit, Department of Medicine, The University of Chicago, Chicago, Illinois 60637 and the **Department of Neurology, Harvard Medical School, Boston, Massachusetts 02115

We isolated and characterized the human β-Trace protein (βTP) gene promoter. βTP, also known as prostaglandin D₂ synthase, is a lipocalin secreted from the choroid plexus and meninges into cerebrospinal fluid. Basal transcription of the βTP gene is directed from a core promoter found within the first 325 bases of the 5’-flanking sequence. The βTP gene promoter is responsive to thyroid hormone (3,3’,5-triiodothyronine, T₃) and efficiently repressed by unliganded human thyroid hormone receptor β (TRβ). Functional analysis of the βTP promoter in TE671 cells revealed that responsiveness to T₃ occurs in sequences 2.5 kilobase pairs 5’ of the start site. Within the hormone-responsive region we identified a thyroid hormone response element (TRE) located from –2576 to –2562 base pairs relative to the transcription start site. The βTP TRE is composed of two directly repeated consensus half-sites separated by a 3-base pair spacer (DGR3). The βTP TRE forms specific complexes with TRβ. We have shown that a gene active in the choroid plexus and meninges is responsive to T₃, T₃ may play a role in the regulated transport of substances into the cerebrospinal fluid and ultimately the brain.

β-Trace protein (βTP)* is a component of human cerebrospinal fluid (CSF) and one of very few proteins found in CSF not also present in serum. In human CSF, βTP is present at 2.6 mg/dl, ranking it among the major CSF proteins (1). βTP, identified by Clausen in 1961 (2), is primarily expressed in the choroid plexus (CP). βTP is also expressed to a lesser extent in meninges and oligodendrocytes (3, 4). Other than the CNS, the major site of βTP expression is the epididymis (4, 5).

A protein with similar distribution to βTP has been identified as prostaglandin D₂ synthase (PDS) in rats (6, 7). PDS catalyzes the conversion of prostaglandin H₂ to prostaglandin D₂ (PGD₂). A role for PGD₂ in regulation of sleep induction has been proposed (8, 9). Recently, βTP and PDS were shown to be the same protein (10, 11). In prior studies we have referred to βTP/PDS as PDS but, in deference to precedence, we now refer to it as βTP (12).

The human βTP message encodes a 180-residue polypeptide that is a member of the lipocalin superfamily. Lipocalins are secretory proteins that transport hydrophobic ligands (13, 14). Lipocalin genes appear to have arisen by gene duplication, with most of them clustered in the q34 region of chromosome 9 in man and in the syntenic b-c region of chromosome 4 in the mouse (15). In previous work we localized the human βTP gene to the lipocalin gene cluster on q934. The βTP gene bears a striking resemblance to other lipocalin genes, suggesting a role for βTP in transport (12).

CSF, primarily produced by the CP, can be viewed as an ultra filtrate of serum with protein levels approximately 0.5% those in serum. Exchange of proteins and other substances between CSF and the extracellular fluid of the brain is free (16). The CP secretes highly specialized transporters that carry essential substances into the CSF and then to the brain. The primary function of the meninges is the maintenance of the blood-CSF barrier, but it also contributes to CSF and many substances enter into CSF equally well from either the meninges or CP. Cultured meningeal cells secrete many of the same transport proteins as the CP (17). Several CSF transporters have been characterized including transthyretin, transferrin, and ceruloplasmin; they carry thyroxine, iron, and copper, respectively (18, 19).

Garcia-Fernández et al. (20) found that levels of βTP mRNA in the CNS of adult rats decrease following chemically induced hypothyroidism. The mechanism by which thyroid hormone (T₃) influences βTP gene expression is unknown. T₃ exerts its effects through binding to thyroid hormone receptors (TR), which are widely distributed in the CNS (21). In the CP, T₃ augments transport function; hypothyroid rats have reduced Na⁺–K⁺-ATPase activity, a marker for transport processes (22).

To better understand mechanisms of βTP gene regulation, we subcloned the human βTP gene promoter and analyzed its expression in the human rhabdomyosarcoma cell line TE671. We identify a small core promoter that directs basal gene transcription at high levels and a distal element that determines T₃ responsiveness.

MATERIALS AND METHODS

Isolation and Sequencing of the βTP Promoter—The 3.8-kb XhoI-XhoI fragment from the βTP genomic clone pGACS86 (12) was inserted...
Regulation of the β-Trace Gene

FIG. 1. Deletion analysis of the βTP promoter. Schematic on the left represents the promoter deletion constructs fused to the CAT gene. Stippling denotes the first 65 bases of the βTP 5'-untranslated region fused to the CAT gene. The start site of each construct relative to the start site of transcription is shown to the left. pCAT495R has the first 500 bp of the βTP promoter cloned in the reverse orientation. Restriction sites used to generate clones are marked with down arrows. Bar graphs on the right represent the CAT activity in TE671 cells of the clones shown schematically on the left. Data are expressed as cpm/min/unit β-GAL activity and are reported as the mean ± S.E. from three separate transfections. The CAT activity of all clones was significantly different from the full-length clone, pCAT2759 (p < 0.05).

into the Smal site of pBSKS+, and approximately 1 kb of 3’ sequence was excised using the Exo III/mung bean nuclease system (Stratagene). The resulting fragment, spanning from −2759 to +65 bp, was subcloned into the CAT vector pFFCAT (25) to generate clone pCAT2759 (Fig. 1). Exo- and endonuclease deletions of pCAT2759 produced clones with successive 5’ deletions. Sequence was analyzed as described previously (12).

βTP-Thymidine Kinase (TK) Promoter Fusions—Clone pCAT235 was produced by subcloning the region between −2759 and −2080 bp of the βTP promoter into pBSKS+. Small internal deletions were introduced into pCAT235 by digestion with Styl and EcoNI followed by Klenow fill-in or mung bean nuclease digestion to remove one or both of the βTP TRE half-sites, respectively. To liberate the inserts from the pBSKS+ vector, the constructs were opened with BanHI, made blunt-ended with Klenow, and subsequently digested with SauI. Gel-purified fragments were subcloned into the pBLCAT2 vector that had been opened first at the SaII site of pBSKS+; the three clones thus produced were as follows: 1) pTK680F, with the 680-bp fragment of pCAT235 in the forward orientation, 2) pTK33’ with a 104-base internal deletion which removes the 3’ half-site of the TRE, and 3) pTKΔ5’ + 3’, with a 108-base internal deletion which removes both half-sites of the TRE.

Clone pTK100, spanning bases −259 to −2464 of the βTP promoter, was produced by deleting 356 bp of 3’ sequence from clone pTK680F by double digestion with EcoNI and SalI. Clone pTK680R was produced by subcloning the 680-bp BanHI-SaII fragment of pCAT235 into the corresponding sites of pBLCAT2. Clone pTKJK3 was produced from overlapping oligonucleotides cloned into the HindIII-XhoI sites of pBLCAT2. Clone pTK100R was produced by PCR amplification of bases −2590 to −2518 bp of the βTP promoter using pCAT2759 as template and oligonucleotides that introduced a 5’ HindIII site and a 3’ XhoI site. PCR product was digested with HindIII and XhoI and subcloned into the corresponding sites in pBLCAT2.

Northern Blot Analysis—Total RNA was isolated from adult rat brain or TE671 cells using the method of Chomczynski et al. (24). Total RNA was electrophoresed through 1% agarose gels containing 3% formaldehyde, capillary blotted onto a GeneScreen nylon membrane (Du Pont NEN), and probed as described previously (12).

Transient Transfections and Cell Culture—The host cell line used in these studies was the human rhabdomyosarcoma cell line TE671 (ATCC CRL 8605) (25, 26). Cells were passaged in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum (BioWhittaker) and 40 μg/ml gentamicin (Life Technologies, Inc.). For TR cotransfections, Dulbecco’s modified Eagle’s medium with 10% charcoal/dextran-treated fetal bovine serum (HyClone) was used.

On the day preceding transfection, 4 × 10⁵ cells were seeded into 60-mm culture dishes. Plasmid DNA was transfected into cells using the calcium phosphate co-precipitation method (27). For each dish, 1 pmol of CAT construct was co-transfected with 2 μg of β-galactosidase (β-GAL) expression vector pRSV-β-GAL (28) as an internal control. For TR cotransfections 2 μg of hTRβ1 expression vector was used (29). pBSKS+ was added to bring the total DNA in each dish to 12 μg. Medium was replaced 18 h after transfection. Where necessary, T3 or hormone vehicle were introduced into the fresh medium at a final concentration of 100 nM. After 48 h the cells were harvested, pSV_CAT (30) was used throughout as a positive control vector for CAT expression, and pTK83 was used for T3/TR responses (31). The negative control for CAT expression was the promotorless CAT vector pFFCAT1 and for T3 responses, pBLCAT2, which contains the TK promoter (32).

Reporter Gene Assays—To correct for variations in transfection efficiency, cell extracts were assayed for β-GAL activity (33). After adjusting for β-GAL levels, CAT activity was determined using a variation of the diffusion assay (34). All transfections were repeated at least four times. Data, reported as mean ± S.E., except where noted, are from three separate transfections.

Histochemistry—Transfected TE671 cells were fixed with paraformaldehyde and overlaid with a solution of 0.5 mg/ml 5-bromo-4-choro-3-indolyl-β-D-galactosidase, 2.5 mM ferri/ferricyanide, 1 mM MgCl₂, 15 mM NaCl, and 50 mM Tris-HCl, pH 7.5. The reaction proceeded overnight in the dark at 37 °C.

Gel Retardation Assay—Complementary oligonucleotides (OLG) spanning the regions shown in Fig. 7A were used for gel retardation of the βTP TRE and IR1 elements. The βTP TRE OLGs were 5’-AGCGGCGGATGGCCCTTGCACTCTTAAAGGTGCA-3’ and the complementary strand 5’-TGCGCTCCTACACCATGAGGTCTCAGAAGCCTACTCCCC-3’. The mutant TRE, 4DR3, is similar to βTP TRE but introduces C → A or C → T mutations at bases −2573, −2572, −2564, and −2563. The IR1 OLGs were 5’-TGGACACAGGGGACTTGAGGATCGCTGTCTCGTA-3’ and the complementary strand 5’-TCGGGTACCGGGACTCTGCTCGTCTGTA-3’. The control probe for TR binding was the rat malic enzyme promoter (rME) TRE (35). Complementary OLGs were hybridized and 5’ overhangs filled in with Klenow polymerase and [α-32P]dCTP. Labeled duplexes, purified on G50 columns, had a specific activity greater than 1.7 × 10⁶ cpm/pmol. Recombinant human TRβ1 and RXRα were prepared as described by Sakurai et al. (36).

Binding reactions contained 10 fmol of labeled TRE (approximately 17,000 cpm), 20 μM HEPES, pH 8.0, 50 mM KCl, 0.1% Nonidet P-40, 10% glycerol, 1 mM diithiothreitol, 28 mg/ml poly(dI-dC), 20–100 fmol of TRβ1, and/or 20–100 fmol of RXRα. Reactions proceeded at room temperature for 20 min. In supershift experiments, complexes were permitted to form for 20 min. 1 μl of anti-hTRβ1 polyclonal antibody β62 (37) was then added followed by incubation for a further 20 min at room temperature. DNA-protein complexes were resolved on non-denaturing 5% PAGE gels run at room temperature.

RESULTS

Isolation and Identification of the βTP Core Promoter—The βTP promoter was isolated from the genomic clone pG4CS86
Regulation of the β-Trace Gene

To localize regions of the promoter important to βTP transcription, a set of 10 promoter-CAT gene fusion constructs with increasing 5′ deletions was produced, the 5′ termini ranging from −2759 to +16 bp in the untranslated region (Fig. 1). The human rhabdomyosarcoma cell line TE671 (25, 26) expresses βTP mRNA at high levels and transfects efficiently (Fig. 2). Parallel transfections of the 10 deletion constructs into TE671 cells revealed the βTP gene promoter to be highly active, generating CAT activity at a level comparable to the positive control vector pSV5-CAT (30). The βTP core promoter region is small, deletions from −2759 bp to −595 bp had minimal effects on βTP promoter activity. Deleting the bases between −595 and −325 actually increased βTP promoter activity 1.8-fold. Deletions within the 325-bp core promoter region results in major loss of activity (Fig. 1). The −80-bp clone is inactive, which localizes the sequences necessary for maximal basal activation of the βTP gene between −325 and −80 bp of the promoter.

Nucleotide Sequence of the βTP Gene Promoter—The sequence of the core promoter is presented in Fig. 3. The region from −227 to −180 bp of the βTP promoter has high sequence identity with regions of the human luteinizing hormone subunit β promoter (LH-β) (68% identity, bases −239 to −192) (38) and the human insulin-like growth factor II P4 promoter (80% identity, bases −318 to −287) (IGF-II) (39). The IGF-II P4 promoter is active in the CP (40). A 20-bp near-perfect palindrome (PAL I, bases −176 to −157) bears extended homology to the AP4 site originally identified in the SV40 enhancer (41) and to the cAMP response element, ENKCRE-2, found within the proenkephalin gene promoter (42). However, the βTP promoter is only mildly responsive to forskolin (data not shown).

The βTP Gene Promoter Is Responsive to Thyroid Hormone (T3) and Human Thyroid Hormone Receptor β (TRβ)—In vivo analysis has shown that βTP mRNA expression is regulated by T3. To determine if the human promoter mounted transcriptional responses to T3 and TRβ, we studied T3 effects on the βTP promoter in TE671 cells. Results from reporter constructs (Fig. 4, top) and Western blot analysis (data not shown) indicate that TE671 cells do not express thyroid hormone receptors. Thus to determine if the human βTP gene responds to T3 and TRβ, the full-length clone, pCAT2759, was cotransfected with a TRβ expression vector (29) into TE671 cells and cultured in the presence or absence of 100 nM T3. The results reveal that the βTP promoter is strongly regulated by TRβ in a T3-dependent manner (Fig. 4). βTP transcription is elevated 4-fold over basal levels in the presence of T3 and TRβ. Unliganded TRβ (no T3) represses the activity of the βTP promoter 12-fold compared with basal levels. When both effects are considered, βTP promoter activity is stimulated 45-fold by T3 over the level observed with unliganded TRβ alone. Fig. 4 also shows that the response of pCAT2759 to T3 and TRβ is in the range observed with the strong TRE of the rME-positive control, pTK83 (31). Varying the amount of TRβ expression vector cotransfected with CAT constructs did not alter the result (data not shown). As shown in Fig. 4, two heterologous promoter-CAT constructs, pSV5-CAT and pBLCAT2, have responses to TRβ and T3 different from those observed for the βTP promoter, indicating that βTP promoter responses do not result from effects on cell viability or transcriptional competence.

T3-responsive Region of the βTP Gene Promoter—To identify the T3-responsive region of the βTP promoter, the deletion constructs (Fig. 1) were cotransfected with a TRβ expression vector or pBSKS sham control and cultured with 100 nM T3. Only the full-length clone, pCAT2759, shows activation by T3 and TRβ, localizing the T3 responsive region to the sequence between −2759 and −2018 bp (Fig. 5A). There was no significant activation by T3 alone (pBSKS sham) over basal levels for any of the deletion constructs examined (data not shown). To account for the repressive effects from unliganded TRβ, the activity of the deletion constructs in the presence of T3 and TRβ was compared with that from TRβ alone. The results, shown in Fig. 5B, again demonstrate that only the −2759-bp clone possesses major responses to T3.

Similar experiments were performed to identify the region responsible for TRβ-mediated repression. Deletion of the region responsible for T3 activation (−2759 to −2018 bp) barely altered repression by unliganded TRβ (Fig. 5C). Repression by unliganded TRβ was alleviated by deletions inward from −1423 bp. Thus, repression occurs at alternative or additional

![Fig. 2. βTP mRNA is highly expressed in host cell line TE671.](image)

![Fig. 3. The 5′-flanking region of the human βTP gene.](image)
sites to those responsible for $T_3$-mediated activation.

Identification of a Thyroid Hormone-responsive Element (TRE) in the $\beta$TP Gene Promoter—To characterize further the $T_3$-responsive region of the $\beta$TP promoter, 680 bp of upstream sequence (−2759 bp to −2080 bp) was cloned upstream of the minimal thymidine kinase (TK) promoter fused to the CAT gene as contained in the pBLCAT2 vector. $T_3$ stimulates an 8-fold increase of CAT activity when the 680-bp fragment was cloned in the forward direction and 9.5-fold when cloned in the reverse orientation (Fig. 6). Thus the $\beta$TP promoter $T_3$-responsive region also confers strong $T_3$ induction on the heterologous TK promoter in an orientation independent manner. To delineate the $T_3$-responsive region of the upstream fragment, two constructs were produced that successively removed 5′ and 3′ sequence. In the first construct a deletion was introduced on the 3′ end of the 680-bp fragment, leaving the 285 bp of 5′ sequence (pTK300 in Fig. 6). The construct, pTK300, is nearly as active as the original 680-bp fragment (7.6- versus 8-fold activation), indicating that the 3′ sequence makes a negligible contribution to the $T_3$ response. The second construct further narrowed the sequence on both 5′ and 3′ ends of the 300-bp construct, encompassing 102 bp from −2620 to −2518 bp of the $\beta$TP promoter (pTK100 in Fig. 6). The 102-bp construct is as effective as the 295-bp construct (7.5- versus 7.6-fold) and nearly as effective as the 680-bp construct, indicating that the $T_3$-responsive region is located within sequence spanning −2620 to −2518 bp.

The sequence between −2620 and −2518 bp was searched for half-sites that conformed to the general consensus 5′-PuGG(A/T)CPu-3′ (where Pu indicates a purine nucleoside) and that possessed the number and spacing of half-sites consistent with known TREs. Using this approach a TRE was identified between bases −2576 and −2562 bp (Fig. 7A), which is composed of two directly repeated half-sites separated by 3 bp (DR3). To test the role of the $\beta$TP TRE in directing the $T_3$ responses, deletion analysis was used to remove the 3′ half-site and subsequently both half-sites of the TRE from the 680-bp fragment. Deletion of the 3′ site and 3′-flanking sequence results in a drop in activation by $T_3$ and $T_3\beta$ from 8.0- to 2.9-fold (pTKΔ9′ in Fig. 6). A similar deletion of both half-sites of the $\beta$TP TRE results in the loss of the $T_3$ induction (pTKΔ5′+3′ in Fig. 6). Thus deletion of the $\beta$TP TRE results in the loss of the $T_3$ responses identified in the upstream fragment of the $\beta$TP promoter.

Gel shift assays were used to determine if the $\beta$TP TRE formed specific complexes with TRβ. The binding of TRβ to the $\beta$TP TRE was compared with that of the DR4 type TRE from the rME promoter (43). An IR1 type element, between bases −2110 and −2088 bp, which was determined not to contribute to $T_3$ responses (data not shown), was used as a negative control (Fig. 7A). The $\beta$TP TRE binds and shifts with TRβ homodimers and more intensely when the RXRα accessory protein is present (Fig. 7B). The shifted bands are at levels of intensity similar to those obtained when the rME element is present in combination with RXRα. This result is consistent with the known ability of TRβ homodimers to form complexes with RXRα (52).
FIG. 6. Deletion analysis of the thyroid hormone-responsive region of the βTP promoter. Bases −2759 to −2080 of the T3-responsive region of the βTP promoter were cloned upstream of the heterologous TK promoter fused to the CAT gene. Schematic on the left represents the promoter constructs fused to the TK-CAT gene of the pBLCAT2 vector. The two squares represent each half-site of the TRE element, where the solid square represents the 3’ half-site with perfect match to consensus. The hatched squares represent mutated half-sites as described under “Results.” The oval represents an inverted repeat of consensus half-sites identified in the upstream region. Gaps within the schematic represent internal deletions introduced into the sequence. TK fusion constructs were cotransfected with a TRα expression vector and cultured with 100 nM T3 or with hormone vehicle alone. Bar graph on the right represents the fold activation of CAT activity in TE671 cells of the clones shown schematically on the left. Data are expressed as CAT activity in the presence of TRα and T3 divided by CAT activity in the presence of TRα alone. Data are reported as the mean ± S.E. from three separate transfections.

used, indicating formation of high affinity complexes between the βTP TRE and TRβ/RXRα. As expected, the IR1 element failed to bind TRβ and shifted only faintly in the presence of TRβ/RXRα (Fig. 7C).

To further characterize the βTP TRE, cold competitions were performed using the βTP TRE element itself or the rME element. As expected, unlabeled βTP TRE competes with labeled βTP TRE (Fig. 8A). The rME element competes effectively with the βTP TRE element for binding to TRβ indicating that βTP TRE forms complexes in the same fashion as rME (Fig. 8B).

Within TRE half-sites, loss of one or both of the two conserved G nucleotides substantially reduces TR binding and TRE function (44, 45). To test the role of these nucleotides, a mutant βTP TRE was constructed (ΔDR3) in which the G residues at bases −2573–74 and −2564–63 were changed to T or A, respectively (Fig. 7A). The reconfigured element, ΔDR3, failed to bind TRβ homodimers and bound TRβ/RXRα heterodimers only faintly (Fig. 8C) proving that residues known to be crucial for TR binding in other TREs are also necessary for TR binding to the βTP TRE. Confirming the results from gel shift, placement of the mutant βTP TRE element upstream of the TK promoter within the pBLCAT2 vector failed to confer T3 responses to the TK-CAT construct in TE671 cells (construct pTKΔDR3 in Fig. 6). Thus, the intact sequence of the βTP TRE is necessary to bind TRβ and to activate transcription in response to T3 and TRβ.

To probe the composition of the DNA-protein complexes, anti-hTRβ antibody was used to super-shift DNA-protein complexes that had formed with TRβ. βTP TRE-protein complexes, formed in the presence of TRβ or TRβ/RXRα, were shifted by antibody, demonstrating that the complexes with βTP TRE were formed by TRβ binding (Fig. 8D).

FIG. 7. The TRE from the βTP promoter binds TRβ. A, the sequence of the βTP TRE is presented as is that of the IR1-negative control. Potential TRE half-sites are overlined by arrows. Shown below the βTP TRE sequence are the nucleotide changes introduced to produce the ΔDR3 element. B and C, gel shift analysis. The βTP TRE and IR1 regions shown in A were labeled with 32P. The rME promoter TRE probe was similarly labeled and served as the positive control for receptor binding. 10 fmol of labeled probe was incubated with purified recombinant TRβ, RXRα, or both. Protein from a sham receptor preparation (Mock) was used to control for nonspecific protein binding. The rME TRE complexes were resolved on polyacrylamide gels alongside the βTP TRE and IR1 complexes. H, denotes TRβ/RXRα heterodimers, D, denotes TRβ homodimers, and F denotes unbound probe.

DISCUSSION

Basal transcription of the β-Trace gene is directed from a small and highly active core promoter. The core promoter is found within the first 325 bp of upstream sequence and directs CAT gene expression in TE671 cells at a level similar to the pSV5-CAT-positive control vector. Regions of the core promoter bear striking sequence identity to the P4 promoter of the IGF-II gene (39), which is active in the choroid plexus (40), and to the β-LH gene, which is active in the CNS (38).

The human βTP gene is regulated by TRβ in a T3-dependent manner. T3 and TRβ substantially elevate βTP promoter activity, whereas unliganded TRβ effectively represses the promoter. The level of T3-dependent activation observed is comparable to that observed using a classical TRE from the rME promoter (43), indicating that the overall response of the βTP promoter to T3 is strong (Fig. 4).

Deletion analyses indicate that the βTP thyroid hormone-responsive region lies between −2759 and −2018 bp. When placed upstream of the TK minimal promoter in either orientation, this region confers T3 regulation on the heterologous TK promoter. Further deletion analysis of βTP-TK promoter fusions allowed the T3-responsive region to be localized to the sequence between −2620 and −2518 bp, a region in which we have identified a TRE composed of two consensus half-sites separated by a 3-bp spacer (DR3-type). The 3’ half-site of the TRE exactly matches the general consensus half-site, and its sequence between bases −2518 and −2508 defines the TRE consensus which is lost with mutation of the two conserved G nucleotides within the 3’ half-site of the TRE.

Deletion of both half-sites completely abolishes T3 induction. Deletion of both half-sites completely abolishes T3 induction. As with other TREs, T3 induction from the βTP TRE is lost with mutation of the two conserved G nucleotides within each half-site.

Gel shift experiments demonstrate that the βTP TRE forms specific complexes with both TRβ homodimers and TRβ/RXRα heterodimers. We used cold competitions with the rME TRE to test the hypothesis that βTP TRE binds to and interacts with TRβ in a manner consistent with other TRE sequences.

The βTP TRE is well upstream of the core promoter. This
organization places the \( \beta TP \) promoter in a growing family of genes distinguished by TRE elements distal to the core promoter. These include the human insulin gene where the TRE is located at \(-1 \) kb (46), the rat \( S_{14} \) gene which has multiple TREs located in a 200-bp region around \(-2.6 \) kb (47), and the rat \( ucp \) gene which has two TREs located in the region around \(-2.3 \) kb (48, 49). Both the \( \beta TP \) TRE and one of the \( ucp \) TREs, the downstream TRE, are composed of two directly repeated half-sites separated by a 3-bp spacer. Directly repeated half-sites with three base pair separations are commonly associated with vitamin D receptors in accordance with the 3,4,5 rule (44, 50). However, the rat \( ucp \) downstream TRE is unresponsive to induction by vitamin D receptors, indicating that DR3-type TREs are capable of \( T_{3} \)-specific responses (48). Additionally, in vitro analyses have shown that DR3 elements can bind TR and direct \( T_{3} \) responses at or near the level observed with the more common DR4 spacing (51). The sequences flanking the half-sites may prove to be more important in honing the \( T_{3} \) response of the \( \beta TP \) TRE than the spacings between the half-sites. Koenig et al. (52) have identified an extended consensus half-site sequence which functions as an equally strong TRE regardless of whether the spacing between the half-sites is 3, 4, or 5 bp. A similar dependence on half-site and flanking sequence, independent of half-site spacing, has been noted for the closely related retinoic acid receptor elements (53).

The \( \beta TP \) promoter shows considerable \( T_{3} \)-independent repression by TRβ (54). Repression appears to be specific for the \( \beta TP \) promoter as two heterologous promoters used in this study (\( SV_{2} \) and \( TK \)) are only mildly affected by unliganded TRβ. Deletion of the sequences responsible for \( T_{3} \) activation does not alleviate repression. Repression may result from TR binding to TRE half-sites located elsewhere in the promoter. The \( T_{3} \) sites can comprise a functional element in TRE-mediated repression (55, 56). Additionally, unliganded TR might disrupt protein-protein interactions necessary for transcription as has been observed in the human glycoprotein hormone \( \alpha \) gene (57).

\( \beta TP \) starts express \( \beta TP \) but not TRβ. These properties permitted the examination of basal \( \beta TP \) promoter activity in the absence of the potentially confounding effects of TRβ. Subsequent expression of TRβ in TE671 cells allowed dissection of the repressive effects of TRβ on \( \beta TP \) basal transcription from the more familiar role of TRβ in activation. The basal activity of the \( \beta TP \) promoter observed in TE671 cells in the absence of TRβ may be physiologically relevant. Although TR expression is widespread it is not universal (37, 58).

The increase in the activity of the \( \beta TP \) promoter by \( T_{3} \) and TRβ and its repression by TRβ alone, as shown here, agrees with and provides a dual mechanism for the reduced \( \beta TP \) mRNA levels observed in thyroidectomized rats (20). The thyroid hormone responsiveness of the \( \beta TP \) promoter may also imply \( T_{3} \) control of PGD2 synthesis. However, the high levels of \( \beta TP \) and TRβ promoter activity should be contrasted to the low levels of PGD2 observed in human CSF (59). Perhaps TRβ functions as a ligand transporter within the CNS, as is the case for other proteins secreted by the CP and meninges. The structural similarity between \( \beta TP \) and other lipocalin transporters provides indirect support for a role in lipid transport. Further support for the role of \( \beta TP \) in transport processes has recently been provided by the work of Hoffmann et al. (60) who, in a careful in vivo analysis of \( \beta TP \) expression in the developing mouse, have observed \( \beta TP \) expression at or near a number of blood-tissue barriers, hinting at a role for \( \beta TP \) in transport across these barriers.

The present study indicates that \( T_{3} \) exerts a measure of control over \( \beta TP \) gene expression. If \( \beta TP \) functions to transport a specific ligand into CSF then \( T_{3} \) potentially exerts a general level of control over the availability of the ligand in the CNS. Important questions regarding the regulation of \( \beta TP \) transcription remain to be addressed. Foremost among them is whether the expression of \( \beta TP \) is regulated in a tissue-specific manner, implying the use of different enhancer elements within the \( \beta TP \) promoter or different combinations of tissue-specific transcription factors. Additionally, the role of \( T_{3} \) on \( \beta TP \) expression must be examined in the context of the other tissues in which it is expressed.

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