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### Research Article

Type 3 iodothyronine deiodinase (D3) catalyzes the conversion of T4 and T3 to inactive metabolites. It is highly expressed in placenta and thus can regulate circulating fetal thyroid hormone concentrations throughout gestation. We have cloned and expressed a 2.1-kb human placental D3 cDNA which encodes a 32-kD protein with a  $K_m$  of 1.2 nM for 5' deiodination of T3 and 340 nM for 5' deiodination of reverse T3. The reaction requires DTT and is not inhibited by 6-n-propylthiouracil. We quantitated transiently expressed D3 by specifically labeling the protein with bromoacetyl [ $^{125}I$ ]T3. The  $K_{cat}/K_m$  ratio for 5' deiodination of T3 was over 1,000-fold that for 5' deiodination of reverse T3. Human D3 is a selenoenzyme as evidenced by (a) the presence of an in frame UGA codon at position 144, (b) the synthesis of a 32-kD  $^{75}Se$ -labeled protein in D3 cDNA transfected cells, and (c) the presence of a selenocysteine insertion sequence element in the 3' untranslated region of the mRNA which is required for its expression. The D3 selenocysteine insertion sequence element is more potent than that in the type 1 deiodinase or glutathione peroxidase gene, suggesting a high priority for selenocysteine incorporation into this enzyme. The conservation of this enzyme from *Xenopus laevis* tadpoles to humans implies an essential role for regulation of thyroid hormone inactivation during embryological development.

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# Type 3 Iodothyronine Deiodinase: Cloning, In Vitro Expression, and Functional Analysis of the Placental Selenoenzyme

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## Abstract

**Type 3 iodothyronine deiodinase (D3) catalyzes the conversion of T<sub>4</sub> and T<sub>3</sub> to inactive metabolites. It is highly expressed in placenta and thus can regulate circulating fetal thyroid hormone concentrations throughout gestation. We have cloned and expressed a 2.1-kb human placental D3 cDNA which encodes a 32-kD protein with a K<sub>m</sub> of 1.2 nM for 5 deiodination of T<sub>3</sub> and 340 nM for 5' deiodination of reverse T<sub>3</sub>. The reaction requires DTT and is not inhibited by 6*n*-propylthiouracil. We quantitated transiently expressed D3 by specifically labeling the protein with bromoacetyl [<sup>125</sup>I]T<sub>3</sub>. The K<sub>cat</sub>/K<sub>m</sub> ratio for 5 deiodination of T<sub>3</sub> was over 1,000-fold that for 5' deiodination of reverse T<sub>3</sub>. Human D3 is a selenoenzyme as evidenced by (a) the presence of an in frame UGA codon at position 144, (b) the synthesis of a 32-kD <sup>75</sup>Se-labeled protein in D3 cDNA transfected cells, and (c) the presence of a selenocysteine insertion sequence element in the 3' untranslated region of the mRNA which is required for its expression. The D3 selenocysteine insertion sequence element is more potent than that in the type 1 deiodinase or glutathione peroxidase gene, suggesting a high priority for selenocysteine incorporation into this enzyme. The conservation of this enzyme from *Xenopus laevis* tadpoles to humans implies an essential role for regulation of thyroid hormone inactivation during embryological development. (*J. Clin. Invest.* 1995; 96:2421–2430.)** Key words: iodide peroxidase • thyroxine • thyroid hormones • selenium • selenocysteine

## Introduction

Thyroid hormone is critical to the normal development of the human central nervous system (CNS).<sup>1</sup> Despite the presence

of thyroxine (T<sub>4</sub>) and thyroid follicles in the fetal thyroid by 10–12 wk of gestation as well as the potential availability of maternal thyroid hormone, the free concentration of the active thyroid hormone, 3,5,3'-triiodothyronine (T<sub>3</sub>), is less than half that of maternal levels up to the time of delivery (1–3). The physiological rationale for this circumstance is not well understood but is thought to permit the precise timing and regulation of T<sub>3</sub> delivery to the CNS by coordination of the expression and action of the type 2 5'-iodothyronine deiodinase (for review see reference 4). This enzyme uses tissue T<sub>4</sub> as a substrate to produce T<sub>3</sub> locally and is the primary source of T<sub>3</sub> for this organ (5). It is also possible that normal circulating T<sub>3</sub> concentrations could have deleterious effects on immature tissues or could enhance the metabolic requirements of the fetus.

There are two principal mechanisms by which the circulating fetal T<sub>3</sub> concentration is maintained at low levels. One is that the type 1 iodothyronine deiodinase (D1) in fetal liver is expressed at low levels relative to those in adult life (for review see references 6 and 7). This reduces the extrathyroidal T<sub>3</sub> supply from this source. The second important factor in maintaining low serum T<sub>3</sub> concentrations is the expression of high levels of the type 3 deiodinase (D3) in placenta of all species so far examined (for review see references 4, 6–8). This deiodinase catalyzes the inner ring deiodination of T<sub>3</sub> and T<sub>4</sub> inactivating circulating iodothyronines as well as minimizing transplacental passage of maternal hormone. D3 activity is also expressed in brain, especially in the rat fetus, in fetal rat skin and intestine, as well as in embryonic chick liver (6, 7). In humans, placental D3 is sufficiently potent that instillation of 700 μg of T<sub>4</sub> into amniotic fluid at term causes insignificant increases in the neonatal serum T<sub>3</sub> concentration assessed 24 h later (9).

The cDNAs encoding the D1 enzymes of several species have been cloned, and all have been shown to contain the rare amino acid selenocysteine (10–12). The requirement for selenium in the active center of this enzyme for maximum activity can explain the increased ratio of T<sub>4</sub> to T<sub>3</sub> in the circulation and the significant decrease in D1 activity in the liver and kidney of rats made selenium deficient (13, 14). However, selenium deficiency has no effect on placental D3 activity nor can a selenium-labeled protein be identified in rat placental microsomes (15, 16). This has led to the conclusion that this enzyme does not contain selenocysteine. On the other hand, St. Germain et al. recently identified a T<sub>3</sub>-responsive cDNA found in *Xenopus laevis* tadpoles as one encoding a D3 enzyme (XD3) (17). The XD3 protein is 50% identical to rat D1 and contains an in frame UGA codon and selenocysteine insertion sequence (SECIS) element in the 3'-untranslated region of the mRNA (17). A SECIS element is a stem loop sequence which is required for suppression of the stop codon function of UGA and the insertion of selenocysteine (18). We undertook the present studies to identify the human D3, to establish whether it is, or is not, a selenoenzyme and analyze its tissue expression and catalytic function.

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1. Abbreviations used in this paper: BrAc, bromoacetyl; CNS, central nervous system; D1, type 1 iodothyronine deiodinase; D3, type 3 iodothyronine deiodinase; GH, growth hormone; GTG, gold thioglucose; h, human; HEK, human embryonic kidney; nt, nucleotide; PE, phosphate/EDTA buffer; PTU, 6*n*-propylthiouracil; SECIS, selenocysteine insertion sequence; SEI D, selenophosphate synthetase, TK, thymidine kinase; ut, untranslated.

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## Methods

**Materials.** Two cDNA libraries were used. The first was a human placenta cDNA library in a CDM-8 vector prepared according to the methods of Aruffo and Seed (20), and kindly provided by Dr. Brian Seed (Massachusetts General Hospital, Boston, MA). The second, a human placenta cDNA library in  $\lambda$ -Zap II, was purchased from Stratagene Inc. (La Jolla, CA). Bromoacetylchloride was from Aldrich Chemical Co. (Milwaukee, WI).  $^{125}\text{I}$ -labeled 3,3',5'-triiodothyronine ( $\text{rT}_3$ ) and [ $^{125}\text{I}$ ] 3,5,3' triiodothyronine were from DuPont-NEN (Boston, MA). [ $^{75}\text{Se}$ ]-Selenite was a generous gift of Dr. Dolph Hatfield (National Institutes of Health, Bethesda, MD) and was originally obtained from Dr. Kurt Zinn (University of Missouri Research Reactor, Columbia, MO). All other chemicals were of reagent grade.

**Isolation of a human D3 cDNA.** Based on sequence homology between rat D1 cDNA and *Xenopus laevis* D3 cDNA, we prepared two degenerate oligos, sense No. 1 AT(CT)TT(CT)GG(AGCT)(AT)(G-C)(AGCT)TG(CT) AC(ACGT)TG(CT)CC and antisense No. 2 (AG)TG(AGCT)GC(CT)TC(CT) TC(AGT)AT(AG)TA(AGT)-AT(AGT)AT. We used these oligos in a reverse transcriptase-PCR reaction with 2  $\mu\text{g}$  of human placental poly(A) $^+$  RNA as template. Total RNA was prepared from human placenta using the guanidium method (19). Poly(A) $^+$  RNA was isolated by chromatography on oligo-(dt) cellulose type 7 (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ). We amplified a fragment 120 bp long using the above oligos and subcloned this into pBluescript (pBS, Stratagene Inc.). The nucleotide sequence of this fragment had 72% identity to the XD3. Based on this sequence, two specific oligos (sense No. 3 and antisense No. 4) were synthesized for use with the placental library to obtain the full-length cDNA. The initial PCR amplification was with either oligo No. 3 or No. 4 and a CDM-8 vector-specific oligo. This amplification produced a band 812 bp long which contained 580 bp of sequence similar to the 5' portion of the XD3, but the same strategy was not successful for cloning the 3' portion of the cDNA. We therefore screened a different human placental cDNA library (cloned in  $\lambda$ ZAPII; Stratagene Inc.) using the subcloned 812-bp 5' fragment as a probe. From a total of  $\sim 900,000$  recombinants, five positive clones were isolated, one of which corresponded to the full-length cDNA. This was also subcloned into pBS.

**Northern blotting.** A multiple-tissue Northern blot (Clontech Laboratories, Palo Alto, CA) containing 2  $\mu\text{g}$  of poly(A) $^+$  RNA from a number of normal human tissues was hybridized with hD3 full-length cDNA following the directions of the manufacturer and autoradiographed. The brain sample was derived from a whole normal adult human brain processed for mRNA extraction.

**Isolation of a partial human D3 genomic clone and S1 analysis.** A human placenta genomic library in  $\lambda$ -Fix II (Stratagene Inc.) was screened by hybridization using as probe a 180-bp fragment corresponding to the 5' end of the hD3 cDNA (PstI/SacI fragment). DNA from two of five positive clones was mapped after digestion with appropriate restriction enzymes by Southern hybridization. DNA fragments were subcloned into pBS and sequenced by the dideoxy chain termination method using Sequenase Version 2.0 (United States Biochemical Corp., Cleveland, OH). Poly(A) $^+$  RNA from human placenta was purified as described before. The 5' end of the hD3 gene transcript was determined by S1 analysis as described (19). A 23-nucleotide (nt) primer complementary to the -62 to -42 bp sequence 5' to the A of the initiator ATG (CCGACCACCAACCGCGACGTG) was end labeled with [ $\gamma$  $^{32}\text{P}$ ]ATP and  $\text{T}_4$  nucleotide kinase (Pharmacia). The template for the synthesis of the single-stranded probe was a  $\sim 600$ -bp genomic fragment with its 3' border 178 bp downstream of the ATG. After the annealing, the extension reaction and the isolation of a single-stranded 350-nt probe, an amount of probe equal to  $5 \times 10^4$  Cerenkov counts was hybridized with 10  $\mu\text{g}$  placental Poly(A) $^+$  RNA for 14 h. The next day, digestion was performed using S1 nuclease (New England Biolabs Inc., Boston, MA) and the product analyzed on a 6% polyacrylamide, 8.3 M urea gel in parallel with a sequencing reaction generated with the extension primer.

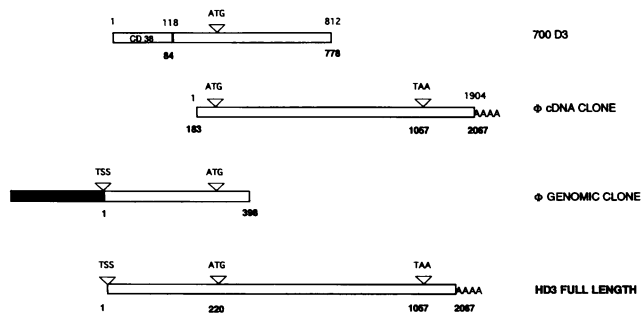
**Preparation of hD3, XD3, and SelD vectors for eukaryotic expression.** To confirm that this clone encoded the type 3 deiodinase, a 1.9-kb insert containing 35 bp of 5' untranslated (ut) region (186–2066) (Fig 1) was excised from pBluescript and inserted into the XhoI/NotI sites of CDM-8, a mammalian expression vector (20). The same construct was then prepared with the *Xenopus* D3 cDNA (XD3), kindly provided by Drs. Robert A. Schwartzman and Donald D. Brown of Carnegie Institution (Baltimore, MD), that was excised from pBS with EcoRI/XhoI restriction enzymes and subcloned into the same site of the D10 eukaryotic expression vector (21).

The human selenophosphate synthetase (Sel D), the homologue of the bacterial enzyme (22), has recently been cloned (23). Its cDNA was also subcloned into CDM-8 as described.

**DNA transfections.** Transfection of human embryonic kidney (HEK)-293 cells was by calcium phosphate precipitation as described previously (24). 2 d after transfection, cells were harvested and sonicated in 0.1 M potassium phosphate, 1 mM EDTA, pH 6.9, (phosphate/EDTA buffer [PE] buffer), containing 25 mM DTT. Transfection efficiencies were monitored by assay of human growth hormone (GH) in the media derived by cotransfecting a constitutive thymidine kinase (TK) promoter-directed human growth hormone-expressing plasmid, pTKGH (25). Kinetic studies and affinity labeling were performed using aliquots of the same sonicates.

**Deiodinase assays.** For 5' deiodinase assay, reactions contained 5–150  $\mu\text{g}$  of cell sonicate protein, 0.2 nM 3,3', [ $^{125}\text{I}$ ]5' triiodothyronine (reverse  $\text{T}_3$  or  $\text{rT}_3$ ) purified by LH-20 chromatography, varying concentrations of unlabeled reverse  $\text{T}_3$  or other reagents as indicated and 10 mM DTT in PE buffer in a final volume of 300  $\mu\text{l}$ . Incubations were for 60 or 120 min at 37°C.  $^{125}\text{I}$  was separated by TCA precipitation after addition of horse serum as described (26). Deiodination was linear with both protein and time and the quantity of protein assayed was adjusted to consume  $< 30\%$  of substrate. All reactions were in duplicate, and all experiments were performed at least twice, with similar results. For the determination of 5' deiodinase activity reaction mixtures contained 1–20 nM [ $^{125}\text{I}$ ] 3,5,3', triiodothyronine, varying concentrations of DTT, unlabeled  $\text{T}_3$ , or other reagents as indicated. [ $^{125}\text{I}$ ] $\text{T}_3$  was purified by chromatography using Sephadex LH20 (Sigma Chemical Co., St. Louis, MO) before use. The labeled compounds present in the reaction mixture were separated for quantification by ascending paper chromatography (27). Data were quantified and values for  $V_{\text{max}}$  and  $K_m$  estimated using double reciprocal plots as previously described (17). Data were subjected to one-way ANOVA, and statistical differences among groups were determined using Duncan's multiple range test (28).

**Bromoacetyl (BrAc) [ $^{125}\text{I}$ ] $\text{T}_3$  affinity labeling and enzyme quantitation.** BrAc [ $^{125}\text{I}$ ] $\text{T}_3$  was synthesized from bromoacetylchloride and  $\text{T}_3$  as described previously (29). The product was purified on LH-20 Sephadex by elution with ethanol and purity verified by thin layer chromatography in ethyl acetate/glacial acetic acid (9:1), and the concentration of the product was determined from the specific activity of the  $\text{T}_3$  used in the starting material as described previously. In some experiments, labeled BrAc $\text{T}_3$  was diluted with 0.01–3.0 pmol of unlabeled BrAc $\text{T}_3$  synthesized in the same manner. In each reaction 0.05 mCi ( $\sim 10$  fmol) of BrAc [ $^{125}\text{I}$ ] $\text{T}_3$  was incubated 10 min at room temperature with 100  $\mu\text{g}$  of cell sonicate protein in 50  $\mu\text{l}$  PE and 10 mM DTT. Reactions were terminated by addition of gel loading buffer containing SDS and  $\beta$ -mercaptoethanol, followed by boiling for 2 min. Samples were analyzed by SDS-PAGE and autoradiography. The regions of the gel corresponding to the D3 enzyme protein were excised and counted. Equivalent regions were counted from gel lanes containing CDM-8 vector-transfected sonicates and the nonspecific counts subtracted from total counts incorporated. The quantity of enzyme was determined by saturation analysis after plotting the ratio of BrAc [ $^{125}\text{I}$ ] $\text{T}_3$  specifically bound to the 32-kD protein to total counts added versus the picomoles of BrAc [ $^{125}\text{I}$ ] $\text{T}_3$  bound per milligram of total protein as previously described (26). Extrapolation of this linear plot to the abscissa gave an estimate of the total enzyme present as pmol/mg protein, assuming 1 mol of BrAc $\text{T}_3$  is bound/mol enzyme. Quantifications were performed at least twice for each sonicate. Densitometric quantification of the



**Figure 1.** Human D3 clones. The numbers over the bars refer to the nucleotide sequence of the particular clone, whereas the bold numbers under each bar correspond to the nucleotide sequence of the predicted hD3 full length cDNA. The position of the transcriptional start site, (TSS), the translation initiation codon (ATG) and the stop codon (TAA) are also indicated. The white field areas correspond to cDNA sequences, the gray to genomic DNA.

autoradiographs was performed by a computing densitometer (Molecular Dynamics Inc., Sunnyvale, CA).

<sup>75</sup>Se *in vivo* labeling. Plasmids were transfected by CaPO<sub>4</sub> coprecipitation into the HeLa cell line, HtTA (21) cells, as previously described (30). 3 d before transfection, these cells were plated onto 60-mm dishes in DME containing 10% FCS. 1 d before transfection the media was changed to DME containing 1% FCS supplemented with 100 pM T<sub>3</sub>, 10 mg/ml transferrin, 20 μg/ml insulin, and 50 nM hydrocortisone to decrease the medium selenium concentrations. The day after transfection, the cells were shocked with 10% DMSO and fresh media containing 5 nM unlabeled Na<sub>2</sub>SeO<sub>3</sub> plus 6 μCi Na<sub>2</sub>[<sup>75</sup>Se]O<sub>3</sub>/dish. After 18 h of incubation, the cells were harvested, washed, resuspended in PE buffer, and sonicated. Aliquots of labeled cell sonicate were analyzed by SDS-PAGE.

*Sequence analysis.* Nucleotide and protein sequence analysis was performed using the Sequence Analysis Software Package from the Genetics Computer Group (University Research Park, Madison, WI).

## Results

*Isolation of a human D3 cDNA.* A combination of PCR and screening by homology was used to isolate a fragment 812 bp long (700D3) from the CDM-8 library containing nucleotides 84–778 of the complete hD3 cDNA (Fig. 1). Since the first 5' 118 nt of 700D3 was found to be 100% identical to the published sequence of the human lymphocyte antigen CD38 (Fig. 1), we suspected that this portion of the cDNA was a product of a cloning artifact. Since the 5' ut region of XD3 extends only 24 nt 5' to the initiator ATG, the identity of the intervening nucleotides (between 119 and 195) in 700D3 was not clear. We next used the 700D3 5' clone to isolate 5 positive D3 clones from a λ-ZAP II library. These contained inserts varying in length from 1500 to 1905 nucleotides. The longest of these clones was subcloned into pBS and sequenced and contained nucleotides 195–2067 of the hD3 full-length cDNA. None of the phage inserts contained sequences 5' to nucleotide 195 (Fig. 2). To define the 5' ut region of human D3 we next screened a human genomic library with a probe from the coding region. We identified several clones containing contiguous sequences identical to those in the 700D3 clone between the CD38 fragment and the 5' border of the sequence homologous to the 5' ut of XD3 (nt 84–200 of the final cDNA), establishing that these were contained within the human D3 gene.

We used the genomic fragment which extended 600 nt 5'

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1  AGCCCAAGATTTC TAGGCATTGGCCGCTGCTGGGTGACTTCGGGCTCAAGTTGCAAG
61  GGGCGGGCCGGCCGGAGGTGGAGTCTCCCGCCAAATTGAAGCTCCGCTATAAATGAA
121  CTCCTGCTACATGCTGAAGCCAGATGCCCTCCGAGCCAGCTCCGGTGTGGTGGCGAG
181  AGGGCGAGGGTCCAGGGGGCTTCGGGCTGCAGCCACCTGCCTCCACTCCCTGCTGCT
      M L H S L L L L
241  TCACCTCTGAGGCTCTGGCCAGACCGCTCGTGCCTCGTCTCTCCCGGCTTCCT
      G S L R L C A Q T A S C L V L F R F L
301  CGGCACGGCTTCATGCTCTGGCTTCGATTTCCTGTGTATCCGCAAGCATTTCCTGGG
      G T A F M L W L D F L C I R K H F L G
361  CGCGCCGCGGGGCAAGCCAGCCGCGAAGTGGAGCTCAACAGTGAAGGGAGGAGGT
      R R R R G K P E P E V E L N S E G E S P
421  GCCTCCGATGACCCGCCATCTGCGTGTCCGACGACAAACCGCTGTCACCCCTGGGCTC
      P P D D P P I C V S D D F R L C T L A S
481  GCTAAGGCGGTGTGGCATGGCAGAACTGGATTCTTCAAGCAGGCGCAGGAGGGGG
      L K A V W H G Q K L D F F R Q A H E G G
541  TCCGGCCCAACTCCGAGGTGGTCTCGCCGACGGCTCCAGAGCCAGCACATCTCTGA
      P A P N S E V V L P D F G F Q S Q I H I L D
601  CTACCGCAAGGAAACCGCCGCTGGTCTCAATTCGCGAGCTGCACCTGCACCCGCTT
      Y A Q G N R P L V L N F R G S C T S e P P F
661  CATGGCGCATGAGGCCCTTCAGCGCTGGTCACTAAGTACCAGCGCAGCTGCAGCT
      M A R M S A F Q R L V Q R D V D F
721  CCTCATCTACATCGAGGAGCCGCTCCGACGGTTCAGCCAGGCTACCTCC
      L I I Y I E E A H P S D G W V T T D S P
781  CTACATCTCCACAGCAGCCGAGCCGAGGCTGAGGCGAGGAGGAGGAGTACTGCA
      Y I I P Q H R S L E D R V S A A R V L Q
841  GCAAGGTGACCCGGCTGCGCTCTGCTCGACACCATGGCCAACTCCAGCAGCTCGGC
      Q G A P G C A L V L D T M A N S S S A
901  CTATGGCGCTACTTCGAGCTCTCTATGTATCCAGAGTGGCACTATTATGTACAGGG
      Y G A Y F E R L Y I Q S G T I M Y Q G
961  CGGCGTGGCCCGGAGGCTACCAGGCTCTGAGCTGGCCACTTGGTGGAAAGCGTATGA
      G R G P D G Y Q V S E L R T W L E Y D
1021  TGAGCAATGCACGGGCTCGGCCCGGAGGGTGAACATCCAACGCAATTCGACTGA
      E Q L H G A R P R R V *
1081  ACTTGGTGGGCTGGCCCTCGAGCCTTCGAAGCCACGTCGAAGCGCTCAAACCAAGTC
1141  ACGCTTGGGACGGCCAGTGACATGTGTGCTGAGCCACCATTCAGACTGAGTCTGCA
1201  CCCTCAGCCACATGAACAATCTCCCTACCTCCGAGACTCTGCTTCTGTAACCTGCTCA
1261  TTACCTGCTGGCTTGCCCTTGCGTGTGCCCCAGAGACTTGGCTCTACAGCTCTTCA
1321  TTACACCCCTCCCGAGCGTCCCTCAGCAAGTGTCTTGGCCGGTGTCTCCCGAGCT
1381  GCAAGAGACTTGGCCAGCCCGCCGCGCTGAGGAGCTGGGTTCAGGAGACTCTC
1441  AGCTCAGCTGAGCTAGTGCCTGGCACCCACTGCTCGCGCGGAGAGGGGGTTCCCTGT
1501  TGCTTTTGTCTGTTCCCTGTCCTGGTGGGGAAAGTATGTCGTGATGGGGAGGGGT
1561  GGGCAGTAGTTCCTCCCGCTGTTTGGGTGACAGGAGCCCACTGCTGATGACGAA
1621  CTATCTTAATGCTGTGACACAGAGCTAGTCTGAATTGCAAGGGCCCTCAAAGCAGCA
1681  CCTAAACTTGAAGGGGAGGGTGTCTGGTTCCTGGTGAAGTAAACCTTAAAAGGGAG
1741  GGAAGTTGGGGTGTCTGCTTTGGGACAGAGGAAAGATAGCTTGAGAGGCTTGGCGAGG
1801  TCGCAGCCCGGAGGAGAGAAAAGCTGAGACTTCCTGGGAAATGACGTTGGGGTATG
1861  GGAGTCCGGGAAAGAGAGGTGGGGGAGAGCTGAGCTGCCAAGTGAAGGGGAGCTCTA
1921  GCGAGAGCTGCTGATTGTGGGGCTGGGAGGTGGAGGGCCCTGATTCGAAGGCCATTTGG
1981  TGAGTGTTTTGTGGAAATATTCCTGTATATAAATTCCTTCAATCTACAATAATAAG
2041  GCTTGAAGTAACTGCAAAAAGGAA

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**Figure 2.** cDNA and predicted amino acid sequence of the human D3. The amino acid selenocysteine is noted as SeC.

to the initiator ATG to map the transcriptional start site using S1 nuclease and human placental poly(A<sup>+</sup>) mRNA. There was one major protected band of ~187 bp ending with the A or C nucleotide 220/221 bp 5' to the translation initiation codon (data not shown).

The hD3 cDNA contains 220 bp of 5' untranslated region, an 834-bp open reading frame which contains an in frame TGA codon at position 650–652, and a 3' untranslated region of 1,012 bp. The open reading frame begins with an ATG codon after a Kozak consensus sequence (CCACC, Fig. 2) and ends with a TAA codon at position 1056. A consensus polyadenylation signal (AATAAA) is present at position 2034–9 (Fig. 2) and is followed by a short poly A tail. The deduced amino acid sequence predicts a protein of 278 residues, with a molecular mass of 31.5 kD assuming the TGA codon at position 650–652 encodes selenocysteine. HD3 is highly homologous to the XD3 enzyme, 66% identity at the nucleotide level and 58% identity (73% similarity) to the XD3 deduced amino acid sequence. The human enzyme contains a 12-amino acid insert in the amino-terminal region and is seven residues longer than XD3 (Fig. 3 A). There is also a potential N-glycosylation site (NXS) in the predicted sequence at residues 222–224 which is conserved in XD3. An hydropathy analysis (Fig. 3 B) revealed a hydrophobic amino-terminal portion consistent with a transmembrane domain.

*Northern blotting.* A <sup>32</sup>P-labeled hD3 full-length cDNA hybridizes to a single mRNA species of ~2.1–2.2 kb in human placenta (Fig. 4 A) consistent with the size of the composite cDNA. No hybridizing band could be detected in RNA from seven other human tissues. A low intensity band of the same

A

```

Human D3 1 MLHSLLLHSRLCAQTASCLVLFERFLGTAFMLWLLDFLCIRKHLGRRR 50
          |||: |. :| .|:|:| | | | | | | | | | | | | | | | | | | |
Xeno D3  1 MLHCAGFHTGKLVKQVAACCLLLEPRFLITGLMLWLLDFQCIRRRVLLTAR 50

51 RGKPEPEVELNSEGEVPPDDPPICVSDNRLCTLASLKAVWHQKLDFF 100
          |:.....| | | | | | | | | | | | | | | | | | | | | | | |
51 .....EESTAHEDPPLCVSDSNRMCTIVESLRAVWHQKLDYF 88

101 RQAHEGGPAPNSEVVLFDGFGSQHLLDYAQQNRPLVNFSGSCT*PPFMAR 150
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
89 KSAHLGCSAPNTEVVMLEGRRLCKLLDFSQGKRPLVNFSGSCT*PPFMAR 138

151 MSAPQRLVTKYQRVDFLILLYIEEAHPSDGMVITDSPYLLIPQHRSLIEDRV 200
          : | : | | | | | | | | | | | | | | | | | | | | | | | | | | | |
139 LQAYRRLAAQHVGADFLLVVIEEAHPSDGLSTDSAYQIIPQHQCLQDRL 188

201 SAARVLQGGAFGCALVLDIMANSSSAYGAYFERLYVIQSGTIMYQGGRG 250
          . | | : : | | | | | | | | | | | | | | | | | | | | | | | | | |
189 AAAQLMLQGGAFGCRVVDIMNNSNAAYGAYFERLYVLEGGKVVYQGGRG 238

251 EDGYQVSELRITWLEFYDEQLHGARPRV..... 278
          | : | | | | | | | | | | | | | | | | | | | | | | | | | | | |
239 PEGYKISELRFMLLEQYQGLMGIKGSGQVVIQV 271
  
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B

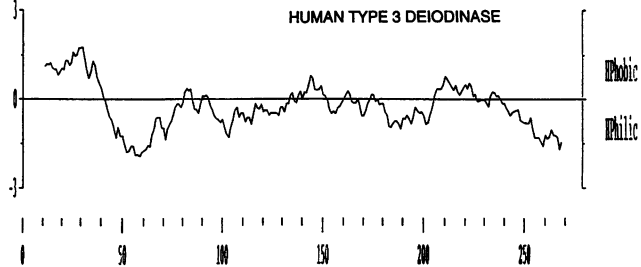
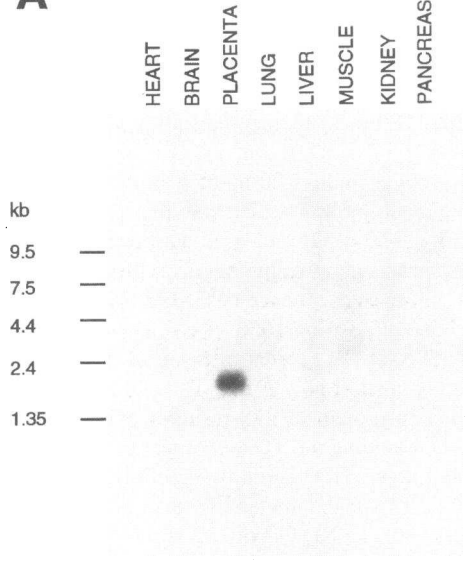


Figure 3. (A) Alignment of the predicted amino acid sequences of human and *Xenopus laevis* D3 proteins. Selenocysteine at position 144 is shown with an asterisk. (B) Hydropathy analyses of human D3 protein using the Kyte-Doolittle algorithm (window = 21) as predicted by the PEPLOT program of University of Wisconsin Genomic Computer Group.

size appeared in lung poly(A)<sup>+</sup> with 5 d longer exposure but not in brain. This pattern of distribution correlates with previous demonstrations of D3 activity in human placenta, but does not show expression of D3 in human brain which would be expected

on the basis of animal studies (6). Probing with a mouse  $\beta$ -actin fragment (Fig. 4 B) confirmed the presence of adequate quantities of well preserved mRNA in all lanes.

A



B

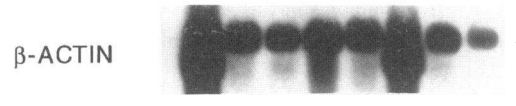


Figure 4. Northern blot analysis of human type 3 deiodinase. The blot was probed with an hD3 (A) or a mouse  $\beta$ -actin cDNA (B). Each lane contains 2  $\mu$ g of human Poly(A)<sup>+</sup> RNA. The sizes of the molecular weight markers are indicated.

Affinity labeling of hD3. Bromoacetyl [<sup>125</sup>I]T<sub>3</sub> labeling of the hD3 transfected HEK-293 cell sonicates was performed to establish if transiently expressed D3 could be specifically labeled with BrAc[<sup>125</sup>I]T<sub>3</sub> and, if so, to determine the size and quantity of the transiently expressed protein. When hD3 cDNA is transfected, a band of ~ 32 kD appears, which is not present in the vector-transfected cells (Fig. 5). The BrAc[<sup>125</sup>I]T<sub>3</sub> labeling of the hD3 protein is specifically blocked by increasing concentrations of cold BrAcT<sub>3</sub> (Fig. 5) and a plot of specifically

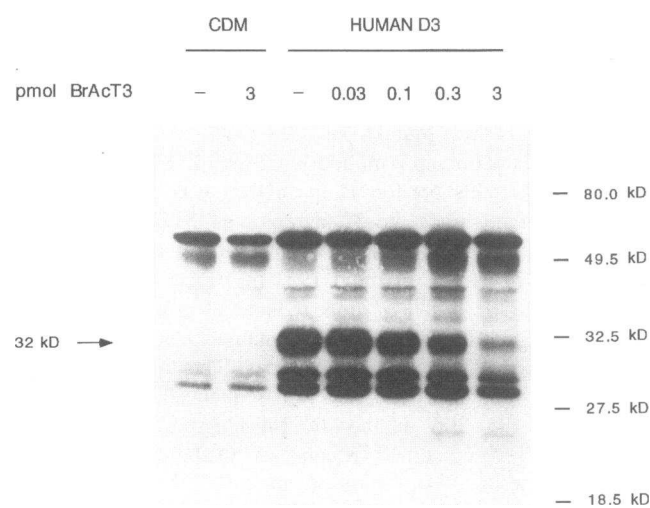
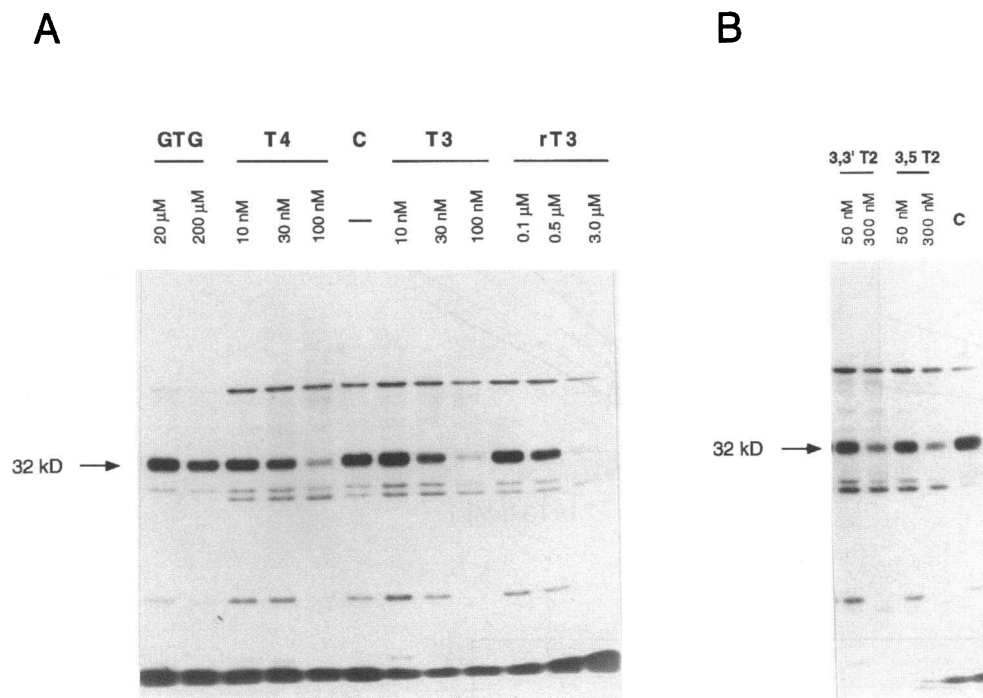


Figure 5. BrAcT<sub>3</sub> labeling of transiently expressed human type 3 deiodinase. Transfection of the CDM-8 vector alone or vector containing the human D3 as indicated. Cell sonicate protein (100  $\mu$ g) was incubated with a constant quantity of BrAc[<sup>125</sup>I]T<sub>3</sub> together with increasing amounts of added unlabeled BrAcT<sub>3</sub> as indicated above each lane. The positions of the molecular weight standards are shown.



**Figure 6.** Inhibition of BrAc[<sup>125</sup>I]T<sub>3</sub> labeling of human D3 by various competitors or substrates. Cell sonicates of D3 cDNA-transfected HEK-293 cells (100  $\mu$ g) were exposed to BrAc[<sup>125</sup>I]-T<sub>3</sub> after 10 min incubation with various competitors or substrates at the indicated concentrations and analyzed by SDS-PAGE. Lanes labeled C are controls.

labeled protein vs [BrAcT<sub>3</sub>] is linear allowing quantitation of the transiently expressed hD3. These results indicate that the hD3 cDNA encodes a protein of the size predicted from the open reading frame if the stop function of the UGA codon at position 650 is suppressed and the UAA at 1055-7 is the true stop codon (Fig. 2).

We have previously shown that covalent labeling of in vitro expressed D1 by BrAc[<sup>125</sup>I]T<sub>3</sub> is blocked by substrates or competitive inhibitors of the deiodinase due to occupancy of the selenocysteine-containing active site. Similarly, BrAc[<sup>125</sup>I]T<sub>3</sub> labeling of the 32-kD protein is blocked in a dose-dependent fashion by D3 substrates (Fig. 6). The estimated order of potency of these compounds to block BrAcT<sub>3</sub> labeling mirrors that expected from the substrate specificities of the human or rat placental enzyme, with T<sub>3</sub> > T<sub>4</sub> >> rT<sub>3</sub> (Fig. 6 A) (6-8). Also 3,5-diiodothyronine (3,5,-T<sub>2</sub>) and 3,3'-T<sub>2</sub> (Fig. 6 B) inhibit the BrAc[<sup>125</sup>I]T<sub>3</sub> incorporation into the 32-kD protein at concentrations comparable to those of T<sub>3</sub> or T<sub>4</sub>. Gold-thiogluco- (GTG), a potent inhibitor of D1 action and BrAc[<sup>125</sup>I]T<sub>3</sub> labeling ( $K_i \sim 10$  nM) (31), is much less efficient as an inhibi-

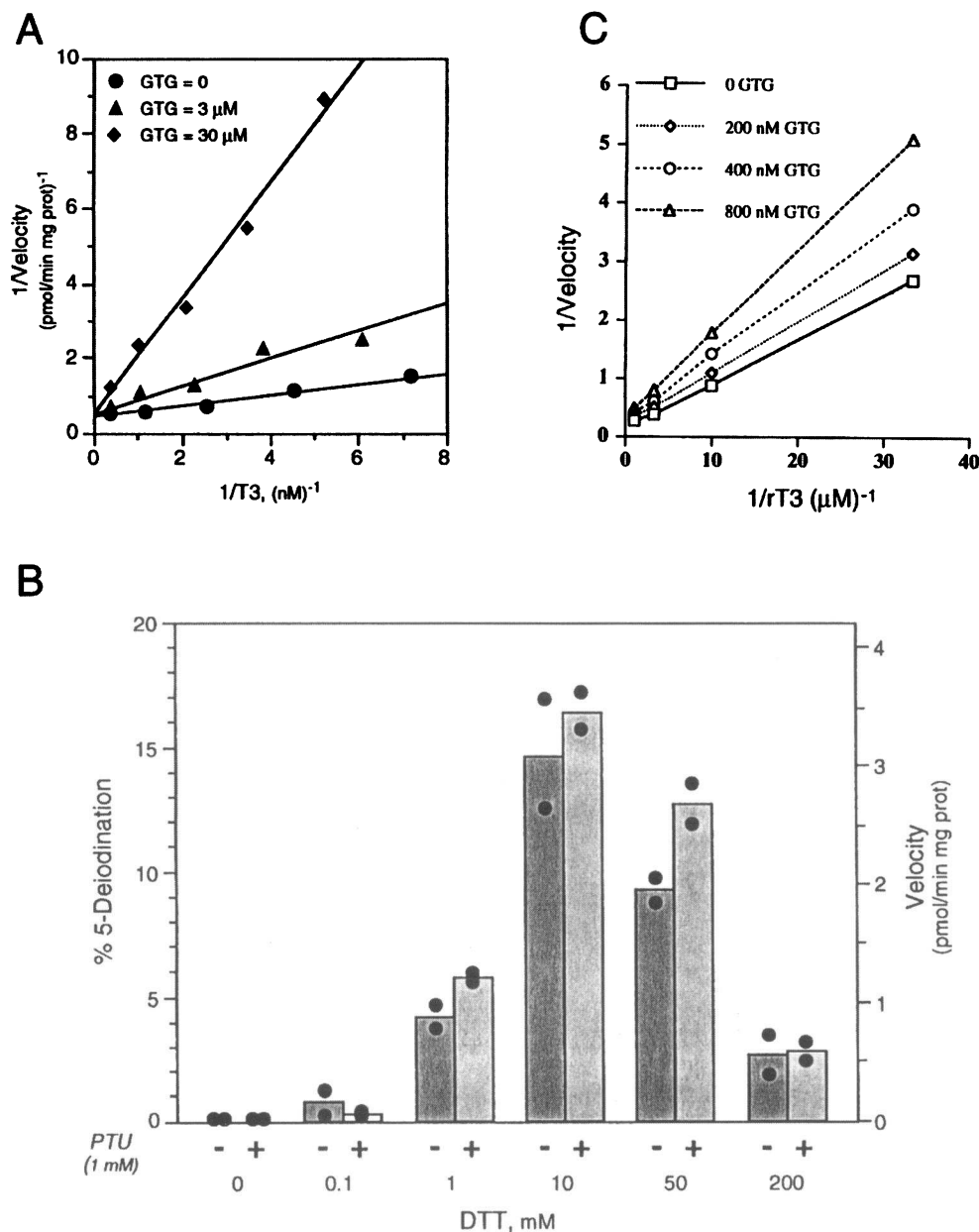
tor of BrAc[<sup>125</sup>I]T<sub>3</sub> D3 labeling. As deduced from labeling reactions in the presence of GTG (Fig. 6 A), 2 mM GTG would be required for a 50% reduction of the BrAc[<sup>125</sup>I]T<sub>3</sub> binding.

*Human D3 catalyzes both 5 and 5' iodothyronine deiodination.* To analyze the reaction kinetics of hD3, we transiently expressed the protein in HEK-293 cells which contain no endogenous D3. Cell homogenates from hD3-transfected cells deiodinate the inner ring of T<sub>3</sub> in a saturable fashion, producing <sup>125</sup>I and 3,3'-diiodothyronine. The apparent  $K_m$  of the hD3 enzyme for T<sub>3</sub> is 1.2 nM and the  $V_{max}$  is 4.0 U (1 U = 1 pmol T<sub>3</sub> deiodinated min<sup>-1</sup> mg<sup>-1</sup> sonicate protein (Table I). The enzyme requires DTT as cofactor in the deiodination reaction, with maximal 5D activity observed at 10 mM DTT (Fig. 7 B). GTG is a competitive inhibitor of T<sub>3</sub> 5 deiodination, with an apparent  $K_i$  of 5.2  $\mu$ M (Fig. 7 A). The enzyme is insensitive to 6*n*-propylthiouracil (PTU) inhibition, with no effect observed up to concentrations of 1 mM at varying DTT levels (Fig. 7 B). Shown in Table I are the values for [E], the picomoles of specifically bound BrAcT<sub>3</sub>/mg HEK-293 cell sonicate protein in the preparation used for kinetic analyses. Using these results,

**Table 1.** Kinetics of 5 and 5' Deiodination by Transiently Expressed hD3

Deiodination activity and substrate	EXP	$K_m$	$V_{max}$	[E]*	$K_{cat}$	$K_{cat}/K_m$	$K_i$ GTG
		$\mu$ M	pmol/min mg prot	pmol mg prot <sup>-1</sup>	min <sup>-1</sup>	min <sup>-1</sup> $\mu$ M <sup>-1</sup>	$\mu$ M
5 deiodinase (T <sub>3</sub> )	1	0.0011	3.96	0.140	28.2	25636	6.4
	2	0.0013	4.08	0.215	19.0	14615	4.1
	mean	0.0012	4.02	0.177	23.6	19666	5.2
5' deiodinase (reverse T <sub>3</sub> )	3	0.333	0.24	0.215	1.1	3.3	0.9
	4	0.337	0.21	0.140	1.5	4.4	—
	mean	0.335	0.23	0.177	1.3	3.8	—

Exp, experiment; prot, protein; \* Enzyme quantification was performed by saturation analysis using BrAc[<sup>125</sup>I]T<sub>3</sub> as described in Methods.



**Figure 7.** Kinetic studies of 5' deiodination of T<sub>3</sub> (A and B) or 5' deiodination of reverse T<sub>3</sub> (C) by transiently expressed human D3. (A) Inhibition by GTG. (B) Effect of incubations with various concentrations of DTT with or without PTU. Dots indicate results of independent kinetic assays and the height of the bars their mean. Activities are expressed either as percent deiodination or as velocity. (C) Effect of GTG on 5' deiodination of reverse T<sub>3</sub>. The Velocity is in the same units as in A. Results are representative of two closely agreeing experiments.

the  $K_{cat}$  for 5' deiodination of T<sub>3</sub> was 23 molecules min<sup>-1</sup> and the  $K_{cat}/K_m$  ratio for T<sub>3</sub> 5' deiodination is 19,660 min<sup>-1</sup> μM<sup>-1</sup> (Table I).

Type 3 deiodinase also catalyzes 5' deiodination of reverse T<sub>3</sub>, although with much lower efficiency. For reverse T<sub>3</sub> to 3,3' T<sub>2</sub> conversion, the  $K_m$  was 0.33 μM and the  $V_{max}$  is 0.24 U (Table I). The  $K_{cat}$  for 5' deiodination of rT<sub>3</sub> is 1.4 molecules min<sup>-1</sup>, about 10-fold lower than that for the 5' deiodination of T<sub>3</sub> with the  $K_{cat}/K_m$  ratio of 3.8 min<sup>-1</sup> mM<sup>-1</sup> markedly lower than that for 5' deiodination (Table I). GTG also inhibits 5' deiodination of reverse T<sub>3</sub>, with an apparent  $K_i$  of 0.9 μM (Fig. 7 C). This reaction was also insensitive to PTU inhibition at concentrations up to 1 mM.

**Labeling of hD3, XD3, and rD1 with <sup>75</sup>Se.** To establish that the transiently expressed hD3 incorporates selenium, HtTA cells were transfected with the hD3 cDNA and incubated with Na<sub>2</sub>[<sup>75</sup>Se]O<sub>3</sub> for 18 h. The hD3 cDNA encodes an ~32-kD <sup>75</sup>Se-labeled protein and its <sup>75</sup>Se-labeling is enhanced 10-fold

by cotransfection with the human selenophosphate synthetase cDNA, SelD (Fig. 8). In the same experiment, we also could identify the <sup>75</sup>Se-labeled rat D1 protein, which appears as a radiolabeled 29-kD band in the rD1 cDNA transfected cells (Fig. 8). With a threefold longer exposure time, a ~30-kD band could be seen in XD3 cDNA transfected cells indicating that the rate of synthesis or the efficiency of selenocysteine incorporation into the *Xenopus* enzyme is much lower than that of hD3.

**The hD3 mRNA contains a SECIS element.** We have previously shown that eukaryotic selenoprotein mRNAs contain SECIS elements in the 3' ut region which are necessary and sufficient for selenocysteine incorporation at UGA codons (18, 32). Transfection of a cDNA in which the 3' ut region (3' to nt 1118) is deleted did not produce a functional deiodinase indicating the presence of SECIS activity in the 3' ut of hD3 (Fig. 9). To confirm this and to compare the potency of the hD3 SECIS element with that of the rat D1 mRNA, we inserted

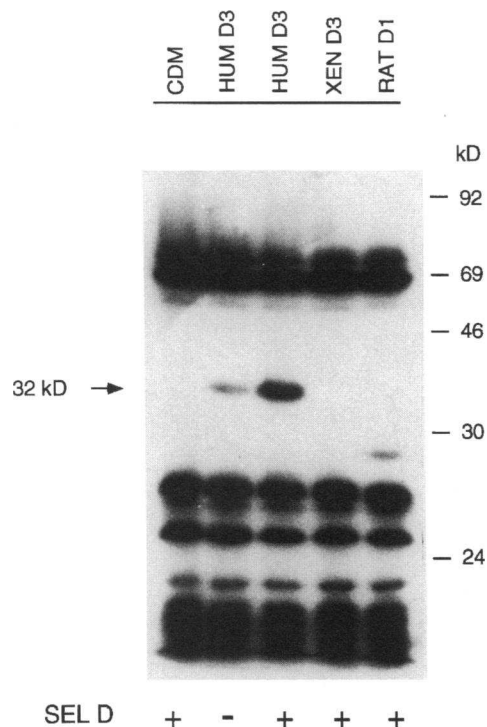


Figure 8. Incorporation of  $^{75}\text{Se}$  into transiently expressed human or *Xenopus* D3 or rat D1 in the presence or absence of coexpressed human SelD in HtTA cells. Cells were incubated in selenium-depleted medium for 1 d before transfection of plasmids expressing the various proteins. CDM indicates cells transfected with the vector alone.

the hD3 3' ut region (nt 1118–1720) downstream of the rat D1 cDNA coding region. In the absence of an intact SECIS element, this construct does not express a functional D1 (Fig. 9). The deiodinase activity of the D1-hD3(SECIS) construct was  $\sim 4.5$ -fold higher than that of the wild-type D1 cDNA, similar to that of the previously characterized D1-selenoprotein P (SECIS) construct (32). These results indicate there is a highly potent SECIS element in 3' ut region of the hD3 mRNA.

Analysis by a folding program was used to determine if the inserted SECIS sequence would be predicted to form a stem-loop structure, similar to that of the D1 SECIS element. Five potential stem loops were predicted by this program, but the segment between nt 1480 and 1720 (Fig. 10 A), had the highest negative free energy ( $-44.5$  kcal). To localize the SECIS activity more precisely we prepared a construct in which these sequences were inserted 3' to the D1 coding region. This sequence is able to drive D1 expression with an efficiency about 50% of the parent D1-D3(SECIS) construct (Fig. 9). Thus, while this mRNA segment still has twice the activity of the D1 element, additional sequences 5' to 1480 are necessary for the full hD3 SECIS potency.

## Discussion

*Human D3 is closely related to Xenopus D3 and human D1.* Type 3 deiodinase catalyzes the conversion of  $\text{T}_4$  to reverse  $\text{T}_3$  and  $\text{T}_3$  to 3,3'- $\text{T}_2$ , both metabolically inactive products. The D3 enzyme thus shares with D1 the capacity to inactivate thyroid hormone though D3 does not accept sulfated iodothyronines which are highly preferred for inner-ring deiodination by D1 (33). The catalytic constants for 5' deiodination of  $\text{T}_3$  and 5'

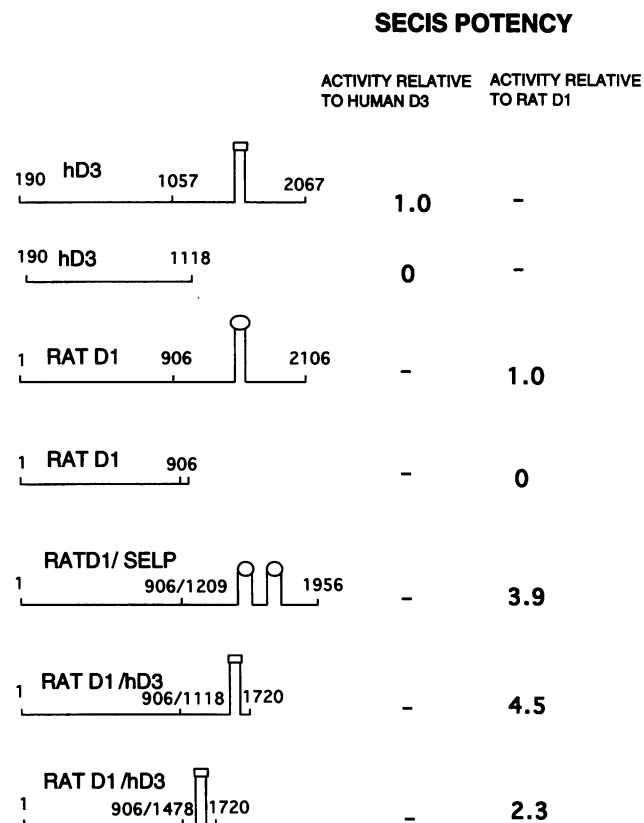


Figure 9. Analyses of the functional potency of the human D3 SECIS element. HEK-293 cells were transfected as described in Methods with CDM-8 expression plasmids containing the constructs schematically presented. Cell sonicates were prepared 48 h after transfection and were assayed for 5' deiodinase activity using reverse  $\text{T}_3$  as substrate. Transfections were performed in the same experiment for each series of constructs. Deiodinase activities were normalized for transfection efficiency by assay of hGH expressed from a cotransfected TKGH plasmid and results expressed as a multiple of the activity of the parent construct (hD3 or rD1) in the same transfection. Results are the mean of three closely agreeing experiments.

deiodination of reverse  $\text{T}_3$  are virtually identical to those for XD3 (17) and to those found for rat tissues (8). While the enzyme catalyzes outer ring deiodination of reverse  $\text{T}_3$  as does D1, the  $K_{\text{cat}}/K_m$  ratio is nearly four orders of magnitude lower than for inner ring deiodination of  $\text{T}_3$  indicating that the latter is a much more efficient reaction. Despite this efficiency, the  $K_{\text{cat}}$  for inner ring deiodination of  $\text{T}_3$  by D3 is 24, 100-fold lower than that for 5' deiodination of reverse  $\text{T}_3$  by D1 (26). This may explain why D3 deiodination is insensitive to PTU since it seems likely from studies of both wild-type and site-directed D1 mutants that an intermediate Se-I complex must be formed for interaction with this agent (8, 12, 34).

Human D3, like the *Xenopus* enzyme and D1, is a selenocysteine-containing protein as evidenced by the presence of an in frame UGA codon, a requirement for an SECIS element for successful translation, and the fact that it incorporates  $^{75}\text{Se}$  in a SelD-dependent fashion. The deduced amino acid sequences of the human and *Xenopus* enzyme are 58% identical (73% similar). In addition, there is 47% similarity with the bifunctional (5' or 5' deiodination) human D1 enzyme which is especially high in the region surrounding the selenocysteine residue (11).

Type 3 deiodinase is an integral membrane protein resistant



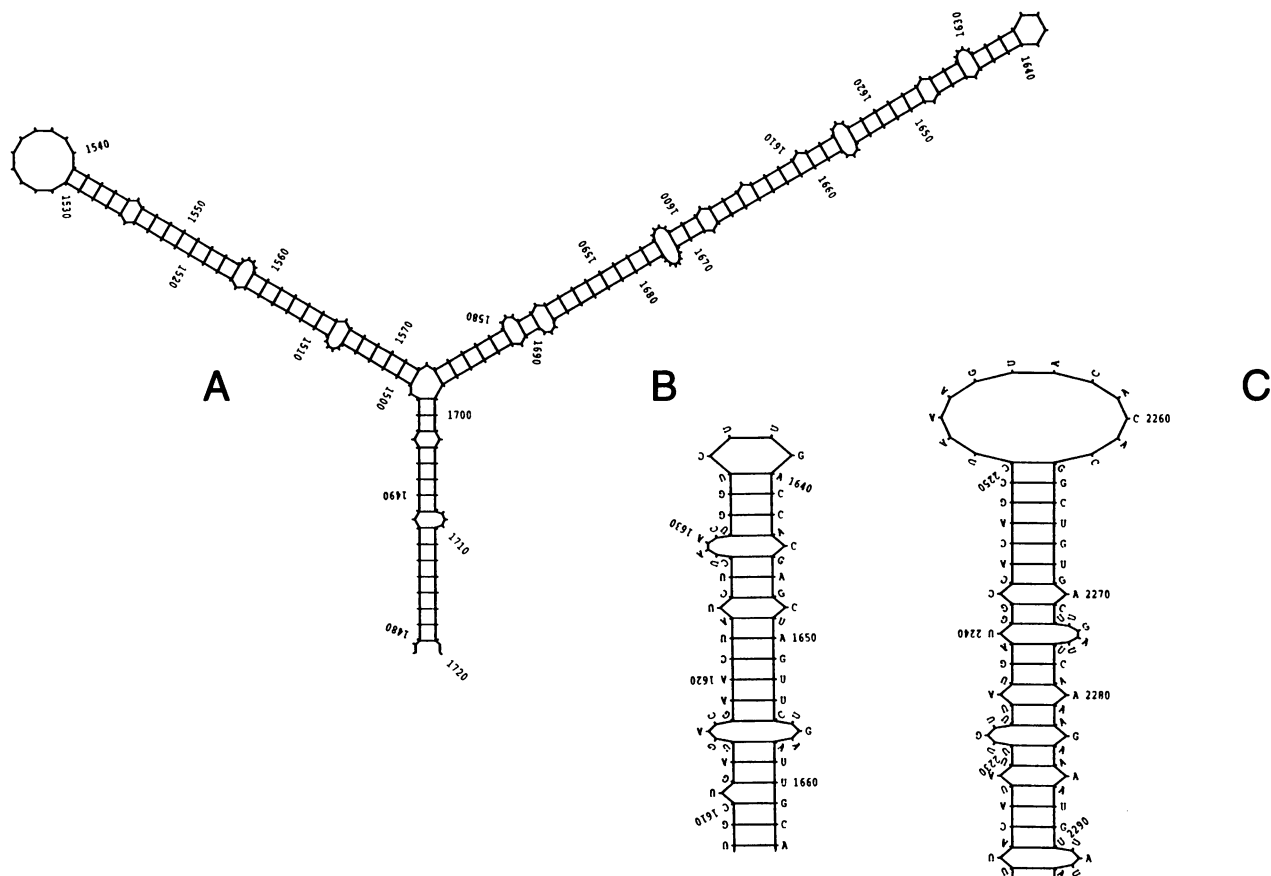


Figure 10. Predicted structures of portions of the 3' ut mRNA of the human D3 (A and B) and D1 (C) mRNAs. The sequence shown in A (nt 1480–1720) has 2.4-fold the SECIS activity of the wild type D1 SECIS element. An enlargement of the terminal portion of the hD3 major stem loop is shown in B for comparison with the human D1 stem loop shown in C. Structural analyses were performed using the FOLD program of the University of Wisconsin Genetics Computer Group Software.

to extraction from microsomal membranes by high pH (35). Hydrophathy analysis of the predicted protein revealed a highly hydrophobic amino terminal region of about 40 amino acids which is the only portion of the protein which can qualify as a membrane spanning domain (Fig. 3 B). The amino-terminal location of this sequence is similar to that found for the D1 enzyme (36).

The most significant difference between hD3 and XD3 is the presence of a 12 amino acid insertion at position 51 in the human protein. The location of this insert, just carboxy-terminal to the hydrophobic domain, suggests that it would be found immediately external to the lipid bilayer. If the topology of D3 is similar to that of D1, this would be located in the cytoplasmic compartment (36). It is a hydrophilic sequence with a number of charged amino acids but its presence does not alter the kinetic characteristics of the human D3 enzyme for either  $T_3$  or reverse  $T_3$  relative to that of XD3 (17).

*Human D3 mRNA is expressed in placenta and lung.* Northern blotting identified a 2.1–2.2-kb transcript in placenta and lung but not in brain. The absence of the expected positive signal in the brain mRNA sample could be explained either by very low mRNA levels in this tissue or by a poor representation of D3 expressing portions of the CNS in the sample examined. In addition, D3 activity is much higher in fetal than in adult rat brain (37), and we are aware of only one study demonstrating D3 activity in normal adult human brain (38). D3 activity has

been identified in human CNS malignancies (39), and more extensive studies will be required before this issue can be resolved.

*Human D3 can be affinity labeled in vitro by BrAcT<sub>3</sub>.* Previous studies have shown that BrAcT<sub>3</sub> is an excellent affinity label for the D1 enzyme (29, 31, 40). On the other hand, similar techniques were unsuccessful in achieving specific labeling of D3 in rat brain microsomes (35) though partial success was obtained with rat placental microsomes (15). Schoenmakers et al. interpreted the 32-kD band found after BrAcT<sub>3</sub> labeling in microsomes of various tissues as being nonspecific since it was present in a number of tissues which did not express D3 activity and was not blocked by incubation with substrate (35). Based on comparisons of  $V_{max}$  estimates, the sonicate from transiently transfected HEK-293 cells has 30–40 times the specific D3 activity of rat brain microsomes explaining the specificity of the labeling we observed (Figs. 5 and 6). Santini et al. also labeled a 32-kD protein in rat placental microsomes but only ~ 50% of BrAcT<sub>3</sub> incorporation could be blocked by incubation with 150  $\mu$ M  $T_3$ . As shown in Fig. 6, a  $T_3$  concentration of 100 nM is sufficient to block completely BrAcT<sub>3</sub> labeling of D3. Thus, even in placental microsomes, a maximum of 50% of the 32-kD BrAcT<sub>3</sub> labeled protein is D3. The identities of the other BrAcT<sub>3</sub> labeled 32-kD protein(s) in placenta, brain, and other tissues are not known.

There is a striking difference between the concentration of

GTG required for inhibition of D3 activity (1 or 5  $\mu\text{M}$ ) and that for blocking BrAcT<sub>3</sub> labeling ( $\sim 2 \text{ mM}$ ). This is in contrast to results with D1 where the  $K_i$  for GTG is  $\sim 10 \text{ nM}$  and 100 nM GTG blocks labeling completely (11, 41). It suggests that the access of BrAcT<sub>3</sub> to the binding site of D3 is markedly favored over that of GTG. Similarly, GTG is an extremely effective inhibitor of type 1 deiodinase activity, whereas 1,000-fold higher concentrations are required to inhibit D3. Thus, while GTG is a competitive inhibitor (Fig. 7), the conformation of the substrate binding site is not favorable to the entry of this compound.

**Synthesis of <sup>75</sup>Se-labeled hD3, XD3, and rD1.** The transiently expressed human D3, *Xenopus* D3, and rat D1 can be labeled with <sup>75</sup>Se in cell culture. The results in Fig. 8 illustrate that while this occurs under standard conditions in selenium-depleted media, <sup>75</sup>Se incorporation is markedly enhanced by coexpression of the human selenophosphate synthetase (h-SelD). This enzyme catalyzes the formation of selenophosphate, the active selenium donor in the eukaryotic selenocysteine incorporation process (23, 42). The quantity of <sup>75</sup>Se-labeled hD3 formed is much greater than that of rat D1 consistent with the presence of a strong SECIS element in the 3' ut of the hD3 mRNA (Fig. 9).

Recent studies have shown that rat placental D3 activity is not reduced by dietary selenium deficiency as is hepatic D1, leading to the speculation that placental D3 is not a selenoprotein (16). Since this is unlikely, other explanations for the lack of effect of selenium deficiency on placental D3 activity must be sought. These include either that the placenta, like the brain and thyroid (43, 44), is resistant to selenium depletion or that selenium incorporation into D3 has a high priority over other pathways of cellular selenocysteine incorporation.

**Human D3 contains highly potent SECIS element.** Deletion studies confirmed that a SECIS element is located in the 3' ut region of hD3 cDNA. Studies with chimeric D1/D3 3' ut constructs showed that this sequence is sufficient for the incorporation of Se into a heterologous (D1) selenoenzyme (Fig. 9). We have previously shown that the SECIS element of rat selenoprotein P mRNA consists of two adjacent stem loops and has four times the potency of that in D1 mRNA (32). The potency of the hD3 SECIS element was comparable to this, suggesting a high priority for selenocysteine incorporation into D3 as well. A portion of this sequence, nt 1478–1720, was less active than the entire 3' ut but still had over twofold the potency of the rat D1 SECIS. This mRNA sequence is predicted to form two stem-loops (Fig. 10 A) though it shares low identity with both the *Xenopus* 3' ut sequence (39%) and the D1 SECIS element (33%). The longer of these (Fig. 10 B) contains two nucleotide sequences conserved in the SECIS elements of D1, GPX, and selenoprotein P (loops 1 and 2) (32). These are YNATGANGR (nt 1611–1619) in the ascending limb of the stem and the unpaired YUGR (nt 1654–1657) on the descending limb (compare Fig. 10, B and C). However, the predicted loop does not contain the A residues which are also conserved in previously analyzed SECIS structures (32). Understanding the mechanism by which functional SECIS potency is preserved without this characteristic feature will require further dissection of this structure.

Taken together, these results show that there is a major similarity between the human D3 enzyme and that of *Xenopus laevis*. In the latter species, the D3 cDNA was identified because the mRNA increased markedly in association with T<sub>3</sub>-induced tadpole metamorphosis. This suggests it provides a control

mechanism to block excessive T<sub>3</sub> formation during metamorphosis (17). Presumably, a similar purpose is served by the presence of D3 in human placenta. Our results show that, despite only 73% similarity and the presence of a 12 amino acid insert in hD3, the catalytic activities of human and *Xenopus* enzymes are virtually identical. The  $K_m$  for 5 deiodination of T<sub>3</sub> (1 nM) is within the physiological range of circulating T<sub>3</sub> concentrations thus indicating the biological relevance of D3 activity. Since we were able to covalently label the transiently expressed protein in a saturable and specific fashion with BrAcT<sub>3</sub> we could quantitate the enzyme turnover number. This is two orders of magnitude lower than that for rat D1 which can explain the marked differences in sensitivity of the reaction to PTU inhibition despite the fact that they both contain selenium in the active site (34). The functional analyses of the 3' ut sequences of D3 mRNA indicate that it is one of the most potent SECIS elements yet identified and that it differs in certain respects from those previously examined. Thus the cloning and functional analysis of human D3 provides the background for both a better comprehension of human maternal-fetal physiology as well as further insights into the biology of mammalian selenoprotein synthesis.

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