

# STRUCTURE UPDATE

## Up to date with human thyroglobulin

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### Abstract

The coding region of the human thyroglobulin (TG) mRNA has been resequenced, and comparison with the TG sequence originally published in 1987 showed many variations. All of the variations were validated in 20–40 other alleles, and this resulted in the revision of 41 nucleotide positions. This review presents the revised wild-type human TG sequence, including all known exon/exon boundaries and additional data on the TG mRNA population, concerning alternative splicing and variability of the polyadenylation cleavage site. The amino acid sequence derived shows one additional, 12 changed, and 10 polymorphic residues. Protein characteristics, such

as acceptor and donor tyrosine residues, *N*-glycosylation sites, cysteine-rich repeats, the proposed receptor domain, and antigenic epitopes, are included, and their relationship to the revised sequence is discussed. Furthermore, all reported TG mutations causing dysmorphogenesis in humans and animals are designated in the nucleotide and amino acid sequences. This up-to-date profile of the human TG molecule presents the features of importance for its complex role in thyroid hormonogenesis, and is the basis for future studies on the structure–function relationship.

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### Introduction

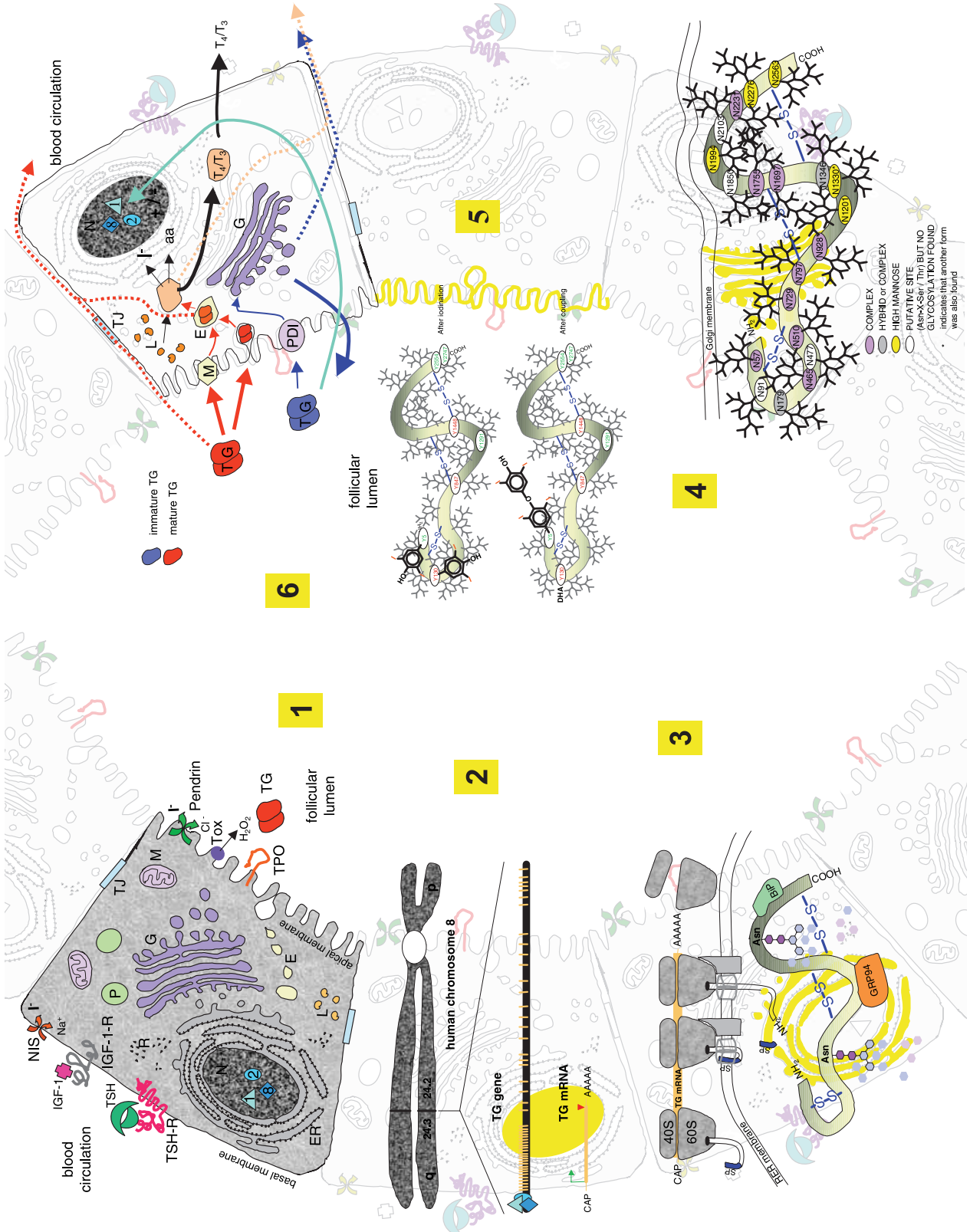
#### *Thyroid gland*

The thyroid gland is responsible for thyroid hormone production and mainly produces the prohormone thyroxine (T<sub>4</sub>), which contains four iodide molecules (Dunn 1999). The gland consists of follicular structures – epithelial cells that border a lumen with their apical membranes and are in contact with the blood circulation through their basal membranes (Fig 1). The most highly expressed protein in the thyroid gland is thyroglobulin (TG), which functions as a scaffold protein for thyroid hormonogenesis and as storage protein for thyroid hormones and iodide. Other thyroidal proteins involved in thyroid hormone synthesis are the receptor for thyrotropin (TSH-R), a seven-transmembrane receptor located in the basal membrane passing the signal that thyroid hormone is needed. Upon thyrotropin stimulation, several processes, including TG synthesis, are upregulated in the thyrocyte cells; all of these processes favor thyroid hormonogenesis. The sodium iodide symporter (NIS), located in the basal membrane, and pendrin, located in the apical membrane,

are responsible for the iodide supply and transport within the gland. Iodination of specific tyrosine residues in TG and coupling of these iodinated residues to form T<sub>4</sub> depend on the thyroid peroxidase (TPO) anchored in the apical membrane and on one or more thyroid oxidases (Tox) that provide the H<sub>2</sub>O<sub>2</sub> (Dupuy *et al.* 1999, De Deken *et al.* 2000) (Fig. 1, cell 1).

#### *Thyroglobulin expression, synthesis and maturation*

Initial transcription of the TG gene (>300 kb) is regulated by thyroid-specific transcription factors TTF-1, TTF-2 and Pax8 (Fig. 1, cell 2). The full-length 8.7 kb mRNA transcript is the most highly expressed mRNA transcript in normal thyrocytes, with an expression level of 2.7% (Pauws *et al.* 2000, 2001). After translation of the mRNA, the post-translational route of TG starts with the signal peptide directing the uptake in the endoplasmic reticulum (ER), where the first mannose and glucose residues are added and the TG protein is folded (Fig. 1, cell 3). At least seven molecular chaperones are known to guide this process to ensure TG quality (Kim & Arvan 1993, 1998).



When TG is misfolded at this stage, stable complexes are formed, and TG is retained in the ER and broken down. TG that 'passes' the ER quality control is directed to the Golgi apparatus, where glycosylation proceeds (Fig. 1, cell 4). At this point, TG molecules have a molecular weight of 300 000, contain 10% carbohydrate structures, and are routed further through the cell to the follicular lumen while homodimers with a molecular weight of 660 000 are formed.

In the follicular lumen, iodination takes place on tyrosine residues in TG, resulting in monoiodotyrosines and di-iodotyrosines (MITs and DITs, respectively). Subsequent coupling of two DIT residues (or DIT and MIT) takes place under oxidative conditions, resulting in iodinated TG molecules carrying T<sub>4</sub> (or tri-iodothyronine) on specific hormonogenic sites (Lamas *et al.* 1989, de Vijlder & Den Hartog 1998) (Fig. 1, cell 5). The efficiency of iodination correlates with completion of carbohydrate structures (Bastiani *et al.* 1995), and poorly iodinated TG molecules (immature TG) can be internalized from the lumen and recycled through the Golgi apparatus (Fig. 1, cell 6). A receptor-binding domain involved in the binding of immature TG molecules to a membrane receptor has been identified (Mezgrhani *et al.* 1997). Protein disulphide isomerase (PDI) is thought to be a candidate receptor (Mezgrhani *et al.* 2000).

The TG–T<sub>4</sub> complexes (mature TG) are stored in a concentrated compact protein form in the follicular lumen (Berndorfer *et al.* 1996). Hormonal secretion to the venous flow requires limited extracellular proteolysis of the stored TG–T<sub>4</sub> complexes, followed by vesicular internalization; subsequent fusion with lysosomes results in breakdown of the TG–T<sub>4</sub> complexes, thereby liberating thyroid hormones from the protein backbone (Brix *et al.* 1996). Vesicular internalization can take place both by non-selective fluid phase uptake and by receptor-mediated endocytosis. Two receptors, located in the apical membrane, have been proposed to participate in the endocytosis: megalin (Zheng *et al.* 1998), and the thyroid asialoglycoprotein receptor (Ulianich *et al.* 1999).

#### Thyroglobulin DNA sequence

The TG gene, mapped to human chromosome 8q24·2–8q24·3 (Baas *et al.* 1985, Bergé-Lefranc *et al.* 1985, Rabin *et al.* 1985), covers at least 300 kb of genomic DNA and contains 8·5 kb coding sequence divided over 48 exons (Mendive *et al.* 1999), separated by introns varying in size

up to 64 kb (Baas *et al.* 1986). In 1987, the primary structure of human TG was deduced from the sequence of its 8448-base mRNA and corresponding coding DNA (cDNA) sequence of 8304 bp (Malthiery & Lissitzky 1987). This sequence has been used since to screen for mutations in patients with hypothyroidism due to putative TG-synthesis defects (Ieri *et al.* 1991, Targovnik *et al.* 1993, 1995). Recently we sequenced the TG cDNA of seven hypothyroid patients in which dysmorphogenesis was caused by a putative TG-synthesis defect (van de Graaf *et al.* 1999a,b). Multiple sequence variations were found relative to the original sequence; these were further investigated using TG mRNA from normal thyroid tissue (van de Graaf *et al.* 1997, 1999a, and this report).

Reverse transcriptase/PCR (RT-PCR) also demonstrated the existence of various wild-type splice variants (Bertaux *et al.* 1991, 1995, Targovnik *et al.* 1992, Mason *et al.* 1995, van de Graaf *et al.* 1999a, Hishinuma *et al.* 1999), which upon translation attributes to a heterogeneous population of TG molecules within one individual.

#### Materials and Methods

##### RNA isolation and complementary DNA preparation

Total RNA was isolated from up to 20 thyroid gland tissue samples (obtained with informed consent) by using TRIzol reagent (Life Technologies BV, Breda, The Netherlands). cDNA was synthesized using random hexamers and reverse transcriptase according to standard procedures.

##### DNA amplification

PCR amplification was performed using 100 ng cDNA as the template in a total reaction volume of 25 µl. For nucleotide sequencing, fragments of 500 bp (with 20–70 bp overlap) were amplified with 2·5 U AmpliTaq DNA polymerase (Perkin Elmer, Norwalk, CT, USA) using the following protocol: 2 min at 94 °C; 35 cycles of 15 s at 94 °C, 1 min at 60 °C, 1 min at 72 °C; 10 min at 72 °C.

Oligonucleotides (van de Graaf *et al.* 1999a) (synthesized using an Expedite nucleic acid synthesis system; Millipore, Bedford, MA, USA) were specific for human TG (for the sequence, see GenBank accession no. U93033) and were coupled to M13 tags.

**Figure 1** Thyroglobulin synthesis and maturation processes within the thyroid follicle. A thyroid follicle is schematically depicted with a general thyrocyte (cell 1) in which different organelles (E, endosome; ER, endoplasmic reticulum; G, Golgi apparatus; L, lysosome; M, mitochondrion; N, nucleus; P, peroxisome; R, bound and free ribosome) and thyroid-specific proteins (IGF-I, insulin-like growth factor-I; IGF-I-R, receptor for IGF-I; NIS, sodium iodide symporter; TG, thyroglobulin; TJ, tight junction; Tox, thyroid oxidases; TPO, thyroid peroxidase; TSH, thyrotropin; TSH-R, receptor for TSH; 1, TTF-1; 2, TTF-2; 8, Pax8) are indicated. In cells 2 to 5, the relevant processes in the different organelles are presented (cell 2, nucleus; cell 3, endoplasmic reticulum; cell 4, Golgi apparatus; cell 5, apical membrane and follicular lumen). Cell 6 illustrates the pathways for TG internalization and the secretion of thyroid hormones and TG.

### Nucleotide sequencing

Both the sense strand and the antisense strand were sequenced using the M13 tags linked to the PCR fragments. Reactions were performed using the Big Dye primer cycle sequencing kit (M13 forward and M13 reverse) and the Big Dye deoxyterminator cycle sequencing kit (in combination with PCR primers) depending on the GC content of the fragment; both kits were from Applied Biosystems/Perkin Elmer, Foster City, CA, USA. After electrophoresis on a sequencing gel, the samples were analysed on an ABI Prism 377 DNA sequencer and aligned with the TG cDNA sequence by using AUTOASSEMBLER software (Applied Biosystems/Perkin Elmer).

### Results and Discussion

In order to update the coding sequence of human TG, we partially repeated and extended our earlier studies. This resulted in the direct sequencing of total cDNA for human TG in a population of 20–40 alleles (8–24 normal alleles and 12–16 from patients with a putative TG-synthesis defect).

We showed that the open reading frame of human TG consists of 8307 bp because of an extra nucleotide triplet (CAG) after position 2952. The open reading frame codes for 2768 amino acid residues, the signal peptide included. The 23 revised nucleotide positions resulted in the change of 12 amino acid residues. One of these, at amino acid position 1024, reduces the original number of tyrosine residues in the TG monomer from 67 to 66. Furthermore, if the study of a Japanese population (Hishinuma *et al.* 1999) is also taken into account, 15 nucleotide positions can be considered polymorphic, 10 of them resulting in amino acid polymorphisms.

All of the changes detected in patients with a putative TG-synthesis defect were either wild type or polymorphic, except for nucleotide G229A, which was homozygously present only in three patients at an allele frequency of 15%.

After the initial screening of 6 alleles (van de Graaf *et al.* 1997), the A-to-G change at position 7589 seemed to be a revision; however, extension of the screening to 66 alleles (Mendive *et al.* 1997) showed that it is a polymorphic nucleotide position.

Figure 2 shows the revised human TG coding sequence of 8307 nucleotides according to the international ubiquity code. The 5' untranslated region up to the TATA box (Parma *et al.* 1987) and the 3' untranslated region up to the polyadenylation cleavage site that was positioned variably within one individual (Pauws *et al.* 2001) have been added to the cDNA sequence. The 41 nucleotide variations compared with the original sequence (insertions, revisions and polymorphisms) are indicated. The genomic organization of 48 exons, recently further unraveled by Targovnik

and co-workers (Parma *et al.* 1987, Mendive *et al.* 1999, Rivolta *et al.* 1999, Moya *et al.* 2000) is presented in Fig. 2. The 11 alternative splice variants from normal alleles that were characterized are also indicated (Bertaux *et al.* 1991, 1995, Targovnik *et al.* 1992, Hishinuma *et al.* 1999, Mason *et al.* 1995, van de Graaf *et al.* 1999a).

The encoded amino acid sequence (a 19-amino-acid signal peptide plus 2749 amino acids) is presented in one-letter code underneath the cDNA, and the 23 amino acid variations are indicated. To complete the complex profile of the TG protein, the human acceptor and the donor tyrosine residues (Lamas *et al.* 1989, Dunn 1999), N-glycosylation sites (Yang *et al.* 1996), the cysteine-residue-rich repeated domains (Malthiery & Lissitzky 1987, Gentile & Salvatore 1993, Molina *et al.* 1996), the acetylcholinesterase homologous domain (Swillens *et al.* 1986), the proposed binding domain to the N-acetylglucosamine receptor (Mezgrhani *et al.* 1997), the recently identified thioredoxin boxes (Klein *et al.* 2000), and the most prominent antigenic epitopes (Henry *et al.* 1992, Mallet *et al.* 1992, Kong *et al.* 1995, Saboori *et al.* 1995, Erregragui *et al.* 1997) have been included.

Previously identified mutations and deletions in human thyroid pathology (Ieri *et al.* 1991, Targovnik *et al.* 1993, 1995, Hishinuma *et al.* 1999, van de Graaf *et al.* 1999b) and homologous positions in animals with hereditary thyroid disorders linked to a TG defect (Dutch goats (Veenboer & de Vijlder 1993), Afrikander cattle (Ricketts *et al.* 1987), *rdw* rats (Hishinuma *et al.* 2000) and *cog/cog* mice (Kim *et al.* 1998)) are indicated in Fig. 2. Other variations in TG mRNA identified in patients with Pendred's syndrome (Mason *et al.* 1995), a variant type of adenomatous goiter (Hishinuma *et al.* 1999), and non-endemic simple goiter (Corral *et al.* 1993) are also indicated (Fig. 2). There are no data on a putative functional role for the TG variations in these types of diseases.

The 11 type-1 cysteine-rich repeats, first described in the bovine TG sequence (Mercken *et al.* 1985), are very well characterized and are even referred to as 'thyroglobulin type-1 domains' when identified in other proteins of different origin and function. It has been shown that these repeats are protein modules, i.e. self-folding structural units, and can act as proteinase inhibitors in other proteins (Lenarcic & Bevec 1998). It is hypothesized that the type-1 repeats in TG could act as pH-dependent binders and reversible inhibitors of the proteases implicated in TG degradation and T<sub>4</sub> release (Molina *et al.* 1996), but up till now no definite function for this type of repeat in TG has been established. No mutations that could shed more light on the role of the cysteine type-1 repeats in TG have been reported in any of the cysteine residues located in the type-1 domains.

The putative function of the acetylcholinesterase homologous domain in TG is not yet clear, but as acetylcholinesterase interacts with cell membranes in the nervous system a similar role for the homologous domain at



↓

┌1

tataaaagctcctg

gccaggggacctagggcaagcagtggtttctcctcctcctcccaggaagggccagaaa

1 atggccttggtcctggagatcttaccctgctggcctccatctgctgggtgctggccaat  
 -19 M A L V L E I F T L L A S I C W V S A N

┌2

61 atcttcgagtaccagtgatgccagcccttcgtccctgtgagctgcagagggaaacg  
 2 I F E Y Q V D A Q P (L R P C E L Q R E T

┌3

121 gcctttctgaagcaagcagactacgtgcccagtgctgagaggatggcagcttcagact  
 22 A F L K Q A D Y V P Q C A E D G S F Q T

181 gtccagtgccagaacgacggcctcctgctgggtgtggtggggtccaacrgcagtgaaagt  
 42 V Q C Q N D G R S C W C V G A N G/SS<sup>®</sup> E V

4

241 ctgggcagcaggcagccaggacggcctgtggcttgtctgtcattttgtcagctacagaaa (1)  
 62 L G S R Q P G R P V A C) (L S F C Q L Q K

301

301 cagcagatcttactgagtggtacattaacagcacagacacctcctacctccctcagtg  
 82 Q Q I L L S G Y I N S T D T S Y L P Q C

361

361 caggattcaggggactacgcgctgttcagtgatgtgcagcaggtccagtgctgggtg  
 102 Q D S G D Y A P V Q C D V Q Q V Q C W C

421

421 gtggacgcagaggggatggaggtgatgggaccgccagctggggaggccaaagcgatg  
 122 V D A E G M E V Y<sup>®</sup> G T R Q L G R P K R C) 5

481 ccaaggagctgtgaaataagaaatcgctcgtcttctccacggggtgggagataagtcacca  
 142 (P R S C E I R N R R L L H G V G D K S P

541 cccagtgttctgcgaggagaggttatgctgtccagtgcaaatttgtcaacaccaca  
 162 P Q C S A E G E F M P V Q C K F V N T T<sup>®</sup>

┌6

601 gacatgatgattttgatctggtccacagctacaacaggtttccagatgcattttgtgacc  
 182 D M M I F D L V H S Y N R F P D A F V T

661 ttcagttccttcagaggaggttccctgaggtatctgggtattgccactgtgctgacagc  
 202 F S S F Q R R F P E V S G Y C H C A D S

┌7

721 caagggcgggaactggctgagacaggtttggagttgttactggatgaaatttatgacacc  
 222 Q G R E L A E T G L E L L L D E I Y D T

781 attttctggtgctggaccttccctccaccttccactgaaaccaccctgtaccggatactg  
 242 I F A G L D L P S T F T E T T L Y R I L

┌8

841 cagagacggttctcgcagttcaatcagtcactctggcagattccgatgccccacaaa (2)  
 262 Q R R F L A V Q S V I S G R F R C) (P T K

901

901 tgtgaagtggagcgggttacagcaaccagctttggtcaccctatgttccaagctgccgc (3)  
 282 C E V E R F T A T S F G H P Y V P S C R

961

961 cgaatggcgactatcagcgggtgcagtgccagacggaagggccctgctggtgtgtggac  
 302 R N G D Y Q A V Q C Q T E G P C W C V D

┌9

1021 gccaggggaaggaaatgcattggaaccggcagcaaggggagcccatcttgtgctgaa  
 322 A Q G K E M H G T R Q Q G E P P S C) (A E

1081 ggccaatcttgtgcctccgaaaggcagcaggccttgtccagactctactttgggacctca  
 342 G Q S C A S E R Q Q A L S R L Y F G T S

1141 ggctacttcagccagcagcactgttctcttccccagagaaaagatgggcctctccaaga  
 362 G Y F S Q H D L F S S P E K R W A S P R

1201 gtagccagatttggccacatcctgccaccacgatcaaggagctctttgtggactctggg  
 382 V A R F A T S C P P T I K E L F V D S G

1261 cttctccgccaatgggtggaggacagagccaacagttttctgtctcagaaaatcttctc  
 402 L L R P M V E G Q S Q Q F S V S E N L L

1321 aaagaagccatccgagcaatttttccctcccgagggtggtcgtcttgccttcagttt  
 422 K E A I R A I F P S R G L A R L A L Q F

1381 accaccaacccaaagagactccagcaaaccttttggagggaaattttggatgattgt  
 442 T T N P K R L Q Q N L F G G K F L V N V

1441 ggccagtttaacttgtctggagcccttggcacaagaggcacatttaacttcagtcaatt  
 462 G Q F **N L S**® G A L G T R G T F **N F S** Q F

1501 ttccagcaacttgggtcttgcaagcttcttgaatggaggagacaagaagatttggccaag  
 482 F Q Q L G L A S F L N G G R Q E D L A K

1561 ccactctctgtgggatttagattcaaattcttccacaggaacccctgaagctgctaagaag  
 502 P L S V G L D S **N S S**® T G T P E A A K K

1621 gatggtactatgaataagccaactgtgggcagctttggcttgaatataacctacaagag  
 522 D G T M N K P T V G S F G F E I N L Q E

1681 aacaaaaatgccctcaaattccttgccttctcctcctggagctccagaattccttctctc  
 542 N Q N A L K F L A S L L E L P E F L L F

1741 ttgcaacatgctatctctgtgcccagaagatgtggcaagagatttaggtgatgtgatggaa  
 562 L Q H A I S V P E D V A R D L G D V M E

1801 acgggtactcagctcccagacctgtgagcagacacctgaaaggctatttgtcccatcatgc  
 582 T V L **S S** Q T C E Q T P E R L F V P S C

1861 acgacagaaggaagctatgaggatgtccaatgcttttccggagagtgtgggtgtgtaat  
 602 T T E G S Y E D V Q C F S G E C W C V N

1921 tcctggggcaaaagacttccaggtcaagagtcagaggtggacagccaaggtgccccaca  
 622 S W G K E L P G S R V R **G G** Q P R C) (P T

1981 gactgtgaaaagcaaagggtcgcgatgcaaagcctcatgggcagccagcctgctggctcc  
 642 D C E K Q R A R M Q S L M G S Q P A G S

2041 acctgtttgtccctgcttgtactagtggaggacatttctgctgtccagtgcttcaac  
 662 T L F V P A C T S E G H F L P V **Q** C F N

2101 tcagagtgtactgtgtgatgtgaggggtcaggccattcctggaactcgaagtgaata **(4)**  
 682 S E C Y C V D A E G Q A I P G T R S A I

2161 gggaagcccaagaaatgccccacgccctgtcaattacagkctgagcaagcttctcagg  
 702 G K P K K C) (P T P C Q L Q **S/AE** Q A F L R

2221 acgggtcaggcctgctctctaactccagcatgctaccacccttccgacacctacatc  
 722 T V Q A L L S **N S S**® M L P T L S D T Y I

2281 ccacagtgcagcacccgatgggcagtgaggagacaagtgcaatgcaatgggcctccyggagcag  
 742 P Q C S T D G Q W R Q V Q C N G P P E Q

2341 gtcttcgagttgtaccaacgatgggaggctcagaacaagggccaggatctgacgcctgcc  
 762 V F E L Y Q R W E A Q N K G Q D L T P A

2401 aagctgctagtggaagatcatgagctacagagaagcagcttccggaaacttcagtctcttt  
 782 K L L V K I M S Y R E A A S G N F S <sup>®</sup> L F

2461 attcaaagtctgtatgaggctggccagsaagatgtcttcccggtgctgtcacaataccct  
 802 I Q S L Y E A G Q Q/ED V F P V L S Q Y P

2521 tctctgcaagatgtcccactagcagcactggaagggaaacggccccagcccagggagaat  
 822 S L Q D V P L A A L E G K R P Q P R E N

2581 atcctcctggagccctacctcttctggcagatcttaaatggccaactcagccaatacccg (5)  
 842 I L L E P Y <sup>°</sup> L F W Q I L N G Q L S Q Y P

2641 gggctcactcagacttcagcactccttggcacattttgatcttcggaactgctgggtg  
 862 G S Y S D F S T P L A H F D L R N C W C

2701 gtggatgaggctggccaagaactggaaggaatgcggtctgagccaagcaagctcccaaca  
 882 V D E A G Q E L E G M R S E P S K L P T

2761 tgtcctggctcctgtgaggaagcaaagctccgtgtactgcagttcattagggaaacggaa  
 902 C) (P G S C E E A K L R V L Q F I R E T E

2821 gagattgtttcagcttccaacagttctcggttccctctgggggagagtttctcggtgcc  
 922 E I V S A S N S S <sup>®</sup> R F P L G E S F L V A

2881 aaggaatccggctgaggaatgaggacctcggccttctcgcctcttcccggcccgggag  
 942 K G I R L R N E D L G L P P L F P P R E

2941 gctttcgggagcagtttctgcgtgggagtgattacgccattcgcctggcggtcagctc  
 962 A F A E Q F L R G S D Y A I R L A A Q S

3001 accttaagcttctatcagagacgccgcttttcccggacgactcggtggagcatccgcc  
 982 T L S F Y Q R R R F S P D D S A G A S A

3061 cttctcgggtcgggcccctactrgccacagtgatgcggttggaagttgggagcctgtg  
 1002 L L R S G P Y M/VP Q C D A F G S W E P V

3121 cagtgccacgctgggactgggcaactgctggtgtgtagatgagaaaggagggttcatcct  
 1022 Q C H A G T G H C W C V D E K G G F I P

3181 ggctcactgactgccgctctctgcagattccacagtgcccgacaacctgagagaaatct  
 1042 G S L T A R S L Q I P Q C) (P T T C E K S

3241 cgaaccagtgggctgctttccagttggaacaggctagatcccaagaaaaccatctcca  
 1062 R T S G L L S S W K Q A R S Q E N P S P

3301 aaagacctgttctgcccagcctgcttagaacaaggagatgaccaggctgcaggcatcg  
 1082 K D L F V P A C L E T G E Y A R L Q A S

3361 ggggctggcacctggtgtggtgaccctgcatcaggagaagagttgaggcctggctcgagc  
 1102 G A G T W C V D P A S G E E L R P G S S

3421 agcagtgccagtgcccaagcctctgcaatgtgctcaagagtgaggctcctctcyaggaga  
 1122 S S A Q C) (P S L C N V L K S G V L S R R

3481 gtcagccaggctatgtcccagcctgcagggcagaggatgggggcttttcccagtgcaa  
 1142 V S P G Y V P A C R A E D G G F S P V Q

3541 tgtgaccagggcccagggcagctgctgggtgtgcatggacagcggagaagaggtgcctggg  
 1162 C D Q A Q G S C W C V M D S G E E V P G  
 17

3601 acgcgcgtgaccgggggcccagcccgcctgtgagagcccgcgggtgcccgtgccattcaad  
 1182 T R V T G G Q P A C) E S P R C P L P F N

3661 gcgtcggagggtggttgggtggaacaatcctgtgtgagacaatctcgggccccacaggctct  
 1202 A S E V V G G T I L C E T I S G P T G S

3721 gccatgcagcagtgccaattgctgtgcccgcagggctcctggagcgtgtttccaccaggg  
 1222 A M Q Q C Q L L C R Q G S W S V F P P G  
 C

3781 ccattgatatgttagcctggagagcggacgctgggagtcacagctgcctcagccccgggc (6)  
 1242 P L I C S L E S G R W E S Q L P Q P R A  
 18

3841 tgccaacggcccagctgtggcagaccatccagacccaagggcactttcagctccagctc  
 1262 C Q R P Q L W Q T I Q T Q G H F Q L Q L

3901 ccgcccgggcaagatgtgcagtgctgactacgggrtttgcctgcagactttccaggttttc  
 1282 P P G K M C S A D Y A G/DL L Q T F Q V F  
 19

3961 atattggatgagctgacagcccgcggtcttctgccagatccaggtgaagacttttggcacc  
 1302 I L D E L T A R G F C Q I Q V K T F G T

4021 ctggtttccattcctgtctgcaacaactcctctgtgcaggtgggtgtctgaccagggag  
 1322 L V S I P V C N N S S V Q V G C L T R E

4081 cgtttaggagtgaaatgttacatggaatcacggcttgaggacatcccagtggtctctctt  
 1342 R L G V N V T W K S R L E D I P V A S L  
 20

4141 cctgacttacatgacattgagagagccttgggtgggcaaggatctccttgggccttcaca  
 1362 P D L H D I E R A L V G K D L L G R F T

4201 gatctgatccagagtggtcattccagcttcatctggactccaagacggtcccagcggaa  
 1382 D L I Q S G S F Q L H L D S K T F P A E

4261 accatccgcttctcccaaggggaccactttggcacctctcccaggacatggtttgggtgc  
 1402 T I R F L Q G D H F G T S P R T W F G C  
 21

4321 tcggaaggattctaccaagtcttgacaagtgaggccagtcaggacggactgggatgcgtt  
 1422 S E G F Y Q V L T S E A S Q D {G L G C V

4381 aagtgcctgaaggaagctattcccaagatgaggaatgcattcctgtcctgttgattc  
 1442 K C P E G S Y S Q} {D E E C I P C P V G F

4441 taccaagaacagggcagggagcttggcctgtgtcccattgctcctgtgggcagaacgaccatt  
 1462 Y Q E Q A G} {S L A C V P C P V G R T T I  
 22

4501 tctgcyyggagctttcagccagactcactgtgtcactgactgtcagaggaacgaagcaggc  
 1482 S A G} A F S Q T H C (V T D C Q R N E A G  
 t

4561 ctgcaatgtgaccagaatggccagtatcgagccagccagaaggacaggggcagtggggaag (7)  
 1502 L Q C D Q N G Q Y R A S Q K D R G S G K  
 \*

4621 gccttctgtgtggacggcgagggggcgaggctgccatggtgggaaacagaggcccctctt  
 1522 A F C V D G E G R R L P W W E T E A P L



┌23

4681 gaggactcacagtggttgatgatgcagaagtttgagaaggttccagaatcaaaggtgatc  
 1542 E D S Q C) L M M Q K F E K V P E S K V I

4741 ttcgacgccaatgctcctgtggctgtcagatccaaagttcctgattctgagttccccgtg  
 1562 F D A N A P V A V R S K V P D S E F P V

┌24

4801 atgcagtgcttgacagattgcacagaggacgaggcctgcagcttcttcaccgtgtccag  
 1582 M Q <C L T D C T E D E A C S F F T V S T

4861 acggagccagagatttctgtgatttctatgcttggacaagtgacaatggtgctgcatg  
 1602 T E P E I S C D F Y A W T S D N V A C M

┌25

4921 acttctgaccagaaacgagatgcactggggaactcaaaggccaccagctttggaagtctt  
 1622 T S D Q K R D A L G N S K A T S F G S L

4981 cgctgccaggtgaaagtgaggagccatggtaagattctccagctgtgtatttgaanaag  
 1642 R C Q V K V R S H G Q D S P A V Y L K K

┌26

5041 ggccaaggatccaccacaacacttcagaaacgctttgaaccactggtttccaaaacatg  
 1662 G Q G S T T T L Q K R F E P T G F Q N M

5101 ctttctggattgtacaaccccattgtgttctcagcctcaggagccaatctaaccgatgct  
 1682 L S G L Y N P I V F S A S G A N L T D A

5161 cacctcttctgtcttcttgcacgacgctgatctgtgttgcatggcttcgctcctcaca  
 1702 H L F >C L L A C D R D L C C D G F V L T

┌27

5221 caggttcaagggtgccatcatctgtgggttgctgagctcaccagtgctcctgctttgt  
 1722 Q V Q G G A I I C G L L S S P S V L L C

5281 aatgtcaaagactggatggatccctctgaagcctgggctaagtctacatgtcctggtgtg  
 1742 N V K D W M D P S E A W A N A T C P G V

5341 acatatgaccaggagagccaccaggtgatattgcgtcttggagaccaggagttcatcaag  
 1762 T Y D Q E S H Q V I L R L G D Q E F I K

┌28

5401 agtctgacacccttagaaggaactcaagacacctttaccaatcttcagcaggtttatctc  
 1782 S L T P L E G T Q D T F T N F Q Q V Y L

┌29

5461 tggaaagattctgacatgggtctcggcctgagctctatgggatgtagaaaaracacagtg  
 1802 W K D S D M G S R P E S M G C R K N/DT V

30

5521 ccaaggccagcatctccaacagaagcaggtttgacaacagaacttttctcccctgtggac (8)  
 1822 P R P A S P T E A G L T T E L F S P V D

5581 ctcaaccaggtcattgtcaatggaaatcaatcactatccagccagaagcactggcttttc  
 1842 L N Q V I V N G N Q S L S S Q K H W L F

31

5641 aagcacctgttttcagcccagcaggcaaacctatgggtgcctttctcgttggtgagcaggag  
 1862 K H L F S A Q Q A N L W >C L S R C V Q E

5701 cactcttctgtcagctcgcagagataacagagagtgcaccttgtacttcacctgcacc  
 1882 H S F C Q L A E I T E S A S L Y F T C T

5761 ctctaccagaggcacaggtgtgtgatgacatcatggagtccaatgccagggtgcaga  
 1902 L Y P E A Q V C D D I M E S N A Q G C R

5821 ctgatcctgcctcagatgccaaaggccctgttccggaagaaagttatactggaagataaa |32  
 1922 L I L P Q M P K A L F R K K V I L E D K

5881 gtgaagaacttttacactcgctgccgttccaaaaactgatggggatattccattagaat  
 1942 V K N F Y T R L P F Q K L M G I S I R N

5941 aaagtgcccatgtctgaaaaatctatttctaattgggttctttgaatgtgaacgayggtgc (6)  
 1962 K V P M S E K S I S N G F F E > < C E R R / W C

6001 gatgcgaccatgctgcactggcttggatttctaaatgttcccagttaaaggagga  
 1982 D A D P C C T G F G F L N V S Q L K G G

6061 gaggtgacatgtctcactctgaacagcttgggaattcagatgtgcagtgaggagaatgga  
 2002 E V T C L T L N S L G I Q M C S E E N G

6121 ggagcctggcgcattttggactgtggctctcctgacattgaagtccacacctatcccttc  
 2022 G A W R I L D C G S P D I E V H T Y P F

6181 ggatggtaccagaagccattgctcaaaataatgctcccagttttgccccttgggtgtt  
 2042 G W Y Q K P I A Q N N A P S F C P L V V

6241 ctgccttccctcacagagaaagtgtctctggactcgtggcagtccttggccctctcttca  
 2062 L P S L T E K V S L D S W Q S L A L S S

6301 gtggttgttgatccatccattaggcactttgatgttggccatgtcagcactgctgccacc  
 2082 V V V D P S I R H F D V A H V S T A A T

6361 agcaatttctctgctgtccgagacctctgtttgtcggaaatgttcccaacatgaggcctgt  
 2102 S N F S A V R D L > < C L S E C S Q H E A C

6421 ctcatcaccactctgcaaacccaacctggggctgtgagatgtatgttctatgctgatact  
 2122 L I T T L Q T Q P G A V R C M F Y A D T

6481 caaagctgcacacatagtctgcagggtcagaactgccgacttctgcttctgtaagaggcc  
 2142 Q S C T H S L Q G Q N C R L L L R E E A

6541 acccacatctaccggaagccaggaatctctctgctcagctatgaggcatctgtaccttct  
 2162 T H I Y R K P > G I S L L S Y E A S V P S

6601 gtgcccatttccacccatggccggctgctgggcaggtcccaggccatccaggtgggtacc  
 2182 V P I S T H G R L L G R S Q A I Q V G T

6661 tcatggaagcaagtggaccagttccttggagttcyatatgctgccccgccctggcagag  
 2202 S W K Q V D Q F L G V P / L Y A A P P L A E

6721 agggccttccaggcaccagagcccttgaactggacaggtcctctgggatgccagcaagcca  
 2222 R R F Q A P E P L N W T G S W D A S K P

6781 agggccagctgctggcagccaggcaccagaacatccacgtctcctggagtcaagtgaagat  
 2242 R A S C W Q P G T R T S T S P G V S E D

6841 tgtttgatctcaatgtgttcatccctcagaatgtggcccctaacgcgtctgtgctggtg (9)  
 2262 C L Y L N V F I P Q N V A P N A S V L V

6901 ttcttccacaacacccatggacagggaggagagtgaaggatggccggctatcgacggctcc (10)  
 2282 F F H N T M D R E E S E G W P A I D C S

6961 ttcttggtgctgttggcaacctcatcgtggctcactgccagctaccgagtgggtgtcttc  
2302 F L A A V G N L I V V T A S Y R V G V F

| 41

7021 ggcttctgagttctgggtccggagaggtgagtggaactggggctgctggaccaggtg  
2322 G F L S S G S G E V S G N W G L L D Q V

7081 gcggtctgacctgggtgcagacccacatccgaggatttggcggggaccctcggcgctg  
2342 A A L T W V Q T H I R G F G G D P R R V

7141 tccctggcagcagaccgtggcggggctgatgtggccagcatccaccttctcacggccagg  
2362 S L A A D R G G A D V A S I H L L T A R

| 42

7201 gccaccaactcccaacttttccggagagctgtgctgatgggaggctccgactctcccg  
2382 A T N S Q L F R R A V L M G G S A L S P

7261 gccgccgtcatcagccatgagagggctcagcagcaggcaattgcttggcaaaggaggtc  
2402 A A V I S H E R A Q Q A I A L A K E V

7321 agttgccccatgtcatccagccaagaagtgggtgctctgctccgcccagaagcctgccaat  
2422 S C P M S S S Q E V V S C L R Q K P A N

| 43

7381 gtcctcaatgatgccagaccaagctcgtggccgtgagtgcccttccactactgggg  
2442 V L N D A Q T K L L A V S G P F H Y W G

7441 cctgtgatcgtatggccacttctccgtgagcctccagccagagcactgaagaggtcttta  
2462 P V I D G H F L R E P P A R A L K R S L

7501 ygggtagaggtcgatctgctcattgggagttctcaggacgacgggctcatcaacagagca  
2482 W/RV E V D L L I G S S Q D D G L I N R A

| 44

7561 aaggctgtgaagcaatttgaggaaagtcraggcggaccagtagcaaaacagccttttac  
2502 K A V K Q F E E S Q/RG R T S S K T A F Y

7621 caggcactgcagaattctctgggtggcgaggactcagatgcccgctcgaggctgctgct  
2522 Q A L Q N S L G G E D S D A R V E A A A

7681 acatggtattactctctggagcactccacggatgactatgcctccttctccgggctctg  
2542 T W Y Y S L E H S T D D Y I A S F S R A L

| 45

7741 gagaatgccacccgggactactttatcatctgccctataatcgacatggccagtgctgg  
2562 E N A T R D Y F I I C P I I D M A S A W

7801 gcaaagagggcccggaggaaacgtcttcatgtaccatgctcctgaaaactacggccatggc  
2582 A K R A R G N V F M Y H A P E N Y G H G

| 46

7861 agcctggagctgctggcgatgttcagtttgcttggggcttcccttctaccagcctay (11)  
2602 S L E L L A D V Q F A L G L P F Y P A Y

7921 gaggggcagttttctctggaggagaagagcctgtcgctgaaaatcatgcagtacttttcc  
2622 E G Q F S L E E K S L S L K I M Q Y F S

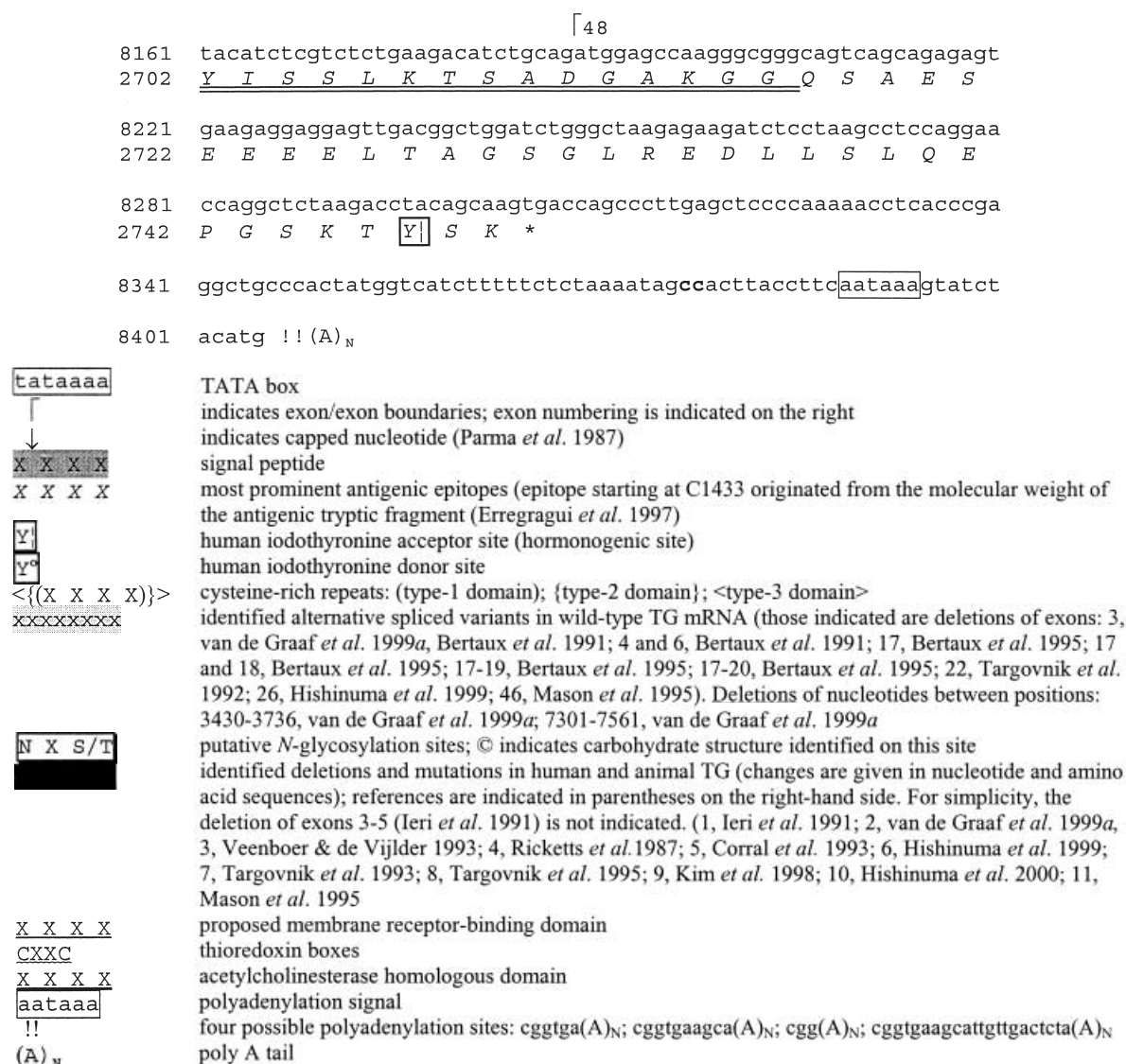
| 47

7981 cacttcatcagatcaggaaatcccaactacccttatgagttctcacggaagtagccaca  
2642 H F I R S G N P N Y P Y E F S R K V P T

\* (when nt 7920 c)

8041 ttgcaaccccctggcctgactttgtaccccgtgctgggtggagagaactacaaggagttc  
2662 F A T P W P D F V P R A G G E N Y K E F

8101 agtgagctgctcccaatcgacagggcctgaagaagccgactgctccttctgggtccaag  
2682 S E L L P N R Q G L K K A D C S F W S K



**Figure 2** Sequence of the human TG gene. The cDNA nucleotide sequence is given in the upper line (nucleotides 1–8307) and the amino acid translation is given below the first nucleotide of each triplet (amino acids 19–2749). Nucleotide polymorphisms are indicated in the nucleotide ambiguity code, and encoded amino acid polymorphisms are separated by forward slashes (/); an asterisk (\*) indicates a stop codon. All variations with respect to the original sequence (Malhière & Lissitzky 1987), in nucleotides and amino acids, are indicated (bold).

the carboxy-terminal end of the TG molecule has been proposed for apical membrane interaction. Unfortunately, the mutation in *cog/cog* mice located in this domain leads to ER storage of apparently misfolded TG molecules (Kim *et al.* 1998) and therefore does not directly contribute to the elucidation of the function of this domain.

The heparin-binding domain consists of non-basic/non-acidic amino acids (X) alternating with basic amino acids (B) and is shown to have different formats, for example XBBXBBX or XBBXBX (Cardin & Weintraub 1989). In rat TG, the heparin-binding domain 693

RRLKRP 699 is proposed to bind to megalin that participates in the endocytosis of TG–T<sub>4</sub> complexes from the follicular lumen (Marino *et al.* 1999). However, in human TG, such a heparin-binding domain is not found in the homologous region, but a possible equivalent could be the 2582 AKRRARG 2587 motif.

Recently, three highly conserved thioredoxin boxes have been identified in mammalian TG, and studies on a bovine TG fragment revealed a role for these boxes in self-assisted disulfide-bond formation leading to the intermolecular cross-linking of luminal TG (Klein *et al.* 2000).

The existence of a wide variation of alternative RNA transcripts for human TG has been described in normal and pathological situations, but only 11 have been mapped exactly in the nucleotide sequence (Fig. 2). Most of these alternative transcripts are found by using sensitive RT-PCR techniques, and their concentration in normal situations is less than that of the wild-type spliced transcript. Only in two pathological situations described is the alternatively spliced transcript concentration increased relative to that of the wild type and linked to the mutation (Targovnik *et al.* 1993, Ricketts *et al.* 1987). The template used in RT-PCR reactions is RNA isolated from thyroid tissue, and usually no histological studies are performed to determine whether all thyrocytes contain these alternatively spliced transcripts or if they show a heterogeneous pattern.

Although nothing is known about the effect of alternative splicing on post-translational processes, one can speculate that these TG molecules are concluded 'misfolded' by the ER quality control and therefore never reach the lumen. On the other hand, these alternatively spliced transcripts may result in a population of alternative TG protein molecules. Such protein molecules could have alternative conformations, leading to a downregulating role in thyroid hormonogenesis (because the conformation is less optimal for T<sub>4</sub> formation) or to an increase in TG degradation and T<sub>4</sub> release in the case of spliced out cysteine-rich repeated domains. Alternative TG protein molecules might have a lower degree of iodination and could be involved in the recently proposed feedback suppressor function of TG (Suzuki *et al.* 1998, 1999). These studies describe a suppressive effect of poorly iodinated follicular TG protein molecules on the transcription of thyroid-specific transcription factors TTF-1, TTF-2 and Pax8, serving as a feedback autoregulator of TSH-stimulated thyroid function. This novel regulatory pathway awaits further confirmation.

In the normal human population, the existence of alternative spliced mRNAs lacking exon 4 of the TG gene has been described (Bertaux *et al.* 1991). The implications of this for TG function are difficult to assess, because excision of the important donor tyrosine residue 130 and the second type-1 cysteine-residue-rich repeated domain (both of which are encoded by exon 4) is linked to thyroid dysmorphogenesis (Ieri *et al.* 1991).

In addition to its biochemical role, TG serves as the target for an autoimmune response both in healthy subjects and in subjects with autoimmune diseases associated with thyroid dysfunction (Salvi *et al.* 1988). Serological studies have shown that there are at least 40 antigenic epitopes on human TG (Roitt *et al.* 1958). Recently, Tomer discussed the three general approaches used in these studies and their specific results (Tomer 1997). He concluded that while the epitopes recognized by anti-TG antibodies found in healthy individuals are polyclonal, the autoimmune thyroid-disease-related epitopes are different, and are

restricted to specific epitopes that are mainly conformational and probably located in non-hormonogenic regions of the molecule. Another study, using 26 monoclonals in cross-inhibition and epitope-linkage experiments, resulted in a tentative map of human TG epitopes, providing information on the three-dimensional arrangement of functional domains (Erregragui *et al.* 1997). Since alternatively spliced transcripts are defined which, upon translation, lack part of an antigenic region (Fig. 2), one could be critical of the use of tryptic digests in combination with N-terminal amino acid sequencing and cross-inhibition in order to map antigenic epitopes.

In view of this, the update of the human TG coding sequence includes essential reference data necessary to screen for mutations and to map epitopes, and structure-function subsequently for further relationship studies on thyroglobulin.

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