Genomic organization of the 5′ region of the human thyroglobulin gene

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(C M Moya and F M Mendive contributed equally to the study)

Abstract

Objective: The purpose of the present work is to establish the intron–exon organization from exon 12 to exon 23 of the human thyroglobulin gene and to construct a physical map of the 5′ terminal half of the gene.

Design: Screening of a genomic library and subsequent restriction map, hybridization and sequencing methods have been employed to characterize the recombinant positive phages.

Methods: A human genomic DNA library was screened by in situ hybridization. Southern blotting experiments were performed to characterize the phage inserts. Intron/exon junction sequences were determined by the Taq polymerase-based chain terminator method. Finally, the thyroglobulin gene was mapped using the Gene Bridge 4 radiation hybrid clone panel.

Results: We isolated and characterized four λ phage clones that include nucleotides 3002 to 4816 of the thyroglobulin mRNA, encompassing exons 12 to 23 of the gene. The exon sizes range between 78 and 219 nucleotides. We found that the GT-AG splicing sequences rule was perfectly respected in all the introns. A total of 7302 intronic bases was analyzed. Hormogenic tyrosine 5 and 1291 are encoded by exons 2 and 18. Also, seven alternative spliced variants are associated with the 5′ region.

Thyroglobulin gene maps to 5.5 centiRays from the AFMA053XF1 marker, in chromosome 8.

Conclusions: The present study shows that the first 4857 bases of thyroglobulin mRNA are divided into 23 exons and the four phages isolated include 32.6 kb genomic DNA, covering 1815 nucleotides of exonic sequence distributed in 12 exons, from exon 12 to 23.

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Introduction

Synthesis of tri-iodothyronine (T₃) and thyroxine (T₄) follows a metabolic pathway that depends on the integrity of the thyroglobulin (TG) structure (1, 2). This large glycoprotein is a homodimer of 660 kDa synthesized and secreted by the thyroid cells into the lumen of the thyroid follicle. The human TG is coded by a large gene about 300 kb long (3), located on chromosome 8q24.2-8q24.3 (4–6). The number of exons has been estimated to be above 48 (7), but intron/exon junctions in the human gene are only known for the first 11 exons (8–10) and the last 13 exons (7, 11).

The mRNA encoding human TG is 8.5 kb long (12–14). The general organization of the mRNA sequence showed a 41-nucleotide (nt) 5′-untranslated segment preceding an open reading frame of 8307 bases. The preprotein monomer is composed of a 19-amino acid signal peptide followed by a 2749-amino acid polypeptide, including 66 tyrosine residues of which 3–17 are iodinated in vivo. Analysis of the primary structure of the TG protein for internal homology led to its division into three regions (12): type-1 repetitive region which contains eleven type-1 repeat elements located between positions 12 and 1191 and between 1492 and 1546 (12, 15, 16); type-2 repetitive region composed of three type-2 elements located between amino acids 1437 and 1484 (12); and type-3 repetitive region with five elements between residues 1584 and 2168 (12). This internal repetitive protein organization makes TG an example of gene evolution by intragenic duplication events and gene fusions (9).

In contrast, the last 581 C-terminal residues show no internal homology and contain remarkable sequence similarity with Torpedo californica acetylcholinesterase (17).
The TG maturation process occurs within thyrocytes and includes intramonomer disulfide bonding formation, glycosylation, phosphorylation and sulfatation (18). The oxidation, iodination and coupling reactions are mediated by thyroperoxidase at the thyrocyte apical cell surface (19). The native three-dimensional TG structure is necessary for the coupling of idotyrosyl residues to form T4 and T3. Five hormonogenic acceptor tyrosines have been identified and localized at positions 5, 1291, 2554, 2568 and 2747 in human TG (20) and several tyrosines have been proposed as donor sites by transferring an idophenoxyl group to an acceptor idotyrosine. After coupling, the acceptor residues are converted to hormones and the donor residues are converted to dehydroalanine. The last stages of the biosynthesis of the thyroid hormones follow TG internalization from the lumen and its proteolytic cleavage in the lysosomal system.

Recently, two probable molecular control mechanisms of thyroid hormonogenesis were identified in the N-terminal half of the sequence (21, 22). The first suggests that an apical endogenous membrane receptor interacts with the secreted immature TGS and prevents their premature lysosomal transfer and degradation (21). Molina et al. (22) propose that the 11 type-I repeats regulate the mature TG degradation by a selective and reversible inhibition of the lysosomal proteases.

Reverse transcriptase-polymerase chain reaction (RT-PCR) demonstrates the existence of 11 alternative splice transcripts for the TG gene (14, 23–27). Whether these must be considered as ‘noise’ originating from inaccuracy of the splicing machinery or are endowed with some function in thyroid hormonogenesis remains to be determined.

Knowledge of the structural organization of the human TG gene will help to elucidate the function of the different domains of the protein. On the other hand, the efficient detection of mutations in TG-linked families requires a more thorough description of the genomic structure of TG gene, including precise intron/exon boundaries.

In the present work, we report the establishment of the intron–exon borders of exons 12 to 23 of the human TG gene, including sequence data from splicing signals and the flanking intronic regions. Additionally, a physical map covering the majority of the 5′ region of the gene was also constructed.

Materials and methods

Probe amplification by RT-PCR

Four RT-PCR fragments: PCR 2.2, PCR 3.1, PCR 3.2 and PCR 3.3, previously described as 1.2F–2R, 3F–1.3R, 1.3F–2.3R and 2.3F–3R respectively (28), were used in the screening of a human genomic library and in Southern blot analysis. These four RT-PCR probes map in the central region of the TG mRNA and together encompass nt 3012 to 6010 (according to new cDNA numbering (13)).

Total RNA was prepared from human thyroid tissue by the method of Chomczynski and Sacchi (29). Two micrograms total RNA were first reverse transcribed with 200 U Moloney murine leukemia virus reverse transcriptase (Gibco BRL, Life Technology, Gaithersburg, MD, USA) and 20 U Rnase inhibitor (Rnasin, Promega, Madison, WI, USA) in 20 μl solution containing a standard reverse transcription buffer (Gibco BRL), 1 mmol/l of each dNTP (dATP, dCTP, dTTP and dGTP) and 50 pmol of the reverse primer during 1 h at 42 °C. The reverse transcriptase was inactivated at 95 °C.

The PCR reactions were performed in 100 μl, using a standard PCR buffer (Gibco BRL) containing the 20 μl RT reaction, 2.5 mmol/l MgCl2, 4% dimethyl-sulfoxide, 2 U Taq polymerase (Gibco BRL) and 50 pmol of each reverse and forward primers. No dNTP were added in the PCR reaction, so, as the RT reaction is diluted 1/5, the final concentration of nucleotides was 200 μmol/l. The samples were subjected to 40 cycles of amplification. Each cycle consisted of denaturation at 95 °C for 30 s, primer annealing at 55 °C for 30 s, and primer extension at 72 °C for 1 min. After the last cycle, the samples were incubated for an additional 5 min at 72 °C to ensure that the final extension step was complete.

The DNA sequences of each of the oligonucleotides used for RT-PCR and the positions of the first and last nucleotide of each probe are shown in Table 1 and Fig. 1 respectively. The amplified products (PCR 2.2: 1076 bp, PCR 3.1: 681 bp, PCR 3.2: 683 bp and PCR 3.3: 884 bp) were analyzed in a 1.5% agarose gel.

Screening of a human genomic library

An aliquot of human genomic library constructed with λ Dash II phage vector (Stratagene, La Jolla, CA, USA) was used to infect Escherichia coli XL1-Blue MRA. Infected bacteria were plated on petri dishes and screened by the filter replica method using PCR 2.2, PCR 3.1, PCR 3.2 and PCR 3.3 as hybridization probes.

Prehybridization and hybridization were carried out at 42 °C in 50% formamide, 5 × Denhardt’s solution, 5 × SSPE (1 × SSPE is 0.15 mol/l NaCl, 0.01 mol/l NaH2PO4 (pH 7.7), and 0.001 mol/l EDTA), 0.5% SDS and 100 μg/ml sonicated denatured salmon sperm DNA.

The probes were labeled with α[32P]dATP by random priming. Filters were washed with 2 × SSC (1 × SSC is 0.15 mol/l NaCl and 0.015 mol/l sodium citrate, pH 7.0), 0.1% SDS, followed by 1 × SSC, 0.1% SDS, and finally 0.1 × SSC, 0.1% SDS, twice each at 65 °C. The filters were exposed to X-ray film at −70 °C with an intensifying screen.
Screening of recombinant positive phages by PCR

The use of PCR provides a rapid and precise screening system to determine the relative localization of recombinant positive phages. This approach was used to identify the phage containing exon 23.

The DNA from each positive phage stock was purified by proteinase K treatment and phenol extraction. PCR reaction and cycles were performed as described for the probe amplification by RT-PCR. The forward (position of 5' end TG sequence: 4700) and reverse (position of 5' end TG sequence: 4762) primers are shown in Table 2. The amplified products, 63 bp, were analyzed in a 15% acrylamide gel.

Preparation of \(\lambda\) phage DNA

Bacteriophage DNA was prepared with the Wizard lambda preps DNA purification system (Promega). After elution from the mini column, DNA was extracted twice with phenol-chloroform, salt concentration was adjusted to 2 mol/l with ammonium acetate and the DNA was precipitated with ethanol.

Southern blot analysis of recombinant positive phages

Restriction and blotting experiments were carried out using standard procedures (30–32). One microgram \(\lambda\) phage DNA was digested with 10 U EcoRI endonucleases (Gibco-BRL). Prehybridization, hybridization and washing of the filters were performed as described for the screening of the genomic library, except that washing was performed using 0.5% SDS.

In order to map the recombinant clones, the membranes were hybridized with PCR 2.2, PCR 3.1, PCR 3.2 and PCR 3.3 probes in separate experiments. Recombinant phages DNA were also used as probes to check the possibility of overlapping between contiguous phages.

DNA sequencing

The exon and intron/exon junction sequences were determined by Taq polymerase-based chain terminator method (fmol, Promega) from \(\lambda\) phage clones DNA. Primers were especially designed for each intron–exon junction. Oligonucleotide sequences and the position of their 5' ends are shown in Table 2. Intron–exon borders were characterized by alignment between the cDNA (13) and genomic sequences, using the PC Gene computer program (Intelligenetics, Inc., Geneva, Switzerland).

Chromosomal mapping

A panel of 93 radiation hybrid clones (Genebridge panel, Research Genetics, Huntsville, AL, USA) was used to map the TG gene by PCR screening. PCR were

### Table 1

<table>
<thead>
<tr>
<th>RT-PCR fragments</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR 2.2</td>
<td>CTATCAGAGACGCCGCCTTTTCCCC</td>
<td>CTAAACGCTCCCCTGTCAGACAACCC</td>
</tr>
<tr>
<td>PCR 3.1</td>
<td>GCGGAGCGCTGGGAGATCAGACTGTC</td>
<td>CAGGACATGGGACACAGGCCCC</td>
</tr>
<tr>
<td>PCR 3.2</td>
<td>GGGCCAGCTCATCAGGAGCAAC</td>
<td>TGCTCCAGGAGCTGAGAAC</td>
</tr>
<tr>
<td>PCR 3.3</td>
<td>GGTCTCAAGCTCAGGAGCCA</td>
<td>GGTCCGCATCGACCCGTCGCC</td>
</tr>
</tbody>
</table>

1Forward primer used in the PCR 2.2 probe amplification; 2reverse primer used in the PCR 3.1 probe amplification.

### Table 2

<table>
<thead>
<tr>
<th>Exon number</th>
<th>Position of 5' end</th>
<th>Nucleotide sequence (5' → 3')</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>3012</td>
<td>CTATCAGAGACGCCGCCTTTTCCCC</td>
<td>CTATCAGAGACGCCGCCTTTTCCCC</td>
</tr>
<tr>
<td>13</td>
<td>3149</td>
<td>GTGGTGAGATGAGAAAGGAGGG</td>
<td>CTATCAGAGACGCCGCCTTTTCCCC</td>
</tr>
<tr>
<td>14</td>
<td>3228</td>
<td>GTGCGGAAATCTCAGGACC</td>
<td>CTATCAGAGACGCCGCCTTTTCCCC</td>
</tr>
<tr>
<td>15</td>
<td>3335</td>
<td>GGAATAATTCGGTCGTCG</td>
<td>CTATCAGAGACGCCGCCTTTTCCCC</td>
</tr>
<tr>
<td>16</td>
<td>3446</td>
<td>GCAATGTGCTCAAGAGTGAGGAG</td>
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</tr>
<tr>
<td>17</td>
<td>3686</td>
<td>CAATCTCGTGAGACATACTCGG</td>
<td>CTATCAGAGACGCCGCCTTTTCCCC</td>
</tr>
<tr>
<td>18</td>
<td>3854</td>
<td>AGCTGTGGAGACACTCCAGGACC</td>
<td>CTATCAGAGACGCCGCCTTTTCCCC</td>
</tr>
<tr>
<td>19</td>
<td>4003</td>
<td>GTGAAAGAATTTTGGCAGCCTGGG</td>
<td>CTATCAGAGACGCCGCCTTTTCCCC</td>
</tr>
<tr>
<td>20</td>
<td>4184</td>
<td>TCTCGGTCGCTCCAGACATGCAGC</td>
<td>CTATCAGAGACGCCGCCTTTTCCCC</td>
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<tr>
<td>21</td>
<td>4393</td>
<td>GGAAGCTTATTCCCAAGATGAG</td>
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</tr>
<tr>
<td>22</td>
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<td>GGTGTCAGACTCTGAGAGCC</td>
<td>CTATCAGAGACGCCGCCTTTTCCCC</td>
</tr>
<tr>
<td>23</td>
<td>4700</td>
<td>TGATGACAGAATTTTGAGGAGTTC</td>
<td>CTATCAGAGACGCCGCCTTTTCCCC</td>
</tr>
</tbody>
</table>

1Forward primer used in the PCR 2.2 probe amplification; 2reverse primer used in the PCR 3.1 probe amplification.
performed in 50 µl solution, using Qiagen core kit (Qiagen, GmbH, Hilden, Germany), containing 2.5 mmol/l MgCl₂, 0.2 mmol/l dNTP, 50 ng target DNA, 50 pmol of each of the primers, and 1 U Taq DNA polymerase. The primers used were 5'-cgttctgttcccccacagtt 3' as forward primer (5' end is located 208 nt upstream from exon 1, in the TG promoter region) and 5'-gctccatggcctcagaactt 3' as reverse primer (5' end is located 22 nt downstream from exon 1, in intron 1) (33). In order to allow easy sequencing, M13 sequences have been incorporated into PCR fragments by adding specific sequences at the 5' end of the primers. Cycles were performed as described for probe amplification by RT-PCR. The amplified products (374 bp, 338 of them TG sequences) were analyzed in a 1.5% agarose gel. No amplification product was obtained from hamster DNA used as negative control. The TG specificity of the PCR fragment was established by sequencing, using M13 primers sequences and the Thermo Sequenase Fluorescent Labeled Primer Cycle Sequencing Kit (Amersham Life Science, Amersham, Bucks, UK) followed by analysis on an ABI 370 A automated sequencer. Both strands were sequenced. The PCR results were analyzed using the Rhmapper computer program through the publicly available World Wide Web server of the Whitehead Institute/MIT center (http://carbon.wi.mit.edu:8000/cgi-bin/config/rhmapper.pl).

Results
About 1.9 x 10⁶ phages of a human genomic library were screened by filter hybridization with four human TG cDNA probes (PCR 2.2, PCR 3.1, PCR 3.2 and PCR 3.3) corresponding to 3 kb TG mRNA. Fifty plaques scored positive and two of these were randomly selected and purified to homogeneity. The DNA was prepared from the corresponding phages and digested with EcoRI. Two different restriction patterns were obtained leading to the identification of two recombinant phages termed λ Dash 62 and λ Dash 151 respectively. Southern blot experiments with the same screening probes were used to localize the phage inserts with respect to the mRNA sequence and determine their relative orientation. Figure 1 shows the localization of λ Dash 62 and λ Dash 151 clones according to their positive hybridization fragments.

λ Dash 62 is located 5' to λ Dash 151 since the exonic fragments of λ Dash 62 hybridize with cDNA probes PCR 2.2 and PCR 3.1 and do not hybridize with PCR 3.2, whereas the λ Dash 151 exonic fragments are positive with all three probes and do not overlap with λ
Dash 62. These observations were verified by hybridizing
the EcoRI blot with λ Dash 62 used as probe. The
hybridization of λ Dash 62 and λ Dash 151 with PCR
3.3 was negative, since the region is outside the
sequence covered by this probe.

In order to identify the intron/exon boundaries of the
TG gene and to analyze the regions responsible for pre-
mRNA processing, we performed cycle sequencing
reactions from primers designed according to a
sequencing strategy (i.e. the establishment of one
intron–exon junction allowed us to design the following
primer to sequence the neighbouring intron/exon
border and so on). All investigated intron–exon
junctions and splicing sites were sequenced from exonic
primers (Table 2) heading into the flanking introns,
that is a forward primer, directed to the 5’ donor site
and a reverse primer, directed to the 3’ acceptor site.
Our sequencing results show that λ Dash 62 contains
exons 12 to 18 and that λ Dash 151 contains exons 19
to 21. The DNA sequences surrounding the intron/
exon junctions are shown in Fig. 2. The negative
hybridization of the λ Dash 62 with λHTg5 (8),
containing a segment of intron 11, reveals a gap
between both clones, located in intron 11.

We previously reported on the intron 21/exon 22/
intron 22 junction sequences in the clone named λ
Dash 161 (34), obtained in the screening reported in
this study followed by screening of recombinant
positive phages by PCR. λ Dash 151 and λ Dash
161 displayed similar hybridization patterns, as
shown in Fig. 1. An additional new clone named λ
Dash 121 was obtained using the same approach.
The predicted 63 bp fragment is included in exon 23
and was observed in one of the twenty stocks
investigated by PCR. The presence in λ Dash 121
of a fragment of 0.7 kb which is negative for PCR
3.1 and positive for PCR 3.2 allows localization of
a fragment of 0.7 kb which is negative for PCR
3.1 and positive for PCR 3.2 of the TG monomer
(20). The detailed analysis showed that they are located at positions 5 and 1291 of
the TG monomer within exons 2 and 18 respectively, out of the repetitive region. Comparisons between
the exon organization and type-1 and type-2 cysteine-rich
repetitive elements are summarized in Fig. 3b. Ten of
the eleven type-1 repeat motifs span between exon 2
and 16, while the eleventh repeat is entirely located in
exon 22. The 3’ end of exon 20 and a fraction of exon
21 encode the three type-2 repetitive elements.

The knowledge of exon limits allowed us to
demonstrate that the alternative splice variants char-
acterized by Bertaux et al. (25) consist of a series of
transcripts where complete exons or a group of
consecutive complete exons were skipped. Bertaux
et al. (25) found four transcripts smaller than
previously known TG mRNA. Sequencing reactions
showed that these transcripts were 844, 689, 532 and
313 bp, smaller than normal size. Here, we show that
the regions absent in each of these RNA molecules
correspond to exons 17; 17 and 18; 17, 18 and 19;
and 17, 18, 19 and 20 respectively.

Figure 3c shows the seven alternative spliced
variants, in wild type TG mRNA, that have been
associated with the 5’ region (14, 23–25). Exons 3,
4 + 6 and 22 are also alternatively spliced.

Finally, the TG gene was mapped using the Gene
Bridge 4 radiation hybrid clone panel. The results of the
PCR reactions (data not shown) for the 93 clones of the
panel map the Tg gene 5.5 centiRays (cR) from the
AFMA053XF1 marker, in the D8S557-D8S529 inter-
val (144.8–144.9 centiMorgan (cM)) of chromosome
8.

These results are in perfect agreement with the data
obtained previously by in situ hybridization to human
chromosomes (4–6) and with that of Deloukas (36),
who used forward and reverse primers localized in
exons 47 and 48, respectively, to map TG on the same
radiation hybrid clone panel.

Discussion
In addition to the first 11 intron/exon boundaries
available from previous studies, we now report the
sequencing and characterization of the intron–exon
organization of exons 12 to 23 of the human TG gene.
The corresponding gene region was studied in detail.
All exon borders and intron/exon junctions were localized precisely and sequenced. The positions of the first 23 exons of the TG gene were correlated with the repetitive organization of the protein.

The nucleotide sequence of human TG cDNA and the deduced sequence of the protein showed that 70% of each monomeric chain is characterized by the presence of three families of repetitive units, all of which include strongly conserved cysteine residues (12, 15, 16). Type-1 motif is present in the N-terminal portion of the molecule and is repeated eleven times. A more refined analysis considers two subtypes of the type-1 repeat, 1A and 1B (16). Type-1A contains six conserved cysteine residues. The ninth and eleventh units possess only four cysteines and both units were assigned as type-1B. Up to now, the location of the extremities of the first ten

Figure 2 (Part I)

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repeats was based on the limited available intron–exon
junctions identified by Parma et al. (9) – exons 1 to 11
for the human and exons 12 to 16 for the bovine TG
gene. In this study, we established an alignment of type-
1 repeats according to our results about the first 23
intron–exon junctions of human TG (Fig. 3b) and
based on the criteria proposed by Molina et al. (16): (i)
repeats 2, 4, 7, 10 and 11 are each encoded by a single
exon (exon 4, 8, 10, 16 and 22 respectively), repeats 1
and 9 by two exons (exons 2 and 3, and 14 and 15
respectively) and repeats 3 and 8 by three exons (exons
5, 6 and 7, and 11, 12 and 13 respectively); (ii) repeats
5 and 6 are a fraction of exon 9, the N-terminal limit of
repeat 5 is ambiguous (15, 16); (iii) in repeats 1, 2, 3,
6, 7, 8, and 9 the conserved cysteine located in the C-
terminal segment of the unit is interrupted by an intron
(intron class 1); (iv) in repeats 4, 10 and 11 mapping in
exons 8, 16 and 22 respectively, the conserved C-
terminal cysteines are not interrupted by any intron.

Yamashita and Konagaya (37) reported the isolation
from chum salmon eggs of a potent protease inhibitor
with high sequence similarity with the TG type-1

Figure 2 (Part II) Sequence data of intron–exon boundaries 12 to 23 and their flanking intronic regions. The first and last nucleotides of each exon are indicated by capital letters and their flanking intronic sequences by lower-case letters. Numbers indicate the first and last nucleotides of each exon. The gaps (…….) represent intron regions whose sequences were not determined.
module. This cysteine-rich motif is also found in 32 different proteins (16, 37, 38), suggesting that they arose from an early duplication event followed by additional gene duplications and divergence. However, the role that this repeat sequence plays in these genes and its relationship to proteolytic activity remains unclear. Molina et al. (16) proposed that the 11 type-1 repeats from TG would control the sequential proteolytic events of TG degradation, first generating the selective release of thyroid hormones and then complete TG degradation. These findings support the idea that type-1 motifs selectively and reversibly inhibit the lysosomal proteases. It is possible that the 11 copies present in TG may have specificities for different protease activities.

The three type-2 repetitive elements map between exons 20 and 21. It is interesting to note that exons 17 to 20 disrupt the repetitive organization of TG (Fig. 3b), connecting the first ten type-1 modules with the three type-2 modules. Curiously, this region is the subject of four alternative splicing events (25) (Fig. 3c). Within the TG locus, the conservation of nonrepetitive sequences between two regions that are highly repetitive was hypothesized to be important in the structural organization and expression of TG. It has been speculated that the skipping of exons 17 to 20 by alternative splicing may result in a population of alternative TG molecules with a role in the control of thyroid hormonogenesis. It is conceivable that partial or total excision of this segment of the TG would affect the protein structure and subsequently the coupling mechanism, resulting in a decrease of hormone production. Alternatively, it is possible that elimination by alternative splicing of exons containing type-1 motifs would result in an increase in hormone production secondary to a loss of protease inhibitor activity. In this context, three alternative splicing events were localized in exons 3, 4 + 6, and 22 (14, 23, 24), thus eliminating type-1 repetitive units 1, 2 + 3 and 11 from TG transcripts. In a previous study, we identified a heterozygous nonsense mutation in exon 22 in two siblings with hereditary goiter and markedly decreased tissue levels of TG (34, 39). The point mutation was removed from a fraction of transcripts by preferential accumulation of an alternatively spliced product missing exon 22.

In conclusion, the present study shows that the first 4857 bases of TG mRNA (including a 41-nucleotide 5′-untranslated segment) are divided into 23 exons, whose sizes range between 78 and 1101 base pairs. The GT-AG splicing consensus sequences are rigorously respected in all introns analyzed. The 5′ region contains eleven type-1 and three type-2 repeat motifs and is subjected to seven alternative splicing events, resulting in a heterogeneous nonsense mutation in exon 22 in two siblings with hereditary goiter and markedly decreased tissue levels of TG (34, 39). The point mutation was removed from a fraction of transcripts by preferential accumulation of an alternatively spliced product missing exon 22.

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which could be involved in the regulation of biosynthesis and release of thyroid hormones.

Detailed knowledge of the organization of the TG gene and the availability of the sequence of each of the intron/exon boundaries provide the basis for the investigation of families affected with defective TG synthesis.

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The nucleotide sequence data reported in this paper have been submitted to the GenBank, EMBL and DDJ databases under the accession numbers AF105681, AF105682, AF105683, AF105684, AF105685, AF105686, AF170486, AF170487, AF170488, AF170489, and AF237421.

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