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Cloning from the thyroid of a protein related to actin binding protein that is recognized by Graves disease immunoglobulins

(thyrotropin/microfilament disruption/glycoprotein/autoantigen/Ca^{2+}–calmodulin kinase)

PETER J. LEEDMAN*†, BEVERLEY FAULKNER-JONES*, DAVID S. CRAM*, PAUL J. HARRISON*, JEANETTE WEST*, ELIZABETH O'BRIEN*, RICHARD SIMPSON‡, ROSS L. COPPEL*, AND LEONARD C. HARRISON*§

*Burnet Clinical Research Unit and ‡Joint Protein Structure Laboratory, Walter and Eliza Hall Institute of Medical Research, Victoria, 3050 Australia

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ABSTRACT **Human actin binding protein (ABP) links specific membrane glycoproteins to cytoskeletal actin microfilaments. In human platelets and leukocytes, ABP directly links, respectively, the membrane glycoproteins GPIb and the high-affinity Fc receptor for IgG (FcγIR) to cytoskeletal actin microfilaments. Similar interaction between the thyrotropin (TSH) receptor and ABP in endocrine cells might explain the rapid and profound disruption of actin microfilaments induced by TSH in cultured thyroid follicular cells. By screening a thyroid λgt11 cDNA expression library with serum from a Graves disease patient, we identified a clone encoding a protein, designated truncated ABP (TABP), that shares extensive homology (≈70%) with ABP. TABP is a truncated ABP-like protein with an open reading frame of 195 aa that encodes a protein of ≈21 kDa. TABP lacks an actin binding domain but contains two predicted β-sheet repeats within which is a putative dimerization domain and between which lies a putative glycoprotein binding site containing a consensus site for phosphorylation by Ca^{2+}–calmodulin kinase II. TABP contains a unique C-terminal insertion within which lies a hydrophobic predicted membrane-associated region, absent from ABP. Although TABP mRNA is expressed widely, immunoblot analysis demonstrated the presence of TABP antibodies specifically in the sera of a minority of subjects with autoimmune thyroid disease. A 24-residue sequence of similarity was identified between the TSH receptor and platelet glycoprotein GPIbα that may represent a transmembrane ABP binding site. We suggest, therefore, that signal transduction by TSH in the thyroid involves direct linkage of the TSH receptor to actin microfilaments by ABP and that TABP may interact with ABP to mediate TSH-induced actin microfilament disruption.**

The addition of thyrotropin (thyroid-stimulating hormone, TSH) or cAMP analogues (e.g., 8-bromo-cAMP) to cultured Fisher rat thyroid line (FRTL-5) (1), human thyroid adenoma (2), or bovine embryonic thyroid cells (1) produces a striking and rapid reduction in the number and organization of actin microfilament bundles, accompanied by a change from a "flattened" to a "rounded-up" cell phenotype (1). A well-organized microfilament system reappears, after removal of TSH (or 8-bromo-cAMP). Similar rapid actin microfilament disruption has also been observed in cultured rat ovarian granulosa cells after the addition of follicle-stimulating hormone or cAMP analogues (3). To our knowledge, the mechanism underlying this prominent hormone-induced actin disruption has not been elucidated.

Recently, the manner in which intracellular actin binding proteins and specific membrane glycoproteins interact in the human platelet and leukocyte has been described. Platelets contain a membrane skeleton composed predominantly of actin filaments, an actin binding protein (ABP), and numerous membrane glycoproteins, including GPIb (4). In the platelet, ABP, which was recently cloned (5), serves as a central linkage protein for the direct attachment of actin microfilaments of the membrane cytoskeleton to GPIb (4). Similarly, in the human leukocyte the high-affinity glycoprotein receptor that binds the Fc domain of IgG1 and IgG3 (FcγIR) binds directly to ABP in the absence of IgG (6). Addition of IgG to COS cells transfected with FcγIR rapidly decreases the avidity of FcγIR for ABP, which implies that ABP functions as a dynamic linker between the FcγIR and cytoskeletal actin.

It is possible, therefore, that in the thyroid TSH-induced actin disruption is mediated via ABP, which directly links actin microfilaments and the TSH receptor (TSH-R). This study describes the cloning and characterization of an ABP-like protein¶ that shares extensive homology with ABP, isolated during attempts to clone the human TSH-R. Based on this homology and elements of its sequence, this molecule was designated TABP, for truncated ABP. We hypothesize that TABP may function in concert with ABP in the thyroid to directly link integral membrane glycoproteins, such as the TSH-R, to cytoskeletal actin and thus play a crucial role in signal transduction.

MATERIALS AND METHODS

A λgt11 human thyroid carcinoma cDNA library (Clontech) was screened with a serum (G2) from a patient with untreated Graves disease (7) that had been shown to immunoprecipitate TSH-affinity-purified TSH-R proteins and bind to TSH-R on immunoblots (8). A ^{32}P-labeled oligonucleotide, TSH-R-P1 [5′-CA(A/G)GGN(C/T)TNGGNAAA(T/C)GA(A/G)AT(C/G)NAA(A/G)-3′, N = all nucleotides], based on the amino acid sequence derived from microsequencing of a purified 50-kDa porcine TSH-R protein (9), was synthesized and used to probe the positive clones obtained by immunoscreening (7). TABP cDNA was isolated from the recombinant phage and both strands were sequenced (7). DNA sequences were analyzed using Genetics Computer Group (Madison, WI) sequence analysis software and the EMBL DNA databank (Release 32.0) and the GenBank protein databank (Release 74.0) were searched for sequence homologies.

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Abbreviations: ABP, actin binding protein; CaM kinase, calcium–calmodulin kinase II; TABP, truncated ABP; TSH, thyroid-stimulating hormone; TSH-R, TSH receptor; FcγIR, receptor for the Fc domain of IgG1 and IgG3; GST, glutathionine *S*-transferase.

†Present address: Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Howard Hughes Medical Institute and Harvard Medical School, 75 Francis Street, Boston, MA 02115.
§To whom reprint requests should be addressed.

¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M62994).

Purified TABP cDNA was subcloned into pGEX2 and recombinant affinity-purified glutathione-*S*-transferase–TABP fusion protein (GST–TABP) produced as described (10). Sera were obtained from subjects with untreated or treated Graves disease hyperthyroidism (n = 10), Hashimoto thyroiditis (n = 4), polymyositis (n = 1), mixed connective tissue disease (n = 1), type 1 diabetes mellitus (n = 1), and type 2 diabetes mellitus (n = 1) and from normal subjects (n = 5).

Total cellular RNA was purified from human tissues, Swiss 3T3 fibroblasts, and HeLa cells (7), and poly(A)$^+$ RNA was selected. TABP coding region cDNA was subcloned into pBluescript II (KS+/−), to facilitate production of ^{32}P-labeled antisense RNA transcripts (11). For Northern blot analysis, total RNA samples were electrophoresed through an agarose/formaldehyde gel, transferred to nitrocellulose, and probed with an antisense-labeled coding region TABP RNA probe. For RNase protection assay (11), the transcription vector was linearized to generate a 303-nt RNA probe, including the 3′-terminal 97 nt unique to TABP and 143 nt common to both TABP and ABP. Full-length ^{32}P-labeled transcripts were hybridized with human total and poly(A)$^+$ RNA and analyzed as described (11).

For PCR, first-strand DNA was synthesized from total and poly(A)$^+$ RNA (0.1–1 μg) with Superscript RNase H$^-$ Moloney murine leukemia virus reverse transcriptase and an oligo(dT)$_{12-18}$ primer. Each reverse transcription reaction mixture (5 μl) was mixed with TABP-3 and L-20 primers and *Taq* polymerase to initiate amplification (7). Southern blot analysis was performed on the PCR products using a ^{32}P-labeled internal oligomer (L-30) (7). TABP-3 corresponds to the 5′ end of the TABP cDNA (5′-GGGCTTTATTAACACC-3′), L-20 is nt 524–541 (5′-GCGATTATGTGCTGGCTG-3′), and L-30 is nt 370–390 (5′-GGCCAGAAGAGTTCCTTCCTG-3′).

RESULTS

Cloning and Sequencing of TABP. Screening of a human thyroid follicular carcinoma λgt11 cDNA expression library with a Graves disease serum (G2) yielded 48 positive clones, 5 of which were purified to homogeneity. To determine the clone specificity, they were probed with [^{32}P]TSH-R-P1, and one positive clone, T1, was characterized. The complete nucleotide sequence determined for T1 is shown in Fig. 1. Analysis of the 2007-nt sequence revealed a putative open reading frame of 585 bp, preceded by two methionine residues, which would encode a protein of 195 aa residues with a calculated molecular weight of ≈20,700. The first ATG codon, located 109 nt downstream from the 5′ end of T1, is considered a potential initiation site for translation, because the surrounding sequence is in reasonable agreement with the consensus defined for start codons (12) and because a stop codon is present in-frame 39 bp upstream. Another potential initiation codon is present 13 nt downstream of the first ATG, but the surrounding sequence is less consistent with a start site. The putative noncoding sequence (≈1250 nt) downstream of the open reading frame in the T1 clone does not contain a poly(A) addition site or a poly(A) tail.

Homology of TABP with Human ABP. The sequence of T1 is unique. No significant homology was found with the sequences of the TSH-R (13), lutropin receptor (14), and T1. Significant homology was identified, however, with ABP (5) (Fig. 2). This homology was present over the majority of the

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                                                                    1 GGGCTTTATTAACAC
  16 CACCCGAGCAGGTCCAGGGACATTATCCGTCACCATCGAAGGCCCATCCAAGGTTAAAATGGATTGCAGGAAACACCTGAAGGGTACAAAGTC

 109 ATG TAC ACC CCC ATG GCT CCT GGT AAC TAC CTG ATC AGC GTC AAA TAC GGT GGG CCC
   1 MET Tyr Thr Pro Met Ala Pro Gly Asn Tyr Leu Ile Ser Val Lys Tyr Gly Gly Pro

 166 AAC CAC ATC GTG GGC AGT CCC TTC AAG GCC AAG GTG ACA GGC CAG CGT CTA GTT AGC
  20 Asn His Ile Val Gly Ser Pro Phe Lys Ala Lys Val Thr Gly Gln Arg Leu Val Ser

 223 CCT GGC TCA GCC AAC GAG ACC TCA TGC ATC CTG GTG GAG TCA GTG ACC AGG TCG TCT
  39 Pro Gly Ser Ala Asn Glu Thr Ser Cys Ile Leu Val Glu Ser Val Thr Arg Ser Ser

 280 ACA GAG ACC TGC TAT AGC GCC ATT CCC AAG GCA TCC TCG GAC GCC AGC AAG GTG ACC
  58 Thr Glu Thr Cys Tyr Ser Ala Ile Pro Lys Ala Ser Ser Asp Ala Ser Lys Val Thr

 337 TCT AAG GGG GCA GGG CTC TCA AAG GCC TTT GTG GGC CAG AAG AGT TCC TTC CTG GTG
  77 Ser Lys Gly Ala Gly Leu Ser Lys Ala Phe Val Gly Gln Lys Ser Ser Phe Leu Val

 394 GAC TGC AGC AAA GCT GGC TCC AAC ATG CTG CTG ATC GGG GTC CAT GGG CCC ACC ACC
  96 Asp Cys Ser Lys Ala Gly Ser Asn Met Leu Leu Ile Gly Val His Gly Pro Thr Thr

 451 CCC TGC GAG GAG GTC TCC ATG AAG CAT GTA GGC AAC CAG CAA TAC AAC GTC ACA TAC
 115 Pro Cys Glu Glu Val Ser Met Lys His Val Gly Asn Gln Gln Tyr Asn Val Thr Tyr

 508 GTC GTC AAG GAG AGG GGC GAT TAT GTG CTG GCT GTG AAG TGG GGG AGG AAC ACA TCC
 134 Val Val Lys Glu Arg Gly Asp Tyr Val Leu Ala Val Lys Trp Gly Arg Asn Thr Ser

 565 CTG GCA GCC CTT TTC ATG TCA CAG TGC CTT AAA ACA GTT TTC TCA AAT CCT GGA GAG
 153 Leu Ala Ala Leu Phe Met Ser Gln Cys Leu Lys Thr Val Phe Ser Asn Pro Gly Glu

 622 AGT TCT TGT GGT TGC TTT TGT TGC TTG TTT GTA ATT CAT TTT ATA CAA AGC CCT CCA
 172 Ser Ser Cys Gly Cys Phe Cys Cys Leu Phe Val Ile His Phe Ile Gln Ser Pro Pro

 679 GCC TGT TTG TGG GGC TGA AACCCCATCCCTAAAATATTGCTGTTGTAAAATGCCTTCAGAAATAAGTCCTAGACTGGACTC
 191 Ala Cys Leu Trp Gly

 760 TTGAGGGACATATTGGAGAATCTTAAGAAATGCAAGCTTGTTCAGGGGGCTGAGAAGATCCTGAGTACACTAGGTGCAAACCAGAACTCTTGG
 853 TGGAACAGACCAGCCACTGCAGCAGACAGACCAGGAACACAATGAGACTGACATTTCAAAAAAACAAAACTGGCTAGCCTGAGCTGCTGGTTC
 946 ACTCTTCAGCATTTATGAAACAAGGCTAGGGGAAGATGGGCAGAGAAAAAGGGGACACCTAGTTTGGTTGTCATTTGGCAAAGGAGATGACTT
1039 AAAAATCCGCTTAATCTCTTCCAGTGTCCGTGTTAATGTATTTGGCTATTAGATCACTAGCACTGCTTTACCGCTCCTCATCGCCAACACCCC
1132 CATGCTCTGTGGCCTTCTTACACTTCTCAGAGGGCAGAGTGGCAGCCGGGCACCCTACAGAAACTCAGAGGGCAGAGTGGCAGCCAGGCCCAC
1225 ATGTCTCTCAAGTACCTGTCCCCTCGCTCTGGTGATTATTTCTTGCAGAATCACCACACGAGACCATCCCGGCAGTCATGGTTTTGCTTTAGT
1318 TTTCCAAGTCCGTTTCAGTCCCTTCCTTGGTCTGAAGAAATTCTGCAGTGGCGAGCAGTTTCCCACTTGCCAAAGATCCCTTTTAACCAACAC
1411 TAGCCCTTGTTTTTAACACACGCTCCAGCCCTTCATCAGCCTGGGCAGTCTTACCAAAATGTTTAAAGTGATCTCAGAGGGGCCCATGGATTA
1504 ACGCCCTCATCCCAAGGTCCGTCCCATGACATAACACTCCACACCCGCCCCAGCCAACTTCATGGGTCACTTTTTCTGGAAAATAATGATCTG
1597 TACAGACAGGACAGAATGAAACTCTGCGGGTCTTTGGCCTGAAAGTTGGGAATGGTTGGGGGAGAGAAGGGCAGCAGCTTATTGGTGGTCTTT
1690 TCACCATTGGCAGAAACAGTGAGAGCTGTGTGGTGCAGAAATCCAGAAATGAGGTGTAGGGAATTTTGCCTGCCTTCCTGCAGACCTGAGCTG
1783 GCTTTGGAATGAGGTTAAAGTGTCAGGGACGTTGCCTGAGCCCAAATGTGTAGTGTGGTCTGGGCAGGCAGACCTTTAGGTTTTGCTGCTTAG
1876 TCCTGAGGAAGTGGCCACTCTTGTGGCAGGTGTAGTATCTGGGGCGAGTGTTGGGGGTAAAAGCCCACCCTACAGAAAGTGGAACAGCCCGGA
1969 GCCTGATGTGAAAGGACCACGGGTGTTGTAAGCTGGCCC
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FIG. 1. cDNA and predicted amino acid sequence of TABP. Arrows indicate putative initiation codons. The putative open reading frame extends 585 bp. The CaM kinase consensus sequence is underlined. Dashed line denotes putative N-linked glycosylation site. Boxed dashed line denotes hydrophobic putative-membrane-associated region.

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          0                                                    58
TABP   MYTPMAPGNY LISVKYGGPN HIVGSPFKAK VTGQRLVSPG SANETSCILV ES..VTRSST
        ******* * *** *****  ** ******** *** ****   *  ***      *  *  * *
ABP    .YTPMAPGSY LISIKYGGPY HIGGSPFKAK VTGPRLVSNH SLHETS.... .SBFVD.SLT
       2487                                                       2539

          59                                                     112
TABP   E.TCYS...A I.PK.ASSDA SKVTSKGAGL SKAFVGQKSS FLVDCSKAGS NMLLIGVHGP
         **     *   *  *  ** *** ** ** *** ****** * *******  **** *****
ABP    KATCAPQHGA PGPGPA..DA SKVVAKGLGL SKAYVGQKSS FTVDCSKAGN NMLLVGVHGP
       2540         ▲                                           2597

          113                                                    170
TABP   TTPCKE..VS MKHVGNQQYN VTYVVKERGD YVLAVKNGRN TSLAALFMSQ CLKTVFSNPG
        *****  *   ****   *  * *  *  *  * * ****
ABP    RTPCKEILV. .KHVGSRLYS VSYLLKDKGE YTLVVKNGH. ..........  ..........
       2598                                  2634

          171                                195
TABP   ESSCGCFCCI FVIHFIQ.SP .....PACLN G
       *           * *  **     *
ABP    E.....  ...H.IPGSP YRVVVP.... .
       2635                 2647
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FIG. 2. Amino acid sequence alignment of TABP with ABP. Numbering refers to amino acid position relative to the beginning of respective open reading frames. Asterisks denote identical residues. Residues underlined denote CaM kinase consensus sequence. Dashed overline denotes putative N-linked glycosylation site. Sequence between arrows represents putative glycoprotein binding site.

T1 sequence: i.e., 70% homology for 485 nt (nt 112–597 and nt 7631–8116 in T1 and ABP, respectively) and 64% homology for 146 aa (residues 2–148 and 2487–2633 in the putative open reading frames of T1 and ABP, respectively). In view of this homology, this protein was designated TABP. The only other significant homology was with human vimentin (56% identity in a 122-nt overlap, nt 52–174 and 460–587 in TABP and human vimentin, respectively; data not shown).

The human ABP gene encodes a protein of 2647 aa (280 kDa), represented by a single RNA species of 8 kb (5) (Fig. 3*B*). It contains an actin-binding domain near the N terminus (first 275 aa) whose amino acid sequence is similar to other actin filament binding proteins (5). The remaining 90% contains 24 repeats, each ≈96 residues long, predicted to have stretches of β-sheet secondary structure interspersed with turns. Sequence insertions immediately before the 16th and 24th repeats of 23 and 35 aa, respectively, predict two hinges. The insertion prior to the 24th repeat most likely serves as a binding site for membrane glycoproteins (Fig. 2, amino acid residues between the two arrows, and Fig. 3*B*, right-hand arrow) (5).

Comparison of the sequences of TABP and ABP suggests that TABP is a truncated ABP-like protein that lacks the N-terminal actin binding domain and 22 of the 24 repeats (Fig. 3*B*). However, TABP contains a region similar to the second putative insertion, prior to the 24th repeat in ABP (Fig. 2, residues bounded by the two arrows, and Fig. 3*A*, region b). The sequence homology between TABP and ABP is significantly greater on either side of this putative membrane glycoprotein binding domain. TABP and ABP contain the same consensus sequence for phosphorylation by Ca^{2+}–calmodulin kinase II (CaM kinase) in the putative glycoprotein binding domain [Fig. 2, underlined residues, RLVS (Arg-Lys-Val-Ser), residues 35–38 and 2520–2523 in TABP and ABP, respectively; and Fig. 3*A*, region b]. TABP also contains a putative N-linked glycosylation site (Fig. 2, NET, residues 43–45; and Fig. 3, region b) and shares significant homology with ABP in region c, a region considered important for ABP dimerization (6). The least homology is at the extreme C terminus, where TABP has a unique insertion of 33 aa (region d) (Fig. 2 and Fig. 3*A*, region d).

The signal peptide prediction algorithm of von Heijne (15) shows that TABP protein probably does not contain a signal sequence for secretion. Hydropathy profiles (16) identified a hydrophobic region within the C-terminal insertion (see Fig. 1, denoted by the boxed dashed line). aa 174–186 are strongly hydrophobic. Because aa 187–192 are not hydrophobic, the former region most likely represents a membrane-associated rather than a transmembrane domain. Thus, TABP would appear to contain the last two β-sheet repeats of ABP, between which lies a putative glycoprotein binding domain. Notably, the sequence of the putative glycoprotein binding domain in TABP is significantly different from that of ABP, although the same CaM kinase consensus sequence is present.

Analysis of TABP Fusion Protein. Purified GST–TABP fusion protein was subjected to SDS/PAGE and stained with

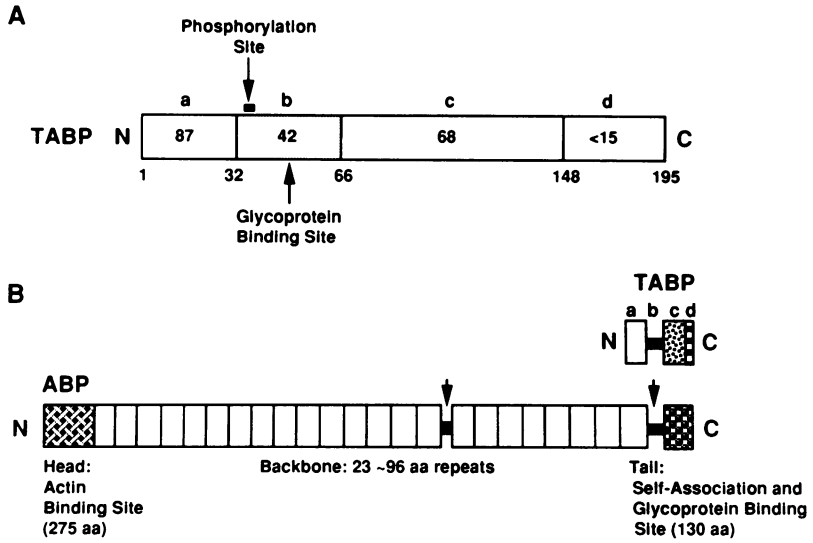

FIG. 3. Structural comparison between TABP and ABP. (*A*) Schematic diagram of TABP. Four regions are defined: a, b, c, and d. Numbers within the regions represent percentage amino acid homology with ABP. Amino acid position at the boundary of each region is shown. (*B*) Schematic comparison of TABP with ABP. The four regions a, b, c, and d correspond to *A*. ABP contains three major regions: head, backbone, and tail. Arrows denote putative amino acid insertions. N, N terminus; C, C terminus.

Coomassie blue (Fig. 4*A*). The intense band at 43–46 kDa represents the GST–TABP fusion protein, which is consistent with the predicted molecular mass of ≈21 kDa for TABP (GST = 26 kDa). The less-intense bands at ≈26 kDa represent the GST moiety and suggest that the fusion protein was not significantly degraded. The bands of higher molecular mass (>50 kDa) represent copurified *E. coli* proteins (see below).

The GST–TABP fusion protein was analyzed on an immunoblot with sera from subjects with Graves disease containing TSH-R antibodies (TRAb), Hashimoto thyroiditis, type 1 diabetes mellitus, type 2 diabetes mellitus, polymyositis, mixed connective tissue disease, and controls (e.g., Fig. 4*B*) (17). A minority of sera from subjects with Graves disease (3 of 10; e.g., Fig. 4, lane G7) and Hashimoto thyroiditis sera (1 of 4, e.g., Fig. 4, lane H1) identified the putative GST–TABP fusion protein at ≈46 kDa. No other sera identified the fusion protein. The band identified at ≈59 kDa (denoted E) was considered to be an *E. coli* protein because it was present in both patient and control sera (e.g., Fig. 4*B*, lane C1). To clearly demonstrate the TABP immunoreactivity with patient sera, the immunoblot was overexposed, which resulted in blurring of the reactivity recognized by a high-affinity anti-GST serum (Fig. 4*B*, lane GST). However, as expected, in shorter exposures of the blot the major GST–TABP (≈46 kDa) and the GST (≈26 kDa) moieties were clearly identified (data not shown).

Tissue Distribution of TABP mRNA. In Northern blot analyses using HeLa and Swiss 3T3 cell RNA, the mRNA for TABP was detected at ≈2.3 kb (data not shown). In addition, an mRNA of ≈8 kb was also detected, which most likely corresponds to ABP mRNA (5). RNase protection analysis indicated that TABP was expressed widely as a protected fragment of the expected size (240 bp) in human lung, liver, thyroid adenoma and normal thyroid (faint but distinct band), and placenta, as well as HeLa 229 cells but not in pancreas RNA (Fig. 5). PCR was also used to detect TABP mRNA expression, utilizing first-strand DNA synthesized from human thyroid and HeLa cell RNA and oligonucleotides designed to prime the amplification of an ≈550-bp fragment of TABP cDNA. An amplified product of ≈550 bp, which was sequenced to confirm that it was TABP cDNA, was detected in both tissues, but only in reaction mixtures that contained

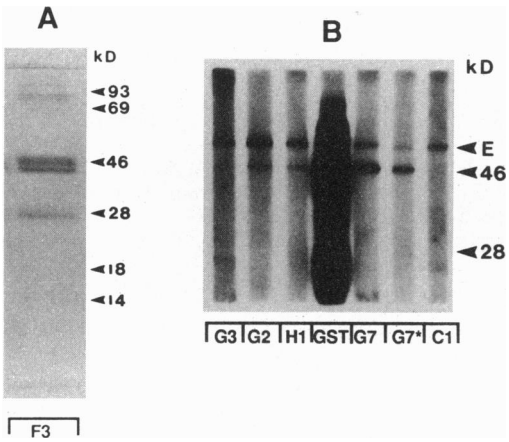

FIG. 4. Analysis of pGEX-TABP fusion protein. Recombinant fusion protein (GST–TABP) was subjected to SDS/PAGE and visualized by Coomassie blue staining in a 12.5% gel (*A*) or analyzed on an immunoblot of an 11% gel (*B*). The gel in *B* was electrophoresed longer than the gel in *A*. ^{14}C molecular weight markers were used. (*A*) F3, GST–TABP fusion protein. (*B*) G2, G3, and G7, three Graves sera containing TRAb; H1, Hashimoto thyroiditis serum; C1, control serum; GST, serum from a rabbit immunized with affinity-purified GST; *, serum was absorbed against *E. coli* protein.

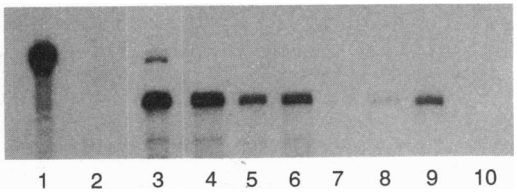

FIG. 5. Analysis of TABP mRNA expression by RNase protection assay. Lane 1 contains undigested TABP antisense RNA probe. Lanes 2–10 contain TABP antisense RNA probe hybridized with tRNA (lane 2), synthetic TABP sense RNA (lane 3), 6 μg of human placental poly(A)$^+$ RNA (lane 4), and ≈45 μg of total RNA from HeLa 229 cells (lane 5), human thyroid adenoma (lane 6), normal human thyroid (lane 7), human liver (lane 8), human lung (lane 9), and human pancreas (lane 10). Undigested probe and protected fragment are 303 and 240 bp, respectively.

reverse transcriptase, not from reaction mixtures in which template DNA was omitted (data not shown).

DISCUSSION

We have cloned and partially characterized a protein, TABP, a truncated member of the ABP family, that would also appear to be an autoantigen recognized by some patients with autoimmune thyroid disease. The high degree of sequence identity between TABP and ABP is consistent with TABP being a truncated form of ABP. However, the sequence of TABP differs from that of ABP in several important respects, which suggests that the two molecules serve different, although associated, functions. By comparison with ABP, we can deduce that TABP neither binds nor crosslinks actin into bundles. The sequence homology between TABP and ABP in the putative glycoprotein binding domain is considerably lower than in the predicted β-sheet repeats that flank this region. This suggests that TABP and ABP have different membrane glycoprotein binding specificities. The C-terminal insertion in TABP is unique and contains a hydrophobic putative membrane-associated domain, which implies that TABP associates with the plasma membrane. In addition, the presence of a self-association domain within the C-terminal β-sheet repeat suggests that TABP has the potential to dimerize. Furthermore, TABP mRNA is expressed widely as is ABP mRNA.

The recent demonstration that the FcγIR binds directly to ABP emphasizes the fact that ABP plays dual roles in some cells. In addition to crosslinking and polymerizing actin filaments (5), ABP links the actin-gel network intimately to the cell membrane by means of direct association with membrane glycoproteins, such as GPIb and FcγIR. The phenomenon of TSH-induced actin disruption implies the existence of an intimate association of the TSH-R with actin microfilaments, presumably via thyroid ABP. The existence of ABP in the thyroid seems likely as our Northern blot analyses with the TABP probe consistently detected mRNA of the correct size (≈8 kb), an ABP has been purified from porcine thyroid tissue (18), and the expression of ABP is ubiquitous (ref. 5; J. Hartwig, personal communication). Recent studies suggest that *in vivo* the TSH-R has two subunits of 53 kDa and 33–42 kDa linked by disulfide bonds, both of which are restricted to the basolateral region of the cell membrane (19). It is relevant, therefore, that we have copurified actin (confirmed by microsequencing) with a 50-kDa porcine TSH-R protein by TSH affinity chromatography (9, 17). Although actin is an abundant cytoplasmic component that could nonspecifically copurify with the TSH-R, γ-actin has also been copurified during purification of the rat FRTL-5 cell TSH-R (20). Moreover, γ-actin contains an epitope recognized by monoclonal antibodies to the purified TSH-R that stimulate cAMP production in a FRTL-5 cell

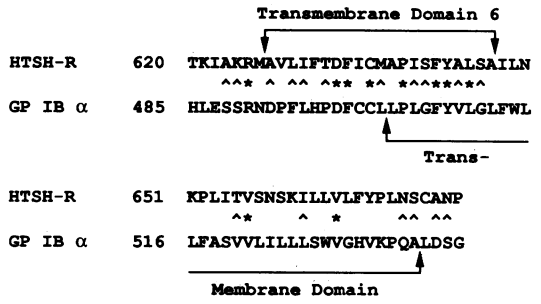

FIG. 6. Amino acid homology between human TSH-R and platelet glycoprotein GPIbα. Amino acid alignment of human TSH-R with GPIbα demonstrates homology in overlapping transmembrane domains. Asterisks denote identical residues; carrots denote conserved amino acid substitutions. Numbering refers to amino acid position relative to the beginning of their respective open reading frames.

bioassay. This suggests that the copurification of actin is not fortuitous and that there is a noncovalent association between thyroid-follicular-cell cytoskeletal actin microfilaments and the TSH-R.

It is not known definitively where in the ABP sequence glycoproteins bind. However, if specific human thyroid, platelet, and leukocyte membrane glycoproteins are directly linked to cytoskeletal actin by ABP, any regions of transmembrane or cytoplasmic sequence homology (for example, between glycoprotein GPIb and the TSH-R) could represent putative ABP-binding sites. Interestingly, one short region of homology is present within the sixth human TSH-R transmembrane domain and the only transmembrane domain of GPIbα (Fig. 6). However, these transmembrane sequences also differ significantly, which suggests that the putative ABP-binding sites may be tissue-specific.

What role could TABP play in the critical linkage between the TSH-R and the cytoskeleton? TABP could be an accessory protein that binds membrane glycoprotein, and in so doing, regulates or modifies the function of ABP. It may also dimerize with ABP and indirectly influence its proposed linkage function. An important aspect of TABP's function may relate to its phosphorylation by CaM kinase. This is relevant as phosphorylation of some proteins targeted by CaM kinase (21), including synapsin I and microtubule-associated protein 2, induces disassociation with, or disassembly of, the cytoskeleton.

Although antibodies to actin microfilament bundles and tubulin are found frequently in patients with both nonthyroid autoimmune disease (22) and autoimmune thyroid disease (23), to our knowledge, this is the first report of autoantibodies to an ABP-like protein component. TABP was initially identified by a serum from a Graves disease subject, which might imply the presence of an epitope for Graves immunoglobulins within the putative glycoprotein binding domain. No homology has been identified, however, between the TSH-R sequences reported to contain epitopes for Graves immunoglobulins (24–26) and TABP. The inability of the GST–TABP fusion protein to be identified on an immunoblot with a majority of Graves disease and Hashimoto thyroiditis immunoglobulins may relate to the denaturation associated with SDS/PAGE and transfer in the presence of SDS. These initial observations support the notion that TABP is an autoantigen, although further work should determine the prevalence and significance of these autoantibodies and the precise subcellular location of TABP.

Elucidation of the interaction between the TSH-R and actin microfilaments should enhance our understanding of cellular transduction in the thyroid and other endocrine tissues. It seems reasonable to propose that in thyroid and ovarian granulosa cells there exists a common pathway of ligand-induced cAMP-dependent actin disruption, which results in a profound alteration in cell phenotype. We propose that this is mediated through ABP-linkage proteins and that accessory proteins such as TABP interact directly with the ligand, receptor, and/or ABP to regulate the process. The cloning and initial characterization of TABP, and the availability of ABP, should facilitate our understanding of this process and enable the functional and potentially immunogenic role of TABP to be evaluated.

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