

# Thyroid Epithelial Cells Produce Large Amounts of the Alzheimer $\beta$ -Amyloid Precursor Protein (APP) and Generate Potentially Amyloidogenic APP Fragments\*

T. L. SCHMITT, E. STEINER, P. KLINGLER, H. LASSMANN, AND  
B. GRUBECK-LOEBENSTEIN

*Institute for Biomedical Aging Research, Austrian Academy of Sciences (T.L.S., B.G.-L.), and  
Bezirkskrankenhaus Hall (E.S.) and the Department of Surgery II (P.K.), University of Innsbruck,  
Innsbruck; and the Research Unit for Experimental Neuropathology of the Austrian Academy of  
Sciences (H.L.), Vienna, Austria*

## ABSTRACT

The Alzheimer  $\beta$ -amyloid precursor protein (APP) is a transmembrane glycoprotein from which the amyloid  $\beta$ -protein is proteolytically derived. The latter is a hydrophobic peptide that can aggregate and forms the core of the senile plaques found in the brains of patients suffering from Alzheimer's disease (AD). In view of the known association between familial AD and thyroid autoimmune disease, the expression pattern and cellular processing of APP in human thyroid cells were investigated.

Cultured thyroid epithelial cells and homogenized thyroid tissue from normal and pathological thyroid samples were analyzed by immunoblotting using specific N- and C-terminal APP antibodies as well as by reverse transcription-polymerase chain reaction in which two sets of oligonucleotide primers were used. The results of these studies demonstrated that APP isoforms 770 and 751 were expressed in fresh thyroid extracts as well as in cultured thyroid epithelial cells, with APP 770 being the predominant form. Compared to other types of

cells, such as lymphocytes and fibroblasts, thyroid epithelial cells produced larger amounts of APP. Most of the mature protein was cleaved within the amyloid  $\beta$  region, as a result of which a large N-terminal APP fragment was released into the culture medium, whereas a C-terminal nonamyloidogenic fragment of 14 kilodaltons (kDa) was retained within the cell. Interestingly, thyroid epithelial cells also contained larger C-terminal APP fragments of 21, 35, and 41 kDa. From the sizes of these fragments it could be deduced that they contained the entire amyloid  $\beta$  sequence and were thus potentially amyloidogenic. The 41-kDa fragment was unique to thyroid cells. These fragments may be released into the circulation after thyroid cell damage. Increased/alterd thyroid APP expression in familiar AD may induce alterations in thyroid epithelial cells and cell damage, and thus explain the frequent occurrence of thyroid autoimmunity in this disease. (*J Clin Endocrinol Metab* 80: 3513–3519, 1995)

**D**EPOSITION of amyloid in the form of senile plaques or diffused in meningo-cerebral vasculature is the characteristic feature of Alzheimer's disease (AD). These lesions, which to a lesser extent also occur in normal aged brain, are suggested to impair synaptic transmission and lead to progressive dementia and neuronal loss. The major proteinaceous component of these plaques is the amyloid  $\beta$  protein ( $A\beta$ ), a hydrophobic peptide of 40–43 amino acids (1, 2). Cloning studies revealed, that  $A\beta$  is derived proteolytically from the much larger amyloid precursor protein (APP), a transmembrane glycoprotein (3). The single copy APP gene comprises 19 exons and is located on chromosome 21. Due to the existence of three alternatively spliced exons (7, 8, and 15), a whole set of related transcripts can be produced (4–7). The corresponding protein isoforms are named according to their amino acid numbers; the three major variants are APP 695, 751, and 770. The two larger isoforms contain exon 7,

which codes for a domain with high homology to Kunitz-type serine protease inhibitors. The recent discovery of two homologous proteins, APLP1 and APLP2 (8), indicates that APP is a member of a larger gene family. The cellular processing of APP is complex. After translation, APP undergoes N- and O-linked glycosylation, phosphorylation, and tyrosine sulfation (9, 10). Two different metabolic pathways have been described. In the secretory pathway, mature APP is cleaved within the  $A\beta$  sequence (at residue 687 of APP 770), and a large N-terminal portion is released by the cell (11). This soluble nonamyloidogenic APP derivative is identical to the serine protease inhibitor protease nexin II (12, 13). In a distinct endosomal/lysosomal pathway, APP is cleaved intracellularly into various fragments (14). C-Terminal fragments frequently contain the intact  $A\beta$  sequence and may thus be amyloidogenic (15, 16). The protease responsible for  $A\beta$  production has not yet been characterized. However, it has recently been demonstrated by several groups, that  $A\beta$  is constitutively secreted by transformed and nontransformed cells in culture (17) and has also been found in body fluids (18).

Whereas early studies concentrated on APP expression in the brain, a growing body of knowledge has been accumulated concerning APP expression and metabolism in extra-neuronal cells such as platelets (19), lymphocytes (20), endothelial cells (21), and fibroblasts (22). The physiological

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Address all correspondence and requests for reprints to: Dr. T. Schmitt, Institute for Biomedical Aging Research, Rennweg 10, A-6020 Innsbruck, Austria.

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importance of APP production in nonneuronal cells is at present difficult to assess, because the biological function of APP is not well understood. A role as a cell surface receptor, a neurotrophic molecule, or a cell adhesion/attachment factor has been suggested (23, 24). It has also been shown to bind to G proteins (25). The presence of amyloid deposits in cerebral blood vessels (1, 26) indicates that extraneuronally produced A $\beta$ P may be transported to the brain and locally precipitate. Whether APP/A $\beta$ P also exerts pathological effects on the different extraneuronal cells in which it is produced is not known. This question is of particular relevance for conditions in which APP/A $\beta$ P production is genetically altered, such as Down's syndrome, which is accompanied by overproduction of APP (27, 28), or familial AD, in which at least one mutation within the secretory cleavage site of APP leads to a predominance of the endosomal/lysosomal metabolic pathway (29). Although patients with Down's syndrome have a wide panel of extraneuronal disturbances (30), which may or may not be due to APP overproduction, there is little evidence of extraneuronal pathology in patients with familial AD. There is, however, one feature common to both conditions, an increased incidence of hypothyroidism and thyroid autoimmune disease (31, 32). This led us to hypothesize that increased or altered  $\beta$ APP production taking place in the thyroid glands of these patients might lead to cell damage and/or antigenic alterations that could consecutively result in thyroid autoimmunity. This assumption seemed of particular interest because it is well known that amyloid derived from other amyloidogenic proteins may be deposited in the thyroid gland (33–35). Apart from a recent study on a rat thyroid cell line (36), APP has not yet been described in thyroid cells, and there is no information about the intrathyroidal occurrence of amyloidogenic APP metabolites. It was, therefore, the aim of this study to analyze intrathyroidal APP in the human system and to investigate its production and metabolism in thyroid health and disease.

## Subjects and Methods

### Patients

Four patients with Graves' disease (GD; 3 women and 1 man; age, 32–57 yr), 5 patients with nonfunctioning solitary thyroid adenoma (all women; age, 46–71 yr), and 32 patients with multinodular nontoxic goiter (NTG; 24 women and 8 men; age, 28–69 yr) were included in the study. Patients with GD had small diffuse goiters and clinical and laboratory evidence of severe hyperthyroidism at the time of diagnosis. All GD patients were treated with methimazole and were euthyroid at the time of surgery. Patients with NTG and solitary thyroid adenomas were untreated before surgery and had normal thyroid hormone concentrations. Four normal thyroid tissue samples were obtained from the contralateral lobe in patients who underwent surgery because of thyroid malignancy. All clinical diagnoses were confirmed by histological examination of surgical specimens.

### Thyroid homogenates

Fresh thyroid samples were obtained from surgically removed thyroid tissue, cut into pieces, and homogenized in phosphate-buffered saline containing 1% Nonidet P-40, 1% Triton X-100, 10  $\mu$ g/mL aprotinin, 2  $\mu$ g/mL pepstatin A, and 5  $\mu$ g/mL leupeptin. The homogenization was performed with an Ultra-Turrax T 25 (Janke and Kunkel, Staufen, Germany) for 30 s at 15,000 rpm on ice. The suspension was centrifuged for 10 min at 5,000  $\times$  g, and the supernatant was collected.

Protein concentrations in supernatants were determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA).

### Cell purification and cell culture

Thyroid epithelial cells (TEC) were purified from human thyroid glands as previously described (37). Briefly, tissue samples were cut into 1- to 2-mm<sup>3</sup> pieces and dispersed with 5 mg/mL collagenase (type IV, Sigma, Deisenhofen, Germany) in RPMI 1640 medium containing 10% fetal calf serum and 1% penicillin/streptomycin at 37 C for 3 h. All tissue culture reagents were obtained from GIBCO (Paisley, UK). The resulting cell suspension was filtered through a 200- $\mu$ m nylon mesh. The red blood cells were lysed with an ammonium chloride buffer (0.82% NH<sub>4</sub>Cl and 0.1% KHCO<sub>3</sub>), and the remaining cells were washed and incubated in RPMI 1640 supplemented with 10% fetal calf serum and 1% P/S in 75-cm<sup>2</sup> culture flasks (Costar, Cambridge, MA) at 37 C in 5% CO<sub>2</sub> and 95% humidity for 24 h. Nonadherent cells were then removed by vigorous washing with medium. The remaining adherent cell population consisted of more than 95% TEC (37). TEC were cultured in RPMI with 5% fetal calf serum. Conditioned supernatants were obtained after a 72-h incubation period from  $1.5 \times 10^6$  cells in 10 mL culture medium. For harvesting, TEC were removed from their plastic support by a 5-min exposure to trypsin-ethylenediamine tetraacetate, washed twice in phosphate-buffered saline, and counted. Cells were sonicated in lysis buffer, and after centrifugation, the protein concentration was determined by the Bio-Rad protein assay.

Skin fibroblasts were obtained and purified as previously described (38). Fibroblast lines were established and used for analysis after 8–12 passages. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood of young healthy donors using a Ficoll-Paque gradient (Pharmacia, Uppsala, Sweden) and washed twice. Cell extracts and conditioned supernatants from fibroblasts and PBMC were obtained in the same manner as described for TEC.

### Antibodies

The monoclonal antibody 22C11, which is specific for an epitope in the N-terminus of APP (39), was obtained from Boehringer Mannheim (Mannheim, Germany). It was used at a concentration of 3  $\mu$ g/mL for immunoblotting experiments. LN27, another monoclonal antibody with N-terminal reactivity, was purchased from Zymed (San Francisco, CA). 6E10, a monoclonal antibody raised against A $\beta$ P, was obtained from K. Kim (NYS for Basic Research, Staten Island, NY). Antisera C7 and C8 were provided by D. Selkoe (Brigham and Women's Hospital, Harvard Medical School, Boston, MA). These antisera specifically recognize the C-terminus of APP (40). For immunoblotting, they were used at a dilution of 1:1000. In some experiments, C7 was preabsorbed with its corresponding peptide C (last 20 amino acids of APP) at a concentration of 6  $\mu$ g/ $\mu$ L, as described previously (40). Another antiserum with C-terminal specificity, 369W, was kindly provided by S. Gandy (Department of Neurology and Neuroscience, Cornell Medical Center, New York, NY).

### Gel electrophoresis and immunoblotting

Protein extracts were boiled in sodium dodecyl sulfate (SDS) lysis buffer, and 70  $\mu$ g protein were applied to the gels, which were run at a constant current of 25 mA. Gels with a polyacrylamide percentage of 8.5%, 10%, or 12.5% were run, depending on the antibodies used. Gels were tank blotted overnight at 95 milliamperes onto 0.2- $\mu$ m nitrocellulose filters (Schleicher and Schuell, Dassel, Germany) in a Tris-glycine buffer, pH 8.3, containing 20% (vol/vol) methanol. Nitrocellulose filters were subsequently blocked in 4% milk powder for 2 h and incubated with the APP antibodies for 2 h. Filters were washed and labeled with alkaline phosphatase-conjugated secondary antibodies (Dianova, Hamburg, Germany). Color development was obtained in a solution containing 0.33 mg/mL NBT and 0.16 mg/mL BCIP in 100 mmol/L Tris (pH 9.6), 100 mmol/L NaCl, and 5 mmol/L MgCl<sub>2</sub>.

In some experiments, the resulting bands were quantified by densitometric scanning (Scanpack, Biometra, Göttingen, Germany).

### Reverse transcription-polymerase chain reaction (RT-PCR)

Total ribonucleic acid (RNA) was prepared by a modified guanidinium thiocyanate-phenol method (41), and yield and purity were determined by UV spectrophotometry (U-2000, Hitachi, Tokyo, Japan). Two micrograms of total RNA were reverse transcribed with system A3500 from Promega Corp. (Madison, WI) using oligo(deoxythymidine) priming. The total reaction volume was 40  $\mu$ l, and the reaction was terminated by heating to 75 C. Four microliters of this sample were used in the PCR, using the PCR Core Kit from Boehringer Mannheim according to manufacturer's instructions. The first pair of primers (comprising exons 7 and 8) was synthesized according to the method of Gardella *et al.* (42), and the second pair (comprising exon 15) was synthesized according to the method of König *et al.* (7). Reaction conditions for both primer pairs were 45 s at 95 C, 45 s at 50 C, and 60 s at 72 C. After amplification for 30 cycles, 20  $\mu$ l reaction mixture were mixed with 5  $\mu$ l loading buffer [50% (vol/vol) glycerol, 0.2% (wt/vol) SDS, and 0.02% (wt/vol) bromophenol blue in Tris-acetate-ethylenediamine tetraacetate buffer] and electrophoresed on a 2.5% agarose gel for 2 h with 70 V in TAE buffer. DNA bands were stained with ethidium bromide and photographed on a UV light table.

### Results

#### APP is produced in the human thyroid gland and expressed and secreted by TEC

Immunoblotting using monoclonal antibody 22C11 revealed the existence of several reactive bands in cultured thyrocytes, conditioned supernatants, and thyroid homogenates (Fig. 1). A double band at 115 kilodaltons (kDa) was visible in cultured TEC (lanes T1 and T2), as well as in extracts from fresh thyroid tissue (lanes E1–E3), but not in conditioned supernatants (S1 and S2). It corresponded to the immature APP holoprotein (43). A slower migrating band at 145 kDa corresponding to the fully glycosylated (mature) protein (43) was also found in cultured cells. These bands were less pronounced in tissue homogenates. The ratios of immature to mature APP, however, were similar in cultured cells and thyroid homogenates. Supernatants conditioned by cultured TEC (S1 and S2) contained only one band at 125 kDa, which corresponds to APP<sub>s</sub> (PN II), the nonamyloidogenic secreted form of APP, which is truncated at the C-terminus (11). An identical staining pattern was found when other

monoclonal antibodies, such as LN-27 or 6E10, were used. All cultured cells and thyroid tissue samples presented in Fig. 1 derived from patients with nontoxic goiters, a condition frequently treated with surgery from which large amounts of tissue are available.

#### APP expression is similar in TEC from normal and pathological tissues

To investigate, whether APP expression was altered in thyroid disease, TEC from normal thyroid glands, GD thyroids, and cold thyroid nodules were compared by Western blotting (Fig. 2). No difference in the expression of APP was found among normal and pathological tissue samples, nor was there a difference among disorders when fresh thyroid extracts were used instead of cultured cells (not shown).

#### TEC express APP isoforms 770 and 751

To analyze in detail which isoforms of APP were produced by TEC, messenger RNA transcripts were investigated using RT-PCR. Total RNA was prepared from cultured TEC and reverse transcribed using oligo(deoxythymidine) priming. The resulting complementary DNAs were amplified by PCR. Two sets of primers were chosen for amplification: one comprising exons 7 (Kunitz-type serine protease inhibitor insert) and 8, and another comprising exon 15. The assay thus allowed the detection of all eight principal APP transcripts that can be generated by alternative splicing.

The first pair of primers was designed to yield DNA fragments of 1) 312 base pairs (bp), containing exons 7 and 8; 2) 255 bp, containing exon 7 but not 8; 3) 144 bp, containing only exon 8; and 4) 87 bp, containing neither exon 7 nor 8. A representative experiment is shown in Fig. 3A. It demonstrates a major band of 312 and a minor one of 255 bp. The dominant APP transcript thus contained both exons 7 and 8, whereas message containing only exon 7 was also present.

The second pair of primers was used for the detection of exon 15. The appearance of one 462-bp band after amplification (Fig. 3B) demonstrated that exon 15 was transcribed by TEC and that APP isoforms lacking exon 15 were not present.

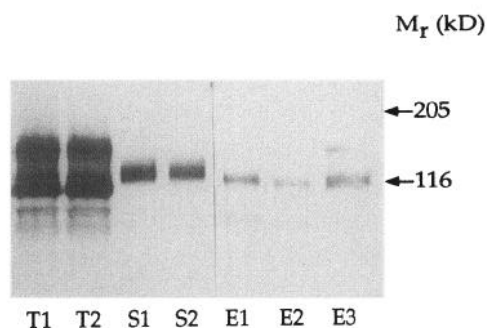


FIG. 1. Identification of APP in thyroid cells by immunoblotting. Extracts from cultured TEC (T1 and T2), the corresponding conditioned supernatants (S1 and S2), and freshly obtained thyroid tissue (E1–E3) were separated on a 8.5% SDS-PAA gel, transferred to nitrocellulose, and stained with the monoclonal antibody 22C11. The 115-kDa band represents the immature form, and the 145-kDa band represents the fully glycosylated form of APP, whereas the 125-kDa band corresponds to the secreted APP form (described in *Results*). The blot represents one of nine experiments.

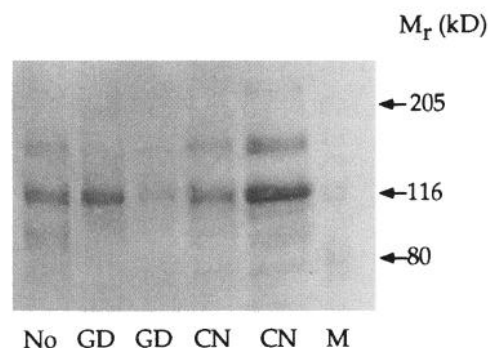


FIG. 2. The expression patterns of APP are similar in normal and pathological thyroid tissue samples. Extracts of TEC derived from normal thyroid tissue (No), GD thyroids (GD), and cold thyroid nodules (CN) were separated on a 10% SDS-PAA gel and immunostained with mAb 22C11. M, Mol wt marker (Bio-Rad). For identification of the bands, see Fig. 1. The blot represents one of three similar experiments.

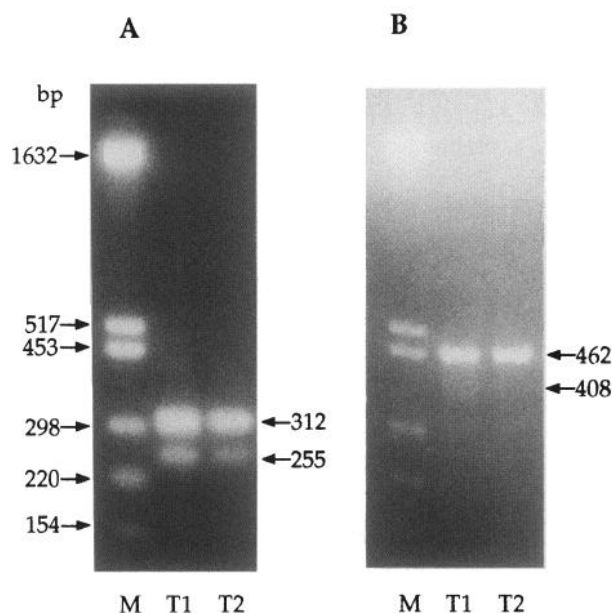


FIG. 3. Identification of APP isoforms by RT-PCR. Total RNA from two different TEC preparations (T1 and T2) derived from NTG tissue samples was reverse transcribed and amplified by PCR using two sets of primers. A, Primer pair 1 enclosing exons 7 and 8 (42). B, Primer pair 2 enclosing exon 15 (7). Amplified DNA fragments were electrophoresed on a 2.5% agarose gel and stained with ethidium bromide. M, Mol wt marker. The expected sizes of the amplified DNA fragments are indicated on the right of the lanes. This PCR analysis represents one of four experiments.

The PCR results thus demonstrated that cultured TEC predominantly produced the APP 770 isoform, which contains exons 7, 8, and 15. The APP isoform 751, which does not contain exon 8, was also present, but was expressed at lower concentrations.

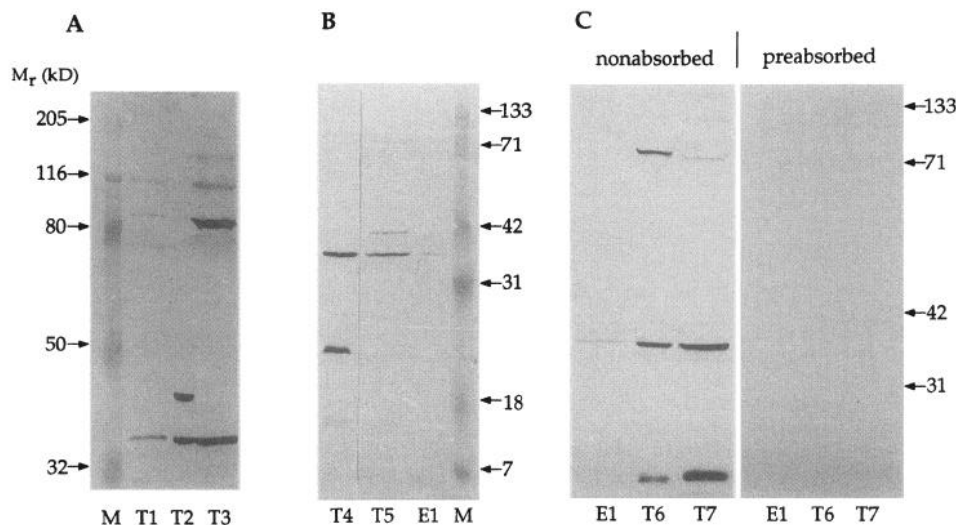
#### TEC produce potentially amyloidogenic fragments of APP

The amyloid region of APP is located close to the C-terminus. To investigate whether potentially amyloidogenic degradation products were formed during APP metabolism in TEC, all samples were analyzed by immunoblotting using

antiserum specific for the C-terminus of APP. A representative experiment using antibody C7 is shown in Fig. 4. This antibody reacted weakly with the full-length mature and immature APP as well as with an inconsistent band at 80 kDa and several bands of lower mol wt (Fig. 4A). Low mol wt bands may correspond to the membrane-bound APP residue, which remains in the cell after secretory APP cleavage and migrates as a 10- or 15-kDa band in the Tris-tricine or the Tris-glycine electrophoresis system, respectively (43). C-Terminal fragments larger than 15 kDa have been shown to contain the entire A $\beta$ P sequence (15, 43). For a better resolution of the low mol wt bands, 12.5% PAA gels were used (Fig. 4B). Two staining patterns were observed. Forty percent of the samples contained three immunoreactive bands at 35, 21, and 14 kDa. A representative example for this protein pattern is shown in Fig. 4B, lane T4. In 60% of the samples, two predominant bands were observed; one at 41 and another at 35 kDa (lane T5 in Fig. 4B). The 14-kDa band was very weak in these samples. No correlation could be found between the presence of the 41-kDa band and a certain clinical or histological condition. Preabsorption of the C7 antibody with its corresponding peptide demonstrated the specificity of the staining (Fig. 4C). Only two faint bands of about 100 and 70 kDa could be detected in some experiments after preabsorption of the antibody. The 41-kDa APP degradation band has so far only been found in TEC, not in other cell types such as PBMC or fibroblasts (Fig. 5B). The same protein fragment was also produced *in vivo*, as it occurred in 60% of fresh tissue homogenates.

A similar reactivity pattern was observed when other antibodies directed against the C-terminus of APP were used. There were, however, minor differences; C8 had a particularly strong reactivity with the 41-kDa band. Neither antibody 369 nor C8 stained the 21-kDa band, which was thus exclusively recognized by C7. This may indicate that it contained an APP epitope not detected by other antibodies or that C7 binding was due to cross-reactivity with a related peptide. No C-terminal APP reactivity could be detected in TEC supernatants (Fig. 5B).

FIG. 4. Detection of C-terminal degradation products of APP in TEC. Immunoblot with the polyclonal antibody C7, specific for the C-terminus of APP (40). A, Transfer from a 10% PAA gel with TEC preparations from three different thyroid glands (T1–T3). B, Improved resolution of smaller C-terminal fragments (41, 35, 21, and 14 kDa) on a 12.5% PAA gel. Extracts are from two cultured TEC preparations (T4 and T5) and from a fresh thyroid gland (E1). C, Control experiment, in which antibody C7 was preabsorbed with its corresponding peptide C. Extracts were from fresh thyroid tissue (E1) and two cultured TEC (T6 and T7). Detailed information on the identity of the bands is provided in Results. The blots represent one of four experiments.





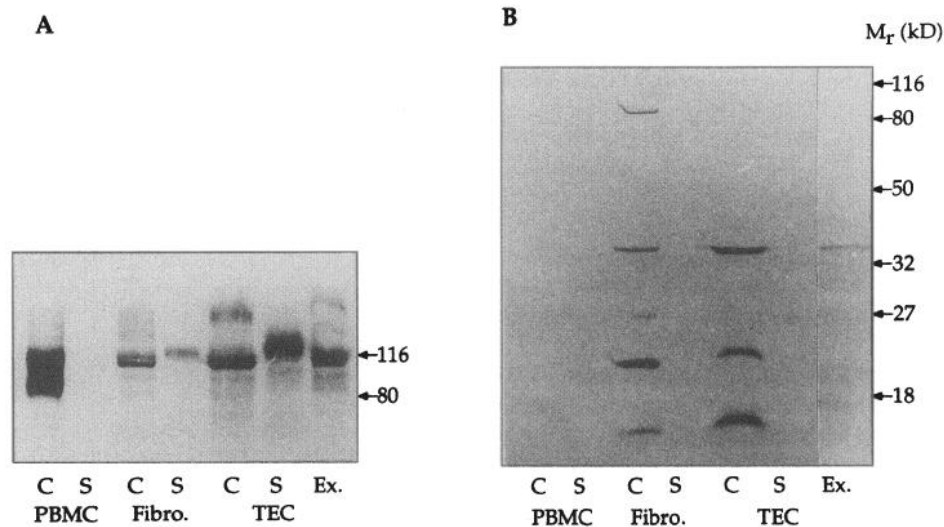


FIG. 5. Comparative analysis of APP immunostaining pattern in resting PBMC, fibroblasts, and TEC. Cells were grown and harvested as described in *Materials and Methods*. Equal amounts of protein from cellular extracts (C) and conditioned supernatants (S) were used; also shown is a fresh thyroid extract (Ex.). Samples were separated on a 8.5% PAA gel and probed with mAB 22C11 (A) or separated on a 12% PAA gel and stained with C7 (B). Densitometer scanning yielded the following arbitrary values for fibroblasts and TEC: 5,270 and 7,571 for the 115-kDa band, 1,323 and 4,442 for the mature 145-kDa band; and 3,255 and 10,589 for the 125-kDa APP<sub>s</sub> (A). The combined value for the 35- and 14-kDa bands in B was 1,538 for fibroblasts and 4,220 for TEC. PBMC contained a 117-kDa band (11,814) and a 90-kDa band (10,008), which presumably correspond to APP 695. They did not secrete APPs and did not contain C-terminal metabolites. The blot represents one of three experiments.

#### APP production/secretion in different cell types

To compare APP expression and metabolism in different types of cells, PBMC, fibroblasts, and TEC as well as their conditioned supernatants were analyzed (Fig. 5). Equal amounts of protein were analyzed by Western blotting with antibody 22C11 (Fig. 5A) and C7 (Fig. 5B), respectively. Extracts from resting PBMC contained large amounts of immature APP770 (115 kDa) as well as a band at 90 kDa, presumably corresponding to APP695, but no APP secretion and no C-terminal metabolites were found. Fibroblasts had a similar intracellular APP expression pattern as TEC, with a prominent double band at 115 kDa, but only small amounts of mature APP were detected. Supernatants conditioned by fibroblasts generally contained relatively low amounts of APP<sub>s</sub>. C-Terminal metabolites were also found in this cell type, with a predominance of the 21-kDa fragment. In all experiments performed, TEC produced larger quantities of mature APP and its metabolic derivatives, the secreted APP<sub>s</sub>, as well as the potentially amyloidogenic C-terminal fragments, than any other cell type investigated.

#### Discussion

The present study for the first time demonstrates that APP is produced by human thyroid cells, both *in vivo* and *in vitro*. By RT-PCR and immunoblotting, APP isoforms 770/751 could be detected. Both isoforms contain exon 7, which codes for a domain with serine protease inhibitor function. Also interesting is the presence of exon 8 in APP 770, which shows homology to the MRC-OX2 antigen found in thymocytes (44). This isoform pattern is typical of most of the extraneuronal cells studied so far. In contrast, neuronal cells produce mainly the 695 isoform. Comparison with other cell types demonstrates that relatively high amounts of APP are pro-

duced by TEC. Especially notable is the rate of APP secretion, which is much higher than that in fibroblasts, a cell type frequently used for secretion assays. The thyroid gland may thus be a major source of APP. High production of APP is followed by increased activity of the lysosomal/endosomal metabolic pathway in many cell types (14). One would thus anticipate that TEC, which are particularly well equipped with late endosomes and lysosomes (45), not only secrete C-terminally truncated APPs, but may also catabolize APP by lysosomal/endosomal degradation. This assumption is supported by our finding of C-terminal APP fragments of different sizes in thyroid homogenates as well as in cultured thyroid cells. From the sizes of these fragments, it can be deduced that they contain the entire A $\beta$ P sequence and are thus potentially amyloidogenic (15, 43). The appearance of a seemingly thyroid-specific band of 41 kDa in 60% of the thyroid tissue samples also suggests that the enzymatic equipment of TEC may favor a tissue-specific APP degradation pattern, as suggested for other types of epithelial cells (46). At present, work is under way in our laboratory trying to define under which conditions the 41-kDa fragment is produced. Studies on APP metabolism in different types of endocrine cells, some of which have been shown to produce APP (47), would also be interesting in this context.

The fate of amyloidogenic APP fragments generated in thyroid cells is of interest. They may be further degraded (by the still unknown  $\beta$ - and  $\gamma$ -secretases), which may lead to the formation of A $\beta$ P. A $\beta$ P-containing fragments or A $\beta$ P could be released into the circulation. This may happen under physiological conditions (17, 18) or in the case of tissue damage. Tissue damage could also affect APP production and metabolism. This must be considered when thyroid destruction is the iatrogen, such as during radioiodine treatment of GD or thyroid cancer. Whether APP fragments released in

these situations may contribute to A $\beta$ P deposition in cerebral blood vessels, as suggested for A $\beta$ P produced by other extraneuronal cell types (18), remains to be elucidated. If not released into the circulation, A $\beta$ P-containing fragments or A $\beta$ P could principally also remain within TEC. Whether this would affect the functional status and the viability of the cell is not known, but it seems conceivable, as C-terminal APP fragments have been shown to evoke neuronal cell death (48). A variety of biological functions have been attributed to A $\beta$ P. A $\beta$ P, for instance, has been shown to bind to various kinds of proteins, such as apolipoprotein E, transthyretin (49), and antichymotrypsin (50). It may also bind to TEC-specific intracellular proteins and affect their functions as well as their antigenic properties. The antigenicity of potential thyroid autoantigens could thus be increased. This could be one of the reasons for the frequent occurrence of thyroid autoimmune disease in conditions with increased/ altered APP metabolism, such as Down's syndrome or familial AD (31, 32). Additional factors of relevance for hypothyroidism and thyroid autoimmunity may be TEC damage due to intracellular A $\beta$ P accumulation or presentation of intracellular APP fragments to autoreactive T cells. The latter possibility is of special interest when seen in the context of the findings that TEC are capable of presenting autoantigenic peptides to autoreactive T lymphocytes of the immune system (51, 52) and that T cells with specific autoreactivity to APP peptides occur even under normal conditions (53).

It would obviously be of great interest to know more about the role of  $\beta$ APP and its metabolites in thyroid physiology. As cell surface expression has not yet been detected, a receptor function seems unlikely. APP binds to heparin and interacts with collagen and laminin (54). It could thus play a role in cell adhesion and in the interactions of cells with components of the extracellular matrix (55). Due to its identity with the protease inhibitor nexin II, a function in coagulation and wound healing has been proposed (56). Future studies, probably using antisense technology, will have to answer which of these mechanisms is of special relevance in the thyroid gland.

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