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Defective thyroglobulin storage in LDL receptor-associated protein-deficient mice

Simonetta Lisi,[1] Roberta Botta,[1] Aldo Pinchera,[1] A. Bernard Collins,[2] Samuel Refetoff,[3] Peter Arvan,[4] Guojun Bu,[5] Lucia Grasso,[1] Vladimir Marshansky,[6] Shaliha Bechoua,[6] Andres Hurtado-Lorenzo,[6] Claudio Marcocci,[1] Dennis Brown,[6] Robert T. McCluskey,[2] and Michele Marinò[1]

[1]*Department of Endocrinology, University of Pisa, Pisa, Italy;* [2]*Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts;* [3]*Departments of Medicine and Pediatrics, University of Chicago, Chicago, Illinois;* [4]*Division of Metabolism, Endocrinology and Diabetes, University of Michigan, Ann Arbor, Michigan;* [5]*Departments of Pediatrics, Cell Biology, and Physiology, Washington University School of Medicine, St. Louis, Missouri; and* [6]*Program in Membrane Biology, Renal Unit, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts*

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Lisi, Simonetta, Roberta Botta, Aldo Pinchera, A. Bernard Collins, Samuel Refetoff, Peter Arvan, Guojun Bu, Lucia Grasso, Vladimir Marshansky, Shaliha Bechoua, Andres Hurtado-Lorenzo, Claudio Marcocci, Dennis Brown, Robert T. McCluskey, and Michele Marinò. Defective thyroglobulin storage in LDL receptor-associated protein-deficient mice. *Am J Physiol Cell Physiol* 290: C1160–C1167, 2006. First published November 23, 2005; doi:10.1152/ajpcell.00382.2005.—The molecular chaperone receptor-associated protein (RAP) is required for biosynthesis of megalin, an endocytic receptor for follicular thyroglobulin (Tg), the thyroid hormone precursor. RAP also binds to Tg itself, suggesting that it may affect Tg trafficking in various manners. To elucidate RAP function, we have studied the thyroid phenotype in RAP-knockout (RAP-KO) mice and found a reduction of Tg aggregates into thyroid follicles. Serum Tg levels were significantly increased compared with those of wild-type (WT) mice, suggesting a directional alteration of Tg secretion. In spite of these abnormalities, hormone secretion was maintained as indicated by normal serum thyroxine levels. Because Tg in thyroid extracts from RAP-KO mice contained thyroxine residues as in WT mice, we concluded that in RAP-KO mice, follicular Tg, although reduced, was nevertheless sufficient to provide normal hormone secretion. Serum TSH was increased in RAP-KO mice, and although no thyroid enlargement was observed, some histological features resembling early goiter were present. Megalin was decreased in RAP-KO mice, but this did not affect thyroid function, probably because of the concomitant reduction of follicular Tg. In conclusion, RAP is required for the establishment of Tg reservoirs, but its absence does not affect hormone secretion.

low-density lipoprotein; knockout mice

THE LDL RECEPTOR-ASSOCIATED PROTEIN (RAP) is an ~39-kDa endoplasmic reticulum (ER)-resident protein that functions as a molecular chaperone for several members of the LDL receptor family, including megalin (6, 23). RAP is present in virtually all cell types that express megalin, where it is localized in the ER and early Golgi compartments via a specialized COOH-terminal ER retention signal (6, 23). In the absence of RAP, folding of megalin is impaired, and as a result, the receptor is retained within the ER with reduced expression on the cell membrane (4, 6, 23–25).

Both megalin and RAP are expressed by thyroid epithelial cells (thyrocytes) (26), where megalin functions as an endocytic receptor for the thyroid hormone precursor thyroglobulin (Tg). Tg is synthesized by thyrocytes and then secreted into the lumina of thyroid follicles, where, in the proximity of the thyrocyte apical membrane, it undergoes hormone formation by the coupling of its tyrosyl residues with iodine (1). Subsequently, hormone-rich Tg molecules can either proceed further into the follicle, where they arrange into aggregates that form the colloid, or can undergo proteolytic cleavage, ultimately resulting in hormone release (1). The latter process occurs mainly in lysosomes after fluid-phase micropinocytosis of Tg by thyrocytes (1), as well as in the lumina of thyroid follicles through the action of extracellular proteases (5, 7). Megalin is expressed on the apical membrane of thyrocytes (26), where it binds to Tg with high affinity, after which Tg is internalized by cells to undergo apicobasolateral transcytosis and is released into the bloodstream (14, 17). The megalin-mediated transcytosis pathway preferentially involves hormone-poor Tg molecules, which, by preventing lysosome engulfment as well as wasteful transcytosis of hormone-rich Tg, renders hormone release more effective (14).

The absence of RAP in thyrocytes should affect megalin expression and function, which might result in a thyroid phenotype similar to that observed in megalin-knockout (megalin-KO) mice, namely, hypothyroidism (14, 15). However, the effects of RAP deficiency may be more complex, because RAP interacts not only with megalin but also with Tg (16). Thus we have recently demonstrated (16) that RAP binds to Tg in solid-phase assays with relatively high affinity and that antibodies against RAP can coprecipitate Tg in thyroid extracts and in cultured thyroid cells; in the latter case, this phenomenon occurs shortly after the beginning of Tg biosynthesis. In addition, transient transfection of differentiated thyroid cells with a secretory RAP-Ig chimera devoid of the ER retention sequence results in impaired Tg release into the cell medium, suggesting that RAP may be involved in the Tg biosynthetic pathway (13). Nevertheless, it is not known how the role of RAP in Tg biosynthesis might affect thyroid function in combination with its role in megalin expression.

Address for reprint requests and other correspondence: M. Marinò, Dept. of Endocrinology, Univ. of Pisa, via Paradisa 2, I-56124 Pisa, Italy (e-mail: m.marino@endoc.med.unipi.it).

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To elucidate the function of RAP in thyrocytes in the present study, we investigated and characterized the thyroid phenotype in RAP-KO mice. Our findings indicate that in the absence of RAP, a defect in follicular Tg associated with slight histological changes resembling early goiter occurs. Hormone secretion was normal, indicating that follicular Tg was still sufficient and sufficiently iodinated to provide hormone secretion.

MATERIALS AND METHODS

Experimental animals. Twenty (10 female and 10 male) 9-wk-old RAP-KO mice (B6.129S7-Lrpap1^{tm1Her}) and 20 (10 female and 10 male) 9-wk-old wild-type (WT) mice (Lrpap1) were purchased from The Jackson Laboratory (Bar Harbor, ME). Animal care and euthanization procedures were performed in accordance with institutional guidelines. Animal protocols and study procedures were approved by the Institutional Animal Care and Use Committees and Institutional Review Boards of Massachusetts General Hospital and the University of Pisa.

Blood samples were collected from 15 RAP-KO (10 female and 5 male) and 15 WT mice (10 female and 5 male). Sera were stored at −20°C until use. The thyroid glands were harvested in all mice and either stored at −80°C for preparation of tissue extracts or fixed with formalin and embedded in paraffin for histological and immunohistochemical studies.

Immunohistochemistry. Four- to five-μm-thick sections from paraffin-embedded specimens were deparaffinized, rehydrated, and subjected to antigen retrieval either in Citra, pH 6.0 (BioGenex, San Ramon, CA), for RAP and Tg detection, or in Tris buffer, pH 10.0, for megalin detection using a decloaking chamber (Biocare Medical, Walnut Creek, CA). Sections were then washed with PBS and incubated with rabbit antibodies against RAP (26), megalin (26), or Tg (Axell, Westbury, NY), followed by biotin-labeled horse anti-rabbit IgG (Vector Laboratories, Burlingame, CA), horseradish peroxidase (HRP)-conjugated streptavidin (Elite ABC reagent; Vector Laboratories), and 3-amino-9-ethylcarbazole (Aldrich Chemical, Milwaukee, WI). Sections were counterstained with Gill's hematoxylin (Fisher Scientific, Fair Lawn, NJ).

Tissue extract preparation and analysis. Tissue samples were washed, minced, and homogenized. Samples were then incubated in lysis buffer [50 mM Tris, pH 8.6, 0.5 M NaCl, 10% Triton X-100, 0.01% deoxycholate, 20 mM EDTA, 0.2% NaN_3, and 10% protease inhibitor cocktail solution (Roche Diagnostics, Mannheim, Germany)] and spun for 30 min at 10,000 *g*. Supernatants were collected and briefly sonicated. To measure poorly soluble Tg aggregates within the pellets, the pellets were collected and subjected to further solubilization by incubation with 6 M guanidine.

Protein concentrations were measured in all samples using the Bradford method. Tg was measured using either Western blot analysis as detailed below or ELISA as follows. Microwell plates coated with the rabbit anti-Tg antibody were incubated with 1 μg of extracts, followed by an unlabeled mouse anti-Tg antibody (Dako, Carpinteria, CA), alkaline phosphatase-conjugated anti-mouse IgG (Sigma, St. Louis, MO) and *para*-nitrophenylphosphate (Sigma). Tg values were calculated on the basis of a standard curve obtained using mouse Tg prepared from frozen mouse thyroids (Pel-Freeze, Rogers, AR).

Total thyroxine (T_4) was measured in thyroid extracts diluted in T_4-, T_3-free human serum using a commercially available ELISA kit (MP Biomedicals, Orangeburg, NY). T_4 residues within Tg molecules were measured by ELISA as follows. Microtiter plates coated with unlabeled rabbit anti-Tg were incubated with thyroid extracts (1 μg), followed by a rabbit anti-T_4 antibody (Accurate Chemical and Scientific, Westbury, NY) labeled with *N*-sulfosuccinimidyl-6-[biotinamido]hexanoate (EZ-Link sulfo-NHS-LC-biotin; Pierce Biotechnology, Rockford, IL) and then by alkaline phosphatase-conjugated streptavidin and *para*-nitrophenylphosphate. The amount of T_4 within Tg was expressed as optical density/ng Tg as measured by ELISA in the same samples.

Western blot analysis. Samples were subjected to SDS-PAGE and blotted onto nitrocellulose membranes. Membranes were incubated with *1*) unlabeled rabbit anti-megalin or anti-Tg antibodies, followed by HRP-conjugated anti-rabbit IgG (Bio-Rad Laboratories, Hercules, CA); *2*) unlabeled mouse anti-Tg antibody, followed by HRP-conjugated anti-mouse IgG (Bio-Rad Laboratories); or *3*) HRP-conjugated mouse anti-Tg antibody (Dako). Bands were detected using ECL, and their density was measured using ImageJ software (National Institutes of Health, Bethesda, MD).

Serum assays. Free T_4 (FT_4) was measured by performing an equilibrium dialysis immunoassay (Nichols, San Juan Capistrano, CA). TSH was measured using a sensitive, heterologous disequilibrium double-antibody precipitation RIA (21). Serum Tg was measured by ELISA as follows. Microwell plates coated with the rabbit anti-Tg antibody were incubated with biotin-labeled mouse Tg alone or in the presence of mouse sera diluted 1:25, followed by alkaline phosphatase-conjugated streptavidin and *para*-nitrophenylphosphate. Tg concentrations were calculated on the basis of a lin/log, competitive, RIA-like curve obtained by incubating anti-Tg antibody-coated wells with biotin-labeled Tg in the presence of various concentrations of unlabeled mouse Tg. The arbitrary value of 1 U/ml was assigned to the result obtained with 1 μg/ml unlabeled Tg.

Data presentation and statistics. Experiments with mouse tissue sections and extracts were performed in at least four mouse pairs. Serum assays were performed in 15 mouse pairs (5 male and 10 female), with the exception of serum TSH, which was measured only in females because TSH values are known to differ between the genders in mice (21) and not enough serum samples were available in males. Serum TSH values in RAP-KO and WT mice were also compared with those obtained in 16 serum samples from a separate series of WT mice (WT2). This additional group of serum samples was selected from our archives and included all samples from mice that shared the same genetic background (B6), gender, and age as the RAP-KO mice and the mice in the first WT group. These mice had been maintained under the same care conditions as the RAP-KO mice and the mice in the first WT group. In all experiments, either the same sample volumes (serum) or the same amount of protein (extracts) were used for comparison. When appropriate, statistical analyses were performed using an unpaired *t*-test or simple regression with StatView software (SAS Institute, Cary, NC). Results are presented as means ± SD.

RESULTS

RAP and megalin expression in the thyroid. RAP-KO mice were previously generated on a C57BL/6J background by disruption of the RAP gene (24, 25). The animals used were purchased commercially and were genotyped systematically by the manufacturer. As reported previously (4, 24, 25), we did not notice any major difference between RAP-KO and WT mice in terms of body size (data not shown), apparent health status, and behavioral features.

As expected on the basis of the knowledge that RAP is an ER-resident protein (6, 25), RAP was found intracellularly by immunohistochemistry in thyrocytes of WT mice (Fig. 1*A*). RAP was not detected in thyroid sections from RAP-KO mice, confirming that the RAP gene had been disrupted successfully (Fig. 1*B*).

In WT mice, megalin was detected by immunohistochemistry on the apical membrane of thyrocytes and, to a lesser extent, intracellularly (Fig. 1*C*). In addition to membrane

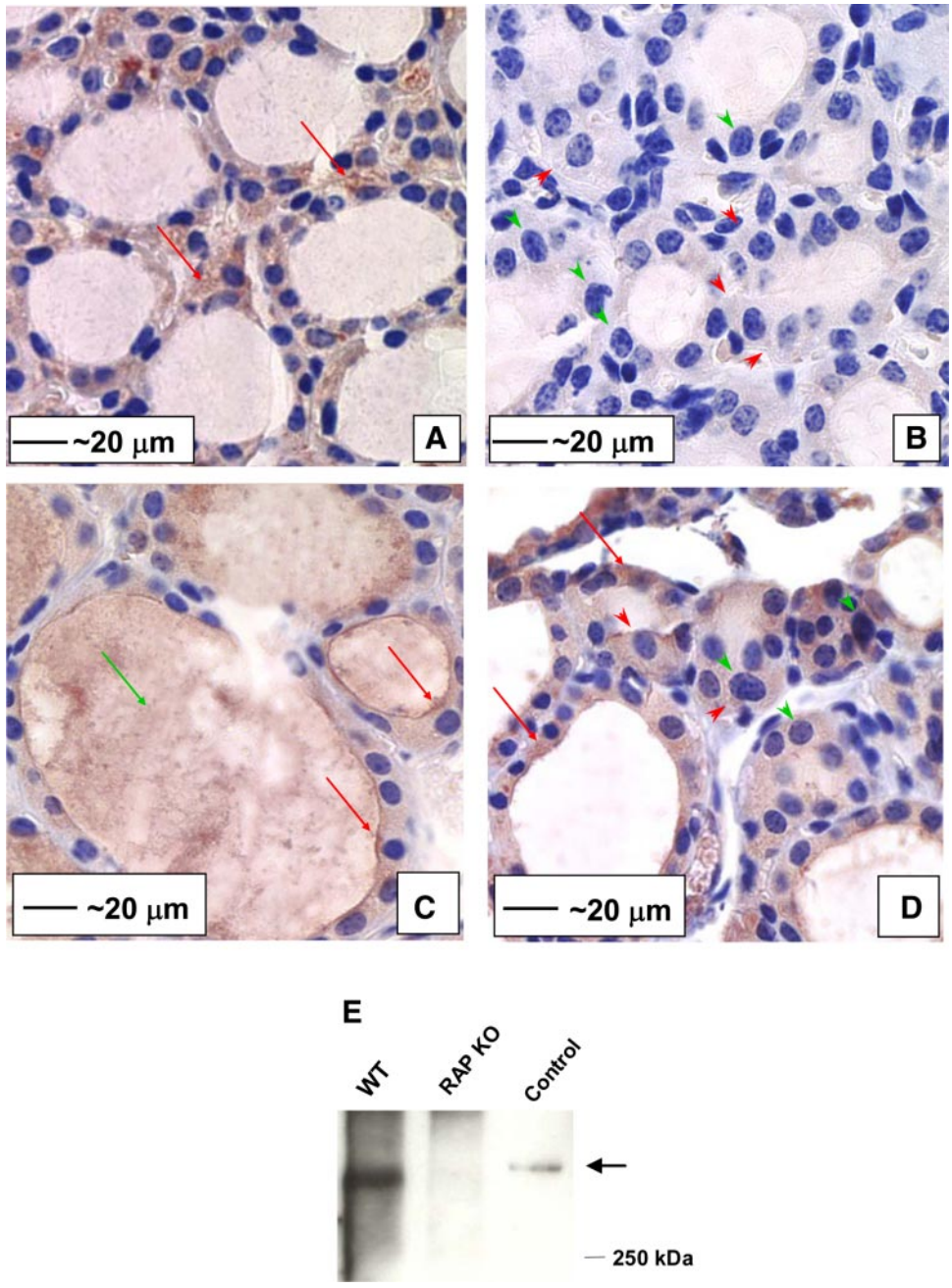

Fig. 1. Receptor-associated protein (RAP) and megalin expression in the thyroid. *A* and *B*: immunohistochemistry performed to examine RAP in thyroid sections. In wild-type (WT) mice (*A*), staining was present intracellularly (red arrows), whereas no staining was observed in RAP-knockout (RAP-KO) mice (*B*). In the thyroid from RAP-KO mice (*B*), thyrocytes were hypertrophic (red arrowheads) and nuclei were enlarged (green arrowheads) compared with those from WT mice. *C* and *D*: immunohistochemistry conducted to assess megalin in thyroid sections. In WT mice (*C*), staining was observed mainly on the apical surfaces of thyrocytes (red arrows) and in the lumina of thyroid follicles (green arrow). In RAP-KO mice (*D*), staining was observed mainly intracellularly (arrows), whereas apical and luminal staining was reduced. In RAP-KO mice (*D*), certain thyrocytes were hypertrophic (red arrowheads) with enlarged nuclei (green arrowheads). *E*: Western blot analysis of megalin in thyroid extracts (7 μg loaded). Arrow indicates full-length megalin. Control, WT mouse kidney extract (1.5 μg loaded).

staining, uniform staining for megalin was observed within the lumina of thyroid follicles, presumably reflecting the release of megalin fragments, which is known to occur in several cells that express the receptor (12, 19, 20). As expected on the basis of the knowledge that RAP is a megalin chaperone (6, 25), intracellular staining for megalin was clearly increased in thyrocytes of RAP-KO mice, suggesting retention of the receptor, whereas staining was markedly reduced in the lumina of thyroid follicles and on the apical membrane of thyrocytes (Fig. 1*D*). Furthermore, on the basis of Western blot analysis, full-length megalin was found to be reduced (in most instances, it was undetectable) in thyroid extracts from RAP-KO mice (Fig. 1*E*).

Reduced follicular Tg in RAP-KO mice. Further immunohistochemical studies in which we used an anti-Tg antibody revealed a reduction of Tg in thyroid follicles in RAP-KO mice. As shown in Fig. 2*A*, in WT mice, abundant staining for Tg was found in the lumina of thyroid follicles, together with some intracellular staining. In contrast, in RAP-KO mice, Tg staining was increased intracellularly, whereas in the lumina of the vast majority of follicles, staining was either undetectable or markedly reduced (Fig. 2*B*). In RAP-KO mice, intense Tg staining also was observed on the apical membrane of thyrocytes, which was not clearly detectable in WT mice.

The overall content of Tg in thyroid extracts from RAP-KO mice was reduced only slightly as assessed by ELISA (Fig. 2*C*)

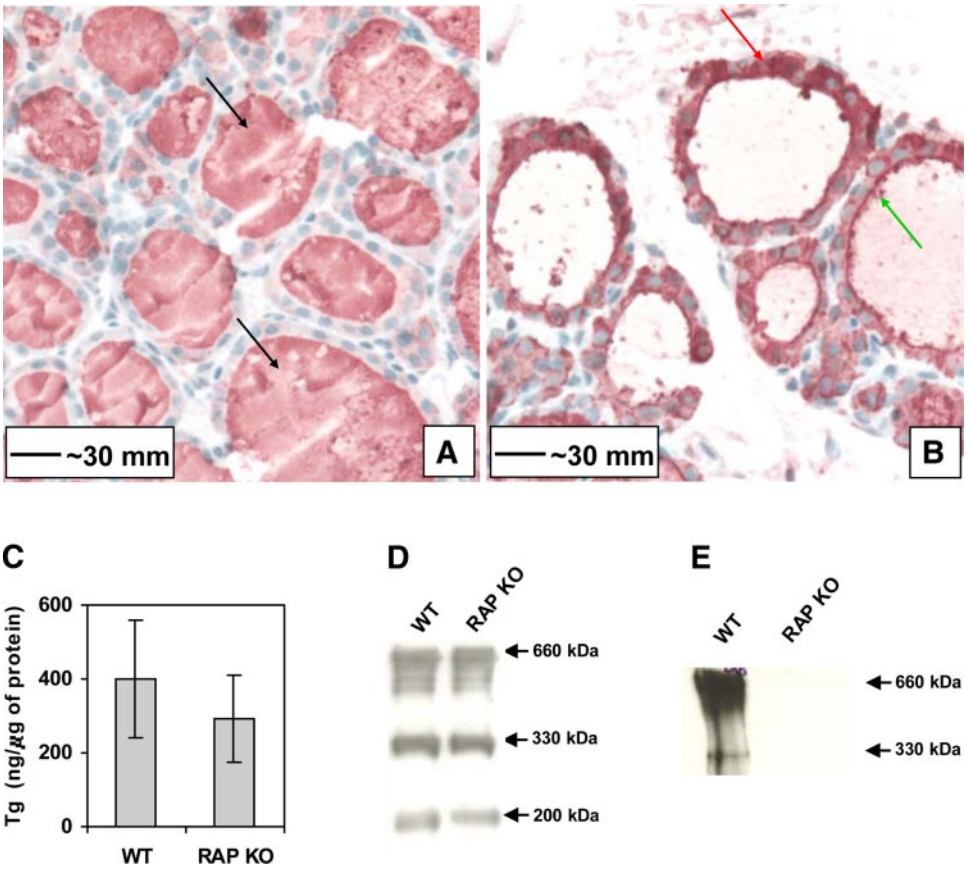

Fig. 2. Reduced follicular thyroglobulin (Tg) in RAP-KO mice. *A* and *B*: immunohistochemical analysis of Tg in thyroid sections. In WT mice (*A*), staining was observed mainly in the lumina of thyroid follicles (arrows). In RAP-KO mice (*B*), staining was present mainly intracellularly (red arrow) and on the apical surfaces of thyrocytes (green arrow), and it was absent or reduced in the lumina of most follicles. *C*: ELISA for Tg in thyroid extracts. *D*: Western blot analysis of Tg in thyroid extracts. Arrows indicate bands corresponding to intact Tg (at ~660 and ~330 kDa) and to a Tg fragment of ~200 kDa. *E*: Western blot analysis of Tg in pellets solubilized after thyroid tissue extraction. Arrows indicate bands corresponding to Tg.

and Western blot analysis (Fig. 2*D*). Thus, although ELISA revealed that Tg in thyroid extracts was ~25% lower in RAP-KO mice, the difference was not statistically significant ($P = 0.4275$; *t*-test). Western blot analysis revealed that 660- and 330-kDa Tg bands (corresponding to intact Tg dimers and monomers) in RAP-KO mice were reduced by only ~20% and ~15%, respectively (mean densities: 660-kDa Tg, 39.1 pixels/cm^2 vs. 48.1 pixels/cm^2 in WT mice; 330-kDa Tg, 39.4 pixels/cm^2 vs. 45.7 pixels/cm^2 in WT mice). Moreover, an additional Tg band migrating at ~200 kDa, presumably representing a proteolytic fragment, was reduced by only ~20% in RAP-KO mice (16.5 vs. 20.9 pixels/cm^2 in WT mice).

Because of the apparent discrepancy between results obtained with thyroid sections and those obtained with thyroid extracts, we considered the possibility that the reduction of Tg in RAP-KO mice observed using immunohistochemistry was due to a reduction of Tg aggregates that may not have been solubilized by standard tissue extraction. Thus follicular Tg can be stored in the form of aggregates (11) that may have been discarded during the extraction procedure, because they are expected to remain within the pellets upon centrifugation of homogenates. To investigate this possibility, the pellets from thyroid homogenates were collected, subjected to solubilization by treatment with 6 M guanidine, and examined using Western blot analysis. As shown in Fig. 2*E*, 660- and 330-kDa Tg bands were detected in the material solubilized from the thyroids of WT mice but not in that from the thyroids of RAP-KO mice, supporting the conclusion that the reduction of follicular Tg in RAP-KO mice reflects a reduction of Tg aggregates that were not solubilized by the standard extraction procedure.

Tg in RAP-KO mice undergoes hormone formation as it does in WT mice. We investigated whether the few Tg molecules found in the colloid in RAP-KO mice, especially those bound to the apical membrane (Fig. 2*B*), could still undergo hormone formation. For this purpose, we first measured the content of total T_4 in thyroid extracts, which was found to be reduced only slightly in RAP-KO mice (Fig. 3*A*), with a difference with WT mice that was not statistically significant ($P = 0.4034$; *t*-test) and with values that paralleled those of Tg in the same extracts (Fig. 2*C*). Thus there was a significant correlation between Tg and total T_4 in thyroid extracts from both WT ($P = 0.0483$; simple regression analysis) and RAP-KO mice ($P = 0.0440$) (Fig. 3*B*). The findings indicate that Tg in RAP-KO mice could still undergo hormone formation. In further support of these findings, the content of T_4 within Tg molecules in thyroid extracts did not differ significantly ($P = 0.4340$; *t*-test) between WT and RAP-KO mice, and interestingly, mean values were slightly greater in RAP-KO mice (Fig. 3*C*). We interpreted the latter finding as possibly due to a likely increased iodine-to-Tg ratio within the colloid in RAP-KO mice, which was in fact expected in view of the reduction of follicular Tg.

Hormone secretion is unaffected in RAP-KO mice. To determine whether RAP deficiency affects hormone secretion, we measured serum FT_4 and TSH. As shown in Fig. 4*A*, mean serum FT_4 was similar ($P = 0.2471$; *t*-test) in the two groups of mice. Although serum TSH values were about twofold those

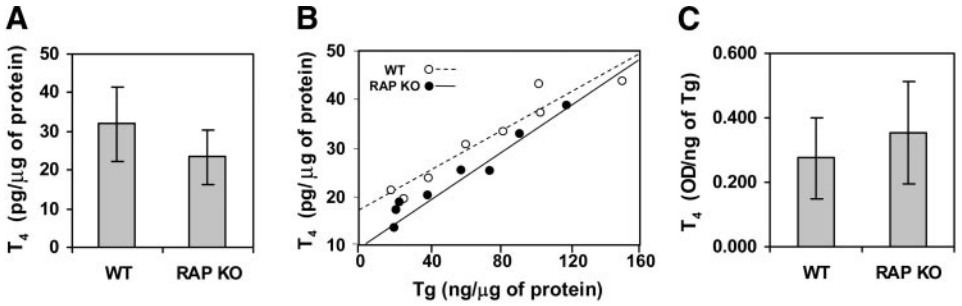

Fig. 3. *A*: total thyroxine (T_4) content in thyroid extracts. *B*: correlation between T_4 and Tg in thyroid extracts. *C*: T_4 residues within Tg molecules in thyroid extracts. OD, optical density.

in RAP-KO mice (Fig. 4*B*), the difference was not statistically significant ($P = 0.2702$; *t*-test). We considered the possibility that the lack of statistical difference between TSH values in RAP-KO and WT mice was due to the relatively small number of samples. To investigate whether this was the case, TSH values in RAP-KO and WT mice were compared with those of a larger group of WT mice (WT2) selected from our archives, including all samples from mice that shared the same genetic background, gender, and age as the RAP-KO mice and the first group of WT mice. As shown in Fig. 4*B*, serum TSH in the WT2 group was similar to that in the first group of WT mice ($P = 0.3502$; *t*-test) but was significantly lower than that in RAP-KO mice ($P = 0.0437$; *t*-test). Serum levels of Tg were significantly higher in RAP-KO mice ($P = 0.0284$; *t*-test) (Fig. 4*C*), which, as discussed below, we interpreted to be due to a directional alteration of Tg secretion by thyrocytes.

Morphological and histological thyroid features in RAP-KO mice. As shown in Table 1, the weight of the thyroid gland did not differ between WT and RAP-KO mice, indicating that the latter group had no goiters. Histological examination of thyroid sections showed a slight degree of thyrocyte and nuclear hypertrophy in RAP-KO mice (Fig. 1, *B* and *D*), and indeed, the mean height of thyrocytes and the mean nuclear area were significantly greater in these mice (Table 1). Cellularity (i.e., number of cells per field) (Table 1), follicular area (Table 1), and vascularity (data not shown) were similar in WT and RAP-KO mice.

DISCUSSION

Because of its large size and high degree of glycosylation, the biosynthesis of Tg is rather complex (1). To achieve its mature three-dimensional structure and to proceed intact through the secretory pathway, nascent Tg molecules interact, both concurrently and sequentially, with several ER-resident molecular chaperones (2, 6, 9). The majority of the reported deletions or mutations of the Tg gene are known to result in intracellular retention and reduced secretion of Tg into follicles, possibly because of impaired interaction of Tg with its chaperones (8, 18).

The major conclusion of the present study is that RAP is required for the establishment of large reservoirs of Tg within thyroid follicles. Thus mice generated by disruption of the RAP gene were found to have a marked reduction of Tg content in thyroid follicles, whereas much of the Tg synthesized by thyrocytes was retained intracellularly or secreted into the bloodstream. The reduction of follicular Tg in RAP-KO mice reflected especially a reduction of poorly soluble Tg aggregates, one of the forms in which Tg accumulates as an iodine and hormone reservoir (1, 11).

RAP-KO mice had significantly higher serum levels of Tg, suggesting that there may have been a directional alteration of Tg secretion, namely, that Tg was secreted from basolateral membranes into the bloodstream rather than from apical membranes into follicles. Other mechanisms through which Tg can reach the bloodstream, namely, transcytosis or leakage from follicles (10), are unlikely to account for the elevated serum Tg levels. Thus megalin-mediated transcytosis, the main route of Tg transcytosis (14, 15, 17), could not be increased, because megalin on the apical membrane of thyrocytes was reduced. In addition, leakage from follicles could be excluded because of the absence of histological evidence of follicle disruption.

In spite of the severe defect of follicular Tg, thyroid function was maintained in RAP-KO mice. Several findings and considerations may explain why RAP-KO mice did not develop hypothyroidism. First, the presence of T_4 in thyroid extracts, the finding that its levels paralleled those of Tg, and more important, the finding that Tg contained T_4 residues indicate that the few Tg molecules secreted into the colloid had undergone hormone formation. Second, Tg within follicles in RAP-KO mice was observed almost exclusively near the apical membrane of thyrocytes, hence in the ideal position for being iodinated rapidly, internalized, and degraded. Thus it is likely that, although not enough Tg was present in the follicles to form large reservoirs, its quantities were sufficient to provide

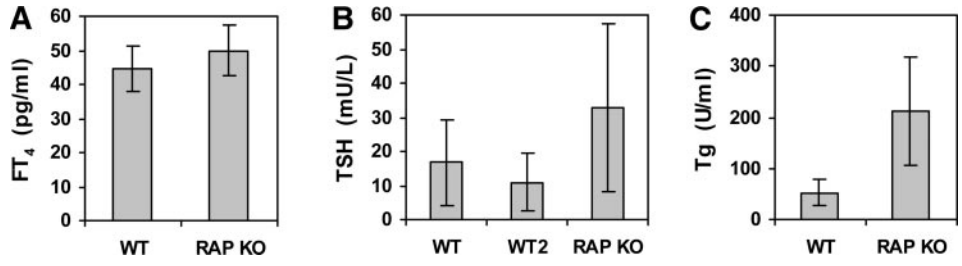

Fig. 4. Serological findings. *A*: free T_4 (FT_4) serum levels. *B*: TSH serum levels. WT2, additional archival serum samples from WT mice of the same age, gender, and phenotype. *C*: Tg serum levels.

Table 1. *Thyroid morphological and histological measurements*

Parameter	WT	RAP-KO
Thyroid weight, mg	8.5±1.6	7.1±0.7
Follicle area, μm^2	15^6±18.5	16.5^6±12.9
No. of cells	148±46.0	154±12.8
Thyrocyte height, μm	3.9±1.5*	5.0±1.8
Nuclei area, μm^2	30.6±19.6†	42.6±6.0

Values are means ± SD. WT, wild type; RAP-KO, receptor-associated protein-knockout. Histological measurements were performed in 1,800-μm^2 tissue sections. The follicle and nuclei areas were measured using the ellipsoid formula. *P = 0.0022 vs. RAP-KO. †P = 0.0175 vs. RAP-KO.

normal hormone secretion. This interpretation is in keeping with the so-called last come, first served hypothesis (22). According to this notion, under normal conditions, the Tg used for hormonogenesis is the last to be secreted into the colloid. These newly secreted molecules would remain near the apical membrane of thyrocytes for a short time, where they would undergo hormone formation and be internalized and/or degraded rapidly. In contrast, the Tg in the center of the follicle would be stored in the form of aggregates serving as an iodine and hormone reservoir, to be used only when the iodine supply is not sufficient for newly secreted Tg to undergo hormone formation. As mentioned above, in RAP-KO mice there was a reduction of Tg aggregates, but Tg secretion probably was sufficient for hormone release.

Serum TSH was increased in RAP-KO mice, but the difference from that of WT mice was not statistically significant. However, when we compared serum TSH values with those of a larger archival series of WT mice (WT2) of the same genetic background, gender, and age, TSH was found to be significantly greater in RAP-KO mice. In fact, certain histological features (thyrocyte hypertrophy and nuclear enlargement) known to be triggered by TSH and to resemble early stages of goiter (3) were observed in RAP-KO mice, which is in keeping with increased TSH secretion. Other features of TSH stimulation, namely, thyroid enlargement, hypercellularity, increased follicle area, and hypervascularity were not observed. Hence, TSH secretion, although increased, probably still was not sufficient to trigger a full-blown goitrous phenotype.

In addition to the defect of follicular Tg, RAP-KO mice also showed a reduction in megalin on the apical membrane of thyrocytes, presumably because of the role of RAP as a

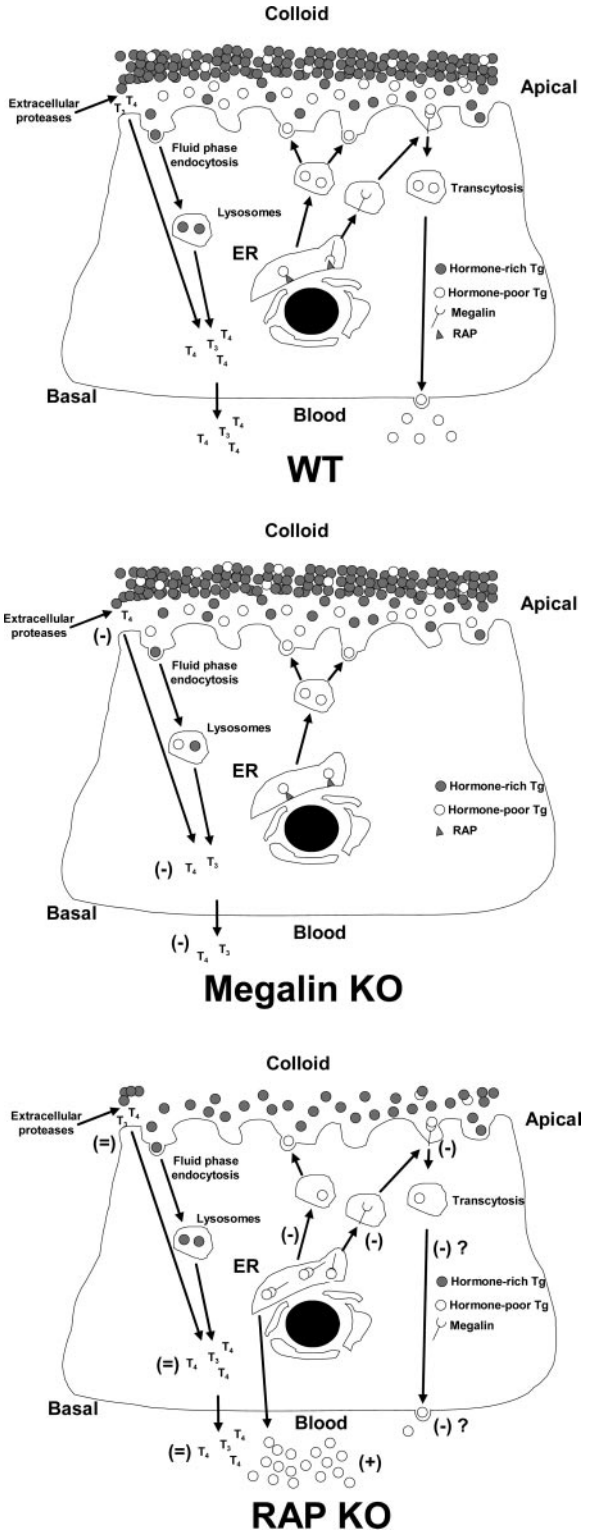

Fig. 5. Schematic representation of how the absence of megalin or RAP may affect Tg trafficking and hormone secretion in the thyrocyte. In the WT thyrocyte, RAP is expressed within the endoplasmic reticulum (ER), where it binds to both megalin and Tg, thereby facilitating apical Tg secretion as well as megalin insertion into the cell membrane. Once within the colloid, Tg undergoes iodination, hormone-rich aggregates accumulate in the center of the follicle, and hormone-poor molecules coexist in varying proportions, depending on iodine intake. Hormone release occurs by lysosomal degradation of hormone-rich Tg after fluid-phase endocytosis or through the action of extracellular proteases. Hormone-poor Tg undergoes megalin-mediated transcytosis. In the megalin-KO thyrocyte, transcytosis cannot take place and hormone-poor Tg undergoes degradation, thereby competing with hormone-rich Tg and reducing hormone release. In the RAP-KO thyrocyte, both megalin and Tg are retained intracellularly. Tg transport is altered, with reduced apical secretion and possibly increased basolateral secretion, thereby resulting in enhanced serum Tg. Tg secreted into the colloid is not sufficient to form aggregates. However, in view of the presumably increased iodine-to-Tg ratio, the hormone content of Tg is likely augmented, thereby providing sufficient quantities of hormone-rich Tg for normal hormone secretion to be achieved. Transcytosis may be decreased because of the reduced megalin expression on the cell membrane; in view of the likely reduction of hormone-poor Tg within the colloid, however, megalin function should be irrelevant under these circumstances.

megalin chaperone (6, 25). Because megalin mediates the transcytosis of Tg with subsequent release of Tg into the bloodstream (14, 17), serum Tg would be expected to be reduced in RAP-KO mice just as it occurs in megalin-KO mice (14, 15). However, RAP-KO mice had increased serum levels of Tg. How could this finding be coupled with the reduction of megalin? An important issue to consider is that although transcytosis is one of the mechanisms by which Tg can reach the circulation, it is not the only one, as mentioned above. Thus Tg also can enter the bloodstream by direct cell secretion or by leakage from disrupted follicles (10). A reduction in transcytosis should cause a decrease in serum Tg only if these two additional mechanisms are not increased. This is the case in megalin-KO mice, in which follicular Tg is normal and there is no follicle disruption (15), but probably is not the case in RAP-KO mice, in which, although there was no follicle disruption, directional secretion of Tg was likely altered. If this were indeed the case, the increased serum Tg might have reflected enhanced basolateral secretion and might have masked reduced transcytosis.

Another important question concerning megalin is why RAP-KO mice did not develop hypothyroidism in spite of the reduced megalin expression. Thus, by mediating transcytosis of hormone-poor Tg, megalin prevents it from competing with hormone-rich Tg for proteolytic degradation, and as a consequence, megalin-KO mice have hypothyroidism (14, 15). A possible explanation for this apparent discrepancy is related to the hormone content of Tg. Transcytosis should have an impact on thyroid function only when there is enough hormone-poor Tg within the colloid (14), which probably was not the case in RAP-KO mice. This hypothesis is supported by the fact that Tg in thyroid extracts from RAP-KO mice contained T_4 as in WT mice and, more important, by the finding that the T_4 content of Tg in RAP-KO mice was slightly greater than that in WT mice, although not to a statistically significant extent. Because extracts comprised both intracellular and colloidal Tg, intracellular Tg was increased in RAP-KO mice, and intracellular Tg is known not to be iodinated, the hormone content of colloidal Tg in RAP-KO mice might have been even greater than that which we measured. Moreover, in RAP-KO mice, the iodine-to-Tg ratio within the colloid was probably increased because of the reduction in follicular Tg, and therefore an enhanced hormone content of Tg was likely to be present. On the basis of these considerations, it is possible that megalin did not affect thyroid function in RAP-KO mice simply because the few Tg molecules within the colloid had a high hormone content. Under the circumstances, transcytosis of hormone-poor Tg probably was not only not possible but also not necessary. A tentative, schematic view of how the absence of megalin or RAP might affect Tg trafficking and thyroid function in the thyrocyte is depicted in Fig. 5.

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