A prominent secretory glycoprotein was detected in the culture medium of porcine thyrocytes which was identified as clusterin by microsequencing. Treatment of thyrocytes with thyroid stimulating hormone revealed a tight regulation of both synthesis and secretion of clusterin, with a distinct fraction of clusterin being always associated with the cells. At least three N-bound glycans were found on each subunit of clusterin, receiving most of the incorporated $^{32}$P-phosphate-label. Binding of clusterin to the immobilized cation-independent mannose 6-phosphate (M6P) receptor indicated that part of the phosphate label was contained in M6P moieties. Immunolabeling of cultured thyrocytes and of thyrocytes in situ showed clusterin on the apical cell surfaces where it colocalized with gp330/megalin, which is known to serve as a binding site for clusterin. The association with the apical plasma membrane, which, in thyrocytes, carries the iodinating system, was confirmed by biosynthetic iodination, an as yet unknown posttranslational modification of clusterin. On the basolateral plasma membranes clusterin was found within distinct, bipartite patches, suggesting that it is a constituent of cell-adhesion complexes and that it participates in cell-cell and cell–matrix interactions.

Key Words: clusterin; iodination; mannose 6-phosphate; gp330/megalin; thyrocytes.

INTRODUCTION

Clusterin is a secretory protein of epithelial cells which is synthesized as a single-chain precursor that is proteolytically cleaved to yield a heterodimeric protein [1, 2]. In many species, including humans, the subunits are of similar size and held together by five disulfide bridges [3, 4]. Depending on the species, clusterin possesses six to seven potential N-glycosylation sites. Another characteristic feature of clusterin is the presence of a dinucleotide binding site which is highly conserved throughout all species [5]. The cDNAs of human, porcine, rat, bovine, and canine clusterin have been cloned and sequenced [6–10]. A variety of functional data have been collected about this glycoprotein. It appears to play an important role in spermatogenesis [11], it is detected in developing epithelia [12], it protects cells from the lytic activity of the complement system [6], it serves as an apolipoprotein [13], it binds specifically to the Alzheimer amyloid $\beta$ peptide [14], and it has been related to apoptosis [1]. Clusterin has been given many names according to its various functions, such as testosterone repressed prostate message 2 [15], cytolysis inhibitor [6], apolipoprotein J [13], sulfated glycoprotein 2 [5], and glycoprotein 80 [10]. Recently, the term clusterin appears to have found wide acceptance describing another important role of this multifunctional glycoprotein, the ability to promote aggregation and adhesion of cells [16]. Finally, endocytic uptake of clusterin is mediated by gp 330/megalin, a large multiligand endocytic receptor which belongs to the family of low-density lipoprotein receptor-related proteins [17]. Gp 330/megalin was detected in a restricted group of epithelial cells, including thyroid epithelial cells, where it was exclusively found at the apical cell surface [18].

Thyroid epithelial cells are known to secrete and iodinate a number of different proteins, the most important and most abundant being TG. In this study we show that clusterin is one of these iodinated proteins contributing to the cellular storage capacity for iodine. Furthermore, we found a second posttranslational modification of clusterin, the presence of mannose 6-phosphate (M6P) on its N-linked glycans commonly known as lysosomal targeting signal. Here, we present evidence that mannose 6-phosphorylation of clusterin does not prevent secretion of clusterin nor does it facilitate association of clusterin with the apical plasma membrane. Instead, colocalization of clusterin with gp330/megalin at the apical plasma membrane of thyrocytes suggests that endocytic uptake of clusterin is mediated by gp330/megalin [17].
MATERIALS AND METHODS

Cell culture. Porcine thyrocytes were obtained from glands of freshly slaughtered pigs. Follicle fragments were prepared as described previously [19] and served to establish two different cell culture systems: (1) Monolayers grown in plastic culture dishes, on Cycloprev polycarbonate filter discs (Becton-Dickinson, Heidelberg, Germany), or on coverslides. (2) Inside-out follicles kept as suspension cultures in hydrophobic Petriperm dishes (Bachofer, Reutlingen, Germany). Cells were maintained in Eagle’s Minimum Essential Medium (MEM) supplemented with 100 U/ml penicillin G, 0.1 mg/ml streptomycin, 0.2 µg/ml amphotericin B, and 10% fetal calf serum (from Gibco/BRL, Berlin, Germany) at 37°C in a 5% CO2 atmosphere.

Materials. L-[3H]Leucine, L-[35S]methionine (in vivo cell labeling grade), [32P]phosphate, [3H]-methylated Rainbow protein molecular mass standards, and the detection kit for enhanced chemiluminescence were obtained from Amersham Buchler (Braunschweig, Germany). Carrier-free [214]NaI was purchased from ICN (Meckenheim, Germany).

The rabbit antiserum specific for human clusterin was kindly donated by Dr. R. W. J. ames, University Hospital, Geneva, Switzerland. Sheep anti-gp330 was kindly provided by Dr. P. Verrouste, Inserm, Paris, France. 5-(4,6-dichlorotriazin-2-yl)aminofluorescein hydrochloride (DTAF)- or tetramethylrhodamine isothiocyanate (TRITC)-conjugated, affinity-purified goat-anti-rabbit or fluorescein isothiocyanate (FITC)-conjugated donkey anti-sheep IgGs were from Dianova (Hamburg, Germany) or Sigma (Munich, Germany). The cation-independent M6P-receptor was isolated from porcine liver and coupled to CNBr-activated Sepharose Cl-4B (Pharmacia, Freiburg, Germany) as described earlier [20]. Peptide-N-glycosidase F (PNGase F) was from Boehringer Mannheim (Germany). MEM, F-12 medium (Ham’s modification), and acrylamide (research grade) were obtained from Serva (Heidelberg, Germany), whereas streptavidin coupled to horseradish peroxidase, reactive blue-2 Sepharose, TSH, and all other reagents were purchased from Sigma.

Biosynthetic labeling. Before labeling with L-[35S]methionine or L-[3H]leucine, cells were kept in serum-free culture medium lacking methionine or leucine for 3–6 h. Labeling was carried out overnight with 15.9–18.5 MBq L-[35S]methionine or 7.5 MBq L-[3H]leucine in serum-deficient medium. Upon labeling with [3H]phosphate, cells were cultured in serum- and phosphate-free MEM for 1.5 h and labeled with 37 MBq [3H]phosphate/ml for 24 h. Labeling with 9–18.5 MBq [125I]NaI was conducted in F-12 medium (Ham’s modification) without fetal calf serum overnight. F-12 medium was chosen because of its low content of phenol red, a known inhibitor for thyroxoperoxidase. Isolation and microsequencing of clusterin. Clusterin was isolated from the culture supernatants of porcine monolayer thyrocytes which were maintained for 3 d in serum-free medium supplemented with 10 µM TSH. Enrichment of clusterin through chromatography on reactive blue-2 Sepharose proved to be very efficient because of its high affinity for dinucleotide-binding proteins. Final purification employed a two-step gel electrophoresis protocol with a first run under nonreducing conditions followed by the gel containing nonreduced clusterin. The gel pieces were then soaked in reducing sample buffer for 20 min at room temperature and boiled for 5 min before loading them into a funnel well gel electrophoresis set [21]. After gel electrophoresis a Cownassie blue R-250 stainable band consisting of about 5–8 kDa pure clusterin was obtained. The band was excised from the gel and subjected to in-gel digestion with Lys C with the resulting peptides being microsequenced by TopLab (Munich, Germany).

PNGase F digestion. Conditioned medium from L-[35S]methionine-labeled porcine inside-out follicles was brought to 0.1% NaNO3, 2 mM EDTA, 0.1% sodium deoxycholate (NaDOC), and 10 U/ml PNGase F and incubated at 37°C in the dark. Twenty-microliter aliquots were withdrawn after 1, 2, 4, and 16 h of incubation and frozen immediately. The same volume of conditioned medium was incubated for 4 h with phosphate-buffered saline (PBS) instead of PNGase F.

PNGase F digestion of conditioned medium from 35P-labeled cells was performed after clearing of medium by centrifugation (5 min at 15,000g) and 10-fold concentration in a Vivapore-chamber (Vivascience Ltd., Binbrook, UK). Samples were then mock-incubated or treated with PNGase F in the presence of 0.5% Triton X-100 instead of 0.1% NaDOC overnight, as described above.

Affinity chromatography with M6P-receptor–sepharose CL-4B. Affinity chromatography of L-[35S]methionine-labeled conditioned medium from porcine inside-out follicles with the cation-independent M6P-receptor coupled to Sepharose CL-4B was performed as described earlier [22].

Gel electrophoretic techniques. SDS-polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to [23] with 12.5% polyacrylamide gels, followed by autoradiography (for 35S-labeled polypeptides) or fluorography (for 3H-, 35S- or 32P-labeled polypeptides) according to [24]. Autoradiography of 35S-labeled polypeptides or fluorography of 32P-labeled polypeptides was performed with intensifying screens.

In order to analyze 32P-clusterin, a two-step gel electrophoretic protocol was followed as described for the identification of clusterin by microsequencing (see above) with the following modification: After PNGase F treatment, three slices were excised from the first gel, covering the molecular mass range of 46–80 kDa which should contain all forms of fully or partially glycosylated clusterin as well as deglycosylated clusterin. These slices were then reduced and loaded into adjacent wells for a second gel electrophoretic run.

Immunoblotting was performed as described earlier [25] with a 1:100 dilution of the primary antibody.

Immunofluorescence microscopy. Porcine thyrocytes were cultured on coverslides or Cycloprev filters for 5–10 days. In the latter case, the formation of confluent monolayers was verified by measuring the transepithelial resistance as described earlier [25]. After treatment with 8 µM TSH for 3 days, cells were washed, permeabilized with 0.2% Triton X-100 for 2 min, and finally fixed for 8 min with methanol and 8 min with acetone, both at ~20°C. After being blocked with 3% ovalbumin 0.1% gelatin in PBS for 1 h at 37°C, cells were washed and incubated for 1.5 h at 37°C with an antiserum specific for human clusterin (diluted 1:100) followed by the incubation with goat-anti-rabbit IgGs coupled to DTAF for 1 h at 37°C. After being washed, cells were mounted on microscope slides and examined with a Leica TCS 4 D confocal microscope (Leica, Bensheim, Germany). Micrographs were taken on Kodak Tmax films (Eastman Kodak Co., Rochester, NY) using a hardcopy device (Fokus Graphics, Oberau, Germany).

Porcine thyroid tissue was infiltrated with polyvinylpyrrolidone for 5 h at room temperature and frozen in liquid propane. Sections were prepared with a cryotome (Reichert-Jung, Wien, Austria) and mounted on microscope slides. After being blocked sections were immunolabeled with anti-human clusterin, TRITC-goat-anti-rabbit IgG, sheep anti-gp330/megalin, and FITC-donkey anti-sheep antiserum. In controls specific antibodies were omitted. Sections were examined with the confocal microscope (see above).

RESULTS

Identification of Clusterin in the Culture Medium of Porcine Thyrocytes as a Prominent, TSH-Regulated, Secretory Polypeptide

Analysis of the secretory products of [35S]methionine-labeled porcine thyrocytes revealed the presence
of TG, which is the major secretory polypeptide, and of another prominent secretory polypeptide with an apparent molecular mass of approximately 43 kDa (Fig. 1A). About 5–8 mg of this polypeptide was isolated from the culture medium of porcine thyrocytes and microsequenced after partial digestion with the protease Lys C. The following sequences were obtained (the one-letter code of amino acids is used starting with the N-terminus of the peptides):

1. S L L S S L E E A K,
2. L Y D Q L L Q S Y Q Q K.

These sequences perfectly matched stretches from both chains of porcine clusterin [7].

For immunoblotting, aliquots of cell lysates and 72-h secretory products from porcine thyrocytes were analyzed by SDS–PAGE under reducing and nonreducing conditions. After being blotted onto nitrocellulose and probed with a rabbit antiserum specific for human clusterin, the characteristic banding pattern of clusterin was obtained (Fig. 1B). Like the human protein, porcine clusterin consists of two disulfide-linked subunits of roughly the same apparent molecular mass.

Porcine monolayers were cultured for 3 days in the absence or presence of 2, 4, 6, 8, and 10 mU/ml TSH. Corresponding amounts of cell lysates and secretory products were subjected to nonreducing SDS–PAGE and immunoblotting for clusterin as above. Whereas TSH induced a slight but steady decrease in cellular clusterin, accumulation of clusterin in the medium was effectively regulated by TSH, reaching its maximum at 6–8 mU/ml TSH (Fig. 1C). Similar results were obtained with cultures stimulated with TSH overnight (not shown).

Clusterin Is Iodinated in Cultured Thyrocytes

Monolayer thyrocytes were labeled with [125I]NaI overnight and aliquots of the cell lysate were analyzed by reducing or nonreducing SDS–PAGE and autoradiography (Fig. 2). Two major iodinated polypeptides were observed. According to their apparent molecular mass and the mobility shift when analyzed under reducing or nonreducing conditions they could be addressed as 125I-clusterin or 125I-TG [25].

We then examined the distribution of cell-associated versus secreted clusterin after an overnight labeling with [125I]NaI. Less than 1% of the radioiodinated clusterin was found in the medium (not shown), indicating that iodinated clusterin remained cell-surface associated.

Clusterin Is Associated with the Plasma Membrane of Cultured Thyrocytes

Immunofluorescence staining of porcine monolayers cultured on coverslides and confocal laser scanning...
microscopy showed a prominent clusterin labeling at the lateral plasma membrane (Fig. 3a). Higher magnifications revealed that clusterin patches lining the cell borders consisted of two half-patches (Fig. 3d), resembling cellular contact sites.

Culture of thyrocytes on porous filters results in the formation of highly polarized epithelial cells. Immunofluorescence staining of filter grown thyrocytes and scanning in the X/Z direction revealed an intense labeling of clusterin at the apical cell surface (Fig. 3b) reminiscent to a strong microvillar labeling (Fig. 3c). Association with the apical plasma membrane brings clusterin in close vicinity to the iodinating system and might explain its strong iodination.

Porcine Clusterin Possesses at Least Three N-Linked Oligosaccharides per Subunit

Porcine inside-out follicles were labeled with L-[35S]methionine overnight and the culture supernatant was collected. Aliquots of the supernatant were subjected to mock or PNGase F digestion for the indicated periods of time and analyzed by SDS-PAGE under reducing or nonreducing conditions (Fig. 4).

Clusterin was again identified by its SH-induced shift in gelelectrophoretic mobility. Digestion with PNGase F resulted in the release of three N-glycans from each subunit of clusterin and the generation of a pair of deglycosylated polypeptides with similar molecular masses.
32P-Label of Clusterin Is Sensitive to PNGase F Digestion

Conditioned medium from porcine monolayers labeled with [32P]phosphate overnight was concentrated and subjected to PNGase F treatment for various periods of time or mock treatment with PBS for 4 h. Aliquots of the incubation mixtures were analyzed by SDS-PAGE under reducing (+SH) or nonreducing (−SH) conditions. The position of clusterin after removal of one, two, or three N-linked glycans (−1 CHO, −2 CHO, −3 CHO) is indicated in the left margin.

Clusterin Carries the M6P-Recognition Signal

Porcine inside-out follicles were labeled with L-[35S]methionine overnight. Aliquots of the medium were incubated with glycin Sepharose CL-4B or M6P-receptor Sepharose CL-4B for 2 h at room temperature while being shaken. Beads were washed and eluted with buffer containing 5 mM M6P. Aliquots of the untreated medium and of the M6P-elutable material were analyzed by SDS-PAGE and fluorography.

From the polypeptides found in the medium of inside-out follicles (Fig. 6, lane 1) only clusterin bound to the immobilized M6P receptor in appreciable quantities (Fig. 6, lane 3). Longer exposure of X-ray films revealed that a small portion of TG was present in the M6P eluate and had bound to the M6P receptor (not shown). 35S-labeled medium incubated with glycin Sepharose CL-4B did not yield any detectable polypeptide in the M6P elution buffer (Fig. 6, lane 2). These results demonstrate that clusterin carries functional M6P-recognition signals on its N-linked glycans. In contrast to minor secretory polypeptides, which were effectively concentrated by M6P receptor chromatography relative to the starting material (large arrowheads), clusterin was hardly concentrated (small arrowhead), indicating that it had a lower affinity for M6P receptors than those minor species.

We then tested whether binding of clusterin to the cell surface was mediated by M6P receptors. As iodination of clusterin involves association with the plasma membrane of thyrocytes (see Fig. 3), the iodination of clusterin was employed to monitor its binding to the cell surface. Monolayer thyrocytes were labeled with [125I]NaI overnight in the presence of 5 mM glucose 6-phosphate (G6P) or 5 mM M6P. Gel-electrophoretic analysis of corresponding amounts of cell lysates under reducing conditions showed an almost identical polypeptide pattern of iodoproteins from G6P- and M6P-treated cells. Selective inhibition of clusterin iodination was not observed (compare lanes 4 and 5 of Fig. 6).

Indirect immunofluorescence staining for clusterin of thyrocyte monolayers cultured in the presence of 5 mM G6P or 5 mM M6P on coverslides yielded a prominent staining of the plasma membrane without any detectable difference in the labeling signal (not shown), indicating that binding of clusterin to the plasma membrane of thyrocytes is not mediated by M6P receptors.

FIG. 5. 32P-labeled secretory proteins from porcine monolayers were subjected to a mock or a PNGase F treatment at 37°C, overnight. After gel-electrophoretic analysis under nonreducing conditions, dusterin-containing regions were excised from the gel, reduced, and subjected to gel-electrophoresis under reducing conditions. 32P-clusterin shifted to a lower molecular mass upon reduction (43 kDa). PNGase F treatment of 32P-clusterin resulted in an almost complete loss of 32P-label, indicating that it was mainly associated with the N-linked glycans of clusterin. The positions of intact dusterin and deglycosylated dusterin (−1CHO, −2CHO, without one or two N-glycans, respectively) are indicated.

FIG. 4. After biosynthetic labeling of porcine inside-out follicles with L-[35S]methionine overnight the conditioned medium of these cells was collected and subjected to PNGase F treatment for various periods of time or mock treatment with PBS for 4 h. Aliquots of the incubation mixtures were analyzed by SDS-PAGE under reducing (+SH) or nonreducing (−SH) conditions. The position of clusterin after removal of one, two, or three N-linked glycans (−1 CHO, −2 CHO, −3 CHO) is indicated.
Inhibition of M6P Receptors in Thyrocytes Does Not Increase Secretion of Clusterin

M6P is known to facilitate transport of newly synthesized lysosomal enzymes from the trans-Golgi network (TGN) to lysosomes. Since clusterin carries M6P on its N-linked glycans we asked whether a portion of clusterin follows the same intracellular M6P-dependent route to lysosomes as newly synthesized lysosomal enzymes. This transport can be inhibited by the lysosomotropic agent NH4Cl [26], which causes newly synthesized lysosomal enzymes to be secreted.

Porcine inside-out follicles were incubated with or without 10 mM NH4Cl while being labeled with L-[3H]leucine overnight. The secretory proteins of these cells were then analyzed by SDS–PAGE and fluorography. The appearance of additional polypeptides in the NH4Cl-induced secretions presumably represent newly synthesized lysosomal enzymes (Fig. 7). The amount of secreted clusterin was the same in normal and NH4Cl-induced secretions, indicating that clusterin is not subject to a direct M6P-facilitated transport from the TGN to lysosomes.

Furthermore, we observed earlier that endocytic uptake of 35S-labeled secretory product from porcine thyrocytes (which mainly consists of TG and clusterin) by

**FIG. 6.** Conditioned medium of L-[35S]methionine-labeled, porcine inside-out follicles was incubated with glycine Sepharose beads (Co) or M6P receptor Sepharose beads (Re). After extensive washing the beads were eluted with washing buffer containing 5 mM M6P. Aliquots of the untreated medium (Me, lane 1) and M6P eluates of glycine Sepharose beads (Co, lane 2) or M6P receptor beads (Re, lane 3) were analyzed by SDS–PAGE under reducing conditions and fluorography. Arrowheads in conjunction with lane 3 are sized according to the ability of M6P receptors to concentrate a polypeptide. In order to test for M6P-mediated association of clusterin with the plasma membrane, porcine monolayer thyrocytes were incubated with [125I]NaI overnight in the presence of 5 mM G6P (lane 4) or M6P (lane 5). Corresponding amounts of the cell lysates were analyzed by SDS–PAGE and autoradiography.

Iodination of Clusterin

Part of the cell surface associated clusterin colocalizes with gp330/megalin, known to serve as a receptor

**FIG. 7.** Porcine inside-out follicles were labeled with L-[2H]leucine in the absence and presence of 10 mM NH4Cl overnight. Corresponding amounts of secretory products were analyzed by SDS–PAGE and fluorography. Secretion of clusterin was not increased by NH4Cl. Additional polypeptides in the NH4Cl-induced secretions most likely represent newly synthesized lysosomal enzymes.

Clusterin Colocalizes with gp330/Megalin at the Apical Cell Surface of Thyrocytes in Situ

Immunofluorescence labeling of cryosections from unfixed porcine thyroid tissue and confocal laser scanning microscopy showed clusterin labeling at the lateral plasma membrane (Fig. 8b, insert). Double labeling experiments demonstrated that clusterin (Fig. 8b, red) and its putative membrane receptor gp330/megalin (Fig. 8a, green) are colocalized (Fig. 8c, yellow) at the apical plasma membrane of thyrocytes in situ.

DISCUSSION

In this report we describe the TSH-regulated synthesis and release of clusterin from thyrocytes, two as yet unknown posttranslational modifications, and the cell surface association of clusterin in cultured thyrocytes as well as in the intact thyroid gland.

Inhibition of M6P Receptors in Thyrocytes Does Not Increase Secretion of Clusterin

Porcine inside-out follicles was not at all inhibited by the presence of 5 mM M6P in the medium, indicating that endocytic uptake of either TG or of clusterin was not dependent on M6P receptors to a large extent (see [22]). Therefore it appears that mannose 6-phosphorylation of clusterin has no major impact on the transport of clusterin in thyrocytes.
for clusterin, on the apical plasma membrane. This localization and the ability of thyrocytes to iodinate polypeptides at the apical plasma membrane [27, 25] are the cellular prerequisites for the biosynthetic iodination of clusterin. The iodination of clusterin and of other polypeptides serves the fixation and storage of the relatively rare element of iodine in the thyroid gland. The apical localization has been described before in FRT cells [28]. However, as FRT cells are unable to iodinate, this posttranslational modification of clusterin escaped its detection as yet. Lamas and co-workers [29] showed that thyroperoxidase-mediated iodination was greatly facilitated by the presence of iodination consensus sequences. From the 12 tyrosine residues appearing in porcine clusterin, only 1 is integrated in an iodination consensus sequence. It is found within the C-terminal chain and is of the Glu-X-Tyr type at Glu-Leu-Tyr$^{327}$ (numbering according to [7]). This suggests that most of clusterin's iodine is added at Tyr$^{327}$ in the C-terminal chain and that this amino acid is exposed at the surface of clusterin where it is accessible to the iodinating system.

Mannose 6-Phosphorylation of Clusterin

Affinity chromatography of the secretory product of thyrocytes with immobilized M6P receptors revealed the presence of M6P-recognition signals within clusterin. Interestingly, some minor species of the secretory product were much better concentrated by the affinity-chromatographic procedure. Because it is known that newly synthesized lysosomal enzymes are secreted to a small extent in spite of possessing free M6P-recognition signals [30], we assume that these polypeptides represent newly synthesized lysosomal enzymes which were concentrated due to their high affinity to M6P receptors. Compared to these polypeptides clusterin expressed a markedly lower affinity to the immobilized M6P receptor. It seems likely that clusterin fails to bind to M6P receptors in the TGN due to the presence of competing lysosomal enzymes. As a result clusterin is efficiently secreted by thyrocytes. This was confirmed by the observation that secretion of clusterin was not increased by treating thyrocytes with...
NH₄Cl, which is known to incapacitate the M6P receptor system and to induce the release of newly synthesized lysosomal enzymes [26]. In addition, iodination of clusterin was not inhibited by the presence of excess M6P, indicating that M6P receptors were not of central importance in mediating attachment of clusterin to the plasma membrane. This task as well as endocytic uptake of clusterin is probably executed by gp 330/megalin [17], which is integrated within the thyroidal apical cell surface [18]. Our observations show that clusterin is an additional member of a group of secretory proteins carrying the M6P residue such as TG, the major secretory protein of thyrocytes. TG has also been shown to possess one M6P residue per subunit and to nevertheless be completely released from thyrocytes [22, 31].

Possible Physiological Role of Clusterin

The physiological role of clusterin in thyrocytes is as yet unknown. It has been proposed to be a marker of TGFβ mediated thyrocyte dedifferentiation [32]. Here, we show the ability of clusterin to become iodinated thereby increasing the storage capacity for iodine. Another possible function in the thyroid gland might be deduced from its appearance within structures resembling desmosomes. Clusterin at the basolateral plasma membrane might be involved in cell adhesion, which is also known from other tissues such as renal epithelial cells [16].

The localization of clusterin on the apical cell surface might have a specific function due to its exposition toward the follicle lumen. Here, it is most probably bound directly to gp330/megalin which has been implicated to operate as an endocytic receptor for TG [33]. It remains to be shown whether uptake of TG by gp330/megalin is affected by the presence of a putative competitive ligand [17] such as clusterin. It has also been shown that clusterin binds the Alzheimer amyloid beta peptide (A beta) [14, 34]. Because thyrocytes express high levels of the precursor protein of A beta [35] and because A beta is barely detectable in thyrocytes, it appears likely that clusterin operates also in the binding and the endocytic removal of A beta, leading to its lysosomal degradation.

The technical assistance of B. Wolf and S. Spürck is greatly acknowledged. We are also grateful to E. Kraemer for photographic work, to B. Naber and T. Dresbach for assistance in the early stages of experimentation, to Dr. W. James, University Hospital of Geneva, Switzerland, for providing the antisem for human clusterin, and to Dr. P. Verrout, Inserm, Paris, France, for the antisem against gp330/megalin. This work was supported by Deutsche Forschungsgemeinschaft, SFB 284, Fonds der Chemischen Industrie, and Bonner Forum Biomedizin. P. Lemansky was supported by a Habilitationsstipendium from the Deutsche Forschungsgemeinschaft and K. Brix by a Habilitationsstipendium of Department of Science and Research, Nordrhein-Westfalen, Germany.

REFERENCES


Received April 6, 1999