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MEGALIN- AND CUBILIN-MEDIATED ENDOCYTOSIS OF PROTEIN-BOUND VITAMINS, LIPIDS, AND HORMONES IN POLARIZED EPITHELIA

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■ **Abstract** Polarized epithelia have several functional and morphological similarities, including a high capacity for uptake of various substances present in the fluids facing the apical epithelial surfaces. Studies during the past decade have shown that receptor-mediated endocytosis, rather than nonspecific pinocytosis, accounts for the apical epithelial uptake of many carrier-bound nutrients and hormones. The two interacting receptors of distinct evolutionary origin, megalin and cubilin, are main receptors in this process. Both receptors are apically expressed in polarized epithelia, in which they function as biological affinity matrices for overlapping repertoires of ligands. The ability to bind multiple ligands is accounted for by a high number of replicated low-density lipoprotein receptor type-A repeats in megalin and CUB (complement C1r/C1s, Uegf, and bone morphogenic protein-1) domains in cubilin. Here we summarize and discuss the structural, genetic, and functional aspects of megalin and cubilin, with emphasis on their function as receptors for uptake of protein-associated vitamins, lipids, and hormones.

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INTRODUCTION

Uptake of nutrients is a main function of polarized epithelia facing such transcellular fluids as the gastrointestinal fluid, renal ultrafiltrate, cerebrospinal fluid, and various secretes. The transport of the substances occurs by nonspecific fluid-phase transfer and by means of specific channels, transporters, and receptors present in the apical membrane of the polarized epithelium. Whereas channels and transporters preferably account for the uptake of small substances (e.g. ions, amino acids, and monosaccharides), larger particles (proteins and lipoproteins) are pinocytosed or specifically endocytosed.

The concept of receptor-mediated endocytosis was established by Brown & Goldstein (12) on the basis of their studies of the uptake of low-density lipoprotein (LDL) and the identification of the LDL receptor. Later, many other endocytic receptors of different classes were identified and the molecular mechanism of endocytosis was characterized in more detail (65). In the plasma membrane, most endocytic receptors are clustered in the clathrin-coated regions, where the internalization is initiated (65). Clathrin heavy and light chains are organized in basket-like structures of the plasma membrane that pinch off the cytosolic side as clathrin-coated vesicles (35). The vesicles, which contain the receptors and their ligands, are subsequently uncoated and delivered to early endosomes that are acidified by ATP-dependent proton pumps. The low pH (<5) causes most ligands and receptors to segregate. The membrane-bound receptors recycle back to the membrane in small vesicular or tubular compartments, whereas the bulk of the volumen containing the ligands fuses with lysosomes. In this late stage of endocytosis, the ligands are degraded into their subcomponents (e.g. amino acids, fatty acids, cholesterol, vitamins, and hormones). Some receptor-ligand pairs deviate from this general scheme of receptor-mediated endocytosis (65). Endocytosis may, for instance, also occur in non–clathrin-coated regions of the plasma membrane (30), and the transport of ligands and receptors may follow other routes after internalization. Transferrin is an example of a ligand not released from its receptor after internalization (65). It recycles back to the membrane after delivery of iron in the endosome. The cation-independent mannose-6-phosphate receptor is an example of a receptor not only recycling back to the membrane but also trafficking between the endocytic apparatus (65) and the *trans*-Golgi network.

Receptor-mediated endocytosis in polarized epithelia (Figure 1) is, in principle, the same as in nonepithelial cells. However, some receptor-ligand systems in

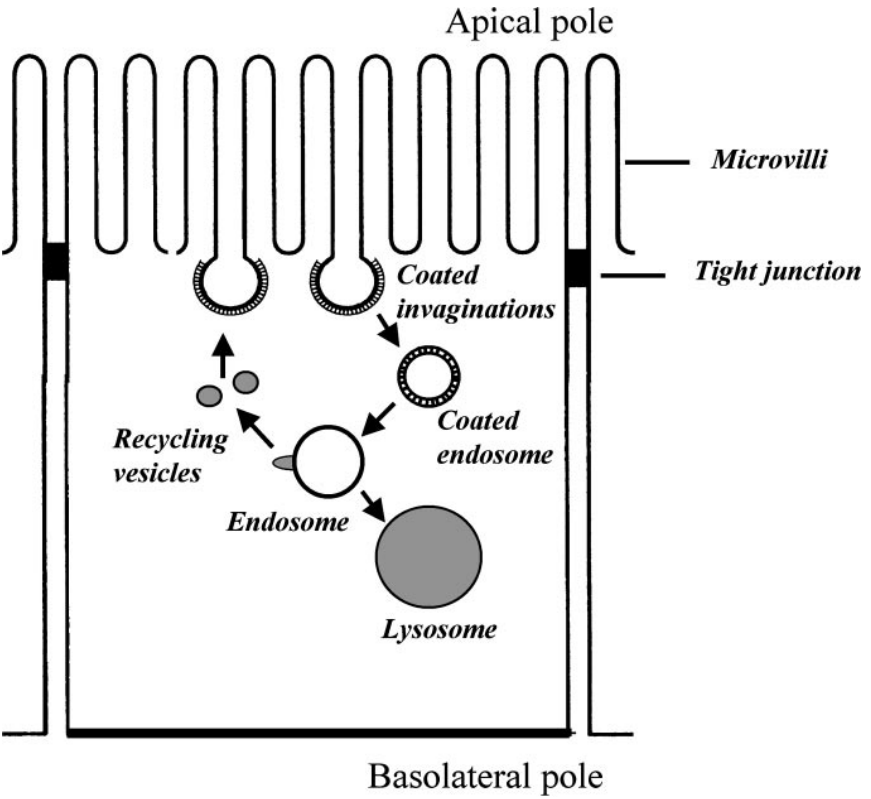

Figure 1 A schematic presentation of the polarized epithelial cell. Megalin and cubilin are apically expressed receptors in the invaginating coated intermicrovillar areas where they mediate uptake of ligands in the tissue fluid facing the apical pole. The receptors recycle via recycling vesicles, which pinch off the endosomes. The bulk of ligands are degraded in the lysosomes.

polarized cells are strictly allocated to either of the two functionally and biochemically distinct areas: the apical and basolateral membranes. The transferrin receptor (3) and the immunoglobulin A receptor (64) are examples of basolaterally expressed receptors, which pick up their ligands on the basolateral surface. Megalin (synonymous with gp330) and cubilin (synonymous with gp280 and IFCR) (59) are examples of membrane receptors expressed in the apical membrane, particularly in the invaginating clathrin-coated regions of the intermicrovillar surfaces (Figure 1).

In this review, we focus on the role of megalin- and cubilin-mediated endocytosis for the epithelial uptake of vitamins, hormones, and lipids bound to protein carriers.

STRUCTURE OF CUBILIN AND MEGALIN

Megalin

Megalin is a 600-kDa transmembrane protein (Figure 2) and the largest known mammalian single-chain receptor (~4600 amino acids) (32, 80). Megalin belongs to the LDL receptor family, which constitutes a small group of receptors that share common structural features (26). The 600-kDa, LDL receptor-related protein is the closest relative to megalin. cDNA cloning of the LDL receptor family proteins in humans and various species has disclosed a very high conservation of the receptors throughout evolution. For instance, a protein with a similar primary structure and almost identical composition of protein modules has been described in the nematode *C. elegans* (94). One common feature of receptors of the LDL receptor family is the presence of a least one ligand-binding region, represented by a cluster of LDL receptor type A repeats flanked by epidermal growth factor (EGF)-type repeats. Ligand interactions occur within the type A repeats, which are ~40–amino acid negatively charged calcium-binding (23) protein modules. Megalin is heterogenously glycosylated with various forms of N-glycans (63). Furthermore,

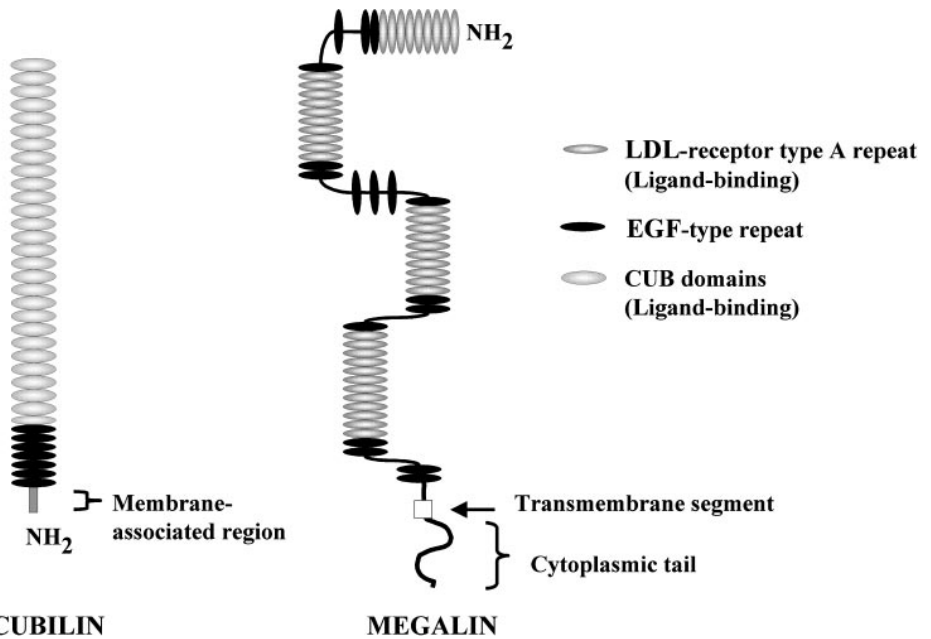

Figure 2 Schematic presentation of the modular structure of the 600-kDa megalin (32, 80) and the 460-kDa cubilin molecules (41, 59). LDL, low-density lipoprotein; EGF, epidermal growth factor; CUB, complement C1r/C1s, Uegf, and bone morphogenic protein-1.

megalin has a rare posttranslational modificationg by carrying the oligo/poly-α2,8-deaminoneuraminic acid (97).

Megalin has a single transmembrane segment, a short cytoplasmic tail, and a large ectodomain containing four ligand-binding regions connected by YWTD propeller repeats and EGF repeats. The cytoplasmic tail of megalin contains three conserved Scr-homology binding regions, three proteinase kinase C phoshorylation sites, seven casein kinase II sites, and three ΨXNPXY motifs (in which Ψ represents a hydrophobic residue) mediating binding to adaptor proteins and the clustering in coated pits. Disabled protein-2, a mitogen-responsive phosphoprotein thought to be an adaptor protein involved in signal transduction, has been shown to bind to the cytoplasmic tail of megalin (68). Whether megalin is signal-transducing is unknown but other receptors of the LDL receptor family have been shown to mediate signal transduction (90).

Cubilin

Cubilin is a 460-kDa glycoprotein (Figure 2) comprising ~3600 amino acids. cDNA cloning of cubilin from rat (59), dog (93), and humans (41) has shown similarity in primary structure and an identical organization of 35 protein modules—eight EGF repeats followed by 27 CUB (complement C1r/C1s, Uegf, and bone morphogenic protein-1) domains. Cubilin has no classical membrane-spanning segment, but the amino-terminal region contains a ~100–amino acid stretch of importance for membrane association (43). A putative amphipatic helix motif (43) and a potentially palmitoylated cysteine (M Kristiansen & SK Moestrup, unpublished data) in the amino-terminal region might be involved in the membrane attachment. The amino-terminal region also has a cleavage site (positioned between residue 10 and 11) for the Golgi proteinase furin (41).

The CUB domain region, which accounts for 85% of the entire protein, represents a region for ligand binding. CUB domains 5–8 and 13–14 have been disclosed as the regions responsible for binding intrinsic factor (IF)–vitamin B_{12} (IF-B_{12}) and receptor-associated protein (RAP) (43). Genetic studies have identified mutations in CUB domains 5–8 as a cause of B_{12} malabsorption (see below) (1, 42). Structural analysis of CUB domains of spermadhesins has shown that the domains consist of two layers of five-stranded β-sheets (77). The crystal structure of dimeric spermadhesin consisting of two CUB domains has revealed that the β-sheet layers from the two CUB domains can face each other (77). If the continuous 27 CUB domains of cubilin are connected with the β-sheet layers facing, the less-conserved β-turn regions may be surface-exposed like the antigen binding hypervariable β-turns in the Fab regions of immunoglobulins. From a structural point of view, the CUB domain region therefore seems to be a well-designed biological matrix for multiple protein interactions.

No other known receptors have close homology to cubilin, which has some structural relationship (59) to a number of EGF and CUB domain-containing proteins (e.g. the bone morphogenic proteins) involved in development.

TABLE 1 Main expression sites of cubilin and megalin[a]

Site	Cubilin	Megalin
Kidney proximal tubule	+	+
Kidney glomerulus[b]	−	+
Ileum	+	+
Lung	+	+
Thyroid	?	+
Thymus	+	+
Parathyroid	?	+
Ependyma	?	+
Eye, ciliary body	?	+
Inner ear epithelium	?	+
Choroid plexus	?	+
Oviduct and uterus	+	+
Epididymis	?	+
Yolk sac	+	+
Syncytiotrophoblast	+	+
Trophectoderm	?	+

[a]From References 28, 38, 50, 78, 81, 95.

[b]Only in rats.

EXPRESSION OF CUBILIN AND MEGALIN

Tissue Distribution

The main tissue expression sites of megalin and cubilin (79) are listed in Table 1. For both proteins, the expression is most abundant in the visceral yolk sac and in the renal proximal tubule cells, in particular the initial segments of the proximal tubule (19). These absorptive epithelia are characterized by high endocytic activity and degradation of the internalized components (14). Their apical poles are exposed to a wide panel of cubilin and megalin ligands. In the remaining tissues, cubilin and megalin is generally less abundant.

Cellular Expression

The subcellular localization of cubilin and megalin has been studied in detail in the proximal tubule cell and the yolk sac (14, 19, 45, 46). The two receptors are strikingly colocalized (59) in the apical endocytic apparatus of the cells.

On the plasma membrane of renal proximal tubule and yolk sac, cubilin and megalin are mainly detected in intermicrovillar areas (19) (Figure 1). Within the

cell, they are present in small and large endocytic vesicles, as well as in specialized electron-dense tubular-vesicular structures, which account for the recycling of internalized plasma membrane and receptors (19).

Processing and Trafficking

The biosynthesis of megalin and cubilin has been described in visceral yolk sac and renal epithelial cells (4, 6, 52, 73–75). As expected for the two high-molecular-weight proteins, the maturation and membrane association are slow, the $t_{1/2}$ being 90 min for megalin and several hours for cubilin. The biosynthesis of megalin is marked by its suggested rapid association (within 30 min) with RAP, the endoplasmic reticulum protein important for the processing of megalin and LDL receptor-related protein (9, 91), another 600-kDa member of the LDL receptor family. RAP binds to multiple sites in the ligand-binding regions of LDL receptor-related protein (LRP) (2) and probably also in megalin (69).

The biosynthesis of cubilin is complex and deviates from that of megalin. Association with RAP during biosynthesis has not been demonstrated, and it is possible (93) that RAP has no importance for cubilin processing because RAP-deficient mice have normal cubilin expression (9). However, genetic analysis of dogs with an inherited lack of functional cubilin indicates that some sort of accessory activity is required for proper cubilin expression. In these dogs, the receptor accumulates in the endoplasmic reticulum, or the early Golgi (24, 25), instead of being processed to the plasma membrane. Genetic analysis has shown no linkage between the processing failure and the cubilin gene.

Pulse-chase studies in yolk sac epithelial cells have suggested that newly synthesized cubilin initially reaches the cell membrane in an immature (endo H sensitive) form, which has not been terminally glycosylated in the Golgi apparatus. Immature cubilin is then presumably recycled by as-yet-unknown pathways through the Golgi before its final expression on the cell surface (4). In the same cells, megalin is addressed to the plasma membrane in a mature endo H–resistant form. Recent studies (92) based on PNGase F and endo H digestion suggest that terminal glycosylation of cubilin may be less extensive in the kidney than in the ileum, indicating an organ-specific process. The posttranslational modification of cubilin may involve cleavage by furin, as suggested by the finding (41) that affinity-purified human cubilin is truncated at the furin cleavage site. Studies in opossum kidney cells suggest that cubilin may be palmitoylated (73).

Little is known about the mechanisms regulating the expression of cubilin and megalin. In vitro up-regulation of megalin by retinoids, vitamin D, or cAMP has been demonstrated in cultured rat kidney proximal tubule cells, human JEG-3 cells, and the mouse embryonic cell line F9 (21, 47). A comparative analysis has shown that cubilin and megalin are expressed at similar levels in F9 cells differentiated by retinoic acid (28).

Although cubilin clearly functions as an endocytic receptor, its primary structure, does not predict any classical transmembrane segment or signals for endocytosis. It is therefore tempting to suggest that cubilin trafficking is assisted

by other structures. The following findings now indicate that megalin has such a function. (*a*) Megalin and cubilin coexpress at the cellular and subcellular level in several epithelia (59) and cell lines (21, 28). (*b*) Cubilin binds in vitro to megalin (59). (*c*) Megalin antibodies inhibit uptake of cubilin ligands (28, 40). (*d*) Megalin-deficient mice have an internalization defect of cubilin (R Kozyraki, J Fyfe, PJ Verroust, C Jacobsen, A Dautry-Varsat, TE Willnow, EI Christensen, SK Moestrup, manuscript in preparation).

LIGANDS FOR CUBILIN AND MEGALIN

Table 2 lists the many ligands reported to bind to megalin and cubilin. This section deals with some general features of ligand binding to the two receptors. More specific aspects of individual ligands relating to ligands specific for various tissues are described in the next section, on the role of the receptors in various epithelia.

The ligands of both receptors are substances of different structure and function. A predominant group is represented by proteins having a carrier function for vitamins, lipids, hormones, and minerals. This group of ligands indicates a broad nutritional function of the receptors. Binding of other proteins, such as albumin and immunoglobulin G light chains, may merely reflect a general protein-rescuing function of the receptors for reuse of the amino acids. The nutritional perspective of this protein uptake applies to the kidney and, in particular, the yolk sac, where the receptors seem to account for a high protein reabsorption. Enzymes, enzyme-protein complexes, and toxins make up other groups of ligands. By scavenging these types of endogenous and exogenous substances, the receptors may indirectly regulate the toxicity of substances in the tissue fluids lining the polarized epithelia.

The ligands of megalin and cubilin bind with a wide range of affinities to the receptors. A high affinity may have a physiological rationale when the receptor density is low in certain tissues. For instance, IF-B_{12} is an intestinal ligand binding with high affinity (K_d = 1–2 nM) to cubilin (8, 41). The high affinity is necessary for effective recognition by cubilin, which has a relatively low expression in the terminal ileum (92). Decreased affinity of the binding of IF-B_{12} is known as a cause of B_{12} deficiency disease (1, 42). On the other hand, a high capacity for uptake may compensate for the low affinity of a ligand-receptor interaction. Albumin reabsorption in the proximal tubules exemplifies such a situation. Cubilin/megalin–mediated albumin uptake (7, 20) accounts for a major part of the protein uptake in the proximal tubule, which has a dense expression of both receptors. Furthermore, the low affinity may cause a more even distribution of albumin uptake along the entire proximal tubule.

Megalin- and cubilin-mediated uptake of receptor-bound ligand ultimately leads to delivery of the bulk of ligand in lysosomes and degradation of the protein components. Nonprotein substances such as vitamins, hormones, drugs, and toxins escape degradation and will either accumulate or be transported out of the lysosomes to the cytosol.

TABLE 2 Megalin and cubilin ligands[a]

	Megalin	**Cubilin**
Vitamin-carrier complexes	TC-B_{12} Vitamin D–binding protein, vitamin D Retinol-binding protein, vitamin A	IF-B_{12}
Lipid-binding proteins	Apo B Apo E Apo J/clusterin Apo H/β_2-glycoprotein-I Apo(a)	Apo A-I
Hormone/hormone-binding proteins	PTH Transthyretin Thyroglobulin Insulin	
Mineral-binding protein		Transferrin[b]
Drugs	Aminoglycosides Polymyxin B Aprotinin	
Toxins	Trichosantin	
Enzymes and enzyme inhibitors	PAI-1 PAI-1-urokinase PAI-1-tPA Pro-urokinase Lipoprotein lipase α-Amylase	
Other	Albumin RAP Ig light chains[c] Ca^{2+} C1q Lactoferrin β_2-Microglobulin EGF Prolactin Lysozyme Cytochrome *c* β_1-Microglobulin PAP-1 Odorant-binding protein Seminal vesicle secretory protein II	Albumin RAP Ig light chains Ca^{2+}

[a]From References 5, 7–9, 13, 16, 17, 20, 29, 36, 37, 39, 40, 46, 56, 57, 60, 61, 66, 67, 70, 71, 76, 81, 83–86, 88, 96. TC, Transcobalamin; B_{12}, vitamin B_{12}; IF, intrinsic factor; Apo, apolipoprotein; PTH, parathyroid hormone; PAI, plasminogen activator inhibitor; tPA, tissue plasminogen activator; RAP, receptor-associated protein; Ig, immunoglobulin; EGF, epidermal growth factor; PAP, pancreatitis-associated protein.

[b]From unpublished data, R Kozyraki, J Fyfe, PJ Verroust, C Jacobsen, A Dautry-Varsat, TE Willnow, EI Christensen, SK Moestrup.

[c]From unpublished data (H Birn, M Leboulleux, SK Moestrup, PM Ronco, P Aucoutner, EI Christensen).

The binding of all megalin and cubilin ligands is dependent on calcium, which binds to the type A repeats of LDL receptor family protein (23) and probably also to the CUB domains of cubilin. Whether calcium is an integrated part of the receptor during the entire endocytic and recycling pathway or whether it is delivered in cells as a ligand is unknown. If a receptor-mediated net transport into the cell of calcium takes place, it might be a part of the nonregulated calcium transport system in the kidney (16). Furthermore, a megalin-mediated calcium transport might explain previous data indicating that megalin is a putative sensor for calcium in the parathyroid gland (32, 51).

The presence of basic motifs (e.g. heparin-binding sites) is a common feature of most ligands binding to megalin, and there is structural and functional evidence that electrostatic interactions between acidic regions of the type A repeats and the basic regions of the ligands are essential for the ligand-receptor recognition (57). Electrostatic interactions are probably also important for the pH-dependent binding of ligands to cubilin.

MEGALIN AND CUBILIN FUNCTION IN VARIOUS EPITHELIA

Intestine

Both megalin and cubilin are expressed in the intestinal epithelium, but only one physiological ligand is known so far—the IF-B_{12} complex (8, 82).

The daily supply of vitamin B_{12} (cobalamin) in food is limited to a few micrograms, which are transported and absorbed by means of specific carriers and receptors (Figure 3). Vitamin B_{12} is initially bound to another vitamin B_{12} binder, haptocorrin, which is degraded in the upper small intestine. Vitamin B_{12} subsequently binds to IF, which is taken up in the terminal ileum by cubilin-mediated endocytosis. Only the vitamin B_{12}-bound form of IF is effectively recognized (8). The importance of IF for vitamin B_{12} absorption is evident from the vitamin B_{12} deficiency state (causing megaloblastic anemia and neurological symptoms) in patients with decreased production of IF. Uptake of IF-B_{12} in the enterocyte is followed by degradation of IF, modification of the cobalamin and transport to the basolateral membrane, where the vitamin is released into plasma in complex with a third vitamin B_{12} binder, transcobalamin (TC). The TC-B_{12} complex is essential for transport and uptake in the organism. Megalin functions as a receptor for TC-B_{12} uptake in the kidney (56), whereas another as-yet-unidentified protein functions as the receptor for TC-B_{12} uptake in the nonepithelial tissues.

In agreement with the terminal ileum being the principal site for cubilin-mediated uptake of IF-B_{12}, a recent study of the segmental distribution of cubilin in the canine intestine has shown that the varied expression of cubilin parallels the IF-B_{12} binding activity (92). Furthermore, the crucial role of cubilin for the

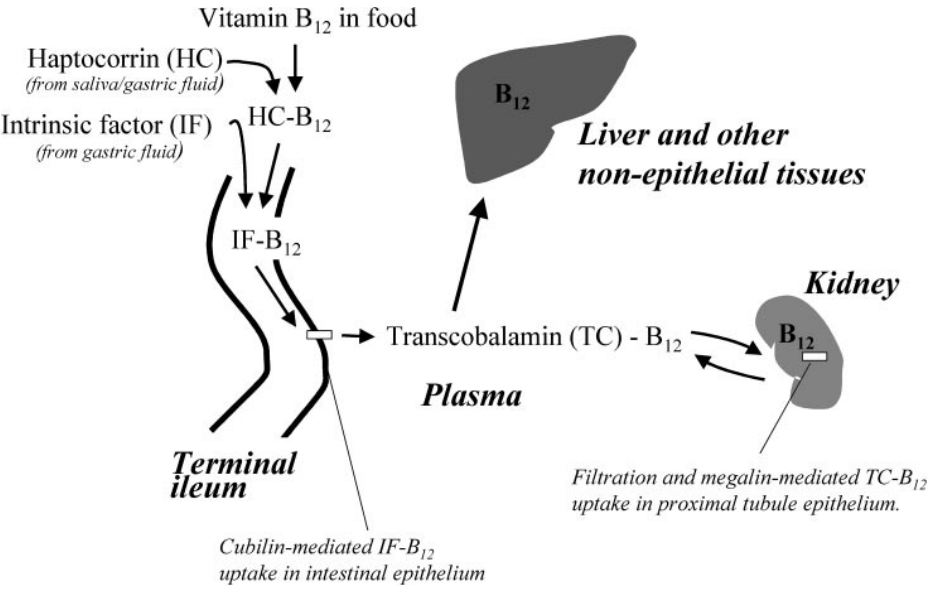

Figure 3 A schematic presentation of the transport of cobalamin (B_{12}) in the human organism. Cubilin accounts for the uptake of IF-B_{12} in the terminal ileum. The vitamin is subsequently secreted into plasma in complex with TC. The TC-B_{12} is essential for the general distribution of B_{12} in the body. Megalin accounts for the uptake of TC-B_{12} complex in the renale proximal tubule, which may function as a short-term storage site for B_{12}.

uptake of IF-B_{12} in the intestine has been settled by the identification of mutations in patients with Imerslund-Gräsbecks disease, a rare condition (∼200 cases reported worldwide) characterized by malabsorption of vitamin B_{12} (27, 33). In many, but not all, cases, the vitamin B_{12} deficiency is accompanied by a vitamin B_{12}–resistant proteinuria (27, 33). Two mutations in the cubilin gene [localized on chromosome 10p (41)] have been characterized in the Finnish population (1). The most prevalent mutation, designated FM1, is a point mutation in the coding region that causes the substitution of Pro_{1297} to Leu in CUB domain 8. This substitution is located within the IF-B_{12} binding region (CUB domains 5–8) of cubilin, and a recent mutagenesis study (42) of the IF-B_{12} binding region of recombinant cubilin has shown that the mutation strongly decreases the affinity of the binding of cubilin to IF-B_{12}. Most of the patients with the FM1 mutation have only a modest proteinuria, indicating that the genetic defect mainly affects the binding of IF-B_{12}. It has been suggested that the other known disease-causing cubilin mutation (FM2), an intronic mutation, results in the in-frame insertion of multiple stop codons in CUB domain 6. One patient and one carrier are known to have the FM2 mutation. Concordant with the suggested truncation of cubilin in CUB domain 6 by the FM2 mutation, the FM2 patient has a strong proteinuria and excretion

of cubilin ligands (40) (SK Moestrup, A de la Chapelle, R Krahe, unpublished data).

Genetic defects in genes other than cubilin may also account for the Imerslund-Gräsbeck disease. There are two lines of evidence for this. (*a*) Genetic analysis of Imerslund-Gräsbeck patients in Norway has not disclosed any mutations in the cubilin gene (1). (*b*) Genetic analysis of a dog model of human Imerslund-Gräsbeck disease (including strong proteinuria) has shown no linkage to the cubilin gene and no mutations in the coding region of the gene (93). However, the affected dogs have an abnormal processing of cubilin, leading to intracellular accumulation and abnormal glycosylation of the protein (25, 92). This finding combined with the genetic findings has led to the conclusion that the dogs are affected by an as-yet-unknown defect in a gene encoding an accessory activity required for cubilin brush-border expression (93).

Kidney

Under normal conditions, the proteins in the glomerular tiltrate are reabsorbed in the proximal tubule (18). Transcellular transport of intact protein is minimal, and it is now well established that the main part of protein undergo internalization and degradation by proximal tubule cells. A number of biochemical and in vivo studies [for a review, see Christensen et al (15)], including analysis of megalin-deficient mice (44) or analysis of dogs with functional cubilin deficiency (7, 43), have provided strong evidence that cubilin and megalin act as key receptors for protein endocytosis by the tubule cells. The spectrum of proteins excreted is not completely defined, but it is evident that cubilin (7) and megalin (44) deficiency lead to excretion of a different but overlapping set of proteins also seen in the urine of patients with renal tubular deficiency (Fanconi syndrome). This observation combined with experimental data on the receptor binding of single ligands suggests three types of binding/uptake properties of the reabsorbed protein. (*a*) Megalin is the main binder. This is the case, for instance, for retinol-binding protein (17), transcobalamin (56), and β_2-glycoprotein-I (61), which are internalized and degraded as described for other ligands of the LDL receptor family. (*b*) Cubilin is the main binder. The most studied examples in this group are transferrin (R Kozyraki, J Fyfe, PJ Verroust, C Jacobsen, A Dautry-Varsat, TE Willnow, EI Christensen, SK Moestrup, manuscript in preparation) and apolipoprotein (apo) A-I (40), the main protein of high-density lipoprotein (HDL). For these ligands, the internalization of the cubilin ligand complex requires megalin. (*c*) Both cubilin and megalin may be able to bind the ligand, as reported for albumin (7, 20).

In conclusion, the megalin- and cubilin-mediated endocytosis in the proximal tubule may be regarded as a highly efficient mechanism for rescuing various nutrients present in the renal ultrafiltrate. Although several grams of filtered protein are reabsorbed, and degraded daily by this mechanism, the rescue of protein-bound components as vitamins may be physiologically more important than the protein itself.

Megalin-mediated uptake of carrier-bound vitamins A, B_{12} (cobalamin), and D is followed by lysosomal degradation of the protein carrier. Vitamin D, which is endocytosed as the 25-OH D_3 form, undergoes a second hydroxylation in the proximal tubule, leading to the active 1,25-$(OH)_2$ vitamin D_3. In addition, the kidney seems to function as a storage organ for B_{12}. The intracellular pathways by which the vitamins, internalized along with their carrier proteins, reenter the circulation are largely unknown, but the transport probably involves vesicular transport. Whatever the exact mechanism, the importance of the megalin-mediated uptake is evidenced by studies of megalin-deficient mice, which in addition to developmental defects (see section on embryonic tissues) have bone malformation (67), loss of the three vitamins in the urine, and no storage of B_{12} in the kidney (10, 17, 67).

The receptors may also rescue lipid by internalizing various apolipoproteins, such as the megalin ligand β_2-glycoprotein-1 (binder of acidic phospholipids) and the cubilin ligand apo A-1 (40, 61), which constitute the major protein of HDL. However, although the kidney is the main catabolic site of apo A-1, it probably represents uptake of lipid-poor apo A-1 rather than lipid-loaded HDL particles (58). In the nonparticle form, apo-A1 has a size, which readily may pass the glomerular filtration barrier. The smallest HDL particles might also to a limited extent pass the barrier, but the physiological significance of this is unclear (58).

The endocytic properties of megalin and cubilin may also have inexpedient consequences, by mediating uptake of various exogenous components, such as drugs and toxins. Some polybasic drugs, for instance the aminoglycosides, are known to bind to the negatively charged megalin (57). Aminoglycosides accumulate in the lysosomes and can in elevated concentrations cause damage of the proximal tubules. Trichosanthin is an example of a plant toxin binding to LDL receptor family proteins, including megalin (13). It inactivates type I ribosomes and is strongly nephrotoxic, probably because of renal filtration and megalin-mediated uptake in the proximal tubules. Although not reported, it seems conceivable that other drugs and toxins may use megalin as a gate for cellular uptake.

Megalin (37) and cubilin (40) can be detected in the normal urine by immunoblotting. The excretion of the receptors is probably due to shedding of the proximal tubule epithelial cells. Detection of the receptors and estimation of their size might show to have diagnostic relevance, for instance for analysis of cubilin defects in Imerslund-Gräsbeck patients (40).

Yolk Sac

The yolk sac is an organ found in all species, although it has markedly different structures and functions. In rodents, it is derived, around embryonic day 7, from the very first endodermal cells that migrate from the inner cell mass to line the blastocoelic cavity. By embryonic day 9 (Figure 4), the yolk sac completely surrounds the embryo in such a manner that its absorptive surface faces the

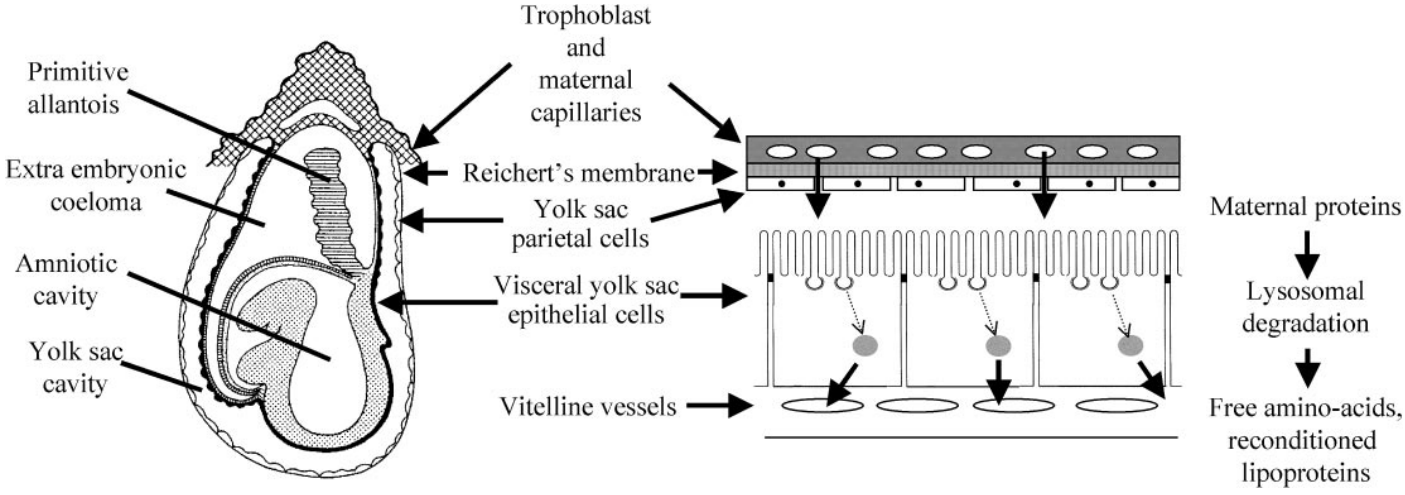

Figure 4 Anatomy and function of the murine yolk sac. (*Left*) Around day 10 of gestation, the yolk sac completely surrounds the embryo and constitutes the sole interface between the fetus and the mother. The parietal epithelium is permeable to maternal proteins. In the current hypothetical model of yolk sac function, megalin and cubilin, expressed at the apical pole of the visceral epithelial cells, can endocytose the proteins, which are present in the yolk sac cavity. Cholesterol freed by degradation of lipoproteins is reconditioned into nascent lipoproteins synthesized by yolk sac epithelial cells and transferred to the embryo.

surrounding decidual tissue in contact with the maternal circulation. The yolk sac remains functional during the entire pregnancy and constitutes the only maternal-fetal interface during organogenesis until establishment of the allantoic placenta around embryonic day 10. There is little, if any, transcellular transport across the yolk sac, and the key exchanges involve receptor-mediated internalization and degradation of proteins present in the maternal plasma(34).

Cubilin and megalin (79) are expressed in the apical yolk sac very early and remain expressed at a high level throughout gestation. As previously suggested by the deleterious effects of anti-cubilin antibodies on fetal development in rats (78), cubilin probably plays a crucial role in the initial phase of this process, i.e. in the uptake of maternal components. Cubilin, in association with megalin, can bind and internalize a variety of plasma proteins, including the most abundant, albumin. It may thus be important for the supply of amino acids used by the embryo, which, as shown elsewhere (11, 48, 49), are produced by degradation of maternal protein. The cubilin-megalin system can also internalize HDL (40) and LDL (85), which suggests that cubilin and megalin may be involved in the transfer to the fetus of maternal cholesterol and other lipids. The as-yet-incompletely defined pathway (31) involves internalization of maternal lipoproteins—largely HDL in rodents—at the apical pole of the visceral yolk sac followed by lysosomal degradation of the protein component (Figure 4). The internalized cholesterol is reloaded into apo B–containing particles secreted into fetal vessels at the basal pole of the visceral yolk sac (22, 72, 87).

Disruption of the megalin gene causes serious defects, including holoprosencephaly, and most megalin-deficient mice die shortly after birth (89). The pathogenesis of the holoproencephaly in megalin-deficient mice is unknown, and it has not been elucidated whether the malformation relates to defective megalin-mediated transport in the yolk sac or in the embryonic tissue, e.g. the megalin-expressing neuroepithelium or kidney epithelium. However, there are many causes of holoprosencephaly (including vitamin A and cholesterol deficiency), the pathogenic mechanisms underlying the neurological malformations in the megalin-deficient mice may be complex.

Thyroid Gland

The polarized epithelium of the thyroid gland has a specialized function. It digests thyroglobulin harboring the thyroid hormones thyroxine (T4) and triodothyronine (T3) [for a review, see Marino & McCluskey (53)]. Thyroglobulin is synthesized by thyrocytes and secreted into the lumen of the thyroid follicles, where it represents the major component of colloid. Here, the thyroglobulin undergoes iodination of tyrosyl residues, leading to the formation of T3 and T4 in the thyroglobulin molecule. Subsequent uptake of iodinated thyroglobulin in the epithelial cells is followed by lysosomal degradation of the major pool of the protein leading to release of T3 and T4. Some thyroglobulin is also transcytosed to the basolateral surface without being degraded, and some thyroglobulin is recycled back to the colloid

fluid. This has suggested the existence of distinct pathways for thyroglobulin—a lysosomal pathway, a transcytosis pathway, and a recycling pathway (53).

Recent work by Marino and coworkers (53–55) has shown that megalin, which is expressed in the apical membrane of the thyroid epithelial cells, binds and mediates endocytosis of thyroglobulin. It has been suggested that in addition to the megalin-mediated endocytosis, nonspecific fluid-phase pinocytosis and endocytosis mediated by a thyroid asioglycoprotein receptor account for uptake of thyroglobulin from the apical surface. The quantitative importance in vivo of each of these uptake pathways remains to be defined (53).

Megalin may play other roles in relation to the thyroid hormones by mediating uptake of transthyretin (84), a carrier of T4. Transthyretin is filtered and reabsorped in the kidney, which is a major organ for conversion of T4 to the active form T3. No renal reabsorption of transthyretin is seen in megalin-deficient mice, and it is therefore conceivable that these mice have a lower renal supply of T4 for conversion to T3.

Cubilin expression and/or function in the thyroid gland has not been investigated.

Other Tissues Expressing Megalin and Cubilin

Megalin and/or cubilin is expressed in a number of polarized epithelia, e.g. type II pneumocytes in the lung, the choroid plexus, the ependyma of the brain, the parathyroid, and the epithelia of the reproductive system (Table 1). Little is known about physiological ligands for the receptors in these tissues. Current knowledge mostly relates to the reproductive system: Apo J (clusterin) has been shown to bind to megalin in the efferent duct and epididymal epithelia rat seminal vesicle (62), and seminal vesicle secretory protein II has been demonstrated as a ligand for megalin expressed by epithelial cells lining the ductal region and the ampulla of the rat seminal vesicle (76). However, the expression of megalin and cubilin in several reproductive organs may suggest other ligands. Interesting candidates are the sex hormone–binding proteins, which have been proposed (90) because megalin is known to bind another steroid carrier, the vitamin D–binding protein (67).

CONCLUSIONS AND FUTURE PERSPECTIVES

Megalin and cubilin are mutually interacting, high-molecular-weight receptors expressed in polarized epithelia. So far, a number of nutritionally important ligands for cubilin and megalin have been identified in various epithelia. In the intestine, cubilin functions as the essential receptor for uptake of vitamin B_{12} in complex with IF. Mutations in the cubilin gene are now known to explain some cases of disease-causing malabsorption of vitamin B_{12} (Imerslund-Gräsbeck's disease). In the kidney, both receptors are involved in the reabsorption of several proteins in the

glomerular ultrafiltrate. The kidney ligands include carrier proteins for vitamins (A, B_{12}, and D) and lipids. A similar multiligand role is suggested for the receptors in the yolk sac epithelium of rodents. Furthermore, cubilin and megalin may account for a substantial uptake of LDL and HDL holoparticles in the yolk sac. In the thyroid, megalin functions as a receptor for uptake of thyroglobulin, which harbors the thyroid hormones.

Future directions of research on the two receptors will probably lead to a more comprehensive and complete ligand list. This may provide new information on the function of the receptors in those epithelia, where their role presently remains obscure. At the molecular level, it will be intriguing to further characterize the processing and trafficking of cubilin and megalin. It might reveal novel basic knowledge on receptor transport and provide new insight into pathogenic mechanisms of diseases with affected cubilin and/or megalin function.

LITERATURE CITED

1. Aminoff M, Carter JE, Chadwick RB, Johnson C, Grasbeck R, et al. 1999. Mutations in CUBN, encoding the intrinsic factor-vitamin B12 receptor, cubilin, cause hereditary megaloblastic anaemia 1. *Nat. Genet.* 21:309–13
2. Andersen OM, Christensen LL, Christensen PA, Sorensen ES, Jacobsen C, et al. 2000. Identification of the minimal functional unit in the low density lipoprotein receptor-related protein for binding the receptor-associated protein (RAP). A conserved acidic residue in the complement-type repeats is important for recognition of RAP. *J. Biol. Chem.* 275:21017–24
3. Anderson GJ, Powell LW, Halliday JW. 1990. Transferrin receptor distribution and regulation in the rat small intestine. Effect of iron stores and erythropoiesis. *Gastroenterology* 98:576–85
4. Baricault L, Galceran M, Ronco PM, Trugnan G, Verroust PJ. 1995. Unusual processing of GP280, a protein associated with the intermicrovillar areas of yolk sac epithelial cells: plasma membrane delivery of immature protein. *Biochem. Biophys. Res. Commun.* 212:353–59
5. Batuman V, Verroust PJ, Navar GL, Kaysen JH, Goda FO, et al. 1998. Myeloma light chains are ligands for cubilin (gp280). *Am. J. Physiol.* 275:F246–54
6. Biemesderfer D, Dekan G, Aronson PS, Farquhar MG. 1993. Biosynthesis of the gp330/44-kDa Heymann nephritis antigenic complex: assembly takes place in the ER. *Am. J. Physiol.* 264:F1011–20
7. Birn H, Fyfe JC, Jacobsen C, Mounier F, Verroust PJ, et al. 2000. Cubilin is an albumin binding protein important for renal tubular albumin reabsorption. *J. Clin. Invest.* 105:1353–61
8. Birn H, Verroust PJ, Nexo E, Hager H, Jacobsen C, et al. 1997. Characterization of an epithelial approximately 460-kDa protein that facilitates endocytosis of intrinsic factor-vitamin B12 and binds receptor-associated protein. *J. Biol. Chem.* 272:26497–504
9. Birn H, Vorum H, Verroust PJ, Moestrup SK, Christensen EI. 2000. Receptor-associated protein is important for normal processing of megalin in kidney proximal tubules. *J. Am. Soc. Nephrol.* 11:191–202
10. Birn H, Willnow TE, Nielsen R, Norden

AG, Moestrup SK, et al. 2001. Megalin-mediated endocytosis of transcobalamin-B_{12} and lysosomal accumulation of vitamin B12 in rat kidney proximal tubule. *Am. J. Phys.* In press
11. Brent RL, Beckman DA, Jensen M, Koszalka TR. 1990. Experimental yolk sac dysfunction as a model for studying nutritional disturbances in the embryo during early organogenesis. *Teratology* 41:405–13
12. Brown MS, Goldstein JL. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science* 232:34–47
13. Chan WL, Shaw PC, Tam SC, Jacobsen C, Gliemann J, Nielsen MS. 2000. Trichosanthin interacts with and enters cells via LDL receptor family members. *Biochem. Biophys. Res. Commun.* 270:453–57
14. Christensen EI, Birn H, Verroust P, Moestrup SK. 1998. Megalin-mediated endocytosis in renal proximal tubule. *Ren. Fail.* 20:191–99
15. Christensen EI, Birn H, Verroust P, Moestrup SK. 1998. Membrane receptors for endocytosis in the renal proximal tubule. *Int. Rev. Cytol.* 180:237–84
16. Christensen EI, Gliemann J, Moestrup SK. 1992. Renal tubule gp330 is a calcium binding receptor for endocytic uptake of protein. *J. Histochem. Cytochem.* 40:1481–90
17. Christensen EI, Moskaug JO, Vorum H, Jacobsen C, Gundersen TE, et al. 1999. Evidence for an essential role of megalin in transepithelial transport of retinol. *J. Am. Soc. Nephrol.* 10:685–95
18. Christensen EI, Nielsen S. 1991. Structural and functional features of protein handling in the kidney proximal tubule. *Semin. Nephrol.* 11:414–39
19. Christensen EI, Nielsen S, Moestrup SK, Borre C, Maunsbach AB, et al. 1995. Segmental distribution of the endocytosis receptor gp330 in renal proximal tubules. *Eur. J. Cell Biol.* 66:349–64
20. Cui S, Verroust PJ, Moestrup SK, Christensen EI. 1996. Megalin/gp330 mediates uptake of albumin in renal proximal tubule. *Am. J. Physiol.* 271:F900–7
21. Czekay RP, Orlando RA, Woodward L, Adamson ED, Farquhar MG. 1995. The expression of megalin (gp330) and LRP diverges during F9 cell differentiation. *J. Cell Sci.* 108(4):1433–41
22. Farese RV Jr, Cases S, Ruland SL, Kayden HJ, Wong JS, et al. 1996. A novel function for apolipoprotein B: Lipoprotein synthesis in the yolk sac is critical for maternal-fetal lipid transport in mice. *J. Lipid Res.* 37:347–60
23. Fass D, Blacklow S, Kim PS, Berger JM. 1997. Molecular basis of familial hypercholesterolaemia from structure of LDL receptor module. *Nature* 388:691–93
24. Fyfe JC, Giger U, Hall CA, Jezyk PF, Klumpp SA, et al. 1991. Inherited selective intestinal cobalamin malabsorption and cobalamin deficiency in dogs. *Pediatr. Res.* 29:24–31
25. Fyfe JC, Ramanujam KS, Ramaswamy K, Patterson DF, Seetharam B. 1991. Defective brush-border expression of intrinsic factor-cobalamin receptor in canine inherited intestinal cobalamin malabsorption. *J. Biol. Chem.* 266:4489–94
26. Gliemann J. 1998. Receptors of the low density lipoprotein (LDL) receptor family in man. Multiple functions of the large family members via interaction with complex ligands. *Biol. Chem.* 379:951–64
27. Gräsbeck R, Gordin RKI, Kuhlbäck B. 1960. Selective vitamin B12 malabsorption and proteinuria in young people. *Acta Med. Scand.* 167:289–96
28. Hammad SM, Barth JL, Knaak C, Argraves WS. 2000. Megalin acts in concert with cubilin to mediate endocytosis of high density lipoproteins. *J. Biol. Chem.* 275:12003–8
29. Hammad SM, Stefansson S, Twal WO, Drake CJ, Fleming P, et al. 1999. Cubilin, the endocytic receptor for intrinsic factor-vitamin B(12) complex, mediates high-density lipoprotein holoparticle

endocytosis. *Proc. Natl. Acad. Sci. USA* 96:10158–63

30. Hansen SH, Sandvig K, van Deurs B. 1991. The preendosomal compartment comprises distinct coated and noncoated endocytic vesicle populations. *J. Cell Biol.* 113:731–41
31. Herz J, Willnow TE, Farese RV Jr. 1997. Cholesterol, hedgehog and embryogenesis. *Nat. Genet.* 15:123–24
32. Hjalm G, Murray E, Crumley G, Harazim W, Lundgren S, et al. 1996. Cloning and sequencing of human gp330, a Ca^{2+}-binding receptor with potential intracellular signaling properties. *Eur. J. Biochem.* 239:132–37
33. Imerslund O. 1960. Idiophatic chronic megaloblastic anemia in children. *Acta. Paediatr. Scan.* 49:1–115
34. Jollie WP. 1990. Development, morphology, and function of the yolk-sac placenta of laboratory rodents. *Teratology* 41:361–81
35. Kirchhausen T. 2000. Clathrin. *Annu. Rev. Biochem.* 69:699–727
36. Kounnas MZ, Argraves WS, Strickland DK. 1992. The 39-kDa receptor-associated protein interacts with two members of the low density lipoprotein receptor family, alpha-2-macroglobulin receptor and glycoprotein 330. *J. Biol. Chem.* 267:21162–66
37. Kounnas MZ, Chappell DA, Strickland DK, Argraves WS. 1993. Glycoprotein 330, a member of the low density lipoprotein receptor family, binds lipoprotein lipase in vitro. *J. Biol. Chem.* 268:14176–81
38. Kounnas MZ, Haudenschild CC, Strickland DK, Argraves WS. 1994. Immunological localization of glycoprotein 330, low density lipoprotein receptor related protein and 39-kDa receptor associated protein in embryonic mouse tissues. *In Vivo* 8:343–51
39. Kounnas MZ, Loukinova EB, Stefansson S, Harmony JA, Brewer BH, et al. 1995. Identification of glycoprotein 330 as an endocytic receptor for apolipoprotein J/clusterin. *J. Biol. Chem.* 270:13070–75; Erratum. 1995. *J. Biol. Chem.* 270(39): 23234
40. Kozyraki R, Fyfe J, Kristiansen M, Gerdes C, Jacobsen C, et al. 1999. The intrinsic factor-vitamin B12 receptor, cubilin, is a high-affinity apolipoprotein A-I receptor facilitating endocytosis of high-density lipoprotein. *Nat. Med.* 5:656–61
41. Kozyraki R, Kristiansen M, Silahtaroglu A, Hansen C, Jacobsen C, et al. 1998. The human intrinsic factor-vitamin B12 receptor, cubilin: molecular characterization and chromosomal mapping of the gene to 10p within the autosomal recessive megaloblastic anemia (MGA1) region. *Blood* 91:3593–600; Erratum. 1998. *Blood* 92(7):2608
42. Kristiansen M, Aminoff M, Jacobsen C, de la Chapelle A, Krahe R, et al. 2000. Cubilin P1297L mutation associated with hereditary megaloblastic anemia 1 causes impaired recognition of intrinsic factor-vitamin B_{12} by cubilin. *Blood* 96:405–9
43. Kristiansen M, Kozyraki R, Jacobsen C, Nexo E, Verroust PJ, Moestrup SK. 1999. Molecular dissection of the intrinsic factor-vitamin B12 receptor, cubilin, discloses regions important for membrane association and ligand binding. *J. Biol. Chem.* 274:20540–44
44. Leheste JR, Rolinski B, Vorum H, Hilpert J, Nykjaer A, et al. 1999. Megalin knockout mice as an animal model of low molecular weight proteinuria. *Am. J. Pathol.* 155:1361–70
45. Le Panse S, Ayani E, Nielsen S, Ronco P, Verroust P, Christensen EI. 1997. Internalization and recycling of glycoprotein 280 in epithelial cells of yolk sac. *Eur. J. Cell Biol.* 72:257–67
46. Le Panse S, Verroust P, Christensen EI. 1997. Internalization and recycling of glycoprotein 280 in BN/MSV yolk sac epithelial cells: a model system of relevance to receptor-mediated endocytosis in the renal proximal tubule. *Exp. Nephrol.* 5:375–83

47. Liu W, Yu WR, Carling T, Juhlin C, Rastad J, et al. 1998. Regulation of gp330/megalin expression by vitamins A and D. *Eur. J. Clin. Invest.* 28:100–7
48. Lloyd JB. 1990. Cell physiology of the rat visceral yolk sac: a study of pinocytosis and lysosome function. *Teratology* 41:383–93
49. Lloyd JB, Beckman DA, Brent RL. 1998. Nutritional role of the visceral yolk sac in organogenesis-stage rat embryos. *Reprod. Toxicol.* 12:193–95
50. Lundgren S, Carling T, Hjalm G, Juhlin C, Rastad J, et al. 1997. Tissue distribution of human gp330/megalin, a putative Ca^{2+}-sensing protein. *J. Histochem. Cytochem.* 45:383–92
51. Lundgren S, Hjalm G, Hellman P, Ek B, Juhlin C, et al. 1994. A protein involved in calcium sensing of the human parathyroid and placental cytotrophoblast cells belongs to the LDL-receptor protein superfamily. *Exp. Cell Res.* 212:344–50
52. Lundstrom M, Orlando RA, Saedi MS, Woodward L, Kurihara H, Farquhar MG. 1993. Immunocytochemical and biochemical characterization of the Heymann nephritis antigenic complex in rat L2 yolk sac cells. *Am. J. Pathol.* 143:1423–35
53. Marino M, McCluskey RT. 2000. Role of thyroglobulin endocytic pathways in the control of thyroid hormone release. *Am. J. Physiol.* 279:C1295–306
54. Marino M, Zheng G, Chiovato L, Pinchera A, Brown D, et al. 2000. Role of megalin (gp330) in transcytosis of thyroglobulin by thyroid cells. A novel function in the control of thyroid hormone release. *J. Biol. Chem.* 275:7125–37
55. Marino M, Zheng G, McCluskey RT. 1999. Megalin (gp330) is an endocytic receptor for thyroglobulin on cultured fisher rat thyroid cells. *J. Biol. Chem.* 274:12898–904
56. Moestrup SK, Birn H, Fischer PB, Petersen CM, Verroust PJ, et al. 1996. Megalin-mediated endocytosis of transcobalamin-vitamin-B12 complexes suggests a role of the receptor in vitamin-B12 homeostasis. *Proc. Natl. Acad. Sci. USA* 93:8612–17
57. Moestrup SK, Cui S, Vorum H, Bregengard C, Bjorn SE, et al. 1995. Evidence that epithelial glycoprotein 330/megalin mediates uptake of polybasic drugs. *J. Clin. Invest.* 96:1404–13
58. Moestrup SK, Kozyraki R. 2000. Cubilin, a high-density lipoprotein receptor. *Curr. Opin. Lipidol.* 11:133–40
59. Moestrup SK, Kozyraki R, Kristiansen M, Kaysen JH, Rasmussen HH, et al. 1998. The intrinsic factor-vitamin B12 receptor and target of teratogenic antibodies is a megalin-binding peripheral membrane protein with homology to developmental proteins. *J. Biol. Chem.* 273:5235–42
60. Moestrup SK, Nielsen S, Andreasen P, Jorgensen KE, Nykjaer A, et al. 1993. Epithelial glycoprotein-330 mediates endocytosis of plasminogen activator-plasminogen activator inhibitor type-1 complexes. *J. Biol. Chem.* 268:16564–70
61. Moestrup SK, Schousboe I, Jacobsen C, Leheste JR, Christensen EI, Willnow TE. 1998. β2-Glycoprotein-I (apolipoprotein H) and β2-glycoprotein-I-phospholipid complex harbor a recognition site for the endocytic receptor megalin. *J. Clin. Invest.* 102:902–9
62. Morales CR, Igdoura SA, Wosu UA, Boman J, Argraves WS. 1996. Low density lipoprotein receptor-related protein-2 expression in efferent duct and epididymal epithelia: evidence in rats for its in vivo role in endocytosis of apolipoprotein J/clusterin. *Biol. Reprod.* 55:676–83
63. Morelle W, Haslam SM, Ziak M, Roth J, Morris HR, Dell A. 2000. Characterization of the N-linked oligosaccharides of megalin (gp330) from rat kidney. *Glycobiology* 10:295–304
64. Mostov KE. 1994. Transepithelial transport of immunoglobulins. *Annu. Rev. Immunol.* 12:63–84
65. Mukherjee S, Ghosh RN, Maxfield FR.

1997. Endocytosis. *Physiol. Rev.* 77:759–803

66. Niemeier A, Willnow T, Dieplinger H, Jacobsen C, Meyer N, et al. 1999. Identification of megalin/gp330 as a receptor for lipoprotein(a) in vitro. *Arterioscler. Thromb. Vasc. Biol.* 19:552–61
67. Nykjaer A, Dragun D, Walther D, Vorum H, Jacobsen C, et al. 1999. An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D3. *Cell* 96:507–15
68. Oleinikov AV, Zhao J, Makker SP. 2000. Cytosolic adaptor protein Dab2 is an intracellular ligand of endocytic receptor gp600/megalin. *Biochem. J.* 347(3):613–21
69. Orlando RA, Exner M, Czekay RP, Yamazaki H, Saito A, et al. 1997. Identification of the second cluster of ligand-binding repeats in megalin as a site for receptor-ligand interactions. *Proc. Natl. Acad. Sci. USA* 94:2368–73
70. Orlando RA, Kerjaschki D, Kurihara H, Biemesderfer D, Farquhar MG. 1992. gp330 associates with a 44-kDa protein in the rat kidney to form the Heymann nephritis antigenic complex. *Proc. Natl. Acad. Sci. USA* 89:6698–702
71. Orlando RA, Rader K, Authier F, Yamazaki H, Posner BI, et al. 1998. Megalin is an endocytic receptor for insulin. *J. Am. Soc. Nephrol.* 9:1759–66
72. Raabe M, Flynn LM, Zlot CH, Wong JS, Veniant MM, et al. 1998. Knockout of the abetalipoproteinemia gene in mice: reduced lipoprotein secretion in heterozygotes and embryonic lethality in homozygotes. *Proc. Natl. Acad. Sci. USA* 95: 8686–91
73. Ramanujam KS, Seetharam S, Dahms NM, Seetharam B. 1994. Effect of processing inhibitors on cobalamin (vitamin B12) transcytosis in polarized opossum kidney cells. *Arch. Biochem. Biophys.* 315:8–15
74. Ramanujam KS, Seetharam S, Seetharam B. 1991. Synthesis and secretion of cobalamin binding proteins by opossum kidney cells. *Biochem. Biophys. Res. Commun.* 179:543–50
75. Ramanujam KS, Seetharam S, Seetharam B. 1992. Leupeptin and ammonium chloride inhibit intrinsic factor mediated transcytosis of [57Co]cobalamin across polarized renal epithelial cells. *Biochem. Biophys. Res. Commun.* 182:439–46
76. Ranganathan S, Knaak C, Morales CR, Argraves WS. 1999. Identification of low density lipoprotein receptor-related protein-2/megalin as an endocytic receptor for seminal vesicle secretory protein II. *J. Biol. Chem.* 274:5557–63
77. Romero A, Romao MJ, Varela PF, Kolln I, Dias JM, et al. 1997. The crystal structures of two spermadhesins reveal the CUB domain fold. *Nat. Struct. Biol.* 4:783–88
78. Sahali D, Mulliez N, Chatelet F, Dupuis R, Ronco P, Verroust P. 1988. Characterization of a 280-kD protein restricted to the coated pits of the renal brush border and the epithelial cells of the yolk sac. Teratogenic effect of the specific monoclonal antibodies. *J. Exp. Med.* 167:213–18
79. Sahali D, Mulliez N, Chatelet F, Laurent-Winter C, Citadelle D, et al. 1992. Coexpression in humans by kidney and fetal envelopes of a 280-kDa-coated pit-restricted protein. Similarity with the murine target of teratogenic antibodies. *Am. J. Pathol.* 140:33–44
80. Saito A, Pietromonaco S, Loo AK, Farquhar MG. 1994. Complete cloning and sequencing of rat gp330/"megalin," a distinctive member of the low density lipoprotein receptor gene family. *Proc. Natl. Acad. Sci. USA* 91:9725–29
81. Seetharam B, Christensen EI, Moestrup SK, Hammond TG, Verroust PJ. 1997. Identification of rat yolk sac target protein of teratogenic antibodies, gp280, as intrinsic factor-cobalamin receptor. *J. Clin. Invest.* 99:2317–22
82. Seetharam B, Levine JS, Ramasamy M, Alpers DH. 1988. Purification, properties,

and immunochemical localization of a receptor for intrinsic factor-cobalamin complex in the rat kidney. *J. Biol. Chem.* 263:4443–49

83. Sim RB, Moestrup SK, Stuart GR, Lynch NJ, Lu J, et al. 1998. Interaction of C1q and the collectins with the potential receptors calreticulin (C1qR/collectin receptor) and megalin. *Immunobiology* 199:208–24
84. Sousa MM, Norden AG, Jacobsen C, Willnow TE, Christensen EI, et al. 2000. Evidence for the role of megalin in renal uptake of transthyretin. *J. Biol. Chem.* 275:88176–81
85. Stefansson S, Chappell DA, Argraves KM, Strickland DK, Argraves WS. 1995. Glycoprotein 330/low density lipoprotein receptor-related protein-2 mediates endocytosis of low density lipoproteins via interaction with apolipoprotein B100. *J. Biol. Chem.* 270:19417–21
86. Stefansson S, Kounnas MZ, Henkin J, Mallampalli RK, Chappell DA, et al. 1995. gp330 on type II pneumocytes mediates endocytosis leading to degradation of pro-urokinase, plasminogen activator inhibitor-1 and urokinase-plasminogen activator inhibitor-1 complex. *J. Cell Sci.* 108(6):2361–68
87. Terasawa Y, Cases SJ, Wong JS, Jamil H, Jothi S, et al. 1999. Apolipoprotein B-related gene expression and ultrastructural characteristics of lipoprotein secretion in mouse yolk sac during embryonic development. *J. Lipid Res.* 40:1967–77
88. Willnow TE, Goldstein JL, Orth K, Brown MS, Herz J. 1992. Low density lipoprotein receptor-related protein and gp330 bind similar ligands, including plasminogen activator-inhibitor complexes and lactoferrin, an inhibitor of chylomicron remnant clearance. *J. Biol. Chem.* 267:26172–80
89. Willnow TE, Hilpert J, Armstrong SA, Rohlmann A, Hammer RE, et al. 1996. Defective forebrain development in mice lacking gp330/megalin. *Proc. Natl. Acad. Sci. USA* 93:8460–64
90. Willnow TE, Nykjaer A, Herz J. 1999. Lipoprotein receptors: new roles for ancient proteins. *Nat. Cell Biol.* 1:E157–62
91. Willnow TE, Rohlmann A, Horton J, Otani H, Braun JR, et al. 1996. RAP, a specialized chaperone, prevents ligand-induced ER retention and degradation of LDL receptor-related endocytic receptors. *EMBO J.* 15:2632–39
92. Xu D, Fyfe JC. 2000. Cubilin expression and posttranslational modification in the canine gastrointestinal tract. *Am. J. Physiol. Gastrointest. Liver Physiol.* 279:G748–56
93. Xu D, Kozyraki R, Newman TC, Fyfe JC. 1999. Genetic evidence of an accessory activity required specifically for cubilin brush-border expression and intrinsic factor-cobalamin absorption. *Blood* 94:3604–6
94. Yochem J, Greenwald I. 1993. A gene for a low density lipoprotein receptor-related protein in the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* 90:4572–76
95. Zheng G, Bachinsky DR, Stamenkovic I, Strickland DK, Brown D, et al. 1994. Organ distribution in rats of two members of the low-density lipoprotein receptor gene family, gp330 and LRP/alpa 2MR, and the receptor-associated protein (RAP). *J. Histochem. Cytochem.* 42:531–42
96. Zheng G, Marino M, Zhao J, McCluskey RT. 1998. Megalin (gp330): a putative endocytic receptor for thyroglobulin (Tg). *Endocrinology* 139:1462–65
97. Ziak M, Meier M, Roth J. 1999. Megalin in normal tissues and carcinoma cells carries oligo/poly alpha2,8 deaminoneuraminic acid as a unique posttranslational modification. *Glycoconjugation J.* 16:185–88