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Editorial Comments

Megalin and cubilin—the story of two multipurpose receptors unfolds

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Introduction

Under physiological conditions, the renal tubular clearance of protein appears to be very efficient. However, the molecular mechanisms responsible for the endocytic uptake of protein in the renal proximal tubule have until recently been largely unknown. Within the last few years, two endocytic receptors, megalin and cubilin, have been shown to be extremely important for this process. The two multi-ligand receptors are strongly expressed in the apical part of epithelial cells in the renal proximal tubule (Figure 1). At the subcellular level they are co-localized in apical clathrin coated pits and endosomes, i.e. in the early endocytic compartments (Figure 2). In addition, they are also detected in the dense apical tubules that provide for the recycling of apical membrane and receptors. Expression in the late endocytic compartments and lysosomes appears more limited. It is interesting to note that both megalin and cubilin are massively expressed in the yolk sac, another epithelial structure in which apical endocytosis of proteins is a crucial physiological function. In this paper we will briefly review the structure of megalin and cubilin as well as the data showing their relevance in the renal tubular reabsorption of not only protein but also vital nutrients, vitamins and different trace elements (Figure 3).

Molecular structure

Megalin

Megalin is a 600-kDa transmembrane protein (Figure 4) belonging to the LDL-receptor family [1]. The complete cDNA sequences have been characterized for rat [2] and human megalin [3]. The extracellular domain contains four clusters of cysteine-rich, complement-type repeats, constituting the ligand binding regions. The ligand binding regions are separated by epidermal growth factor (EGF)-like repeats and cysteine-poor spacer regions containing YWTD motifs, so called propeller repeats, involved in pH-dependent dissociation of receptor and ligands in acidic endosomal compartments [4]. The cytoplasmic tail contains two NPXY motifs, which mediate the clustering in coated pits and thereby initiate the endocytic process. These and other cytoplasmic motifs are possibly involved in signalling functions.

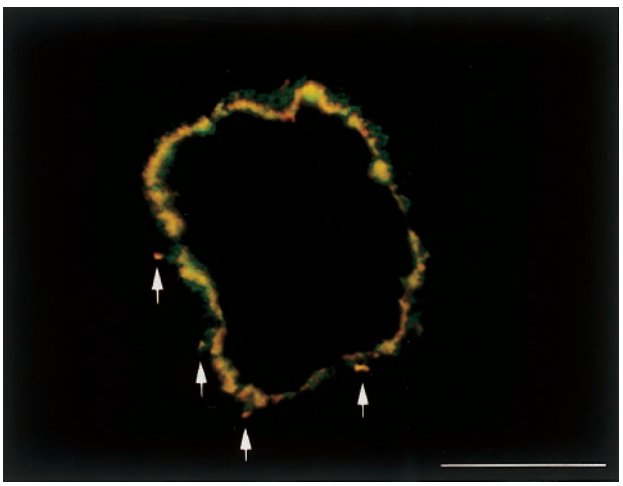

Fig. 1. Double-labelling immunofluorescence for megalin (green) and cubilin (red) of semi-thin cryosection from rat renal proximal tubule. The yellow colour illustrates the co-localization of the two receptors in the apical part of the cells. Labelled endosomes are marked with arrows. Bar = 20 μm.

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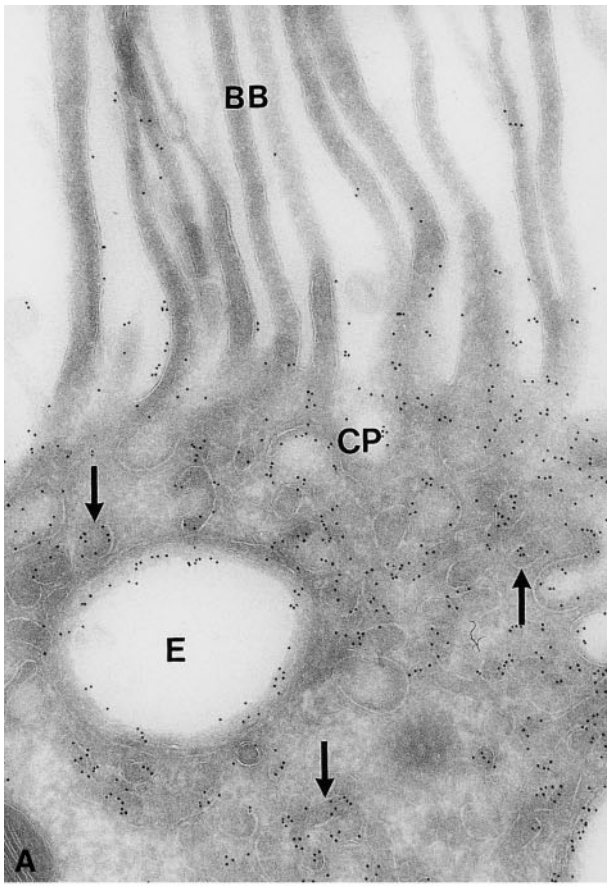

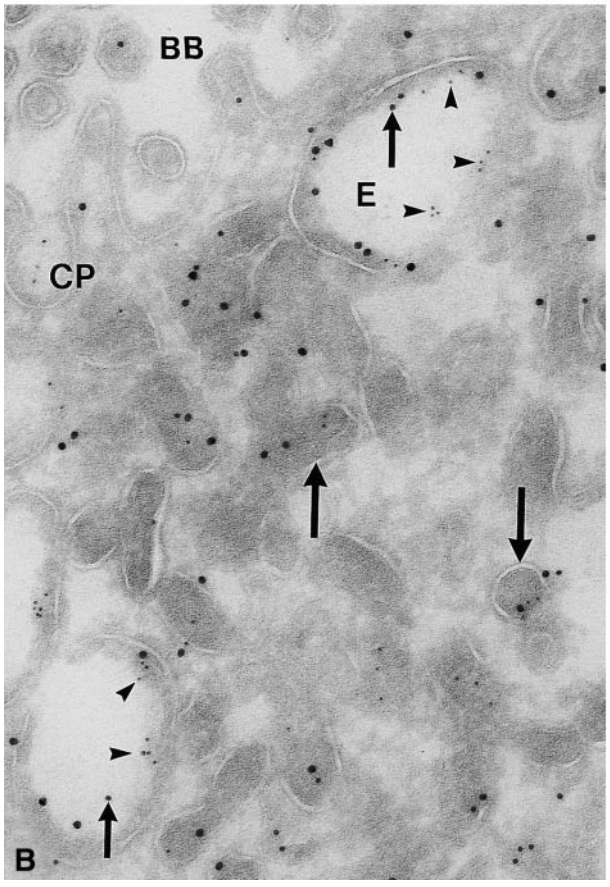

Cubilin

Cubilin is a 460-kDa peripheral membrane protein, previously referred to as gp280, and identical to the intrinsic factor-vitamin B_{12} receptor known from the small intestine. Its primary sequence, determined in rat [5], man [6] and canine [7], is conserved with an overall homology of 69% between rat and human cubilin and 83% between canine and human cubilin. Its structure consists of a 110 amino acid N-terminal stretch, followed by eight EGF and 27 CUB (Complement C1r/C1s, Uegf and Bone morphogenic protein-1 [8]) domains. Each CUB domain consists of 110 amino acids. The structure of CUB domains, which has been determined on spermadhesins [9] (a family of sperm proteins which consist of a single CUB domain), is characterized by two layers of five anti-parallel β-sheets connected by β-turns which include the least conserved regions and likely ligand-binding sites. Interestingly enough, a single spermadhesin can bind simultaneously two distinct ligands. The CUB domains can form dimers by piling up via the β-sheets, in a manner that may favour the exposition of β-turns to the surface. Therefore, the least conserved regions of the β-turns will be preferentially exposed and available for interaction with ligands. This accumulation of CUB domains suggests that cubilin may interact with a variety of ligands.

Cubilin is a peripheral protein and its membrane association depends on the 110 amino acids at the N-terminus stretch [10] and may involve a putative amphipathic helix as well as palmitoylation. Biochemical and immuno-morphological data suggest that the internalization of cubilin is, at least in part, carried out by megalin [5,11].

Expression

While megalin is expressed in many epithelial cells, it appears at present that the expression of cubilin is more restricted (for a review see [12]). The two receptors are co-localized in the proximal tubule, the small intestine, the visceral yolk sac and the cytotrophoblast of the placenta. In addition, megalin has been demonstrated in glomerular podocytes, type II pneumocytes, thyroid and parathyroid cells, the choroid plexus, the

Fig. 2. (**A**) Immunogold labelling for megalin in segment 1 rat proximal tubule. Labelling is seen in apical coated pits (CP) in endosomes (E) and in dense apical recycling tubules (arrows). Rather little labelling is found in the brush border (BB) of the proximal tubular segment 1 (×45 000). (**B**) Triple immunogold labelling for megalin (15-nm gold particles), cubilin (5-nm gold particles) and endogenous retinol binding protein (RBP) (10-nm gold particles) in apical part of rat renal proximal tubule. Small arrows in endosomes (E) indicate labelling for RBP, arrowheads labelling for cubilin and large gold particles labelling for megalin. Large arrows show dense apical tubules labelled for cubilin and megalin. CP and microvilli of the BB are seen in the upper part of the electron micrograph (×55 000).

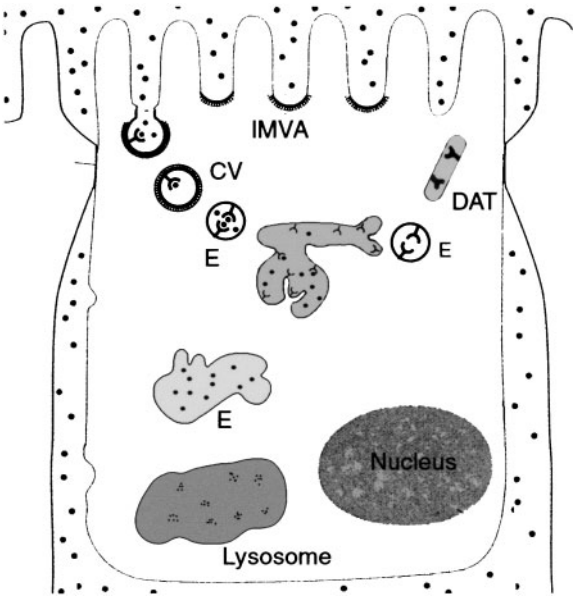

Fig. 3. Schematic drawing illustrating the megalin/cubilin-mediated endocytic process in the renal proximal tubule. Ligands are internalized through apical clathrin-coated pits in intermicrovillar areas (IMVA) into coated vesicles (CV) and subsequently to endosomes in which the ligands dissociate from the receptors. The ligands are transferred through endosomal compartments (E) to lysosomes for degradation and further processing. The receptors are returned to the apical plasma membrane through dense apical tubules (DAT). While the proteins are degraded in lysosomes, vitamins and different trace elements are returned to the circulation by so far poorly defined pathways.

endometrium, the oviduct, epididymis, ependymal cells, labyrinthic cells of the inner ear and the ciliary epithelium of the eye. The intracellular traffic of megalin and cubilin is complex. Megalin requires receptor-associated protein (RAP), a chaperone/escort protein [13,14] that interacts with all members of the LDL-receptor family. Indeed, in RAP-deficient mice, overall expression was reduced to ~23% of control animals; an increased amount of megalin seems to be retained in the rough endoplasmic reticulum and in the smooth paramembranous reticulum [15], although in the same mice, cubilin is only affected to a limited extent. However, as described below, in some dogs with functional cubilin deficiency, cubilin is retained intracellularly and fails to be inserted in the apical plasma membrane [16]. However, in these dogs, the disease and the cubilin gene are not linked, suggesting that additional protein(s) is (are) required for the normal processing of cubilin [7].

Functions

Both receptors are important for normal reabsorption of proteins in the renal proximal tubule as visualized by the proteinuria seen in megalin gene-deficient mice [17], and in dogs lacking functional cubilin [16]. As indicated in Table 1, some proteins bind both receptors, which in addition recognize specific ligands. It is most likely that megalin can both bind and internalize its ligands, whereas the cubilin–ligand complexes need megalin to be internalized. The ligand binding is Ca^{2+} dependent. The binding affinity varies considerably from one ligand to another and it is likely that the efficiency of the overall process is related to the high expression levels of megalin and cubilin in the proximal tubule, which thus constitutes a high capacity system. Some of the ligands attract special attention such as the vitamin-carrier proteins and transferrin. Thus, it has been demonstrated that the megalin/cubilin-mediated reabsorption of vitamin D binding protein is responsible for the renal conversion of 25(OH)D3 to $1,25(OH)_2$ D3 [20,21] in the proximal tubule. For transcobalamin (TC) and retinol-binding protein (RBP), the reabsorption appears to preserve vitamin B_{12} [23] and vitamin A [24], respectively, for the organism. Likewise, iron is being captured by the cubilin/(megalin)-mediated reabsorption of transferrin [11] and haemoglobin [22], a process which under pathological conditions with increased glomerular filtration may be harmful to the kidney. It has been proposed that megalin, which binds calcium strongly [25], could act as a calcium sensor in the parathyroids [26]. It may also be involved in the transport/processing of thyroid hormones [27]. Cubilin and megalin bind lipoproteins (HDL [28,29] and LDL [30], respectively) but their role in cholesterol metabolism is not firmly established, although the dogs with cubilin-deficient expression have hypercholesterolaemia. In contrast, there is strong evidence that cubilin is the physiological receptor for intrinsic factor-vitamin B12 complexes (IF-B12) [31].

Pathology in patients with juvenile megaloblastic anaemia, which have the rare autosomal recessive vitamin B_{12} malabsorption syndrome known as Imerslund–Gräsbeck (I-GS) [32,33], are most probably accounted for by abnormal cubilin gene. Two distinct mutations of the cubilin gene have been identified in Finnish patients with I-GS [34]. The first mutation (FM1) consists of a point mutation in CUB domain 8, which binds the intrinsic factor vitamin B_{12} complexes. The FM2 mutation, so far only detected in a single patient, is an intronic mutation within CUB domain 6, which probably results in the synthesis of a truncated and/or rapidly degraded protein. The dogs that fail to insert cubilin in their apical membrane [16] also have evidence of B_{12} deficiency.

Patients as well as dogs with IG-S have, in addition to the intestinal vitamin B_{12} malabsorption, a B_{12}-resistant proteinuria consistent with the implication of cubilin in protein reabsorption by the proximal tubule. The cubilin ligands, with the exception of intrinsic factor, are massively excreted by I-GS patients and dogs, confirming the hypothesis that cubilin is essential in renal protein reabsorption.

The physiological role of cubilin and megalin expressed by materno–fetal interfaces is unknown but probably crucial as indicated by the teratogenic effect of anti-cubilin antibodies [35] and the developmental

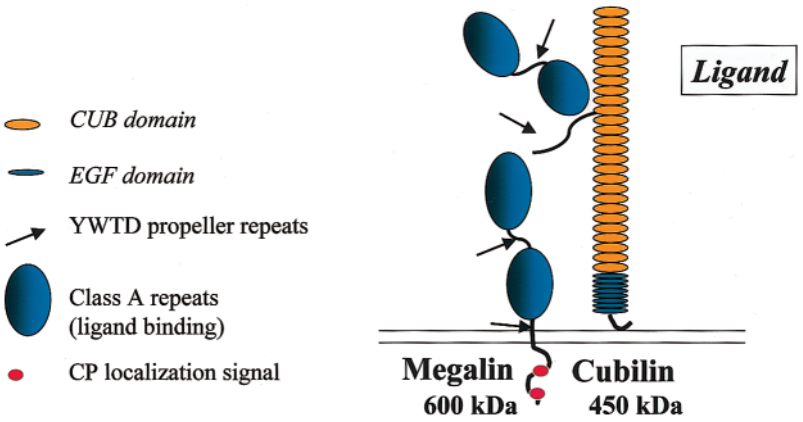

Fig. 4. Schematic presentation of the two endocytic receptors, megalin and cubilin.

Table 1. Ligands to megalin and cubilin

Common to megalin and cubilin	Cubilin specific	Megalin specific
DBP	Clara cell secretory protein	Transcobalamin-vitamin B_{12}
Ig light chains	Apolipoprotein A-I	RBP-vitamin A
Haemoglobin	Transferrin	Apolipoprotein H
Albumin	*HDL*	α_1-Microglobulin
	IF-vitamin B_{12} complexes	Transthyretin
	RAP	α-Amylase
		PTH
		Peptide hormones
		UPA-PAI-I
		Ca^{2+}
		Apo-B
		LPL
		RAP

Ligands shown in italic are not normally found in the circulation or in the PCT lumen. For a review of ligands see [18]. Since then, the following additional ligands listed in the table have been identified for cubilin: Clara cell secretory protein [19] and transferrin [11]; and for both receptors: DBP [20,21] and haemoglobin [22].

defects seen in megalin-deficient mice [17]. Given their wide variety of ligands, cubilin and megalin may be essential in providing the embryo with vital substances, e.g. cholesterol, iron and vitamins.

Conclusion

Cubilin and megalin thus appear as novel multi-ligand receptors which bind distinct but overlapping sets of ligands in different epithelia. Their crucial role in physiology and possibly in pathology outlined above may be even clearer as additional ligands and expression sites are identified. Furthermore, megalin- and cubilin-deficient mice and cubilin-deficient dogs will be important tools for studying tubular and interstitial lesions induced by proteins and other substances reabsorbed by the proximal tubule.

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Halting progression of renal failure: consideration beyond angiotensin II inhibition

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Keywords: ACEI; ARB; chronic kidney disease; ESRD; non-renal factors; renal failure progression

Over the last decade the number of patients receiving treatment for end-stage renal disease (ESRD) has steadily increased, partly due to an increase in the rate of ESRD incidence [1,2]. An increase in diabetes and poorly controlled hypertension can only partly account for the increase. The role of other risk factors for progressive loss of renal function other than factors directly linked to kidneys may provide additional explanation. That these factors that are seemingly unrelated to the kidneys such as patients' physical characteristics, genetics, environment, race, education, socioeconomic status, drug dependence and health care utilization could have important implication for renal failure progression is not widely appreciated. After a terse remark on the role of angiotensin converting enzyme (ACE) inhibition in renal failure progression, this commentary will focus entirely on non-renal risk factors.

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