Megalin and cubilin in proximal tubule protein reabsorption: from experimental models to human disease

Rikke Nielsen[1], Erik Ilsø Christensen[1] and Henrik Birn[1,2]

[1]Department of Biomedicine, Aarhus University, Aarhus, Denmark; and [2]Department of Renal Medicine, Aarhus University Hospital, Aarhus, Denmark

Proximal tubule protein uptake is mediated by 2 receptors, megalin and cubilin. These receptors rescue a variety of filtered ligands, including biomarkers, essential vitamins, and hormones. Receptor gene knockout animal models have identified important functions of the receptors and have established their essential role in modulating urinary protein excretion. Rare genetic syndromes associated with dysfunction of these receptors have been identified and characterized, providing additional information on the importance of these receptors in humans. Using various disease models in combination with receptor gene knockout, the implications of receptor dysfunction in acute and chronic kidney injury have been explored and have pointed to potential new roles of these receptors. Based on data from animal models, this paper will review current knowledge on proximal tubule endocytic receptor function and regulation, and their role in renal development, protein reabsorption, albumin uptake, and normal renal physiology. These findings have implications for the pathophysiology and diagnosis of proteinuric renal diseases. We will examine the limitations of the different models and compare the findings to phenotypic observations in inherited human disorders associated with receptor dysfunction. Furthermore, evidence from receptor knockout mouse models as well as human observations suggesting a role of protein receptors for renal disease will be discussed in light of conditions such as chronic kidney disease, diabetes, and hypertension.

Kidney International (2016) **89,** 58–67; http://dx.doi.org/10.1016/j.kint.2015.11.007

KEYWORDS: albuminuria; endocytosis; Fanconi syndrome; proximal tubule

Correspondence: *Henrik Birn, Department of Biomedicine, Aarhus University, Wilhelm Meyers Allé 3 (building 1234), DK-8000 Aarhus, Denmark. E-mail: hb@ana.au.dk*

Received 11 June 2015; revised 17 August 2015; accepted 19 August 2015

The renal proximal tubule uptake of filtered proteins is essential for the tubular recovery of vitamins, hormones, enzymes, and drugs. By efficient uptake of virtually all filtered proteins, potentially biologically active substances are cleared from the ultrafiltrate, and consequently later segments are not exposed to these substances. The multiligand endocytic receptors megalin and cubilin have been identified as essential receptors in this process, each binding a variety of filtered proteins. The physiological and pathophysiological role of these receptors in the proximal tubule has been investigated by the use of knockout animals and cubilin-deficient dogs. Later, human allelic syndromes were identified, allowing reassessment of the findings obtained in the models. This, as well as observations in models of acquired renal disease, has suggested important pathophysiological implications of receptor dysfunction. In the present Review we will discuss data concerning the functional and developmental role of the receptors obtained from animal models of inherited and acquired receptor dysfunction and compare this with the findings in humans suffering from dysfunction of either of the receptors.

Megalin and cubilin

Megalin was identified as the autoantigen in Heymann nephritis in 1982[1] and later characterized as a large glycosylated receptor of 600 kDa (4655 amino acids) belonging to the low-density lipoprotein receptor family.[2–4] The extracellular domain of megalin consists of 4 clusters of cysteine-rich complement-type repeats, which are believed to be involved in ligand binding,[3–6] and a variety of ligands have been identified[7] (Table 1). Megalin contains a single transmembrane domain (23 amino acids), and the receptor holds an intracellular C-terminal cytoplasmic tail of 209 amino acids. The cytoplasmic domain of megalin regulates receptor trafficking and endocytosis. Two NPXY motifs gather adaptor proteins such as clathrin, AP-2, Dab2, and ARH, which are involved in coated pit formation.[8–11] Megalin is abundantly expressed in the apical membranes of the proximal tubule; more specifically, it is located in the brush border, endocytic vesicles, dense apical tubules (Figure 1), and to some degree in lysosomes.[1,12–14] Even though megalin initially was identified as the Heymann nephritis antigen in rats and has been localized and shown to be endocytically active in human podocytes,[15] its expression in the glomerulus of other species

Table 1 | Ligand table for megalin and cubilin

Megalin	Cubilin
Vitamin carrier proteins	***Vitamin carrier proteins***
Transcobalamin–vitamin B_{12}	Intrinsic factor vitamin B_{12}
Vitamin D–binding protein	Vitamin D–binding protein
Retinol-binding protein	
Folate-binding protein	
Other carrier proteins	***Other carrier proteins***
Albumin	Albumin
Myoglobin	Myoglobin
Hemoglobin	Hemoglobin
Lactoferrin	Transferrin
Selenoprotein P	
Metallothionein	
Neutrophil gelatinase–associated lipocalin	
Odorant-binding protein	
Transthyretin	
Liver-type fatty acid–binding protein	
Sex hormone–binding globulin	
Lipoproteins	***Lipoproteins***
Apolipoprotein B	Apolipoprotein A-I
Apolipoprotein E	High-density lipoprotein
Apolipoprotein J/clusterin	
Apolipoprotein H	
Apolipoprotein M	
Hormones and signaling proteins	***Hormones and signaling proteins***
Parathyroid hormone	Fibroblast growth factor
Insulin	
Epidermal growth factor	
Prolactin	
Thyroglobulin	
Sonic hedgehog protein	
Angiotensin II	
Leptin	
Bone morphogenic protein 4	
Connective tissue growth factor	
Insulin-like growth factor	
Survivin	
Enzymes and enzyme inhibitors	***Enzymes and enzyme inhibitors***
Plasminogen activator inhibitor type I	
Plasminogen activator inhibitor type I–urokinase	
Plasminogen activator inhibitor type I–tissue plasminogen activator	
Pro-urokinase	
Lipoprotein lipase	
Plasminogen	
α-Amylase	
Lysozyme	
Cathepsin B	
α-Galactosidase A	
Cystatin C	
Recombinant activated factor VIIa	Recombinant activated factor VIIa
Immune- and stress-related proteins	***Immune- and stress-related proteins***
Ig light chains	Ig light chains
Pancreatitis-associated protein 1	Clara cell secretory protein
α_1-Microglobulin	α_1-Microglobulin
β_2-Microglobulin	
Drugs and toxins	***Drugs and toxins***
Aminoglycosides	Aminoglycosides
Polymyxin B	
Aprotinin	
Trichosanthin	
Colistin	
Receptors	***Receptors***
Cubilin	Megalin
Transcobalamin II–B_{12} receptor	
Others	***Others***
Receptor-associated protein	Receptor-associated protein
Ca^{2+}	
Cytochrome C	
Seminal vesicle secretory protein II	
Coagulation factor VII	Coagulation factor VII
Coagulation factor VIII	

(Adapted with permission from Christensen EI, Birn H, Storm T, et al. Endocytic receptors in the renal proximal tubule. *Physiology (Bethesda)*. 2012;27:223–236.[7])

has been difficult to establish. Megalin is expressed in a number of extrarenal, absorptive epithelia such as the choroid plexus, the lung alveoli, the ciliary body and retinal pigment epithelium of the eye, the gall bladder, the placenta, the parathyroid glands, and the thyroid gland. Important physiological functions regulating organ development, lung function, and calcium homeostasis have been suggested (see below).[12,16–19]

Cubilin, identified as the target of teratogenic antibodies in 1992 and later as the intrinsic factor vitamin B_{12} receptor in 1982,[20,21] is coexpressed with megalin in the apical endocytic compartments of the proximal tubule,[20–22] and has also recently been demonstrated in rat and human podocytes.[23] Cubilin is a large 460-kDa glycosylated extracellular protein composed of 27 C-terminal CUB domains (complement C1r/C1s, Uegf [epidermal growth factor–related sea urchin protein], and bone morphogenic protein 1)[21,24–26] believed to be responsible for ligand binding.[27–30] The N-terminal part of cubilin consists of 8 epidermal growth factor repeats and a 110–amino acid stretch.[25,26]

Cubilin is an extracellular protein and interacts with other membrane proteins for membrane localization and endocytosis. In the proximal tubule it is believed to interact with megalin, forming a multireceptor complex with megalin driving internalization of the complex and bound ligands (Figure 1).[18,26,31,32]

For correct membrane localization cubilin also depends on the 38- to 50-kDa single transmembrane protein amnionless (AMN).[33,34] AMN is responsible for delivery of cubilin to the membrane, and if AMN is not present, cubilin is retained in intracellular structures.[32,33,35,36] Similarly, AMN is retained intracellularly if cubilin is absent.[31,37] Interactions between the EGF domains in cubilin and AMN are believed to be responsible for this interdependence.[33,38]

The spatial and temporal expression of the receptors during development is very similar, and both receptors are evident in the rodent blastocyst from the 8-cell stage with a cytoplasmic, vesicular appearance that changes to an apical localization in the trophectoderm at the 32-cell stage.[39] In the