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Characteristics of Mammalian Rh Glycoproteins (SLC42 transporters) and Their Role in Acid-Base Transport

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Abstract

The mammalian Rh glycoproteins belong to the solute transporter family SLC42 and include RhAG, present in red blood cells, and two non-erythroid members RhBG and RhCG that are expressed in various tissues, including kidney, liver, skin and the GI tract. The Rh proteins in the red blood cell form an "Rh complex" made up of one D-subunit, one CE-subunit and two RhAG subunits. The Rh complex has a well-known antigenic effect but also contributes to the stability of the red cell membrane. RhBG and RhCG are related to the NH_4^+ transporters of the yeast and bacteria but their exact function is yet to be determined. This review describes the expression and molecular properties of these membrane proteins and their potential role as NH_3/NH_4^+ and CO_2 transporters. The likelihood that these proteins transport gases such as CO_2 or NH_3 is novel and significant. The review also describes the physiological importance of these proteins and their relevance to human disease.

Keywords

SLC42; RhAG; RhBG; RhCG; NH_3; NH_4^+ Transport; CO_2 Transport

1. Overview

Rh glycoproteins

Rh glycoproteins, belonging to solute transporter family SLC42, have long been identified and studied in human blood cells for their immunogenic characteristics and importance in pregnancy (Avent and Reid, 2000). Table 1 summarizes some of the known properties of these membrane proteins. In red blood cells, the Rh antigens exist as a hetero oligomeric "Rh complex" of membrane polypeptides that include one D subunit, one CE subunit and two glycosylated ***RhAG*** **(SLC42A1)** subunits each with 12 transmembrane domains TM (Avent et al., 1996; Conroy et al., 2005; Ridgwell et al., 1992). RhAG has been expressed in heterologous systems (e.g. oocytes) independently of RhCE or D subunits. In addition to its antigenic property, the Rh complex is thought to contribute to the membrane stability and structure of red blood cell. Its exact function has not been determined.

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RhCG

Marini and co-workers made the original observation that human Rh antigens share sequence homology to the MEP NH_4^+ transporters of the yeast (Marini et al., 1997). A subsequent study, (Marini et al., 2000) identified a kidney homologue originally named RhGK (for Rh glycoprotein kidney) and later RhCG (SLC42A3). The RhCG protein shows close identity to RhAG (50%) and sequence homology (24%) with the MEP/Amt ammonium transporters. *RHCG* cDNA was amplified from human kidney RNA and successfully expressed in yeast cells (Marini et al., 2000). Liu et al., (Liu et al., 2000) subsequently cloned human (*RHCG*) and mouse (*Rhcg*) homologues with cDNAs sequences of 1952 and 2097 bp respectively and overall identity of 68.8%. At the protein level, *RHCG* encodes a 53 KDa, 479 amino acid polypeptide, whereas *Rhcg* encodes a 55 KDa, 498 amino acid polypeptide and they are highly conserved (77.2% identity and 90.4% similarity). Hydropathy analysis indicated that they share identical topology of 12 transmembrane domains with intracellular N and C termini (Fig 1). Their 12 TM structure has conserved regions shared with other Rh homologues, especially RhAG, but differ from RhAG with a much-elongated C – terminal and a unique N – terminal. *RHCG* was mapped to the 15q25 chromosome whereas *Rhcg* was mapped by linkage analysis to a locus (D7Xrf229) on the long arm of chromosome 7.

RhBG

Cloning and biochemical characteristics of Rh type B glycoprotein (SLC42A2) were initially accomplished by Liu et al., (Liu et al., 2001). *RHBG* and *Rhbg* have open reading frames of 1377 and 1368 bp respectively, which encode polypeptides of 458 and 455 amino acids respectively. Human and mouse RhBG are 85% identical and 94% similar at the protein level, less similar to RhCG (58% - 53%) and notably different from RhAG (43%). Both proteins have a molecular mass of 49.3 KDa, are negatively charged at physiological pH and have a single N-glycosylation motif. Like RhCG and RhAG, RhBG is a polytopic protein with 12 predicted trans-membrane domains. *RHBG* resides at 1q21.3 of human chromosome 1 and *Rhbg* is on mouse chromosome 3 on a site where many markers are similar to those of human 1q21 containing *RHBG*. The Rh proteins are closely related to the ammonia transporters family (Amt) and seem to have branched off from Amt ancestors in prokaryotes (Huang and Ye, 2010). The two families show different patterns of distribution but they also coexist in a variety of organisms indicating a divergent and independent evolution. Even in organisms that have Amt and Rh proteins their genes cluster independently suggesting a functional distinction between the two families.

Transport of NH_3/NH_4^+

The original link between Rh proteins and NH_3/NH_4^+ transport was provided by studies (Marini et al., 2000) on yeast mutants, termed (Triple-MEPΔ), rendered incapable of NH_4^+ transport by deletions of 3 endogenous NH_4^+-transporter genes. Yeast, like many other fungal species as well as bacteria, efficiently scavenge NH_4^+ which is required as a nitrogen source, without which growth is greatly hampered. Expressing RhAG enabled the Triple-MEPΔ cells to grow in low NH_4^+ medium and resulted in resistance to toxic concentrations of methyl ammonium suggesting a role in NH_4^+ transport. Although the evidence for NH_4^+ transport by Rh proteins is still actively debated, it is intriguing that this class of membrane proteins may be the elusive NH_4^+ transporter. This is very important in tissues, such as the kidney where transport of NH_3/NH_4^+ is critical for regulation of acid-base balance (Heitman and Agre, 2000).

In mammals, normal acid-base homeostasis is critically dependent on renal excretion of NH_4^+ in the urine (Knepper et al., 1989). Renal ammoniagenesis and NH_4^+ transport are highly regulated and NH_4^+ excretion increases several fold during chronic acidosis

(Knepper et al., 1991). In physiological fluids (pH 7.4 – 7.5), NH_3/NH_4^+ exists predominantly as NH_4^+ (~99%) because the pKa of the equilibrium reaction $NH_3+H^+ \leftrightarrow NH_4^+$ is high (~9.2). Whereas NH_3, a small neutral molecule, is assumed to mainly diffuse through the cell membrane, NH_4^+ has to be transported by membrane transporters or channels (Attmane-Elakeb et al., 2001; Good et al., 1984; Knepper et al., 1989). Some K^+ transport pathways may serve as NH_4^+ carriers because of the similarity of the two ions in size and charge.

The renal proximal tubule generates NH_3/NH_4^+ which is then secreted predominantly into the luminal fluid (Hamm and Simon, 1990). This occurs by both NH_3 diffusion into the more acid luminal fluid and by NH_4^+ transport in exchange for sodium on the Na^+- H^+ exchanger (Knepper et al., 1989). In the loop of Henle, total ammonia may be secreted into the descending limb of Henle but is then reabsorbed in the thick ascending limb (Good and Knepper, 1985; Knepper et al., 1989). NH_4^+ is reabsorbed from the lumen into the cell via substitution for K^+ on both the Na-K-2Cl co-transporter and the apical membrane potassium channel (see (Attmane-Elakeb et al., 2001; Good et al., 1984). In the collecting duct, total ammonia is secreted along the length of the tubule. Most, if not all, of this secretion has been thought to occur by non-ionic diffusion of NH_3 driven by the progressively increasing concentrations of ammonia in the medullary interstitium (Hamm, 1986). Concurrent acid secretion along the length of the collecting duct (by H-ATPase for example) keeps luminal concentrations of NH_3 low, maintaining a favorable NH_3 gradient from interstitium to lumen. The apical membrane of collecting duct cells is one site where rate-limiting rapid NH_3 diffusion has to occur. The basolateral membrane, on the other hand, is the site where NH_4^+ transport occurs presumably by one or more membrane transporters (Hamm et al., 1985; Knepper et al., 1989).

2. Expression of Rh Glycoproteins

In red blood cells, ***RhAG*** is expressed in the membrane as a component of the “Rh complex”, as mentioned above. The “Rh Complex” consists of RhAG in association with the nonglycosylated Rh proteins RhD and RhCE in humans or with Rh30 in non-human mammals. RhAG is an erythrocyte-specific protein not found in other tissues (Cartron, 1999; Liu and Huang, 1999). Expression in heterologous systems showed that RhAG was fully glycosylated and properly trafficked to the plasma membranes of oocytes (Westhoff et al., 2002), yeast cells (Marini et al., 2000) and HeLa cells (Benjelloun et al., 2005). Cell surface expression of the “Rh complex” was shown to be linked to RhAG interaction with spectrin-based ankyrin (Nicolas et al., 2006). It was also implied that in red blood cells (RBC) Rh protein, Band-3 and ankyrin form an integral membrane complex that may modulate transport of HCO_3^- and possibly NH_4^+ (Nicolas et al., 2006).

Expression of ***the RHCG and Rhcg*** **genes** has been assessed by Northern blot analysis, in situ hybridization and immunohistochemistry (Eladari et al., 2002; Liu et al., 2000; Verlander et al., 2003). These studies indicated that in human adult tissues RhCG was abundantly expressed in the kidneys, brain, testis, placenta, skeletal muscle, liver, GI tract, pancreas, and prostate (Handlogten et al., 2005; Liu et al., 2000; Marini et al., 2000; Weiner et al., 2003). In human fetal tissues, RhCG was expressed only in the kidney. In mouse adult tissues, RhCG was expressed in kidney, testis, liver and other tissues. Using RT – PCR on rat micro dissected tubules, RhCG was found in distal convoluted tubules, the connecting segment (CNT), cortical collecting duct (CCD) and outer medullary collecting duct (OMCD) but not the proximal tubule and the thick ascending limb of Henle’s loop. Immuno-localization studies showed labeling of the apical membrane of cells within the cortex, outer medulla and upper portion of inner medulla. RhCG was present in all CCD cells and the intercalated cells of OMCD and IMCD (inner medullary collecting duct).

Another study, however, indicated that RhCG is restricted to the intercalated cells of the CCD cells (Eladari et al., 2002). RhCG was also present in the principal cells of the outer stripe of OMCD. The subcellular distribution of RhCG seemed to differ depending on tissue and species. In the mouse kidney, RhCG was reported to be at the apical membrane (Eladari et al., 2002; Verlander et al., 2003) whereas in the rat RhCG was reported to be either exclusively apical (Eladari et al., 2002) or at both apical and basolateral membranes (Seshadri et al., 2006). In the human kidney, RhCG semed to be restricted to the apical membrane (Eladari et al., 2002; Verlander et al., 2003) but another study reported basolateral expression as well (Han et al., 2006).

RhBG expression was confirmed in non-erythroid tissues only. RhBG was expressed in kidney, skin, sweat glands, GI tract (from duodenum to the colon), liver and, to a lesser extent, ovaries. Using immunohistochemistry in mouse kidney, Verlander et al., (Verlander et al., 2003), localized RhBG to the majority of cells of the connecting segment (CNT) and the cortical collecting duct (CCD). In the OMCD and IMCD, only a subpopulation of cells was labeled. Co-localization studies with carbonic anhydrase II, Na-Cl co-transporter and the anion-exchanger (AE1) demonstrated that RhBG was expressed in all CNT cells and in principal cells of CCD. In CCD, RhBG was also labeled in A-type intercalated cells but not B-type intercalated cells. In OMCD and IMCD only intercalated cells exhibited immunoreactivity to RhBG (Quentin et al., 2003; Verlander et al., 2003). It was demonstrated by double immunostaining that RhCG and RhBG were expressed in the same cell but with distinct apical and basolateral localizations, suggesting different transport properties of the two homologous membrane proteins. In a study on mouse liver, RhBG was localized to the basolateral membrane of hepatocytes surrounding central veins (perivenous) but not in periportal or midzonal hepatocytes (Weiner et al., 2003). RhBG expression has been found to be exclusively restricted to the basolateral membrane of CCD cells (Weiner et al., 2003).

3. Molecular Structure of Rh glycoproteins

An important advancement in the field has been the crystallographic structure of AmtB, the bacterial homologue of Rh (Khademi et al., 2004; Li et al., 2006). The high resolution crystals indicate a trimeric complex of subunits made up of 11 TM helices, each forming a central channel where substrate transport presumably occurs. The crystals showed no conformational changes in the presence or absence of NH_4Cl. The proposed tertiary structure indicates that the individual pore of each monomer is composed of an extracellular vestibule followed by a narrow (1.2 A°) and long pore (20 A°) which is terminated at the cytoplasmic side by a wider vestibule. The upper vestibule is lined by aromatic residues that recruit NH_4^+. The central hydrophobic pore has 2 conserved His residues that promote hydrophobic conduction of substrate. In the absence of resolved structure of RhBG or RhCG the AmtB crystal structure serves as the closest and best model for the Rh protein.

Based on analysis of the 3-dimensional structure of AmtB, a novel transport model was proposed (Fig 2). In AmtB, the upper vestibule recruits NH_4^+ by a cation - π interactions provided by the residues Trp 148 and Ser 219. As NH_4^+ moves toward the hydrophobic pore, it gives up its proton predominately on the side of entry (extracellularly) and NH_3 is transported through the pore. NH_3 in the pore is stabilized by two highly conserved histidines (H168 and H318), which interact with NH_3 at 3 specific locations in the pore. On the cytoplasmic side, NH_3 acquires an intracellular H^+ to re-equilibrate with NH_4^+.

The degress to which the tertiary structure of Amt B resembles that of Rh glycoproteins remains an open question. Constructing homology models for Rh proteins based on AmtB structure must take into account critical differences in sequences that render proper

alignment tricky. Among these differences are putative 12 TM domains in Rh proteins compared to 11 TM domains in Amt B; a long intracellulara C-Terminal α-helix in Rh proteins; and altered critical amino acid residues at sites that that are thought to be key for transport function (Huang and Ye; Lupo et al., 2007). Nevertheless, in the absence of resolved crystal structures or RhCG or RhBG, the structure of AmtB is one of the best models to determine structural determinants of function. Initial studies based on this approach point to important characteristics of function including: a role of C-terminal in gating function and potential binding partners to Rh proteins among cellular proteins (e.g. carbonic anhydrase) and other membrane proteins.

4. Function of Rh Glycoproteins

The role of Rh proteins as NH_4^+ transporters

Most studies provide strong evidence for a role of the Rh proteins in NH_3/NH_4^+ transport. The initial studies to characterize the functions of ***RhAG*** were done in yeast mutant cells deficient in NH_4^+ transporter genes (Triple mepΔ). Triple mepΔ cells expressing RhAG were able to grow in media containing very low concentrations of NH_4^+ (Marini et al., 2000). Another study reported that RhAG expression in yeast cells resulted in resistance to toxic concentrations of methyl ammonium (200mM). Also the rate of extracellular NH_4^+ accumulation was higher in cells expressing RhAG (or RhGK) suggesting that RhAG promotes efflux of NH_4^+. Measurement of methyl-amine uptake in oocytes expressing RhAG suggested that RhAG serves as an electro neutral counter-transporter of methyl amine (and NH_4^+) coupled to H^+ exit from the cell (Westhoff et al., 2002). As such RhAG would result in transport of net NH_3 equivalents. At least one study on HeLa cells suggested that RhAG transports NH_3 and NH_4^+ (Benjelloun et al., 2005). However, RhAG is an erythrocyte membrane protein and its contribution to NH_4^+ transport in other tissues is unlikely.

Several studies on ***RhBG*** expressed in oocytes or mammalian cells report that it can transport NH_3/NH_4^+. However different results are reported by different labs. Some studies report that NH_4^+ transport is electroneutral and coupled to the H^+ gradient and behaving as a NH_4^+- H^+ exchanger (Ludewig, 2004; Mak et al., 2006; Zidi-Yahiaoui et al., 2005). Recent studies indicate that RhBG mediates electrogenic NH_4^+ transport and that MA/MA^+ is transported differently than NH_3/NH_4^+ (Nakhoul et al.; Nakhoul et al., 2005; Nakhoul et al., 2006). The reported affinity of RhBG to NH_4^+ is 2–4 mM. This concentration is within reported values of interstitial NH_4^+ concentrations in the renal medulla which vary between 2.5 and 9 mM and is considerably increased in acidosis. In the kidney, the expression of RhBG at the basolateral membrane and the electrochemical gradient for NH_4^+, are consistent with electrogenic influx of NH_4^+ although electroneutral NH_3 transport may still occur.

Similar uncertainties about ***RhCG*** also persist. Some of the earlier studies did confirm a role in NH_3/NH_4^+ transport. However the questions of electrogenic NH_4^+ transport (Nakhoul et al., 2005), or electroneutral NH_4^+- H^+ exchange (Ludewig, 2004) or simultaneous NH_3 and NH_4^+ transport (Bakouh et al., 2004) are not yet resolved. Studies on reconstituted RhCG in liposomes demonstrated increased NH_3 permeability but had no effect on NH_4^+ permeability (Mouro-Chanteloup et al.). Surface pH measurements in oocytes expressing RhCG indicate transport of NH_3 (Musa-Aziz et al., 2009). The presence of RhCG at the apical membrane of the collecting duct, where electroneutral NH_3/NH_4^+ transport is likely, seems to indicate that the mode of transport by RhCG may be different from that of basolateral RhBG.

The role of Rh glycoproteins as gas channels

Historically it was assumed that gases such as CO_2 and NH_3 cross cell membranes by diffusing through the lipid bilayer. However the discovery that some membranes have low permeability to NH_3 or CO_2 challenged this view (Kikeri et al., 1989; Singh et al., 1995; Waisbren et al., 1994) and suggested that specific membrane proteins may facilitate transport of these gases. Rh and related proteins were investigated as potential transporters of CO_2 or NH_3.

Studies on yeast demonstrated dependence of yeast growth in NH_3/NH_4^+ deficient media on pH and concluded that MEP proteins facilitate diffusion of NH_3 and not NH_4^+(Soupene et al., 2001). Other studies of *E. coli* also indicated facilitated NH_3 diffusion by AmtB (Soupene et al., 2002). It was also suggested that RhAG mediates facilitated transport of NH_3 (Ripoche et al., 2004) leading to trapping of NH_4^+ at higher concentrations in RBC than in plasma.

Other evidence indicates that Rh proteins transport CO_2 (Kaplan et al., 2004; Soupene et al., 2004). Direct studies of CO_2/HCO_3^- transport in RBC indicated that DIDS markedly decreased transport of CO_2 and suggested that Rh and Band-3 may be coupled functionally. Another study on CO_2 permeability in normal and Rh_{null} RBC concluded that CO_2 permeation is mediated in part by Rh/RhAG and by AQP1 (Endeward et al., 2006). Another study demonstrated that expressing RhCG in oocytes increased CO_2 permeability compared to H_2O-injected oocytes (Bakouh et al., 2006). A recent study proposed that NH_3 moves through the monomeric pores of AmtB and RhAG whereas CO_2 could move through the central pores of the trimeric structure (Musa-Aziz et al., 2009). Expressing RhCG in oocytes seemed to enhance CO_2 transport (Bakouh et al., 2006). A possible role of Rh glycoproteins in transport of gases such as CO_2 and NH_3 is physiologically critical and studies to resolve the identity of the transported substrate (CO_2, NH_3 or NH_4^+) are needed.

Substrate specificity and pH dependence

Candidate substrates that can be transported by Rh proteins include NH_4^+ (Liu et al., 2000; Ludewig, 2004; Marini et al., 2000); NH_3 (Palkova et al., 1997), CO_2 (Kustu and Inwood, 2006) or methyl ammonium (Ludewig, 2004; Mak et al., 2006). Methyl amine hydrochloride is often used instead of ammonium chloride because of the similarity of the molecules and because it can be easily radiolabeled. ***The pH sensitivity*** of murine RhCG and other Rh glycoproteins is important for several reasons. First, as putative NH_4^+ transporters, a change in pH will shift the equilibrium of NH_3 to NH_4^+ and thus may affect transport of NH_4^+. Second, it has been proposed that murine RhCG may function as an NH_4^+-H^+ exchanger that is driven by the H^+ gradient (Ludewig, 2004; Mak et al., 2006). (Note that functionally, NH_4^+-H^+ exchange is equivalent to NH_3 transport, a notable feature complicating many studies of total ammonia transport. However, NH_4^+-H^+ exchange and NH_3 transport are not the same and will respond differently to physiologic stimuli.) Therefore, a pH change will also affect transport of NH_4^+ by RhCG. Third, pH sensitivity of these transporters is probably highly significant in acid-base disturbances where renal RhBG and RhCG, for example, may play an important role in the adaptive response to acidosis. Finally, based on the crystal structure of AmtB, a model of NH_3/NH_4^+ transport is proposed where deprotonation of NH_4^+ is critical for NH_3 conduction through the pore. This step is also pH sensitive. A recent study showed that NH_4^+ transport by RhBG in mice was activated by raising extracellular pH but was completely inhibited by decreasing extracellular or intracellular pH (Nakhoul et al.).

5. Physiological Significance and Relevance to Human Diseases

Rh and related proteins are highly expressed in mammalian tissues and primitive species. This suggests that Rh proteins are evolutionarily preserved under high selective pressure, yet their biological function remains poorly defined. Of significance, yet not fully understood, are the following observations: The erythroid RhAG is needed for cell surface expression of the "Rh complex" but it may also be involved in NH_3 or CO_2 transport. The non-erythroid RhBG and RhCG are expressed in tissues that transport or metabolize NH_3/NH_4^+ and their renal distribution correlates well with NH_4^+ excretion. However, it was proposed (Li et al., 2007) that they could be linked to other membrane proteins to form a "transport complex" and may transport CO_2. It was also suggested that RhBG and RhCG may represent a new class of NH_4^+ sensing receptors or "ammonium sensors" acting as mediators to regulate different cellular processes (Van Kim et al., 2006).

The physiological roles of RhBG and RhCG in renal NH_3/NH_4^+ handling are becoming more evident. Studies on RhCG in mice indicated that chronic metabolic acidosis increased RhCG protein expression in the medullary CD (Seshadri et al., 2006) and that the increase was in both intercalated cells and the principal cells (Seshadri et al., 2006). The same study indicated an increase in apical and basolateral RhCG expression and a decrease in cytoplasmic RhCG expression. Metabolic studies demonstrated that mice lacking RhCG had impaired urinary NH_4^+ excretion in response to acid loads (Biver et al., 2008). The same study concluded that *Rhcg* knockout mice had reduced apical NH_3 permeability and transepithelial NH_3/NH_4^+ transport. A recent study on mice with renal collecting duct-specific Rhcg deletion (CD-KO) (Lee et al., 2009) showed that under basal conditions urinary NH_4^+ excretion was less in KO mice than in control mice and that after acid loading CD-KO mice developed more severe metabolic acidosis than controls.

Studies on RhBG showed that genetic deletion of pendrin, an apical Cl^--HCO_3^-, decreased RhBG expression. Although earlier studies on *Rhbg* knockout mice (Chambrey et al., 2005) did not elicit abnormal acid-base balance or ammonium handling, later studies proved differently. Recently, Bishop et al., (Bishop, 2009) generated intercalated cell-specific *Rhbg* KO mice and demonstrated that urinary ammonium excretion was significantly less in KO mice vs controls. The same study showed that in control mice, HCl-induced acidosis increased RhBG protein expression significantly in three days.

Most studies provide strong evidence for a role of the Rh proteins in NH_3/NH_4^+ transport. Renal NH_4^+ excretion is critical for acid-base homeostasis and the mechanism includes NH_3 and NH_4^+ transport components. Yet simple diffusion of NH_3 is limited and NH_4^+-specific transporters had not been known to fully account for the significant renal NH_3/NH_4^+ transport. As shown in this figure (Fig 3), it is likely that RhBG and RhCG, working in tandem, may actually function as the elusive NH_4^+ -specific transporters and/or gas channels for NH_3 to explain how the distal nephron achieves transepithelial NH_3/NH_4^+ transport. Characterizing the functions of these proteins is essential to explain these novel mechanisms and will shed light on understanding acid-base homeostasis and its regulation by the mammalian kidney.

In relevance to human diseases, the association of Rh proteins with red blood cell (RBC) disorders is well established (Huang et al., 2000; Van Kim et al., 2006). Mutations in the RH locus leading to complete absence of RBC RH antigens cause Rh null syndrome (Cherif-Zahar et al., 1998; Huang et al., 1998). Dominant over-hydrated hereditary stomatocytosis (OHSt) or Rh deficiency syndrome is caused by mutations of *RHAG* (Cherif-Zahar et al., 1996; Huang and Ye). OHSt is reported to cause increased permeability of RBC to monovalent cations. A recent study (Stewart et al.) reported a heterozygous RhAG missense

mutation (F65S) in patients with OHSt that caused loss of function of RhAG for amine transport (NH_3/NH_4^+ and MA/MA^+). The physiological importance of RhBG and RhCG are mostly evident in KO mice studies as described above. These studies suggest that loss of function mutation, as shown for *Rhcg*, may cause distal renal acidosis and male infertility (Biver et al., 2008). Moreover, RhBG and RhCG have been suggested to act as tumor suppressor factors given their down-regulation in human esophageal cancers (Chen et al., 2002) and mouse brain tumors (Johansson et al., 2004).

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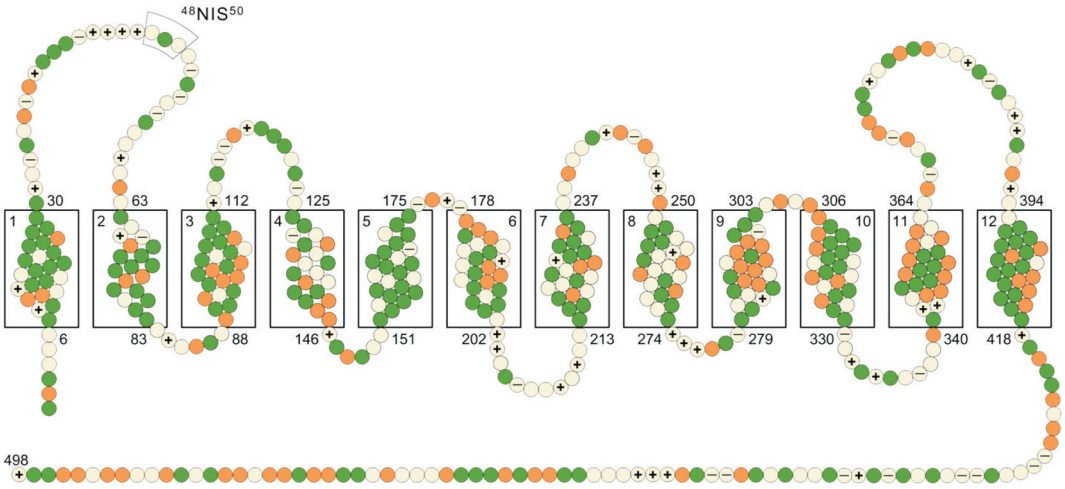

Figure 1. A model of the membrane topology of Mus musculus RhCG

Solid green circles denote the hydrophobic amino acids Phe, Ile, Leu, Met, Val and Trp; orange circles, Gly, Ala and Pro; light yellow circles, polar residues Ser, Cys, Thr, Asn, Gln and Tyr; cicrles marked with + denote the positively charged Lys, Arg and His; and −, negatively charged Asp and Glu. The probability of the location of the transmembrane sequences was predicted by the N-best algorithm (Krogh et al., 2001). The N-glycosylation site (^{48}NIS^{50}), present in the first e4xoloop, is illustrated.

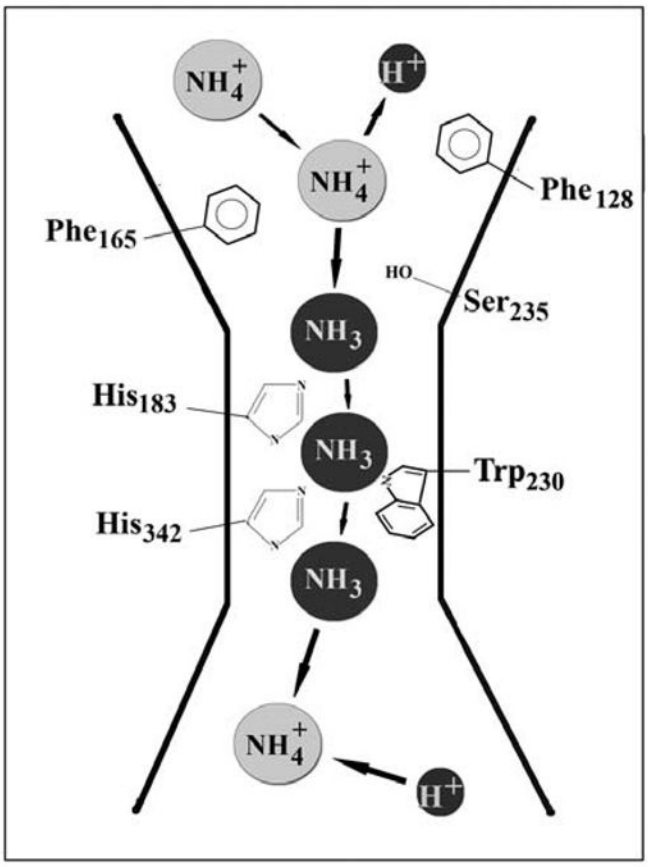

Figure 2. Proposed architecture of the ammonium pore of RhBG based on the crystal structure of AmtB

The model shows the equivalent amino acid residues important for transport of NH_3/NH_4^+ based on alignment of RhBG and AmtB sequences.

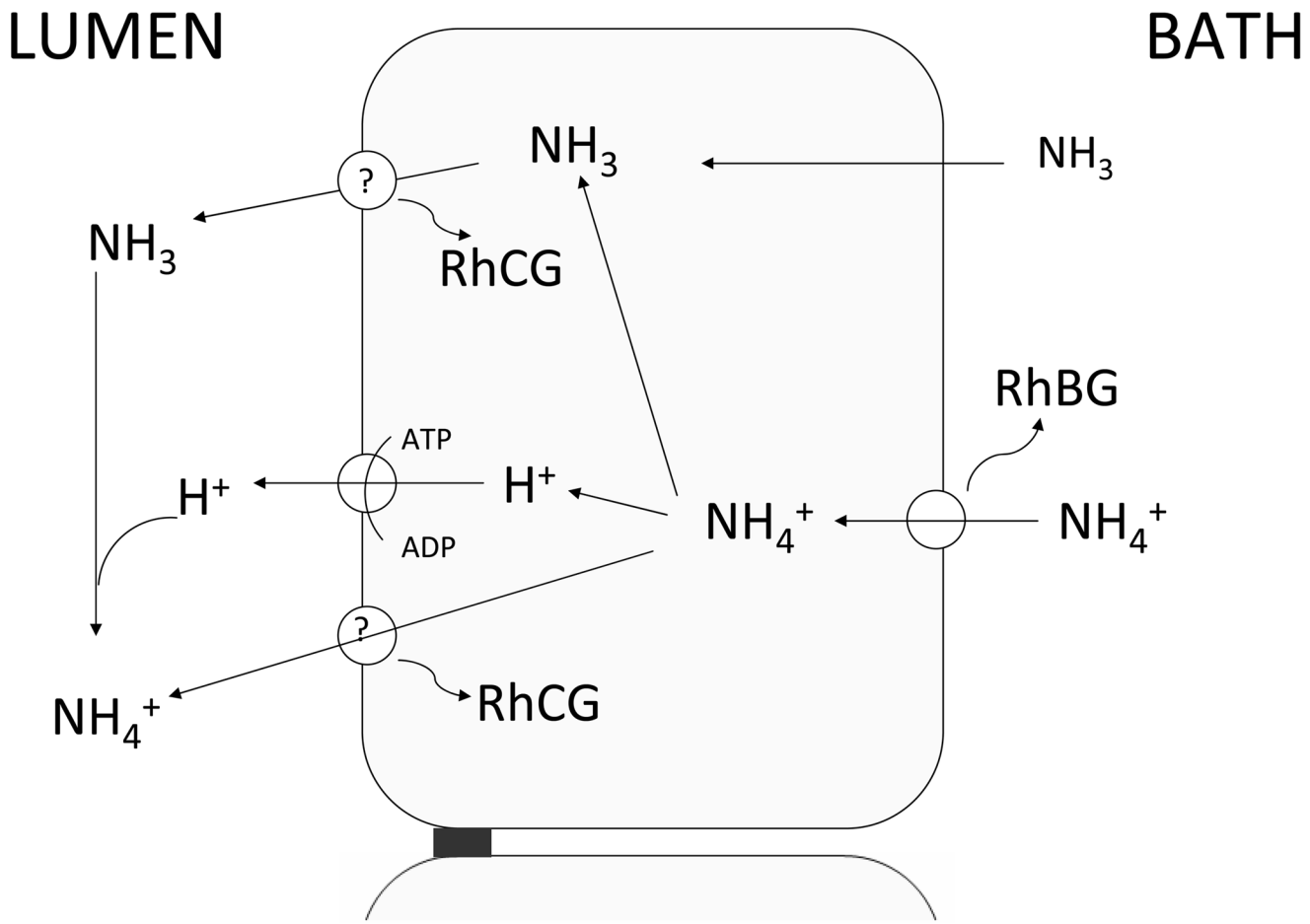

Figure 3. Schematic diagram of a medullary collecting duct cell indicating pathways of NH_3 and NH_4^+ transport

Transporters labeled RhBG and RhCG show the membrane location and putative role of these proteins in transport of NH_3 or NH_4^+.

Table 1

SLC42 - The Human Rh Ammonium Transporter Family

Human Gene Symbol	SLC Symbol	Protein Name	ORF (aa)	Predominent Substrates	Transport type Coupling ions	Tissue distribution & cellular/ subcellular expression	Link to disease	Human gene locus	Sequence Accession ID	Splice Variants and their features
RHAG (Rh50A)	SLC42A1	RhAG	409	NH_4^+, NH_3^+	H^+	Red blood cells, (cell membrane)	Rh_{null}-regulator $Rh_{mod,}$ OHSt *	6p12.3	AF031548	
RHBG	SLC42A2	RhBG	458	NH_4^+, NH_3, Methyl amine Methyl ammonium	Electrogenic No coupled ions	Kidney, Liver, skin, GI tract sweat glands, ovaries (basolateral membrane)	Unknown	1q21.3	AF193807	5 isoforms: Q9H310-1 Q9H310-2 Q9H310-3 Q9H310-4 Q9H310-5 (No experimental confirmation)
RHCG (RhGK)	SLC42A3	RhCG	479	NH_4^+, NH_3	Electroneutral possibly H^+	Kidney (apical membrane) Brain, Testis Placents, Pancreas, Prostate	Renal distal tubular acidosis (?)	15q25	AF193809	Unknown

* OHSt: Dominant over-hydrated hereditary stomatocytosis