

# Expression of Rh Glycoproteins in the Mammalian Kidney

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Ammonia metabolism is a fundamental process in the maintenance of life in all living organisms. Recent studies have identified ammonia transporter family proteins in yeast (Mep), plants (Amt), and mammals (Rh glycoproteins). In mammalian kidneys, where ammonia metabolism and transport are critically important for the regulation of systemic acid-base homeostasis, basolateral Rh B glycoprotein and apical/basolateral Rh C glycoprotein are expressed along the distal nephron segments. Data from experimental animal models and knockout mice suggest that the Rh glycoproteins appear to mediate important roles in urinary ammonia excretion.

**Key Words :** ammonia; kidney tubules, collecting; kidney

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## Introduction

Renal ammonia metabolism and transport are critically important for the regulation of systemic acid-base homeostasis<sup>1-5</sup>. Ammonia exists in two molecular forms, NH<sub>3</sub> gas and NH<sub>4</sub><sup>+</sup> ion. It has traditionally been thought that NH<sub>3</sub> crosses plasma membranes via lipid diffusion and that NH<sub>4</sub><sup>+</sup> requires specific transporter proteins<sup>3-5</sup>. Recent studies have provided new insights into the mechanisms of renal ammonia transport. The traditional theory of simple NH<sub>3</sub> diffusion has been replaced by a regulated model of NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup> transport. Indeed, when dissolved in water, ammonia exists predominantly as the NH<sub>4</sub><sup>+</sup> ion with a pKa of about 9 under physiological conditions<sup>6</sup>. Moreover, there is an increasing amount of evidence that even move-

ment of gaseous molecules such as CO<sub>2</sub> and O<sub>2</sub> requires specific proteins to accelerate the transport where the diffusion is too slow for physiological needs<sup>7, 8</sup>. In the kidney, it has been known that at least two ammonia transporter proteins are expressed.

## Discovery of ammonia transporter family from yeast and plant

Ammonia is an important molecular form of nitrogen, and mediates a key role in nitrogen nutrition for plants and soil microorganisms. It is therefore not surprising that botany-related research discovered the first ammonia-specific transporters. In 1994, simultaneous studies by Marini et al.<sup>9</sup> and Ninnemann et al.<sup>10</sup> cloned the Mep1 gene encoding an ammonium transporter in yeast, *Saccharomyces cerevisiae* and from plants, Amt1, respectively. Both Mep and Amt mediated high affinity ammonium transport activity when expressed in yeast deficient in endogenous ammonium transporters<sup>9, 10</sup>. Their transport activity was insensitive to other cations including sodium and potassium. Subsequent studies identified multiple additional members of the ammonia transporter family. Yeasts have at least

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three members, Mep1, Mep2 and Mep3, and plants have at least two members, Amt1 and Amt2.

### **Rh glycoproteins are mammalian ammonia transporter family members**

The Rh blood group, named after the Rhesus monkey, is very important in the field of transfusion medicine. There are two Rh genes, RHD and RHCE, and numerous genetic rearrangements between them produce a lot of distinct antigens, up to 49. Unlike most cell surface molecules, the RhD and RhCE are not glycosylated but they are closely associated with a glycosylated protein with a previously unknown function, Rh-associated glycoprotein (RhAG).

The first suggestion that Rh glycoproteins might be ammonia transporters was the findings by Marini et al. that the erythroid Rh antigen subunit, RhAG, and a kidney homologue, which they termed Rh glycoprotein-kidney (RhGK), have similar secondary structure to the yeast and plant ammonia transporters and could promote ammonium transport in yeast<sup>11, 12</sup>. In 2000, a separate group (Li et al.)<sup>13</sup> cloned a novel Rh glycoprotein homologue, Rh C glycoprotein, from both human (RhCG) and mouse (Rhcg), which they reported to be predominantly expressed in the kidney, central nervous system and testis. RhGK and RhCG/Rhcg are now known to be identical and RhCG/Rhcg is the term preferred by most investigators. The following year, Li et al., identified another new member of mammalian ammonia transporter family, Rh B glycoprotein (RhBG/Rhbg)<sup>14</sup>. In mammals, three ammonia transporter family members, RhAG/Rhag, RhBG/Rhbg, and RhCG/Rhcg have been identified so far.

### **Molecular structure of ammonia transporter family members**

As Hidden Markov Modeling techniques predicted, the atomic structures of the ammonia transporter family showed that they are integral proteins crossing the membrane 11 times. Khademi et al. resolved the crystallographic structure of a bacterial ammonia transporter, AmtB, at 1.35 Å resolution and revealed that they form symmetric trimers

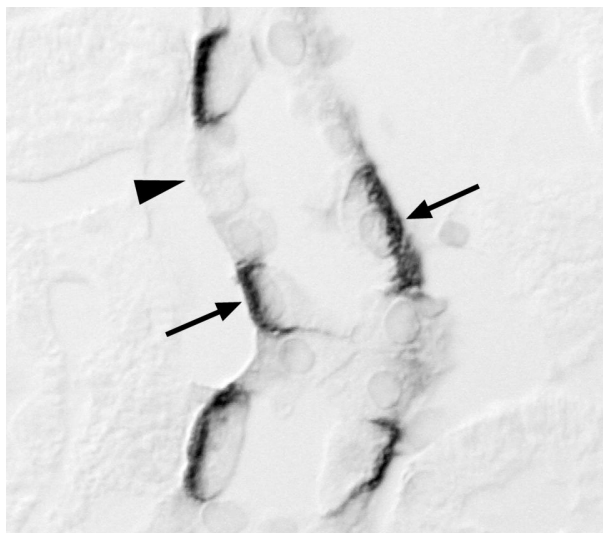
in the membrane<sup>15</sup>. This report identified that AmtB has the same structure in both the absence and presence of ammonia or methylammonia, suggesting that it may be a channel rather than a transporter. However, Zheng et al.<sup>16</sup> reported that slow conformational changes were needed for transient pore opening of AmtB and that its transport rate was rather typical for transporters and far below that of open channels and thus they concluded that AmtB was a transporter, not a channel. At the two ends of the pore, AmtB has recruitment vestibules for  $\text{NH}_4^+$ . These recruitment sites are able to change the pKa of the ammonia buffer reaction, resulting in preferential conversion of  $\text{NH}_4^+$  to  $\text{NH}_3$  and  $\text{H}^+$ . The  $\text{NH}_3$  appears to traverse the protein through a narrow hydrophobic element which allows only the passage of  $\text{NH}_3$  but not the  $\text{NH}_4^+$  ion. Thus, Khademi et al. concluded that AmtB might not mediate the net transfer of protons and not directly alter the membrane potential, although several previous studies showed that ammonia translocation is affected by pH and voltage gradients<sup>4, 5, 9</sup>. Whether other ammonia transporter family members mediate electrogenic ammonia transport or electroneutral  $\text{NH}_4^+/\text{H}^+$  ion exchange remains an active area of investigation. Some bacterial ammonia transporter family members have been shown to mediate electrogenic  $\text{NH}_4^+$  transport. Whether this involves parallel  $\text{NH}_3$  and  $\text{H}^+$  transport or separate  $\text{NH}_4^+$  transport has not been determined.

### **RhAG/Rhag is not expressed in the kidney**

To date, no studies have reported that RhAG/Rhag is expressed in renal tubular epithelial cells. In particular, there is no evidence of RhAG/Rhag expression in the kidney<sup>5</sup>.

### **Basolateral Rhbg is expressed in the mouse and rat kidney**

In the mouse and rat kidney, Rhbg is expressed in the basolateral membrane throughout the distal tubular epithelium (Fig. 1)<sup>17-22</sup>. In 2003, Quentin et al. microdissected nephron segments and performed RT-PCR experiments<sup>19</sup>. The expression level of Rhbg mRNA was highest in the connecting tubule (CNT), cortical collecting duct (CCD),



**Fig. 1.** Basolateral Rh B glycoprotein (Rhbg) immunoreactivity is present in the majority of cortical collecting duct cells (arrows) but is absent in occasional cells (arrowhead).

and outer medullary collecting duct (OMCD) and lowest in distal convoluted tubule (DCT). In both the mouse and rat kidney, Rhbg appeared to be expressed in all cells within the CNT. In the CCD, cells exhibited two distinct patterns of labeling; the principal cells had thin or no basolateral staining, whereas the intercalated cells had more intense basolateral staining. The number of stained cells fell sharply in the inner medulla and none was found in deeper regions of the inner medulla<sup>19</sup>.

The same year, a separate group (Verlander et al.) published a more detailed study in the mouse kidney<sup>22</sup>. The cellular distribution of Rhbg was similar with the report of Quentin et al.. However, Verlander et al. demonstrated that a subpopulation of intercalated cells do not express detectable Rhbg immunoreactivity. Colocalization with pendrin, an apical anion exchanger present in type B and non-A-non-B intercalated cells, revealed that CNT pendrin-positive cells (non-A-non-B intercalated cells) typically exhibited basolateral Rhbg immunoreactivity, whereas CCD pendrin-positive cells (type B intercalated cell) were negative for Rhbg. Subsequent studies also confirmed similar findings in the rat kidney.



**Fig. 2.** Rh C glycoprotein (Rhcg) immunoreactivity is observed in both apical and basolateral membrane (arrow) in the rat cortical collecting duct.

### **RhBG is not expressed at detectable levels in the human kidney**

The distribution pattern of RhBG has not been established in the human kidney. Brown et al., reported, despite RhBG mRNA expression, that they could not detect RhBG protein expression in human tissue lysates or tissue sections, although the same antibodies detected Rhbg in rat tissues<sup>23</sup>. Whether this indicates a lack of RhBG protein expression or unexpected lack of antibody-antigen recognition is not to be determined definitively at present.

### **Species variations of RhCG/Rhcg in mammalian kidney**

In the kidney, RhCG/Rhcg has a similar distribution as Rhbg along nephron segments. Although initial studies reported only apical immunolabeling<sup>22, 24</sup>, recent studies have yielded conflicting results regarding the membrane location of RhCG/Rhcg<sup>17, 18, 20, 21, 25, 26</sup>. Both apical and basolateral immunolabeling have been observed in the kidney of rat, human, and some mouse species (Fig. 2). Kim et al.<sup>26</sup> performed extensive morphological studies using immunogold electron microscopy in Balb/c, C57BL/6, C3H, and Black Swiss 129 mice and showed both apical and basolateral Rhcg expression in all strains, although strain-

dependent differences in the intensity of basolateral Rhcg immunolabel were apparent. Moreover, in the human kidney, RhCG expression is predominantly basolateral, with a lesser degree of apical immunoreactivity<sup>25</sup>). Basolateral Rhcg expression increases in parallel with urine ammonia excretion in experimental animal models, suggesting that basolateral Rhcg may play a role in certain physiological conditions. The trafficking mechanisms of the same protein to both the apical and basolateral plasma membrane remain to be established.

### Renal expression of Rh glycoproteins in experimental animal models

Chronic metabolic acidosis increases net acid excretion through urinary ammonia metabolism. Seshadri et al.<sup>20</sup>) demonstrated that chronic HCl ingestion increased urinary ammonia excretion 25 fold and increased Rhcg protein expression. Rhcg expression was increased in the outer medulla and inner medulla but not altered in the cortex. Chronic metabolic acidosis also altered the subcellular distribution of the ammonia transporter protein<sup>21</sup>). In both intercalated cells and principal cells, apical plasma membrane Rhcg significantly increased, whereas, intracellular Rhcg decreased in response to acidosis. However, chronic metabolic acidosis did not alter Rhbg protein expression or immunoreactivity in the cortex, outer medulla, or inner medulla.

Similar changes in subcellular Rhcg localization were observed in a reduced renal mass-chronic renal failure model<sup>18</sup>). Despite a substantial decrease in nephron number, 5/6 ablation-infarcted kidneys maintained normal ammonia excretion and acid-base homeostasis. This was associated with increased apical and basolateral Rhcg expression in collecting duct cells. In contrast, loss of ammonia transporting cells may contribute to the development of metabolic acidosis. In renal ischemia-reperfusion injury, Rh glycoproteins-positive collecting duct cells specifically detached into the tubular lumen and underwent apoptosis resulting in decreased urinary ammonia excretion<sup>17</sup>). In chronic cyclosporine nephropathy there was development of metabolic acidosis and an accompanying decrease in

Rhcg expression<sup>27</sup>). Moreover, urinary ammonia excretion was inappropriately low, suggesting that the decreased Rhcg expression, by decreasing ammonia excretion, leads to the development of metabolic acidosis.

### Genetic ablation of Rh glycoproteins in mice

Chambrey et al.<sup>28</sup>) inactivated the mouse Rhbg gene by insertional mutagenesis and examined whether the mice displayed disturbed ammonia metabolism. Unexpectedly, the Rhbg knockout mice did not exhibit encephalopathy or renal tubular acidosis and adapted well to a chronic acid-load by increasing urinary  $\text{NH}_4^+$  excretion approximately 3-4 fold. Moreover,  $\text{NH}_3$  or  $\text{NH}_4^+$  entry across the basolateral membrane of collecting duct cells was identical in the knockout and wild-type mice suggesting that Rhbg may not be a critical determinant of ammonia excretion by the kidney. However, one cannot exclude the possibility at present that basolateral Rhcg expression is able to compensate for the lack of Rhbg and thus maintain normal ammonia transport.

In contrast, both global and collecting duct-specific Rhcg deletion altered renal urinary ammonia excretion<sup>29, 30</sup>). Biver et al.<sup>29</sup>) deleted the Rhcg gene in mice and demonstrated that Rhcg absence resulted in marked reduction of ammonia movement across the collecting-duct epithelial cells and a significant drop in blood pH. Male Rhcg-knockout mice also represented a reduction in reproductive capability associated with altered epididymal function. Lee et al.<sup>30</sup>) generated collecting duct-specific Rhcg knockout mice having loxP sites flanking exons 5-9 of the Rhcg gene and expressing Cre-recombinase under control of the Ksp-cadherin promoter. The collecting duct-specific Rhcg knockout mice excreted less urinary ammonia under basal conditions and developed more severe metabolic acidosis after acid loading. Thus, collecting duct Rhcg expression is necessary for normal renal ammonia transport, both under basal conditions and in response to metabolic acidosis.

### Conclusion

Ammonia transporter family members, Rh B glycoprotein and Rh C glycoprotein, are expressed in mamma-

lian kidneys along the distal nephron segments. Basolateral Rh B glycoprotein expression does not change with metabolic acidosis and genetic ablation of Rhb<sub>g</sub> does not alter renal ammonia excretion, either. Rh C glycoprotein exhibits both apical and basolateral membrane localization, albeit with species- and strain-dependent variations in basolateral Rhc<sub>g</sub> immunolabel intensity. Chronic metabolic acidosis increases Rhc<sub>g</sub> protein abundance and Rhc<sub>g</sub> deletion alters renal urinary ammonia excretion. These data suggest that collecting duct ammonia secretion may be mediated, at least, by RhCG/Rhc<sub>g</sub>.

### Foot notes

The abbreviation for human Rh A glycoprotein is RhAG and for non-human Rh A glycoprotein is Rhag. A similar nomenclature is used for Rh B glycoprotein and Rh C glycoprotein.

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