Ammonium Homeostasis and Human Rhesus Glycoproteins

Gabrielle Planelles

Inserm, U806 et Université Paris Descartes, Faculté de Médecine René Descartes, Paris, France

Key Words

Rhesus proteins • Kidney, ammonium production • Renal tubule • Ammonia • Ammonium • Carbon dioxide • Oocyte • Membrane transport

Abstract

The brain ammonium production is detoxified by astrocytes, the gut ammonium production is detoxified by hepatic cells, and the renal ammonium production plays a major role in renal acid excretion. As a result of ammonium handling in these organs, the ammonium and pH values are strictly regulated in plasma. Up until recently, it was accepted that mammalian cell transmembrane ammonium transport was due to NH_4^+ transport by non-specific transporting systems, and to non-ionic NH_3 diffusion, whereas lower organisms (such as bacteria, yeasts and plants) were endowed with specific ammonium transporters (Amts). Sequence homologies between Amts and human Rhesus (Rh) glycoproteins (RhAG, from erythroid cells, and RhBG and RhCG from epithelial cells) raised the hypothesis that Rh glycoproteins act as specific ammonium transporters, further sustained by the polarized distribution of RhBG and RhCG in gut, kidney and liver. Results from functional studies agree that Rh glycoproteins are the first ammonium transporters reported in mammals. However, the nature of the transported specie(s) is much debated: in particular, it is proposed that Rh glycoproteins mediate a direct NH_3 transport, or that they mediate an indirect NH_3 transport (resulting from NH_4^+ for H^+ ex-

change). Direct NH_3 transport (associated or not with NH_4^+ transport) raises the exciting hypothesis that Rh glycoproteins may also transport other gasses than NH_3 (namely, CO_2).

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Introduction

In aqueous ammonium-containing solution, the ionic (ammonium ions, NH_4^+) and the non-ionic (ammonia, NH_3) forms of ammonium¹ are in chemical equilibrium; their respective concentrations regarding the total ammonium concentration depend on the fluid pH. Due to the high apparent pK of $\text{NH}_3/\text{NH}_4^+$, NH_4^+ is the predominant form in mammalian fluids (about 97–98% of total ammonium). In several body epithelial compartments, such as the intestinal or the nephron lumen, the ammonium concentration is in the millimolar range, whereas it is $<100 \mu\text{M}$ in plasma. Any increase in blood ammonium level, for example during hepatic dysfunction, may

¹ In this text, 'ammonium' means 'total ammonium', i.e., $\text{NH}_3 + \text{NH}_4^+$, and is used when ammonia (gas) and ammonium ions are not distinguished. When necessary, the chemical symbols for ammonia (NH_3) and ammonium ions (NH_4^+) are used to distinguish the two forms of ammonium. The equilibrium reaction of $\text{NH}_3/\text{NH}_4^+$ is given by the Henderson-Hasselbach equation applied to ammonium buffer: $\text{pH} = \text{pKa} + \log([\text{NH}_3]/[\text{NH}_4^+])$. In aqueous solutions, $\text{pKa} = 9.246$ at 25°C and 8.891 at 37°C (calculated from Appendix 12.1, table 1 [cf. 1]). In plasma, pKa is 9.01 [2].

severely disturb neuronal function, whereas an increase in urinary ammonium excretion fights against chronic metabolic acidosis (CMA). Before recent studies on Rhesus glycoproteins, it was accepted that the transmembrane transport of NH_4^+ in mammalian cells is mediated by membrane transport systems that are not specific to ammonium, and that NH_3 freely crosses biological membranes. The cloning of the erythroid Rhesus-associated glycoprotein, RhAG (previously named Rh50, due to its molecular weight) was followed by the cloning of two non-erythroid homologues, RhBG and RhCG (previously named RhGK) that are expressed in epithelial structures, but not in erythroid cells. Functional studies from several laboratories revealed that human Rh glycoproteins RhAG, RhCG and RhBG (and/or their mouse orthologs, Rhag, Rhbg and Rhcg, respectively) are ammonium transporters. However, NH_4^+ versus NH_3 transport by Rh glycoproteins is still hotly debated.

Ammonium in the Human Body

Ammonium production is mostly localized to the brain, kidney, and gut. In the central nervous system, the ammonium production is consequent to the metabolism of neurotransmitters. The most important pathway for ammonium production in the brain is the oxidative deamination of glutamate by the glutamate dehydrogenase. A large part of the neuronal production of ammonium is transformed by the astrocytes, thanks to glutamine synthetase (GS): the glutamine released from astrocytes enters into the glutamine-glutamate cycle at the glutamatergic neuronal endings. Also, ammonium diffuses from blood to the central nervous system by crossing the blood-brain barrier, driven by the favorable ammonium chemical gradient (the precise ammonium concentration in the brain is not known with accuracy but is probably physiologically $\leq 50 \mu\text{M}$) and by the pH gradient (brain pH is ~ 7.1). An increase in blood ammonium concentration may rapidly (in particular during metabolic alkalosis) challenge the brain function by overloading the efficiency of the astrocytes to trap ammonium and convert it to glutamine. The subsequent increase in brain ammonium concentration (that may reach up to $\sim 5 \text{ mM}$) has deleterious 'toxic' effects: NH_4^+ in excess permeates into neurons using K^+ and Na^+ channels [3]; this influx induces membrane depolarization, challenges neuronal excitability, disturbs intracellular metabolic and enzymatic reactions, and affects intracellular pH (pH_i) and cell-buffering power. Except for rare iatrogenic or metabolic-induced in-

creases in blood ammonium concentration, hyperammonemic syndromes result from inherited errors in urea cycle enzymes, or from (acute or chronic) acquired failure of the liver functions. In normal conditions, the liver detoxifies the gut ammonium production that is delivered by the portal vein: several grams of ammonium are produced per day in the digestive tract, as a result of protein digestion and deamination processes, and of urea hydrolysis. In the liver, ammonium is enzymatically converted into urea or glutamine, an ammonium detoxification process that is achieved in one single pass. In normal acid-base conditions, the bulk of ammonium (about 90–95%) is converted into urea by the periportal hepatocytes, which are endowed with the complete cycle of urea synthesis (carbamoyl phosphatase synthetase is a low-affinity system for ammonium, with a $K_m \sim 2 \text{ mM}$); during this process, bicarbonate ions (and glutamine) are consumed. Downstream, the ammonium escaping urea conversion is converted into glutamine by GS (a high-affinity system for ammonium, with a $K_m \sim 0.2 \text{ mM}$) in perivenous hepatocytes. During CMA, glutamine synthesis by perivenous hepatocytes is increased at the expense of the upstream ureagenesis: in that way, hepatic HCO_3^- consumption is reduced, whereas removal (conversion) of NH_4^+ is ensured. Thus, during CMA, the glutamine balance in the liver is not neutral: a net release of glutamine from liver to blood occurs. The liver glutamine cycle supports the idea of Haussinger [4] that the liver has a crucial role in acid-base homeostasis².

In the kidney, ammonium production takes place mostly in the proximal tubular cells (endowed with GS). In these cells, ammonium production derived from glutamine that is taken up from blood: glutamine metabolism leads to glutamate or α -ketoglutarate⁻, and to NH_4^+ production. Interestingly, an end product of the α -ketoglutarate⁻ metabolism is HCO_3^- . Thus, renal ammonium synthesis in proximal cells leads to the formation of 'new' bicarbonate ions³, 'replacing' the bicarbonate ions consumed during the hepatic urea formation. HCO_3^- exit from cell to interstitium is ensured by the coupled basolateral transport of Na^+ and HCO_3^- via the renal $\text{Na}^+/\text{HCO}_3^-$ symport,

² It should be emphasized that extrahepatic and extrarenal organs also play a role in acid-base balance and in ammonium homeostasis; in particular, skeletal muscles may metabolize (with low efficiency) ammonium that escapes hepatic detoxification, but can also produce ammonium during exercise.

³ Ammonium synthesis by proximal cells is increased during metabolic acidosis, but also during hypokalemia [7]. Whereas an increased ammonium production in proximal tubular cells is a sparing mechanism during CMA; it participates in maintaining the metabolic alkalosis associated with hypokalemia.

kNBC. Ammonium ions are secreted in the proximal lumen by the apical NHE3, running in $\text{Na}^+/\text{NH}_4^+$ exchange mode [5]. Along Henle's loop, NH_4^+ is reabsorbed from the lumen by taking the place of K^+ on the $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symport, NKCC2. The renal counter-current mechanism concentrates ammonium in the renal inner medulla. Final excretion of ammonium in the distal lumen of the nephron follows NH_3 secretion along the collecting tubules and ducts: this gas diffuses from the renal interstitium to the lumen, due to favorable driving forces (the NH_3 transtubular chemical gradient, and the acid value of luminal pH) across the epithelial cell barrier. In the lumen of the distal nephron, the weak base NH_3 rapidly combines with protons to form the weak acid NH_4^+ as shown in figure 1 [for review, see 6]. This allows not only the secretion of proton equivalents, but also affords a supplemental buffering power to the luminal fluid. With such processes, during CMA, an enhanced proton secretion (mediated by H^+ -ATPases) occurs without inducing a tremendous decrease of luminal pH (deleterious to the renal cells, and also favoring nephrolithiasis), since the enhanced proton secretion is buffered by the enhanced NH_4^+ excretion. Even during CMA, the urinary NH_4^+ has a renal origin (see footnote 3); during this condition, urinary NH_4^+ (increasing to 90 mM) may represent 90% of the renal acid excretion. Thus, urinary ammonium excretion is a major component of the net urinary acid excretion.

Ammonium Transmembrane Transport

Lower organisms are endowed with specific ammonium transporters from the Amt⁴ family. In higher organisms, it was accepted that NH_4^+ is transported in an unspecific way by various transport systems, such as K^+ channels, Na/K -ATPase, Na/K -2Cl co-transport, KCl co-transport, non-selective cationic channels and the colonic H,K-ATPase [8, 9], that transport NH_4^+ in the place of K^+ because hydrated NH_4^+ and K^+ have a similar ionic radius, 1.45 Å [10]. However, these transporters have a lower affinity for NH_4^+ than for K^+ ; thus the *efficiency* of NH_4^+ transmembrane transport depends on the NH_4^+

Fig. 1. Mechanism of NH_4^+ excretion by distal nephron. High epithelium permeability to NH_3 , favorable trans-epithelial NH_3 chemical gradient (due to ammonium accumulation in the renal medulla), and acid pH in the lumen allow NH_3 secretion from the interstitium to the lumen. In the luminal fluid, NH_4^+ formation is consecutive to NH_3 protonation by actively secreted H^+ .

and K^+ respective concentrations in the medium: NH_4^+ transmembrane transport is favored when $[\text{NH}_4^+]$ is high and/or $[\text{K}^+]$ is low. In epithelia involved in ammonium homeostasis, relatively high NH_4^+ concentrations prevail in (at least) one given compartment⁵, making these mechanisms physiologically effective. Also, it was accepted that gasses (including NH_3) freely diffuse across the lipid membranes. This diffusion depends on the gas chemical transmembrane gradient (driving force) and on the lipid properties of the cell membrane (permeability). However, several lines of experimental arguments have challenged the classical view of free diffusion of gas [for review, see 14]: a few cell membranes have a low permeability to gas, and several aquaporins enhance membrane permeability to CO_2 or to NH_3 [14–18].

Our current knowledge of ammonium transport has been revised by the discovery of Rhesus (Rh) proteins. Rh genes (mostly found in invertebrates and in vertebrates) belong to the AMT/RH superfamily. The role in ammonium transport of the Rh proteins that are glycosylated, RhAG, RhBG and RhCG, was first suggested due to sequence homologies with Amt^s [19]. First functional evidence was given by Marini et al. [20] in yeast with the endogenous methylammonium permeases (Mep) removed and transfected with Rh glycoproteins: growth of RhAG- and RhCG-expressing ΔMep yeast was restored in a low ammonium-containing medium. The role of Rh glycoproteins in mammalian ammonium transport was further supported by immunolocalization studies [for review, see 21]: RhBG and RhCG are membrane proteins expressed in tissues involved in ammonium homeostasis and/or transport (in particular the digestive tract, the liv-

⁴ Amt^s (ammonium transporters) are present in microorganisms (archaea, bacteria, fungi, yeasts), plants and invertebrates [11]. The function(s) of Amt^s falls beyond the scope of this review. Contradictory literature has been reported recently as to the function of various Amt proteins and the conduction of ammonium ions, ammonia and/or CO_2 . The recent crystallization of *Escherichia coli* AmtB reveals a channel-like structure presenting an external NH_4^+ binding site; NH_3 conduction is a consequence of NH_4^+ deprotonation along the channel hydrophobic pore [12, 13].

⁵ Only the transcellular ammonium transport is considered here; however, the paracellular transport in epitheliums is of low energetic cost, and contributes to transepithelial transport.

Fig. 2. Cell exposure to an ammonium-containing solution induces intracellular pH changes. Schematic representation of the possible pH_i changes in cells exposed to millimolar concentrations of ammonium. Most cell membranes are highly permeable to NH_3 ; the rapid diffusion of this weak base from the bath to the cell induces immediate cell alkalization, due to the (almost) total NH_3 protonation into NH_4^+ . Line A shows pH_i change in a cell freely permeable to NH_3 but totally impermeable to NH_4^+ . Line B shows the very common feature of pH_i changes in a cell that is permeable to both NH_3 and NH_4^+ : the immediate alkalization (consecutive to NH_3 influx) is followed by a secondary acidification that reflects NH_4^+ influx (cell acidification is related to intracellular NH_4^+ partial dissociation into NH_3 and H^+). The more permeable the cell membrane to NH_4^+ , the more pronounced is the amplitude of the secondary re-acidification. Line C shows the pH_i change observed in a cell that is almost impermeable to NH_3 , but highly permeable to NH_4^+ , as is the case of the *X. laevis* oocyte (for more details on oocyte membrane permeability to NH_3 and NH_4^+ , see Cougnon et al. [8]): the initial alkalization is blunted by an almost immediate acidification.

er, and the kidney). Interestingly, in the liver, RhBG is selectively expressed in the plasma membrane of periportal hepatocytes; because these cells are endowed with the high-affinity GS, they are expected to be endowed with an efficient plasma membrane mechanism allowing ammonium influx. In the kidney, RhBG and RhCG are colocalized along the distal nephron (with a particularly strong expression in acid-secreting A cells, but not in B cells) [21]. They are expressed with opposite cell polarity (RhBG in the basolateral membrane, RhCG in the apical membrane), supporting a coordinate role in net ammonium transcellular transport.

Studying the Function of Rhesus Glycoproteins

From the first report from Marini et al. [20], several functional studies confirmed the role of Rh glycoproteins in ammonium transport. With the exception of a study

performed in red cell ghosts [22], most functional studies were performed after the heterologous expression of Rh glycoproteins. As detailed below, all studies agree to conclude that Rh glycoproteins are the first specific ammonium transporters reported in mammals. However, the mechanism of transport (transporter, channel, channel-like protein) mediated by Rh glycoproteins and the nature of the transported substrate(s) (NH_4^+ and/or NH_3 or H^+) are controversial.

There is no direct approach to monitor a protein-mediated transport of ammonium. Functional approaches measured (i) methylammonium fluxes, (ii) ammonium-induced pH_i changes, and/or (iii) ammonium-induced current in voltage-clamped *Xenopus laevis* oocytes. All of them have technical or conceptual limitations. Labeled methylammonium, $CH_3NH_2/CH_3NH_3^+$ (MeA), used as a surrogate for ammonium fluxes, is not a perfect tracer for ammonium (transporter affinities for methylammonium and ammonium differ, and pK_a for MeA is higher than for ammonium, 10.65 versus 9.25). In several studies (see below), a lack of effect of voltage change on MeA flux was considered as a strong argument in favor of electro-neutral ammonium transport, but this may be an over-interpretation. A pH effect on MeA flux may be due to a direct effect, to a change in transmembrane proton gradient, or to a change in the neutral form concentration of MeA (at the same pH_o and for the same MeA and ammonium total concentration, $[CH_3NH_2]$ is $<[NH_3]$ whereas $[CH_3NH_3^+] \sim [NH_4^+]$, due to the difference in respective pK_a). Analysis of ammonium-induced changes in intracellular pH is the most widely used approach to assess NH_3 and NH_4^+ fluxes. Figure 2 shows schematically the various pH_i changes (determined using a pH fluorescent probe or pH-selective microelectrodes) during cell exposure to millimolar concentrations of ammonium. Ammonium-induced initial pH_i changes as a function of time (dpH_i/dt) are an index⁶ of NH_3 influx (alkalizing phase) and of NH_4^+ influx (acidifying phase) from bath to cell. Accuracy of this determination depends on the sensitivity and rapidity of the technique used to measure pH_i changes. Also, ammonium-induced inward currents in voltage-clamped oocytes (measured at negative holding potentials, in general -50 mV) are used as an index of NH_4^+ transport. A main concern in the interpretation of ammonium-induced current is to discriminate between a current related to NH_4^+ transport directly mediated by the expressed protein, a current specifically associated to

⁶ Ammonium flux, Φ_{Amm} , is $\Phi_{Amm} = dpH_i/dt \cdot \beta$, where β is the cell-buffering power.

the expressed protein, and the activation of endogenous conductance.

Evidence for Ammonium Transport Mediated by the Human Erythroid Rh Glycoprotein A, RhAG

RhAG function was investigated in various cell models. Ammonium transport in RhAG-expressing *X. laevis* oocytes was first reported by Westoff et al. [23]: at extracellular pH, $\text{pH}_o = 7.5$, RhAG-expressing oocytes exhibited a ≈ 10 -fold increase in MeA uptake, compared to control oocytes. Saturable MeA uptake was inhibited by ammonium ($\text{IC}_{50} = 1.1 \text{ mM}$ at $\text{pH}_o 7.5$), pH_o -dependent (increased by alkaline pH_o and reduced by acid pH_o), and enhanced by maneuvers that acidify the cell. High K^+ medium (used to depolarize oocytes) had no effect on MeA flux. This finding, and an only small increase in RhAG-expressing oocyte conductance (but ammonium-induced currents were not checked), was considered as a reliable argument that that RhAG does not mediate an electrogenic ammonium transport. The authors concluded that RhAG mediates a coupled electroneutral NH_4^+/H^+ exchange, and that experimental data did not support NH_3 transport [23]. By studying ΔMep yeast transfected by RhAG, the same group confirmed their conclusion that RhAG mediates a reversible NH_4^+/H^+ exchange [24].

At variance, Ripoché et al. [22] concluded that RhAG enhances NH_3 influx in erythrocytes. To study RhAG function, control red ghost cells and in *Rhnull*⁷ red ghost cells were bathed in an ammonium-containing solution while pH_i changes were measured using stopped-flow spectrofluorometry analysis (using the pH-sensitive probe BCECF). This approach allows measurement of very fast changes in pH_i . The initial rate of ammonium-induced pH_i alkalization was slower in *Rhnull* compared with control cells, consistent with a reduced NH_3 influx in *Rhnull* cells. Also, a less pronounced secondary cell acidification during ammonium exposure was observed, but its mechanism was not further investigated. The authors concluded that RhAG expression facilitates NH_3 transport in red blood cells [22].

In our laboratory, we expressed RhAG in HeLa cells. The pH_i changes induced by a 10 mM NH_4Cl -containing solution were monitored using a video imaging system coupled to BCECF fluorescence. Compared to control

cells, RhAG-expressing cells exhibit a faster initial alkalization, and an enhanced secondary acidification. This supports RhAG-induced enhanced NH_3 and NH_4^+ cell membrane respective permeabilities. The pH_i recovery after withdrawal of a MeA-containing solution also supported enhanced efflux of the neutral form in RhAG-expressing cells. We concluded that RhAG promotes a bidirectional transport of NH_3 , and enhances NH_4^+ transport [26]. Interestingly, when we expressed RhAG in *X. laevis* oocytes, low ammonium concentrations⁸ ($<1 \text{ mM}$) induced inward currents in voltage-clamped cells (-50 mV), agreeing with NH_4^+ -mediated current [27]. This observation is at variance from other reports using the same cell model [28, 29], but the reason(s) of this discrepancy is still unclear.

Evidence for Ammonium Transport Mediated by the Human Non-Erythroid Rh Glycoproteins B and C, RhBG and RhCG

The first report concerning the function of human epithelial Rh glycoprotein was obtained in our laboratory [30]. In RhCG-expressing oocytes, but not in control oocytes, submillimolar ammonium concentrations (from 0.1 mM) induced inward currents, consistent with NH_4^+ influx from bath to the cell. The amplitude of ammonium-induced current was voltage- and pH_o -dependent (increased at alkaline pH_o and reduced at acid pH_o). Kinetic analysis of ammonium transport showed a saturable, high-affinity process: K_m for NH_3 was $\sim 8 \mu\text{M}$, not dependent on pH_o or on NH_4^+ concentration, consistent with RhCG-mediated NH_3 transport. Interestingly, pH_i measurements (using pH-sensitive microelectrodes) revealed that low ammonium concentration induced an initial alkalization, followed by acidification in RhCG-expressing oocytes. The biphasic pH_i change does not support simple NH_3 - NH_4^+ co-transport, and suggests that RhCG-mediated NH_3 and NH_4^+ fluxes vary in an independent manner. This favors the hypothesis of uncoupled fluxes of NH_3 and NH_4^+ or of a bidirectional NH_3 flux with NH_4^+ influx [30]. Our conclusion is that RhCG mediates NH_3 transport and induces NH_4^+ transport. Further investigations confirmed that RhCG mediates bidirectional (i.e., influx and efflux) NH_3 transport. Specificity of ammonium currents was also supported by the inhibitory effect of a mercurial derivative [Planelles et al., unpubl. results].

⁷ In the erythrocyte membrane, RhAG (Rhesus-associated glycoprotein) is associated with the proteins encoded by RHCE and RHD that carry RhD and RhCcEe antigens. Because RhAG expression is mandatory to surface expression of these antigens, some mutations in RHAG result in the *Rhnull* 'regulator' phenotype. For further details on *Rhnull* phenotypes and on Rh proteins in red blood cells, see Le Van Kim et al. [25].

⁸ In *X. laevis* oocytes at physiological pH_o , an ammonium concentration $>1 \text{ mM}$ induces the activation of several endogenous transport systems, in particular a non-selective cationic conductance [for details, see 8].

In RhBG- and in RhCG-transfected HEK cells, fast changes in pH_i were monitored by others using stopped-flow analysis [31]. RhBG and RhCG expression enhances the initial cell alkalinization, a result fully consistent with an enhanced NH_3 influx, induced by the expression of the human epithelial Rh glycoprotein. As it was the case when studying RhAG in red cells [22], the authors did not investigate the mechanism of the secondary acidification that they observed in HEK cells [31].

In RhBG-expressing oocytes a saturable, pH_o -dependent MeA uptake, inhibited by ammonium was reported. In this study, ammonium exposure induced inward currents in voltage-clamped oocytes, but it was concluded that these currents were related to the activation of endogenous NH_4^+ channels, not to RhBG per se. Exposing RhBG-expressing and BCECF-loaded oocytes to a solution containing a submillimolar concentration of ammonium alkalinizes them in a stable manner (within minutes) without altering pH_i in control oocytes [32]. Basically similar observations were obtained in RhCG-expressing oocytes by the same group and led the authors to conclude that RhBG as RhCG mediate an electroneutral NH_4^+/H^+ exchange [33].

Conclusions

Despite the bulk of recent knowledge, the precise function(s), substrate(s) and the mechanism(s) of transport of human Rh glycoproteins remain to be established. Up to now the studies of mouse Rh glycoproteins orthologs have not clarified the field: Rhbg and Rhcg have been reported to mediate electrogenic NH_4^+ transport [34, 35] or electroneutral NH_4^+/H^+ exchange [36]. Several major questions will need to be addressed in the future. It will be important to establish whether or not Rh glycoproteins share the same function and to determine if they mediate *direct* NH_3 transport (with or without associated or coupled NH_4^+ transport) or *indirect* NH_3 transport. It has been proposed that NH_4^+/H^+ counter-transport mediated by Rh glycoproteins is equivalent to net NH_3 transport: the NH_4^+/H^+ exchange would consist in external NH_4^+ binding to the transport protein, followed by its deprotonation and the proton being displaced into the external fluid [29, 36]. This proposal, which needs further analysis, is an attempt to reconcile NH_4^+/H^+ exchange by Rh glycoproteins with the results of the analysis of the Amt crystal structure (see footnote 4 and references 12 and 13). Conclusions from our laboratory that Rh glycoproteins mediate direct NH_3 transport associ-

ated with NH_4^+ transport [26, 30] also need to be investigated further. Such a mechanism would be of interest, since the glutamine transporter SN1 is associated with a proton current that is associated not only with SN1 expression, but also with its physiological function [37]. Lastly, if Rh glycoproteins mediate the direct transport of NH_3 , it is possible that they mediate the transport of other gases. Recent results suggest that CO_2 permeability is increased in RhCG-expressing oocytes [27] and that Rh-null red blood cells have a lower CO_2 permeability than control cells [17]. These results are consistent with the possibility that Rh glycoproteins can mediate CO_2 transport and the concept that Rh glycoproteins have a wide physiological role. Future work, including exploration of transgenic animals (up to now only Rhbg^{-/-} mice have been studied [38] and Rhbg disruption does not lead to an increase in plasma levels of ammonium or to renal distal tubular acidosis), will advance our understanding of these novel proteins.

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