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A courier service for ammonia

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Abstract

Physiological studies in knockout mice demonstrate a surprising role for a kidney protein related to the Rh-factor protein of red blood cells – an ammonia channel critical to maintenance of body fluid pH.

The completion of the Human Genome Sequencing Project spawned a new era of biological research, with many declaring that we have now entered the ‘post-genomic’ era. This declaration is premature, not least because of the roughly 20,000 protein-coding genes in the human genome¹, comprehensive functional annotations are lacking for most. Without this knowledge, we cannot gain a thorough understanding of human disease. So a critical task ahead is to discover the *in vivo* function of each gene product. By investigating the function of the protein encoded by a gene called *RhCG* using classical physiological techniques in knockout mice, Biver, Marini and colleagues² (page 000 of this issue) provide a model for how this goal can be effectively pursued.

We are constantly facing an acid threat. Excess dietary proteins produce strong acids, namely sulphuric acid and phosphoric acid, when oxidized in the body, posing the danger of a lethal drop in blood pH. But our kidneys prevent our demise by removing the strong acids through the excretion of hydrogen ions (H⁺). Most of the excreted H⁺ appears in the urine as ammonium (NH₄⁺ = NH₃ + H⁺), which is made by the cells of the proximal tubule in the kidney from the amino acid glutamine. From the proximal tubule, NH₄⁺ is transferred to the final urine via a series of NH₄⁺ transport processes culminating with a critical transport step across the cells of the collecting duct³ (Figure 1). In this final step, NH₄⁺ seems to enter the cells by direct NH₄⁺ transport⁴, but moves from the collecting-duct cells to the urine by a mechanism involving the parallel transport of H⁺ and ammonia (NH₃)⁵. An ion pump called V-ATPase mediates the H⁺ transport. As for the ammonia, because of its small size (17 Daltons), it was believed to freely diffuse across the membrane of collecting-duct epithelial cells into the urine, independent of a transporter protein⁵. But is it really the case? Biver et al suspected otherwise, testing the hypothesis that a protein encoded by *RhCG* was responsible for this critical ammonia transport process.

The encoded protein, Rhcg, is related to the Rh antigen protein of red blood cells that is used to determine immunological compatibility before blood transfusions. An earlier study by Marini *et al.*⁶ showed that the amino-acid sequences of mammalian Rh-family proteins are similar to those of known ammonia-transport proteins of bacteria, fungi, plants and invertebrate animals. Indeed, when investigators expressed mammalian Rhcg in cells that do not normally express it, ammonia transport was observed⁷. But this observation is not a definitive proof for the physiological role of Rhcg at its natural levels in mammalian tissues. What’s more, when *RhBG* —the gene for another Rh-related protein of the collecting duct — was deleted in mice, no measurable defects in ammonia transport are detected in their kidneys⁸.

Undaunted by the negative result with *RhBG* deletion, Biver *et al.*² deleted the *RhCG* gene in mice. They demonstrate that, contrary to the free diffusion hypothesis⁵, the absence of Rhcg

results in a two-third reduction in the rate of ammonia movement across the collecting-duct epithelial cells, indicating that, at most, only one third of ammonia crosses the lipid membrane by diffusion. So Rhcg seems to function as a membrane channel, allowing the passage of ammonia across the lipid membrane separating the urine from the inside of the collecting-duct cells in the kidney (Fig. 1). The absence of the Rhcg ammonia channel in the knockout mice resulted in a decrease in urinary ammonia excretion and a marked fall in blood pH, thereby clearly establishing a key role for Rhcg in the regulation of the pH of body fluids².

Biver et al also found a reduction in reproductive capability in male *RhCG* knockout mice associated with evidence for altered epididymal function. The epididymis, like the renal collecting duct, acidifies its lumen, and it does so via the same V-ATPase H⁺ pump⁹. However, in the epididymis, acidification plays a different role— an acid environment is necessary for sperm maturation and to immobilize spermatozoa until they are ‘awoken’ by the alkaline environment of the seminal vesicles and prostate. In this context, extensive ammonia secretion into the lumen would be detrimental, since it would prematurely alkalize the epididymal fluid.

The authors suggest that Rhcg may instead be responsible for scavenging potentially toxic ammonia molecules from the lumen to the cells where it can be converted to glutamine.

Trying to identify functional differences between knockout and normal mice is technically challenging in part because of the small size of mice compared to traditional research animals. What helped Biver *et al.* in their quest was their ability to adapt some classical physiological techniques to the mouse, including measurements of the composition of body fluids, measurement of intracellular pH with fluorescent dyes, and solute transport measurements in isolated microperfused renal tubules. These approaches have been part of the physiological armamentarium for years, but have been gradually been displaced by modern techniques for molecular and cell biology in physiological laboratories throughout the world. For example, the isolated perfused tubule technique developed in the 1960 by Maurice Burg¹⁰ — like many other intricate physiological methods — has not been used extensively since 1980s. With expertise in many physiological techniques in decline, Biver and colleagues’ work is a testament that such methods are still of enormous value. It will be interesting to see whether physiologists’ new role as functional annotators of the genome will spur a resurgence of training in traditional physiological techniques.

The paper by Biver and colleagues² resolves a longstanding question in kidney physiology — namely, what is the molecular basis of ammonia transport into the urine? With the identification of Rhcg as the collecting duct ammonia channel, new questions can now be raised about whether and how this channel may be regulated, and about the role of this channel in disease processes involving failure to regulate blood pH. And reproductive physiologists will not doubt soon be addressing the specific roles of Rhcg in epididymal function and spermatozoa maturation.

References

1. Clamp M, et al. Proc. Natl Acad. Sci. USA 2007;104:19428–19433. [PubMed: 18040051]
2. Biver S, et al. Nature 2008;XXX:000–000.
3. Knepper MA, Packer R, Good DW. Physiol. Rev 1989;69:179–249. [PubMed: 2643123]
4. Wall SM, Davis BS, Hassell KA, Mehta P, Park SJ. Am. J. Physiol 1999;277:F866–F874. [PubMed: 10600933]
5. Pitts RF. Fed. Proc 1948;7:418–426. [PubMed: 18867436]
6. Marini AM, Urrestarazu A, Beauwens R, Andre B. Trends Biochem. Sci 1997;22:460–461. [PubMed: 9433124]

7. Westhoff CM, Wylie DE. Transfus. Clin. Biol 2006;13:132–138. [PubMed: 16563829]
8. Chambrey R, et al. Am. J. Physiol. Renal Physiol 2005;289:F1281–F1290. [PubMed: 16077082]
9. Pastor-Soler N, Piétrement C, Breton S. Physiology (Bethesda) 2005;20:417–428. [PubMed: 16287991]
10. Burg MB, Grantham JJ, Abramow M, Orloff J. Am. J. Physiol 1966;210:1293–1298. [PubMed: 5923067]

Figure 1. Ammonium excretion

Acids generated from metabolism of excess dietary protein are excreted largely as urinary ammonium (NH_4^+) produced by the proximal tubule cells of the kidney. NH_4^+ is ultimately transported into the urine from collecting duct cells via parallel movement of hydrogen ions (H^+) and ammonia (NH_3). Biver *et al.*² show that not all ammonia moves by free diffusion—as thought previously—and that most of it crosses through the Rhcg protein, functioning as an ammonia channel. The V-ATPase mediates H^+ transport into the urine, recombining with NH_3 to form NH_4^+ in the urine. As for the initial NH_4^+ entry into these cells, an ion pump called $\text{Na}^+-\text{K}^+-\text{ATPase}$, which can carry NH_4^+ in lieu of potassium, has been proposed to be involved⁴.