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JB Commentary

Riboflavin transporter is finally identified

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Riboflavin or vitamin B_2 is one of the constituents of energy drinks. Although this compound is known to be absorbed in the intestine and that it circulates throughout the body and is excreted in urine, the transporter(s) responsible for the process was only recently identified. Yamamoto *et al.* identified this transporter through functional expression of rat orthologues of a putative bacterial riboflavin transporter.

Keywords: Absorption/Intestine/Energy drink/Riboflavin/Transporter.

Everyone notices that urine turns to be yellow after drinking an energy drink. The compound that renders the yellowish colour is riboflavin, a water-soluble vitamin known as vitamin B_2, which was discovered to be a constituent of the so-called old yellow enzyme by a Nobel prize winner, Professor Otto Warburg in 1925. Its active forms are flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which function as cofactors for a number of redox enzymes and play essential roles in the transfer of electrons in biological oxidation–reduction cycles (*1*, *2*). Riboflavin deficiency during pregnancy and adolescence may be related to developmental abnormalities and may increase the risk for anaemia, cancer, cardiovascular disease and neurodegeneration (*1*, *2*). However, humans and other mammals cannot synthesize riboflavin *de novo*, and thus must acquire it in their diet (*3*). This is one reason why riboflavin is an ingredient of energy drinks.

Let us consider the path of riboflavin after its intake. Riboflavin is absorbed at the luminal surface of the small intestine, enters the blood stream by way of epithelial cells, is distributed throughout the body and reaches target cells (Fig. 1A) (*4–12*). In the brain, riboflavin penetrates blood/brain barrier and is taken up by neurons and astrocytes. Excess amounts of riboflavin are filtered into glomerular filtrate, and excreted primarily through the urine. Some riboflavin is reabsorbed at the luminal surface of renal urinary ducts. Thus, appearance of riboflavin in the urine is a very nice and convenient example for estimating the real-time processing of the absorption, circulation and excretion of drugs. Previous studies have indicated the involvement of transport activities during this process (*4–12*). It is very difficult to estimate how many cells riboflavin enters and how many transporters handle this process before excretion. I am sure that these numbers are enormous. Surprisingly, however, what type of transporter(s) is responsible for riboflavin transport was unknown until recently.

In an article published in 2009 (*13*), Yuasa and his colleagues identified a novel riboflavin transporter RFT2 that is responsible for riboflavin absorption in the small intestine of rodents. Based on information obtained from combined BLAST searches of NCBI databases with the amino acid sequences of *impX*, the gene of the putative riboflavin transporter of *Fusobacterium nucleatum* (*14*), Yuasa and his colleagues succeeded in the isolation of the cDNA rRFT2, which is homologous to rRFT1 that had been originally identified as rat G protein-coupled receptor 172B (rGPR172B) and its human orthologue (hGPR172B). Both are suggested to belong to a group of porcine endogenous retrovirus A receptors but their normal functions are unclear (*13*). rRFT2 is not an ABC-type transporter or SLC-type transporter. rRFT2 consists of 469 amino acids with 11 potential membrane spanning domains with a large extracellular loop containing a putative *N*-glycosylation site. rRFT2 is ubiquitously expressed, but the highest level of expression is observed in the small intestine and colon. When expressed in cultured cells, rRFT2 takes up riboflavin in a saturable manner with respect to its concentration with a V_{max} value of 11 pmol/min/mg protein and a K_m of 0.21 μM. The riboflavin uptake is insensitive to Na^+ or K^+, indicating that it is not a Na^+ co-transporter. rRFT2 is relatively insensitive to many kinds of cations and anions, but highly sensitive to riboflavin derivatives such as lumiflavin, FMN and FAD. The properties of riboflavin uptake are similar to those of membrane vesicles from brush border membrane vesicles of the small intestine, providing credence that the rRFT2 protein is a riboflavin transporter itself. A subsequent study by Yuasa and his group showed that human RFT2 (hRFT2) possesses a transport phenotype similar to that of rRFT2 (*15*). Taken together, these results clearly indicate that RFT2 acts as a riboflavin transporter.

In contrast to RFT2, hRFT1 is thought to have 10 putative transmembrane helices and exhibits very low transport activity with an apparent V_{max} of about 0.08 pmol/min/mg protein and a K_m of 64 nM when expressed in Caco-2 cells (*16*). Other properties such as the expression pattern as well as transport properties in regards to sensitivities to anions, cations, pH and structural analogues are similar to those of

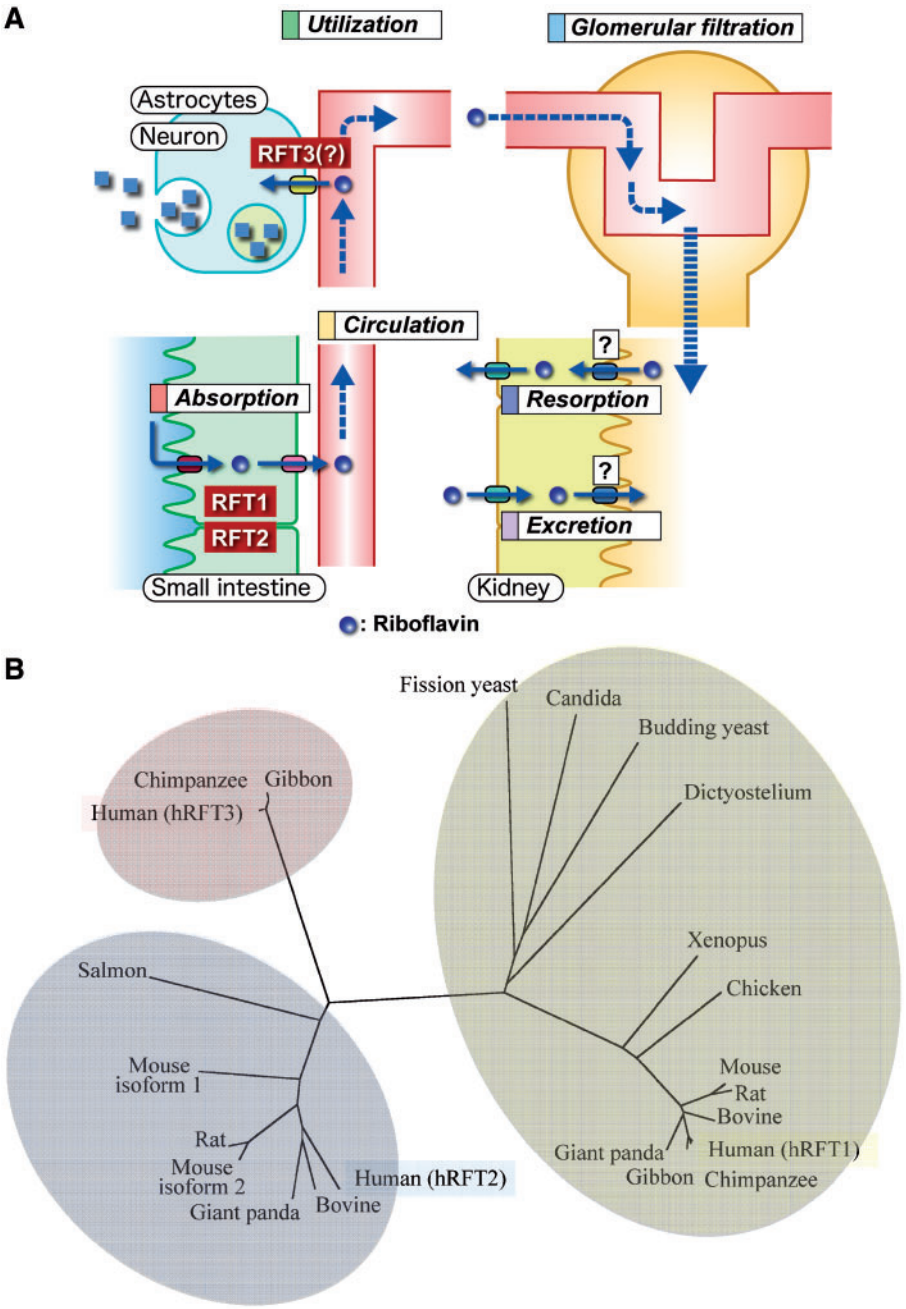

Fig. 1 Expression and distribution of RFT and its orthologues. (A) Proposed function of RFTs. Riboflavin is taken up by intestinal epithelia, is passed through the cells, distributed throughout the body and excreted into the primary urine. Some riboflavin may be reabsorbed through urinary ducts. RFTs may be involved in these processes. It is also possible that an unknown transporter(s) is involved in the urinary excretion and blood/brain barrier transport. '?', unidentified transporter. (B) A phylogenetic tree of mammalian RFTs. Three types of RFTs are classified.

rRFT2. In addition, hRFT3 which has properties similar to those of hRFT1 is present in brain tissue (*17*). Thus, the specific activity of hRFT1 (and also hRFT3) is much lower than that of rRFT2. Hence, it is reasonable to believe that RFT2 plays a central role in intestinal absorption of riboflavin. A recently observed maternal riboflavin deficiency, leading to transient neonatal-onset aciduria type 2, was caused by a microdeletion in the RFT2 gene, *GPR172B*, supports this conclusion (*18*). A phylogenetic tree of the three types of riboflavin transporter identified so far is shown in Fig. 1B.

Characterization of the RFTs as well as the mechanism of riboflavin transport is still at an early stage. The driving force for RFT-mediated riboflavin transport is not known. More importantly, the lack of a specific antibody against RFTs makes it difficult to determine the precise localization of the transporters at cellular and subcellular levels. Information about the function and localization of the RFT will give us insights into the general features of riboflavin transport, since a transporter(s) that is responsible for transcellular movement of a molecule in many organs has not been identified up until now.

Conflict of interest
None declared.

References

1. Powers, H.J. (2003) Riboflavin (vitamin B-2) and health. *Am. J. Clin. Nutr.*. **77**, 1352–1360
2. McCormick, D.B. (1972) The fate of riboflavin in the mammal. *Nutr. Rev.* **30**, 75–79
3. McCormick, D.B. (1989) Two interconnected B vitamins: riboflavin and pyridoxine. *Physiol. Rev.* **69**, 1170–1198
4. Said, H.M. (2004) Recent advances in carrier-mediated intestinal absorption of water-soluble vitamins. *Annu. Rev. Physiol.* **66**, 419–446
5. Daniel, H., Wille, U., and Rehner, G. (1983) In vitro kinetics of the intestinal transport of riboflavin in rats. *J. Nutr.* **113**, 636–643
6. Middleton, H.M. 3rd (1990) Uptake of riboflavin by rat intestinal mucosa in vitro. *J. Nutr.* **120**, 588–593
7. Tomei, S., Yuasa, H., Inoue, K., and Watanabe, J. (2001) Transport functions of riboflavin carriers in the rat small intestine and colon: site difference and effects of tricyclic-type drugs. *Drug Deliv.* **8**, 119–124
8. Daniel, H. and Rehner, G.I. (1992) Sodium-dependent transport of riboflavin in brush border membrane vesicles of rat small intestine is an electrogenic process. *J. Nutr.* **122**, 1454–1461
9. Casirola, D., Gastaldi, G., Ferrari, G., Kasai, S., and Rindi, G. (1993) Riboflavin uptake by rat small intestinal brush border membrane vesicles: a dual mechanism involving specific membrane binding. *J. Membr. Biol.* **135**, 217–223
10. Said, H.M. and Arianas, P. (1990) Transport of riboflavin in human intestinal brush border membrane vesicles. *Gastroenterology* **100**, 82–88
11. Said, H.M. and Ma, T.Y. (1994) Mechanism of riboflavine uptake by Caco-2 human intestinal epithelial cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* **266**, G15–G21
12. Said, H.M., Ortiz, A., Moyer, M.P., and Yanagawa, N. (2000) Riboflavin uptake by human-derived colonic epithelial NCM460 cells. *Am. J. Physiol. Cell Physiol.* **278**, C270–C276
13. Yamamoto, S., Inoue, K., Ohta, K., Fukatsu, R., Maeda, J., Yoshida, Y., and Yuasa, H. (2009) Identification and functional characterization of rat riboflavin transporter 2. *J. Biochem.* **145**, 437–443
14. Vitreschak, A.G., Rodionov, D.A., Mironov, A.A., and Gelfand, M.S. (2002) Regulation of riboflavin biosynthesis and transport genes in bacteria by transcriptional and translational attenuation. *Nucleic Acids Res.* **30**, 3141–3151
15. Fujimura, M., Yamamoto, S., Murata, T., Yasujima, T., Inoue, K., Ohta, K.Y., and Yuasa, H. (2010) Functional characterization of the human orthologue of riboflavin transporter 2 and riboflavin-responsive expression of its rat ortholog in the small intestine indicate its involvement in riboflavin absorption. *J. Nutr.* **140**, 1722–1727
16. Yonezawa, A., Masuda, S., Katsura, T., and Inui, K. (2008) Identification and functional characterization of a novel human and rat riboflavin transporter, RFT1. *Am. J. Physiol. Cell Physiol.* **295**, C632–C641
17. Yao, Y., Yonezawa, A., Yoshimura, H., Masuda, S., Katsura, T., and Inui, K. (2010) Identification and comparative functional characterization of a new human riboflavin transporter hRFT3 expressed in the brain. *J. Nutr.* **140**, 1220–1226
18. Gladys, H., Yonezawa, A., Masuda, S., Inui, K., Sim, K.G., Carpenter, K., Olsen, R.K.J., Mitchell, J.J., Rhead, W.J., Peters, G., and Christodoulou, J. (2011) Maternal riboflavin deficieny, resulting in transient neonatal-onset glutaric aciduia type 2, is caused by a microdeletion in the riboflavin transporter gene GPR172B. *Hum Mutat.* **32**, E1976–E1984