The Journal of Physiology

PERSPECTIVE

A proline-tyrosine motif, endocytosis and low salt – how to link protein functions to organ physiology

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Handling Editors: Peying Fong & Pawel Ferdek

The peer review history is available in the Supporting Information section of this article (https://doi.org/10.1113/JP287335#support-information-section).

Physiology studies the functioning of the human body and aims at a comprehensive understanding of the physical and chemical processes that define the function of organs and their cooperation in a healthy body. An important application of physiology is – via improved understanding of disease pathophysiology and disease compensatory processes – the development of novel and improved treatment options for human diseases. It is obvious that such goals can only be achieved by studying cell and organ functions at a molecular level. Proteins direct virtually all cell functions, and rational treatment options will either have to correct protein dysfunctions as the disease cause or to stimulate or inhibit other proteins that may compensate for the disease-causing dysfunction.

In the last decades, we have witnessed amazing progress in molecular physiology: structural biology has allowed identification of primary, secondary and tertiary structures from a large number of proteins; and biochemistry, cell physiology and computer-based simulation have clarified the mechanisms of their function at almost atomistic resolution. This breath-taking success almost made us forget the difficulties of the next step – to link detailed molecular understanding of single protein function to cell and organ behaviour. In this issue of *The Journal of Physiology* Clara Mayayo-Vallverdú and colleagues provide an example of scientific work that exactly addresses this important task (Mayayo-Vallverdú et al., 2024)

The authors study a particular anion channel, ClC-K–barttin, which is predominantly expressed in the kidney and in the inner ear. ClC-K–barttin channels contribute to NaCl resorption in the loop of Henle and to K^+ secretion by the stria vascularis. They are assembled as multi-subunit complexes, consisting of the pore-forming ClC-K subunit and the accessory barttin subunit. Although neither the stoichiometry nor the architecture of the complex is known, its formation is obligatory for channel function. ClC-K is non-conducting without barttin (Fischer et al., 2010) and cannot traffic to the surface membrane. Barttin allows ClC-K to exit from the endoplasmic reticulum and insert into the plasma membrane and changes its function by modifying voltage-dependent gating processes (Fischer et al., 2010). ClC-K proteins cannot be detected in cells lacking barttin (Rickheit et al., 2010), most likely because barttin is required for complex glycosylation and thus protein stability of ClC-K subunits (Janssen et al., 2009). Mutations in the gene encoding barttin, *BSND*, cause Bartter syndrome IV, with impaired urinary concentration and sensory deafness, with clear correlation between barttin dysfunction and clinical symptoms (Janssen et al., 2009).

This latest paper by the Estevez group is based on a barttin point mutation, Y98A, that was identified decades ago to increase surface membrane insertion of CLC-K–barttin channels. They demonstrate that this mutation indeed affects an endocytic YxxØ motif and use expression in *Xenopus* oocytes and mammalian cells to link increased surface membrane expression to increased stability of the ClC-K–barttin complex. The authors then went on to generate a $Bsnd^{Y95A/Y95A}$ knock-in mouse to study the effects of mutations in the YxxØ motif *in vivo*. Surprisingly, the mutation left protein expression levels and subcellular distribution unaffected under control conditions.

However, barttin was reported to affect phosphorylation of NCC transporters under a high-salt and low-potassium diet (Nomura et al., 2018), and Mayayo-Vallverdú et al. thus tested whether a high-salt and low-potassium diet results in an increased ClC-K–barttin function that is not visible under control conditions. They found NCC phosphorylation levels comparably increased in $Bsnd^{Y95A/Y95A}$ and in WT. However, a high salt and low potassium diet induces hyperplasia of distal collecting tubule only in WT, but not in knock-in animals. The authors conclude that mutations in the endocytic YxxØ motif cause ClC-K–barttin gain-of-function and that ClC-K–barttin channel currents can be regulated by modifying this motif. Since these channels play important roles in urinary concentration and in formation of the endocochlear potential, such modifications might be helpful for treating certain forms of hypertension or hearing impairment.

This paper is a beautiful example of linking knowledge about molecular behaviour to organ functions to reach multi-scale physiology. Many more examples of such approaches are required to understand organ function at molecular resolution. The Y89A mutations was first reported two decades ago, illustrating the effort and the persistence that was required for this publication. However, recent methodological progress will certainly speed up future work. One might think of novel organoid preparation techniques and improved live cell imaging. We are eagerly awaiting the next steps in physiology at atomic resolution.

References

Fischer, M., Janssen, A. G., & Fahlke, C. (2010). Barttin activates ClC-K channel function by modulating gating. *Journal of the American Society of Nephrology*, **21**(8), 1281–1289.

Janssen, A. G., Scholl, U., Domeyer, C., Nothmann, D., Leinenweber, A., & Fahlke, C. (2009). Disease-causing dysfunctions of barttin in Bartter syndrome type IV. *Journal of the American Society of Nephrology*, **20**(1), 145–153.

Mayayo-Vallverdú, C., Gaitán-Peñas, H., Armand-Ugon, M., Muhaisen, A., Prat, E., Castellanos, A., Elorza-Vidal, X., de Heredia, M. L., Alonso-Gardón, M., Pérez-Rius, C., Vecino-Pérez, M., Mallen, A., Errasti-Murugarren, E., Hueso, M., Artuch, R., Nunes, V., & Estévez, R. (2024). Regulation of ClC-K/barttin by endocytosis influences distal convoluted tubule hyperplasia. *The Journal of Physiology*, **602**(17), 4291–4307.

 DOI: 10.1113/JP287335

Nomura, N., Shoda, W., Wang, Y., Mandai, S., Furusho, T., Takahashi, D., Zeniya, M., Sohara, E., Rai, T., & Uchida, S. (2018). Role of ClC-K and barttin in low potassium-induced sodium chloride cotransporter activation and hypertension in mouse kidney. *Bioscience Reports*, **38**(1), BSR20171243.

Rickheit, G., Wartosch, L., Schaffer, S., Stobrawa, S. M., Novarino, G., Weinert, S., & Jentsch, T. J. (2010). Role of ClC-5 in renal endocytosis is unique among ClC exchangers and does not require PY-motif-dependent ubiquitylation. *Journal of Biological Chemistry*, **285**(23), 17595–17603.

Additional information

Competing interests

No competing interests declared.

Author contributions

Sole author.

Funding

None.

Acknowledgements

Open access funding enabled and organized by Projekt DEAL.

Keywords

anion channel, barttin, ClC, nephron function, renal physiology

Supporting information

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