**EDITORIAL FOCUS | Fluid and Electrolyte Homeostasis**

**Molecular physiological exploration beyond the transcriptome.** Focus on “Molecular mechanisms underlying active desalination and low water permeability in the esophagus of eels acclimated to seawater”

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_FISH, AN AQUATIC VERTEBRATE GROUP_, offer many advantages for investigating how vertebrates respond and adjust their physiological processes to cope with environmental changes in terms of comparative and evolutionary physiology. Pioneers in the fields of fish physiology have endeavored to delineate the mechanisms underlying the adaptive and regulatory physiology of fish by using traditional physiological approaches in various species, including many nonmodel species (3, 11). The extensive advances during the past decade in functional genomics have been gained through employing a variety of approaches from suppression subtractive hybridization, to microarrays, to current RNA sequencing, have enabled fish physiologists to interrogate many possible molecular/cellular mechanisms underlying physiological regulation (2, 4, 10). These functional genomic approaches have led to an improved understanding of the major genes and gene networks that may be associated with the targeted physiological processes (2, 4, 10), but most of those proposed mechanisms or pathways are inferential, and they are mainly based on gene expression profiles and subsequent bioinformatic analyses. The mechanisms or pathways inferred from such transcriptome analyses are plausible. However, to substantially enhance our understanding of the targeted physiological functions, such analyses must be supplemented by physiological or other approaches. A search of the most recent 20 articles found in PubMed using the key word of “transcriptome analysis and fish salinity” (that were published after 2013) reveals only articles that provide transcriptomic and the bioinformatics analyses. Yet in all cases, the authors propose many possible connections to the targeted regulatory responses or physiological processes. Very few studies have extended the data from transcriptomic analyses to further physiological, molecular, and/or cell biological investigation to reveal a novel physiological process (1, 15). In contrast, the article by Takei et al. in the current issue, “Molecular mechanisms underlying active desalination and low water permeability in the esophagus of eels acclimated to seawater” (14), is a paragon to integrate RNA sequencing with physiological, pharmacological, molecular, and cell biological approaches to uncover an important physiological mechanism and enhance our current knowledge of the mechanisms underlying adaptation of fish to salinity.

Overcoming the imbalance in body fluid water is one of the most critical issues in a euryhaline fish upon seawater (SW) challenge. Euryhaline teleosts have to drink SW and absorb water in the digestive tract and simultaneously secrete excess salt in the gills in order to maintain body fluid ionic and osmotic homeostasis during acclimation to a SW environment (5, 8, 11). After SW is ingested, the first step is desalination (absorbing NaCl with minimal water transport) in the esophagus, and the resultant isosmotic fluid is moved toward the intestine for water absorption following active NaCl uptake (6, 9, 12). As such, desalination in the esophagus is the first critical process in adaptation of euryhaline teleosts to salinity. This important process, as a traditional issue in fish physiology, had been investigated, and the basic outline was described earlier (6, 9, 12); however, the exact molecular mechanism of esophageal desalination has not been as well characterized as that of intestinal salt uptake/water absorption (5). In Takei’s elegant and comprehensive study (14), they first characterized the basic transport function of NaCl in eels acclimated to fresh water (FW, control) and SW by using an esophageal sac preparation and demonstrated that the Na⁺/Cl⁻ -coupled desalination is enhanced over 10-fold after SW acclimation. Further pharmacological experiments with various transporter inhibitors showed possible involvement of several transporters, including the Na⁺/Cl⁻ cotransporter (NCC), Na⁺/H⁺ exchanger (NHE), anion exchanger (AE), Na⁺/K⁺-ATPase (NKA), and chloride channel (CLC), in this NaCl uptake mechanism. To identify the exact transporters (and the specific isoforms), Takei et al. (14) took advantage of RNA sequencing to compare esophageal transcriptomes between of FW- and SW-acclimated eels and narrowed the candidate genes on the basis of criteria, such as the targets of transporter blockers, substantial expression of transporters, and/or SW-affected transporters. The selected genes were further analyzed by quantitative PCR and in situ hybridization, and the results reinforced those of the physiological and pharmacological experiments. Integrating all of these data, therefore, Takei et al. (14) proposed a model of desalination by absorbing NaCl accompanied with reduced water permeability in the SW eel esophagus. Apical NHE3, SLC26A3 and SLC26A6, NCC, basolateral NKAα1c and NKA3α, CLCN2, and Na⁺/HCO₃⁻-cotransporter are responsible for NaCl uptake, while downregulated aquaporin (AQP1a and AQP3) and upregulated claudin (CLDN15A) suppress water transport. Further studies are necessary to see whether the proposed model of desalination in the eel esophagus is applicable to...
other species. The esophagus and intestine collaborate in tandem to achieve the mechanism of NaCl uptake and water absorption in teleosts in a manner that maintains body fluid ionic and osmotic homeostasis. Interestingly, the esophagus and intestine adopt NCC and NKCC/NCC, respectively, for NaCl uptake, and this subtle difference reflects the distinct patterns of Na\(^+\) and Cl\(^-\) transport in the two regions of the digestive tract. That is, the esophagus transports equal amounts of the two ions, while the intestine transports much more Cl\(^-\) than Na\(^+\) (5). Other differences, like acid and base transport among the two organs, are also important components in terms of body fluid homeostasis during acclimation to salinity. In the intestine, there is a substantial base loss for the formation of CaCO\(_3\), and this is mainly achieved by apical SLC26A6 and membrane and cytosolic carbonate anhydrases (5). It is still unclear whether a net secretion of acid or base occurs in the esophagus, where apical NHE3 and SLC26A3 and SLC26A6 are supplied with H\(^+\) and HCO\(_3\)\(^-\), respectively, by cytosolic CA2a, according to Takei’s model. This remains to be clarified in the future.

The findings of Takei et al. (14) are important because they depict a comprehensive and clear picture of the mechanism mediating esophageal desalination, which enables us to further study the transport functions and their regulations by precisely targeting the specific ions or water and the specific transporters or enzymes. Their discovery also provides important materials to study the transport functions and their regulations by precisely mediating esophageal desalination, which enables us to further depict a comprehensive and clear picture of the mechanism in the future.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

P. P. H. drafted manuscript; P. P. H. edited and revised manuscript; P. P. H. approved final version of manuscript.

REFERENCES


