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 non-model species (3, 11). The extensive advances during the past decade in functional genomics employing a variety of approaches from suppression subtractive hybridization, microarray 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 mainly based on gene expression profiles and the 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 keyword of “transcriptome analysis and fish salinity” (published after 2013) reveals that they all provide only transcriptomic and the bioinformatics analyses. Yet in all cases the authors propose massive 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 paper by Takei and colleagues 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 the most critical issues in a euryhaline fish upon seawater (SW) challenge. Euryhaine teleosts have to drink SW and absorb water in the digestive tract and simultaneously secret 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 1st 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) in the present issue, they first characterized the basic transport function of NaCl in eels acclimated to fresh water (FW, control) and SW by using an esophagheal 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 and colleagues took the advantage of RNA sequencing to compare esophageal transcriptomes between FW and SW-acclimated eels, and narrowed down the candidate genes based on the criteria such as the targets of transporter blockers, substantial expression of transporters and/or SW-affected transporters. The selected genes were further analyzed by qPCR and in situ hybridization and the results reinforced that of the physiological and pharmacological experiments. Integrating all these data, Takei and colleagues therefore proposed a model of desalination by absorbing NaCl accompanied with reduced water permeability in the SW eel esophagus; apical NHE3, SLC26a3/-6, and NCC and basolateral NKA (NKA1\(\alpha_{1c}^{-}/\alpha_{3}^{-}\), CLCN2 and Na\(^+-\)HCO\(_3^{-}\)-cotransporter (NBCe1) are responsible for NaCl uptake while down-regulated aquaporin (AQP1a/-3) and up-regulated claudin (CLDN15a) suppress water transport.

Further studies are necessary to see if 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 2 regions of the digestive tract. That is, the esophagus transports equal amounts of the 2 ions while the intestine transports much more Cl\(^-\)
than Na⁺ (5). Other differences, like acid and base transport among the 2 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 CaCO₃, and this is mainly achieved by apical SLC26a6 and membrane and cytosolic carbonate anhydrases (CA) (5). It is still unclear whether a net secretion of acid or base occurs in the esophagus where apical NHE3 and SLC26a3/-6 are respectively supplied with H⁺ and HCO₃⁻ by cytosolic CA2a according to Takei’s model. This remains to be clarified in the future.

The findings of Takei and colleagues are important because they depict a comprehensive and clear picture of the mechanisms mediating esophageal desalination which enables us to further study the transport functions and their regulations by precisely targeting the specific ion(s) or water and the specific transporter(s) or enzyme(s). Their discovery also provides important materials to compare with the transport mechanisms in mammalian renal tubular cells (13) and fish gill ionocytes (7, 16) that adopt similar transporters for NaCl uptake in terms of evolutionary and comparative physiology.

Grants

The author’s work is funded by grants from the Academia Sinica and the Ministry of Science and Technology of Taiwan.

Disclosure

P.P.H. has co-authored a book chapter (Homeostatic responses to osmotic stress. In:
Fish Physiology Volume 35, Biology of Stress in Fish. Academic Press, pp 208-249, 2016) with the author, YT.

Author contributions

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


