A Review on Zebra fish as an Animal Model to Study Ion Uptake

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***Abstract*: Zebrafish (Danio rerio) possesses several advantages as an experimental organism, including the applicability of molecular tools, ease of in vivo cellular observation and functional analysis, and rapid embryonic development, making it an emerging model for the study of integrative and regulatory physiology and, in particular, the epithelial transport associated with body fluid ionic homeostasis. Zebrafish inhabits a hypotonic freshwater environment, and as such, the gills (or the skin, during embryonic stages) assume the role of the kidney in body fluid ionic homeostasis. Four types of ionocyte expressing distinct sets of transporters have been identified in these organs: H+-ATPase-rich, Na+- K+-ATPase-rich, Na+-Cl− cotransporter-expressing and K+- secreting cells; these ionocytes perform transepithelial H+ secretion/Na+ uptake/NH4 + excretion, Ca2+ uptake, Na+/Cl− uptake, and K+ secretion, respectively. Zebrafish ionocytes are analogous to various renal tubular cells, in terms of ion transporter expression and function. During embryonic development, ionocyte progenitors develop from epidermal stem cells and then differentiate into different types of ionocyte through a positive regulatory loop of Foxi3a/-3b and other transcription factors. Several hormones, including cortisol, vitamin D, stanniocalcin-1, calcitonin, and isotocin, were found to participate in the control pathways of ionic homeostasis by precisely studying the target ion transport pathways, ion transporters, or ionocytes of the hormonal actions. In conclusion, the zebrafish model not only enhances our understanding of body fluid ion homeostasis and hormonal control in fish but also informs studies on mammals and other animal species, thereby providing new insights into related fields.**

***Keywords*: Ion uptake, Acid–base regulation, Trans Epithelial transport, Hormone, Zebra Fish.**

Introduction:

All terrestrial and some aquatic vertebrates have successfully evolved mechanisms to regulate ionic and osmotic homeostasis of their body fluids, which enables cellular activities and physiological processes to operate normally in these animals. In terrestrial vertebrates, these regulatory mechanisms are mainly performed by the kidneys. In aquatic vertebrates such as fish, the gills are the major extra-renal organ involved in body fluid ionic/osmotic homeostasis [28, 51, 52]. As a model organism, zebrafish (Danio rerio) has a number of advantages, including the availability of genetic databases, applicability of forward/reverse genetic manipulation and cell biological approaches, transparent embryos with rapid development, and short life cycle. As such, zebrafish is an emerging and competent model for developmental biology, neurobiology, and human diseases. Furthermore, several research groups have applied the zebrafish model to the study of integrative and regulatory physiology and, in particular, the epithelial transport that is associated with body fluid ionic/osmotic homeostasis. Zebrafish, a freshwater teleost fish, inhabits a hypotonic freshwater environment with low ionic concentrations (compared to those in the plasma) and fluctuant pH and therefore has to actively absorb ions from and secrete acid (or base) and ammonia to the environment, in order to maintain body fluid homeostasis. The majority of these tasks related to ion regulation are performed by the gills, and ionocytes within the gills are the major cell types involved in epithelial ion transport [28]. Recently, four types of zebrafish ionocyte were identified to be responsible for the transport of various ions by distinct sets of ion transporters (Fig. 1), and these ionocytes are analogous to the transporting cells in different segments of the mammalian kidney, in terms of the expression and function of ion transporters. During the embryonic stages before the gills are fully functional, ionocytes initially develop in the embryonic skin, becoming functional at around 24 h post-fertilization (hpf) (zebrafish embryos usually hatch between 48 and 72 hpf at 28.5 °C). Consequently, zebrafish embryos serve as a powerful in vivo model in which to study epithelial ion transport physiology and regulation, for the following reasons: First, ionocytes in embryonic skin are directly exposed to the environment, and their morphology and ion transporter expression can be readily observed in situ without further anatomic manipulation; second, ion fluxes (representing ion transporter function) can be directly examined by noninvasive electrophysiological approaches or the use of tracers (fluorescent probes or radioisotopes); third, the effects of transporter (or ionocyte) loss- or gain-of-function can be analyzed within 1–5 days post-fertilization, at which time the injected morpholinos or mRNAs are effective; and finally, incubation of zebrafish embryos (or adults) in media with different ionic compositions (including changes in Na+, Cl−, Ca2+, NH4+, pH, or others) can facilitate investigation of transport function and regulation of a specific ion by the target transporter.

Methods:

Using combinations of keywords such as “ion uptake” “tans epithelial transport” “zebra fish” etc. a search was performed on different search engines for articles on the topic related to ion transport in zebra fish The search was limited to English language articles published previously. Resulting abstracts were reviewed and articles were excluded if the focus was not on the present topic in hand. Articles representing a developing body of literature were limited to the most recent date.

Results:
A total of 317 articles were retrieved through various search engines such as Science Direct, Google Scholar, Springer and Elsevier. We found 243 article titles that match the aspects of our study. Abstracts of 104 were reviewed 62 of which were selected and reviewed here.

Discussion:
HR, NaR, NCC, and KS ionocytes in zebrafish embryonic skin and gills express distinct sets of ion transporters and thus perform transepithelial H+ secretion/Na+ uptake/NH4 + excretion, Ca2+ uptake, Na+/Cl− uptake, and K+ secretion, respectively. Validation of the proposed zebrafish model requires analysis of the transport kinetics and stoichiometry of several apical transporters (e.g., NCC, pendrin), to understand how these transporters operate in a hypotonic freshwater environment with thermodynamically unfavorable chemical gradients. Basolateral transporters, such as ClC and KCC, await identification and functional analysis. The search for new transporters and transport functions in identified ionocytes or in new types of ionocytes is still in progress, and this may lead to the development of a more comprehensive model. In the model for the molecular pathways of ionocyte development, ionocyte progenitors develop from epidermal stem cells and then differentiate into NaR and HR ionocyte types through a positive regulatory loop involving Foxi3a/-3b and other transcription factors. Although more studies are required to fully elucidate these pathways, the proposed model has helped to pave the way for studies into the role of cell proliferation and differentiation in the functional regulation of ionocytes. Several hormones have been found to participate in the regulatory pathways of ionic homeostasis in zebrafish; detailed studies into the target ion transport pathways, ion transporters, or ionocytes of hormonal actions have been performed, particularly for cortisol, vitamin D, STC-1, CT, and isotocin and their specific receptors, and similar approaches could be applied to other hormones in the future. The four types of ionocyte in zebrafish are analogous to various renal tubular cells, in terms of their ion transporter expression and function. Zebrafish is highly suited for experimental analysis, in terms of the applicability of molecular tools, ease of in vivo cellular observation and function analysis, and rapid embryonic development, and these advantages enable this model to not only enhance our understanding of body fluid ion homeostasis and hormonal control in fish but to also inform studies on mammals or other animal species, thereby providing insights into related fields. However, the drawbacks of the zebrafish model need to be taken into consideration. While pharmacological and loss-of-function approaches are comparatively easy and efficient methods with which to assay the ion regulatory roles of ion transporters, hormones, and hormone receptors in zebrafish in vivo, the necessary reagents or morpholinos affect whole zebrafish embryos; as such, interpretations of cause and effect should be made cautiously, pending further experiments to tease apart the compensatory responses of different transporters or isoforms and the effects of interactions between systemic and local hormones, different receptors, and other hormones.

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