Cell-based homologous expression system for in-vitro characterization of environmental effects on transmembrane peptide transport in fish.

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Abstract

All organisms encounter environmental changes that lead to physiological adjustments and drive evolutionary adaptations. These, in turn, induce behavioral, physiological and molecular changes that affect each other. Deciphering the role of molecular adjustments in physiological changes will help to understand how multiple levels of biological organization are synchronized during adaptations. Transmembrane transporters are prime targets for molecular studies of environmental effects, as they facilitate the ability of cells to interact with the external surrounding. Fish are subjected to fluctuations of environmental factors of their aquatic surrounding and exhibit different coping mechanisms. To study the molecular adjustments of fish proteins to their unique external surrounding, suitable experimental systems must be established. Mozambique tilapia (Oreochromis mossambicus) is an excellent model for environmental stress studies due to its extreme osmotolerance. We established a homologues cellular-based expression system, and an uptake assay, that allowed us to study effects of environmental conditions on transmembrane transport. We applied it to study the effects of environmental conditions on the activity of PepT2, a widely studied transporter due to its importance in absorption of dietary peptides and drugs. We created a stable, modified fish cell-line, exogenously expressing the tilapia PepT2 and tested the effects of temperature and water salinity on the uptake of fluorescent di-peptide, β-Ala-Lys-AMCA. While temperature affected the Vmax of the transport, salinity affected both the Vmax and the Km. These assays demonstrate the importance of suitable experimental systems for fish ecophysiology studies. The presented tools and methods can be adapted to study other transporters in-vitro.

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