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Localization of aquaporin 1 and 3 in the gills of the rainbow wrasse *Coris julis*

Elvira Brunelli[a,*], Angela Mauceri[b], Fasulo Salvatore[b], Alessia Giannetto[b], Maria Maisano[b], Sandro Tripepi[a]

[a]*Department of Ecology, University of Calabria, Via P. Bucci, 4B I-87036 Rende, Cosenza, Italy*
[b]*Department of Animal Biology and Marine Ecology, University of Messina, Contrada Sperone 31 I-98166 S. Agata, Messina, Italy*

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Summary

Ultrastructural and immunohistochemical studies were conducted on the gill epithelium of the Mediterranean rainbow wrasse (*Coris julis*). We analysed the immunolocalisation of aquaporin 3 (AQP3) and aquaporin 1 (AQP1) in the gills using confocal microscopy. The ultrastructural features of the gill were investigated using transmission and scanning electron microscopy. The *C. julis* gill apparatus showed structural characteristics typical for Teleostei. Immunolocalization revealed differential localization of AQP1 and AQP3 in the gill epithelium. Double immunolabelling for Na^+/K^+ ATPase with AQP1or AQP3 revealed that AQP1 is localised in chloride cells, whereas AQP3 is localized in both the chloride cells and the accessory cells. This result suggests an active role of these cells in water/glycerol transport in saltwater fish.

Introduction

Water movement across the cell membrane is regulated by aquaporins (AQPs) or major intrinsic proteins (MIPs), which constitute a family of highly conserved transmembrane proteins (Agre, 1997; Borgnia et al., 1999). Aquaporins are expressed in a wide variety of tissues in plants, microorganisms and vertebrates and are required to transport water and non-ionic compounds (Agre et al., 1993; Connolly et al., 1998; Heymann and Engel, 1999). Different subgroups of aquaporins have been described on the basis of transport specificity. For example, members of the water channel or channel forming integral protein (CHIP) group function exclusively as water channels, whereas members of the water/glycerol channels or glycerol intrinsic proteins (GLPs) are permeable to water, glycerol

*Corresponding author. Tel.: +39 984492975; fax: +39 984492986.
E-mail address: brunelli@unical.it (E. Brunelli).

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and urea (Zheng and Bollinger Bollag, 2003; Borgnia et al., 1999; Hatakeyama et al., 2001; Verkman and Mitra, 2000; Shapiguzov, 2004). AQP1 was the first member of this family to be found in erythrocytes and renal tubules (Preston and Agre, 1991) and is thought to be highly selective for water, whereas AQP3 belongs to the aquaglyceroporin group and is expressed in most types of epithelial cells of the urinary, digestive and respiratory tracts, as well as in the epidermis.

In teleost fish, the role of AQPs in the gill remains contentious because of contradictory results, which are attributable to either different organ-tissue preparations and/or possible species specificities (Cutler et al., 2007; Giffard-Mena et al., 2007; Hirata et al., 2003; Lignot et al., 2002; Tse et al., 2006). Water transport mechanisms in the gills have been investigated in both freshwater and euryhaline fish (Cutler and Cramb, 2002; Giffard-Mena et al., 2007; Hirata et al., 2003; Lignot et al., 2002; Watanabe et al., 2005), but little is known about the role of aquaporins in marine non-euryhaline fish. An integrated ion and water transport mechanism in the gills is important to compensate for osmotic water loss and dehydration in marine teleosts.

The gill apparatus is one of the major osmoregulatory organs in fish. Thus, in addition to their respiratory function, the gills represent an important site for osmotic and acid–base regulation (Claiborne, 1997; Evans et al., 1999; Laurent, 1984). These different roles explain the structural complexity of this organ (Evans et al., 2005; Wilson and Laurent, 2002). The gill epithelium comprises specialised ion-transporting cells: the chloride cells (CCs) (Evans, 2002; Foskett and Scheffey, 1982; Karnaky, 1986), which are also called mitochondria-rich cells. CCs of marine fish and those of freshwater fish differ in their function and morphology (Hirose et al., 2003; Laurent, 1984; Perry, 1997). Pisam et al. (1987) described two subtypes of CCs – the β CCs and the α CCs – that are found in freshwater and seawater fish, respectively (for a review, see Pisam and Rambourg, 1991).

The structure of fish gills has been the subject of several studies in different species (for recent reviews see Wilson and Laurent, 2002), but only a few studies have focused on marine species. In the work reported herein, we conducted preliminary histological and ultrastructural analyses to provide detailed anatomical information about the gill apparatus of the Mediterranean teleost *Coris julis*. We hypothesized that the localization of two members of the AQP family, an aquaporin and an aquaglyceroporin, would prove to be useful in assessing the role of this family of proteins in maintaining the water balance of marine fish and in determining the mechanisms of regulation and osmoreception in the gills.

Materials and methods

Animal collection and maintenance

We collected samples of wrasse, *C. julis*, from the Tyrrhenian coast (S. Lucido, Italy) using baited traps. Captured fish were kept alive in an aerated water tank, transferred to the laboratory, and placed in two 150 l aquaria filled with natural seawater and equipped with filter and oxygenation systems. The animals were acclimatized for 8 days at a temperature of 18–24 °C under a natural photoperiod (light/dark) cycle and were fed daily with a commercial fish food (Tetramin). Water quality parameters in the aquaria were determined before and during the experimental period: salinity (35%), density (1.027–1.028 g/cm^3), temperature (18–24 1 °C), nitrite and nitrate concentrations, dissolved oxygen (8.0–8.6 mg/l), hardness (100 mg CO_3Ca/l) and the absence of heavy metals.

Sampling

Adult sea wrasses ($n = 8$; weight 11–15 g; length ranging from 12 to 16 cm) were randomly selected as experimental animals. The animals were anaesthetised with 2–4 g/l tricaine methane sulphonate (MS 222, Sandoz, Sigma, St. Louis, MO) and killed by spinal cord transection. Animal manipulation was performed according to Ethical Committee recommendations and under the supervision of authorised investigators.

Light and electron microscopy

Tissue samples were fixed in 4% paraformaldehyde (Electron Microscopy Sciences) in phosphate-buffered saline (PBS, pH 7.1, Electron Microscopy Sciences) for 24 h, and then were postfixed in 1% osmium tetroxide (Electron Microscopy Sciences) in the same buffer. The samples used for transmission electron microscopy (TEM) observation were dehydrated in graded ethanol, soaked in propylene oxide and embedded in Epon–Araldite. Semi-thin sections (1–2 μm) were stained with Grimley's dyes (toluidine blue, malachite green and acid fuchsin) and observed using a light microscope (Leitz Dialux EB 20). Ultrathin sections were stained with uranyl acetate in ethanol and lead citrate, coated in Edwards EM 400 and then examined and