The development of correlative microscopy techniques to define ultrastructure and morphology in chloride cells of Nile tilapia yolk-sac larvae

Sophie Fridman*, James E. Bron & Krishen J. Rana
Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, U.K.
*Corresponding author: scf1@stir.ac.uk

1. Introduction

The Nile tilapia (Oreochromis niloticus L.), the predominant farmed species in African aquaculture, displays an ability to thrive in low salinity waters not otherwise used for culture of conventional freshwater fish. The capacity for early life stages to osmoregulate effectively initially on chloride cells which are located predominantly on the body surface of larvae. These specialised cells are responsible for the trans-epithelial transport of ions, thus being achieved using the transport protein Na+/K+-ATPase.

In this study, chloride cells on yolk-sac larvae of Nile tilapia that had been incubated and reared in brackish water were visualised with an anti-Na+/K+-ATPase antibody and a secondary labelling probe FluoromTag® using a combination of microscopy techniques – confocal scanning laser microscopy and transmission electron microscopy. Scanning electron microscopy was also used to confirm the appearance of the structure of the chloride cells on the surface of larvae.

Correlative microscopy, in which a single sample can be examined by multiple imaging techniques e.g. fluorescence and electron microscopy, offers an integrated approach to microscopy and provides valuable insight into biological questions that cannot be gained from a single imaging procedure.

2. Methods

Nile tilapia yolk-sac larvae, hatched and reared in a brackish water environment (20 ppt) were sampled at 3 days post-hatch. They were fixed and immunostained with 2μg/ml of Na+/K+-ATPase antibody using standard protocols, followed by secondary antibody labelling with 5μg/ml Alexa Fluor 488 FluoromTag®; a new immunoprobe comprising a 1.4 nm nanogold particle to which a specific antibody fragment and a fluorochrome had been conjugated (Figure 3).

Half of the samples were visualised on a confocal scanning laser microscope (CSLM) and the remaining samples were enhanced with GoldEnhance® for 10 minutes and processed for transmission electron microscopy (TEM). Samples were also processed for scanning electron microscopy (SEM).

3. Results and Discussion

• This novel and versatile technique, used for the first time on whole-mount fish larvae, offers complementary visualisation of the cellular staining pattern of the anti-Na+/K+-ATPase antibody both by fluorescence microscopy (Figure 2 A and B) and, after gold enhancement, by transmission electron microscopy (Figure 2 C) allowing the specific localisation of the antibody binding sites at an ultrastructural level. Na+/K+-ATPase is usually considered to be interspersed in the tubular system throughout the cytoplasm and localised to the basolateral aspect of the cell (Figure 2 D), but a novel observation made here is that it can also be seen to line the chloride cell wall adjoining the surrounding pavement cells to the apical opening (Figure 2 E) which would explain the full cellular fluorescent imaging.

• This correlative technique has also allowed us to demonstrate, for the first time, ramifying tubular extensions emanating from active chloride cells in fish larvae; Figure 3 A shows a weakly stained fluorescent outcrop underlying an active chloride cell and Figure 3 B shows nanogold particles that confirm the presence of immunolabelled Na+/K+-ATPase.

• Scanning electron microscopy identifies structures highlighted by fluorescent labelling (Figure 4 B) allowing morphology of the deep surface pores, typical of actively secreting chloride cells i.e. cells in contact with external environment, to be defined (Figures 4 A and C).

4. Impact Statement

• This integrated approach - used here for the first time on whole-mount specimens – offers valuable insight into cellular distribution of specific antigen and localisation of its binding sites at the ultrastructural level.

• The knowledge created in this work will assist our understanding of the role of chloride cells in the development of osmoregulatory capacity during critical early hatchery stages in tilapia.

• This research contributes towards the development of sustainable tilapia aquaculture in brackish water and areas of saline groundwater.

References

Acknowledgements
The authors would like to thank Mr Linton Brown for his excellent assistance with the electron microscopy.

Figure 1: Brackish water type chloride cells in 3 day post-hatch Nile tilapia larvae (A) 3-D TEM micrograph of developing cilia of yolk-sac larva showing deep pores of active chloride cells (arrow) (Bar = 50 μm) (B) chloride cells as detected by triple staining (anti-Na+/K+-ATPase (red), actin staining phalloidin (green) and ultrastructural detail of cell wall indicating actin rings around pores (E) 3-D TEM micrograph of deeply-ridged pavements and deep pore typical of a brackish water type chloride cell (Bar = 1 μm).

Figure 2: Positive staining of anti-Na+/K+-ATPase transporter antibody located in brackish water type chloride cells. 3-D images of fluorescent labelled antibody in single (A) and multiple (B) chloride cells using CSLM (Q) transmission electron microscope of ultrathin section; white arrows indicate GoldEnhanced® immunogold labelling of Na+/K+-ATPase transporter (D and E) high magnification detail of labelling sites.

Figure 3: Positive staining of anti-Na+/K+-ATPase transporter antibody located in brackish water type chloride cells (A) 3-D image of fluorescently labelled antibody showing a single chloride cell with ramifying tubular extensions (CLSM) (B) TEM micrograph displaying corresponding nanogold particles in outposts of chloride cell.