Progress towards a method of targeted protein knockdown in *Fundulus heteroclitus*

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The isolated opercular epithelium of killifish, *Fundulus heteroclitus,* has been an electrophysiological model for elucidating fundamental mechanisms of ion transport in vertebrate secretory epithelia [1]. When mounted in an Ussing chamber, active Cl^- secretion by this tissue can be monitored as short-circuit current [4]. The molecular identity of the major ion transport proteins in this tissue have been identified (Na/K-ATPase, NKCC, and CFTR), but a comprehensive understanding of the molecular mechanisms that coordinate transport by these proteins is lacking. Molecular identification of signaling components in this model tissue will require the ability to alter gene expression. In this study, we investigated whether morpholino oligonucleotides, which bind to homologous mRNA and block protein synthesis [3], could be delivered to the cytosol of opercular cells. We specifically tested two prerequisites for mopholino-mediated inhibition of protein synthesis. 1) Could isolated opercula remain viable in culture media long enough for protein turn-over to reduce protein levels? And, 2) could morpholino oligonucleotides be delivered into opercular cells?

Opercular epithelia were removed from killifish that were maintained in seawater as described previously [2]. Isolated epithelia were immersed in culture media and stored at 18°C. To assess cell viability, 10 μM calcein AM and ethidium-1 were added to the media of one set of epithelia for up to one hour. To assess morpholino delivery, 20 μM fluorescein-labeled control morholino and 20 μM of Endo-porter delivery agent (Gene Tools, Philomath, OR) were added to the media of a second set of epithelia for 24 h. All treated tissues were washed for at least 10 minutes in opercula Ringer [2], and imaged with confocal microscopy (Olympus).

Opercular epithelial cells remained viable for up to 72 h in culture (>10:1 Calcein:ethidium-1 staining ratio, not shown), and labeled-morpholinos were readily delivered to the cytosol of operlular cells by co-inbubation with Endoporter for 24 h. Large, ovoid cells up to 20 μm in diameter contained morpholinos, suggesting that delivery occurred in ion secreting Cl^- cells. Therefore, the opercular epithelium is amenable to morpholino delivery and protein knockdown. Future experiments will quantify knockdown of killifish gill proteins and determine if morpholino-treated opercula can generate short-circuit currents. A method to specifically and potently knock-down protein expression would make the opercular epithelium of killifish a powerful new model for defining the molecular details of epithelial ion transport signaling.

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