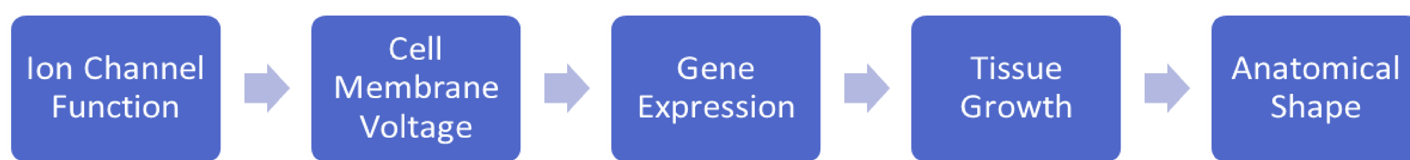
How Certain Ion Channel Proteins Contribute to the Development of the Face in *Xenopus*

Miriam E. Gladstone & Dany S. Adams, Department of Biology, Tufts University

Introduction

- Membrane voltage results from the separation of charged particles, or ions, across the cell membrane
- Ion channels open and close to control ion movement and, thus, membrane voltage
- Patterns of membrane voltage across cells, named the 'electric face', have been shown to precede and control cell movement and development in *Xenopus*
- Channelopathies, diseases caused by the malfunctioning of ion channels, disrupt the electric face and have been found to cause similar craniofacial abnormalities (jaws, eyes, gills / inner ear) in humans and in *Xenopus*



- Neural crest cells originate above the developing spinal cord and develop into the jaw, eye and gills

Objectives

My research aim is to answer two questions:

What is the normal development of *Xenopus*?

- Create a photo atlas of images of the normal craniofacial growth and development of *Xenopus* embryos
- This photo atlas will be available online for other researchers to use and contribute to

How do certain ion channels control craniofacial development?

- Understand how faulty ion channel functioning effects membrane voltage, gene expression and tadpole development

This summer, I also learned many laboratory techniques that will be used during my research throughout the 2015—2016 school year.

Materials & Methods

- Normal craniofacial development was documented by photographing *Xenopus* embryos at various stages of development
- Embryos were injected with mRNA that encodes for a fluorescent protein that is controlled by a neural crest promoter. Fluorescent microscopy was used to photograph the normal expression of neural crest DNA
- Embryos were then embedded in agarose and sectioned using a Vibratome, which slices embedded embryos into thin sections, to obtain a better view of fluorescent neural crest proteins
- Ion flux was changed or blocked by microinjection of fluorescent mRNA & DNA into untreated embryos. This changed the function of ion channels, including Arch-NFP

Material & Methods Continued

- Ion flux was also altered by exposing untreated embryos to certain drugs which changed ion channel function, including Benzoquinonium Dibromide
- The patterns of membrane voltage were monitored using membrane voltage reporting dyes, which move in and out of a cell depending on its membrane voltage
- The expression of key genes was monitored using immunohistochemistry and in situ hybridization
- Embryos were then grown up in MMR and scored for abnormal craniofacial phenotypes
- Abnormal embryos were fixed in paraformaldehyde to be compared to the images of the pattern of electric signals in the cells of the face
- Abnormal embryos were stained with Alcian Blue then dissected to observe skeletal structure

Results

One of the primary results from this research is the beginning of an atlas of edited photos, as shown below, of embryos in various stages of development.

Figure 1. Normal *Xenopus* embryos at approximately stage 12 in profile, vegetal and animal views respectively.

The normal expression of neural crest DNA was photographed and will be compared to neural crest DNA expression in embryos whose ion flux has been disrupted to understand how membrane voltage effects neural crest cells.

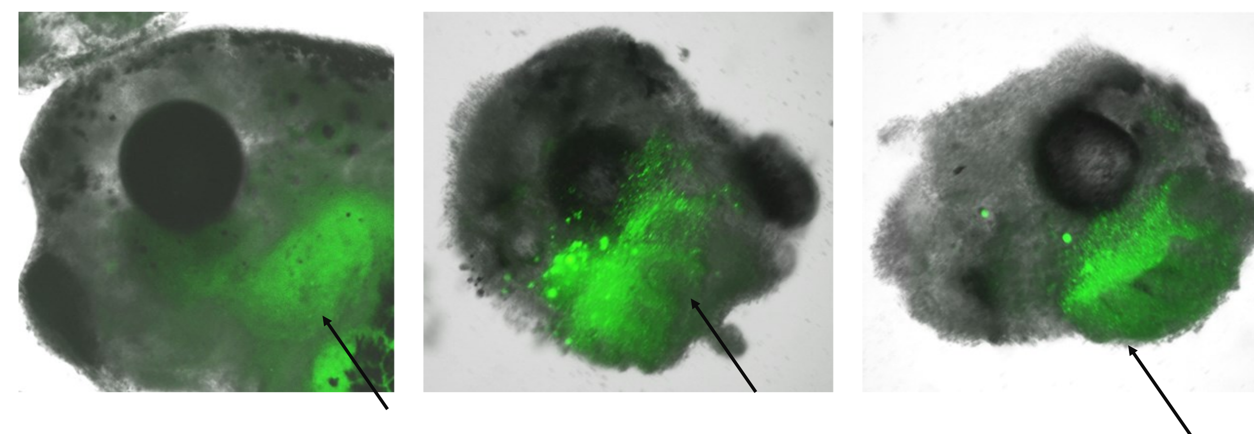


Figure 2. Fluorescent microscopy images of one whole and two dissected (left to right) embryos injected with tol2-slug + transposase which fluoresces where the neural crest promoter is being expressed. The branchial arches (indicated), which are derived from neural crest cells, were glowing.

Patterns of membrane voltage were photographed after injection of Arch-NFP, which reverses H^+ pumps, using membrane voltage reporting dyes.

Results Continued

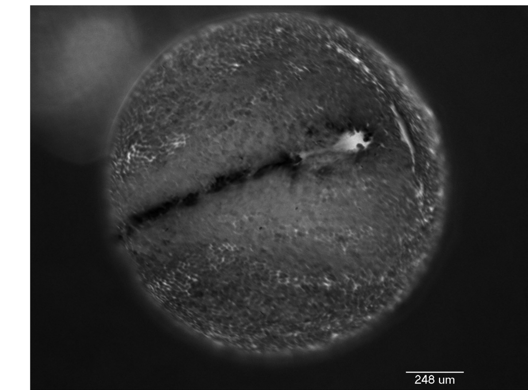


Figure 3. Patterns of cell membrane voltage in an embryo that had been injected with Arch-NFP, which reverses H^+ pump direction. CC2/DMPE was used as a membrane voltage reporting dye pair.

- Embryos that were exposed to Benzoquinonium Dibromide were scored for craniofacial abnormalities. There was not a higher frequency of craniofacial abnormalities among treated embryos compared to no treatment embryos.
- In one dish of treated embryos, the skulls of many embryos were malformed and their brains were breaking out of a small hole at the top of their heads. However, this phenotype was not present in another dish of identically treated embryos.

Conclusions

The normal patterns of development were thoroughly documented. The location and movement of neural crest cells was also photographed. These images will serve as a comparison for when ion channels are manipulated.

The phenotype of the Benzoquinonium Dibromide treated embryos is hypothesized to be due an infection that caused the malformation in these embryos because the identically treated embryos showed no phenotypes.

In the future, more genes, that turn off or upregulate certain ion channels, will be injected and embryos will be exposed to different drugs will alter ion channel function. The electric face of the embryo will be imaged using membrane voltage reporting dyes. In-situ hybridization and immunochemistry will be used to monitor the expression of key genes and the tadpoles scored for craniofacial defects. In this way, the effect of ion channels on the cell membrane voltage, gene expression and morphology will be determined.

References

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