The face of a frog: Time-lapse video reveals never-before-seen bioelectric pattern

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For the first time, Tufts University biologists have reported that bioelectrical signals are necessary for normal head and facial formation in an organism and have captured that process in a time-lapse video that reveals never-before-seen patterns of visible bioelectrical signals outlining where eyes, nose, mouth, and other features will appear in an embryonic tadpole.

The Tufts research with accompanying video and photographs will appear July 18 online in advance of publication in the journal *Developmental Dynamics*.

The Tufts biologists found that, before the face of a tadpole develops, bioelectrical signals (ion flux) cause groups of cells to form patterns marked by different membrane voltage and pH levels. When stained with a reporter dye, hyperpolarized (negatively charged) areas shine brightly, while other areas appear darker, creating an "electric face."

"When a frog embryo is just developing, before it gets a face, a pattern for that face lights up on the surface of the embryo," said senior author Dany S. Adams, Ph.D. Adams is a research associate professor in the Department of Biology in the Tufts School of Arts and Sciences and a member of the Tufts Center for Regenerative and Developmental Biology. "We believe this is the first time such patterning has been reported for an entire structure, not just for a single organ. I would never have predicted anything like it. It's a jaw dropper."

Tufts Post Doctoral Associate Laura N. Vandenberg, Ph.D., was first author of the paper entitled "V-ATPase-dependent ectodermal voltage and pH regionalization are required for craniofacial morphogenesis." Ryan D. Morrie, a biology major in the School of Arts and Sciences, was second author.

Scientific Serendipity

The discovery was a case of scientific serendipity. Adams has spent years studying bioelectrical patterning and left-right developmental differences. Her frequent research tool is a camera hooked up to a microscope that sends images to a computer.

One evening in September 2009 Adams was making time-lapse movies of early stage tadpole development. The images were coming out particularly clearly—no small achievement when filming tiny living creatures. She decided to leave the camera on overnight even though she anticipated that as the developing embryos began to move, the images would likely become too blurred to be useful.

When Adams arrived the next morning, the image on the computer monitor was out of focus as expected. But when she finished processing the rest of the images, she found they were clear. The movies were, she says, "unlike anything I had ever seen. I was completely blown away. I think I thought something like, 'OK, I know what I'll be studying for the next 20 years.'"

The imagery revealed three stages, or courses, of bioelectric activity.

First, a wave of hyperpolarization (negative ions) flashed across the entire embryo, coinciding with the emergence of cilia that enable the embryos to move. Next, patterns appeared that matched the imminent shape changes and gene expression domains of the developing face.

Bright hyperpolarization marked the folding in of the surface, while both hyperpolarized and depolarized regions overlapped domains of head patterning genes. In the third course, localized regions of hyperpolarization formed, expanded and disappeared, but without disturbing the patterns created during the second stage. At the same time, the spherical embryo began to elongate.

The Tufts team found that disrupting bioelectric signaling by inhibiting ductin (a protein that is part of the machinery that transports hydrogen ions) correlated with craniofacial abnormalities. Some embryos grew two brains rather than one; others had thickened optic nerves or lacked normal nasal or jaw development. Interrupting the ion flux also altered the bioelectric patterns on the embryos' surface and expression of important face patterning mRNAs (messenger RNA that acts as a blueprint for proteins).

"Our research shows that the electrical state of a cell is fundamental to development. Bioelectrical signaling appears to regulate a sequence of events, not just one," said Laura Vandenberg. "Developmental biologists are used to thinking of sequences in which a gene produces a protein product that in turn ultimately leads to development of an eye or a mouth. But our work suggests that something else – a bioelectrical signal - is required before that can happen. "

Adams and Vandenberg note that more research is needed to discover if bioelectrical signaling works the same in frogs as in other animals, including people, and if an "electric face" exists in human development. However, they believe that study of such signaling holds great potential.

"Studying bioelectrical signaling has led us to a different, and broader, way of thinking about diseases like cancer, birth defects and tissue regeneration," Adams notes. "Potentially we can find electrical switches that turn on entire developmental cascades rather than having to find many specific tools that turn on many specific genes within that cascade, as is the current approach with gene therapy. After all, we already have tools for regulating some of these bioelectrical signals, such as drugs that prevent acid reflux by controlling potassium and hydrogen ions."

Provided by Tufts University