Researchers discover that changes in bioelectric signals cause tadpoles to grow eyes in back, tail

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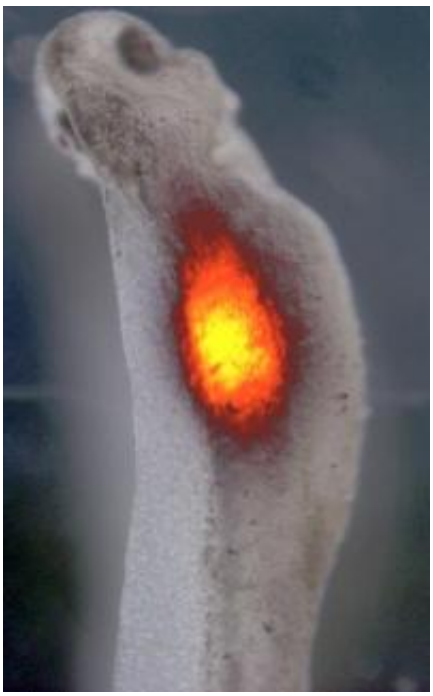

Changing the bioelectric voltage in embryonic frog cell in tadpole's back caused cell to develop into a functioning eye. Credit: Michael Levin and Sherry Aw

For the first time, scientists have altered natural bioelectrical communication among cells to directly specify the type of new organ to be created at a particular location within a vertebrate organism. Using genetic manipulation of membrane voltage in Xenopus (frog) embryos,

biologists at Tufts University's School of Arts and Sciences were able to cause tadpoles to grow eyes outside of the head area.

The researchers achieved most surprising results when they manipulated membrane voltage of cells in the tadpole's back and tail, well outside of where the eyes could normally form. "The hypothesis is that for every structure in the body there is a specific membrane voltage range that drives organogenesis," said Pai. "These were cells in regions that were never thought to be able to form eyes. This suggests that cells from anywhere in the body can be driven to form an eye."

To do this, they changed the voltage gradient of cells in the tadpoles' back and tail to match that of normal eye cells. The eye-specific gradient drove the cells in the back and tail—which would normally develop into other organs—to develop into eyes.

These findings break new ground in the field of biomedicine because they identify an entirely new control mechanism that can be capitalized upon to induce the formation of complex organs for transplantation or regenerative medicine applications, according to Michael Levin, Ph.D., professor of biology and director of the Center for Regenerative and Developmental Biology at Tufts University's School of Arts and Sciences. Levin is senior and corresponding author on the work published in the journal *Development* online December 7 2011, in advance of print.

"These results reveal a new regulator of eye formation during development, and suggest novel approaches for the detection and repair of birth defects affecting the visual system," he said. "Aside from the regenerative medicine applications of this new technique for eyes, this is a first step to cracking the bioelectric code."

Tufts post-doctoral fellow Vaibhav P. Pai Ph.D., is first author of the

paper, entitled "Transmembrane Voltage Potential Controls Embryonic Eye Patterning in Xenopus laevis." .

Eye developed in midsection of tadpole. Credit: Michale Levin and Sherry Aw

Signals Turn On Eye Genes

From the outset of their research, the Tufts' biologists wanted to understand how cells use natural electrical signals to communicate in their task of creating and placing body organs. In recent research, Tufts biologist Dany S. Adams showed that bioelectrical signals are necessary for normal face formation in the Xenopus (frog) embryos. In the current set of experiments, the Levin lab identified and marked hyperpolarized (more negatively charged) cell clusters located in the head region of the frog embryo.

They found that these cells expressed genes that are involved in building the eye called Eye Field Transcription Factors (EFTFs). Sectioning of the embryo through the developed eye and analyzing the eye regions under fluorescence microscopy showed that the hyperpolarized cells contributed to development of the lens and retina. The researchers hypothesized that these cells turned on genes that are necessary for

building the eye.

Changing the Signals Lead to Defects

Next, the researchers were able to show that changing the bioelectric code, or depolarizing these cells, affected normal eye formation. They injected the cells with mRNA encoding ion channels, which are a class of gating proteins embedded in the membranes of the cell. Like gates, each ion channel protein selectively allows a charged particle to pass in and out of the cell.

Using individual ion channels that allow, the researchers changed the membrane potential of these cells. This affected expression of EFTF genes, causing abnormalities to occur: Tadpoles from these experiments were normal except that they had deformed or no eyes at all.

Further, the Tufts biologists were also able to show that they could control the incidence of abnormal eyes by manipulating the voltage gradient in the embryo. "Abnormalities were proportional to the extent of disruptive depolarization," said Pai. "We developed techniques to raise or lower voltage potential to control gene expression."

Electric Properties of Cells Can Be Manipulated to Generate Specific Organs

The researchers achieved most surprising results when they manipulated membrane voltage of cells in the tadpole's back and tail, well outside of where the eyes could normally form.

"The hypothesis is that for every structure in the body there is a specific membrane voltage range that drives organogenesis," said Pai. "By using a specific membrane voltage, we were able to generate normal eyes in

regions that were never thought to be able to form eyes. This suggests that cells from anywhere in the body can be driven to form an eye."

Levin and his colleagues are pursuing further research, additionally targeting the brain, spinal cord, and limbs. The findings, he said "will allow us to have much better control of tissue and organ pattern formation in general. We are developing new applications of molecular bioelectricity in limb regeneration, brain repair, and synthetic biology." Additional authors include post-doctoral fellow Sherry Aw, Tufts Postdoctoral Associate Tal Shomrat, and Research Associate Joan M. Lemire. Funding for this research came from the National Institutes of Health.

More information: "Transmembrane voltage potential controls embryonic eye patterning in Xenopus laevis," Vaibhav P. Pai, Sherry Aw, Tal Shormat, Joan M. Lemire, *Development*, published online before print December 20, 2011,doi:10.1242/dev.073759

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