Voltage gated calcium channels 'read' electric patterns in embryos to create cartilage and bone

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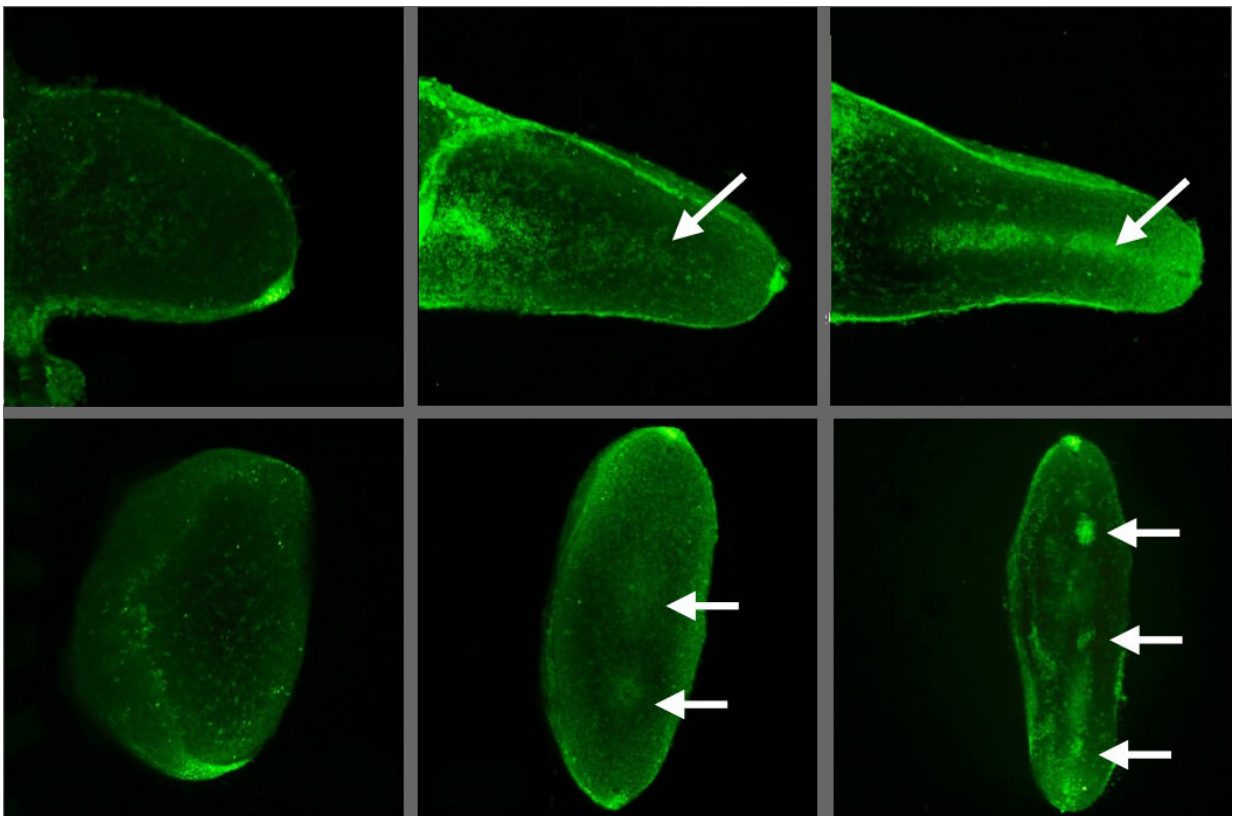

Voltage sensitive dye reveals regions of depolarization correlating with initial stages of cartilage and bone formation in the developing embryo (top, longitudinal; bottom, cross sectional views; left to right: embryo days 10.5, 11.5 and 12.5) Credit: Yu Wang & Mike Levin, Tufts University

Scientists at Allen Discovery Center at Tufts University and Harvard Medical School have revealed how, in the case of limb formation, the electrical patterns formed within an embryo initiate a cascade of molecular changes that culminate in the development of cartilage and bone. The study, published today in *Proceedings of the National Academy of Sciences (PNAS)*, helps answer a central question in developmental biology: "How do immature cells in the developing embryo differentiate and organize into a body?"

Prior studies have shown remarkable electrical patterns in developing embryos, some of which act like blueprints of the tissues and organs that eventually take shape as the embryo matures. The electrical patterns are created by cells pumping charged ions into and out of the cell membrane , creating a voltage potential across the membrane barrier. In analogy to cells of the nervous system, the membrane voltage potentials spread via electrical synapses and provide a way for cells to communicate with their neighbors and coordinate activity.

The new study, conducted in mouse and chicken embryos, demonstrates that a voltage gated calcium channel (VGCC) embedded in the cell membrane, and triggered by voltage to allow calcium ions (Ca2+) to flow into the cell, sets off the expression of genes that guide differentiation to mature cells.

Voltage gated ion channels are known to have a role in the formation of tracheal cartilage, and mutations in VGCCs are also correlated with malformations in humans, including syndactyly (fusion of fingers) in Timothy syndrome patients. The study now points to a role for VGCCs in 'reading out' the bioelectric patterns of an embryo to set the genetic and protein expression machinery in play for body development.

The researchers looked at the developing limbs of both mice and chicken embryos and found that while the limb bud is initially

hyperpolarized, with 1000-fold more calcium ions outside cells than inside, the core of the growing limb becomes depolarized as cartilage forms; (calcium ions flow into the cells to neutralize voltage). They found that the depolarization is mediated by VGCCs, allowing calcium ions to flow inward, which in turn activate a genetic transcription factor called NFATc1, which in turn initiates expression of other genes required for differentiation into mature cartilage cells. The VCGG studied (Cav1.2) was found to be essential for normal cartilage formation, while NFATc1 was confirmed to promote the differentiation of cartilage cells in vitro.

Looking at the role of Cav1.2 more closely, the researchers discovered that it primarily plays a part in the early stages of cell differentiation into cartilage. Other VGCCs may be involved at later stages of development, and other transcription factors may be activated by the increase in intracellular calcium during the process of cartilage and bone formation, according to the researchers.

"It's clear that while we have discovered an essential role for voltage gated calcium channels in reading the embryo's bioelectric pattern, we will need to continue work to understand the role of many different ion channels and genetic factors throughout all stages of development," said Cliff Tabin, associate of the Allen Discovery Center at Tufts and chairman of the Depertment of Genetics in the Blavatnik Institute at Harvard Medical School.

"We are just beginning to understand how the 'software' of embryonic development (the electrical patterns) are created and interpreted by the 'hardware' (the cells' genes and proteins) to enable the cells to cooperate and organize into a highly-patterned body," said Michael Levin, Vannevar Bush Professor of Biology in the School of Arts & Sciences and director of the Allen Discovery Center at Tufts. "Our collaboration with the genetics team in the Tabin lab extends this field to a very

important model, the vertebrate limb, and is a great demonstration of how we get to a better understanding of physics in biology."

More information: Yuji Atsuta el al., "L-type voltage-gated Ca2+ channel CaV1.2 regulates chondrogenesis during limb development," *PNAS* (2019). www.pnas.org/cgi/doi/10.1073/pnas.1908981116

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