Forsyth scientists gain greater understanding of how embryos differentiate left from right

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Researchers at the Forsyth Institute have discovered a new mechanism responsible for early left/right patterning, the process by which organs locate themselves on the left or right side of the body. The discovery of this novel mechanism, garnered through the study of three different vertebrates (frogs, chickens and zebrafish), marks the first time that a single common mechanism has been identified in left-right patterning in three distinct species. Such a discovery may have far-reaching implications for the understanding of craniofacial development, right-left hand preference, right/left brain dominance and a variety of birth defects in humans.

A team of Forsyth Institute scientists, led by Michael Levin, PhD, Director of the Forsyth Center for Regenerative and Developmental Biology, examined the molecular and genetic factors that control left/right asymmetry and identified a novel component: an ion transporter that creates strong natural voltage gradients and pH changes. The pump that normally acidifies subcellular compartments was shown to control embryonic laterality at very early stages. Their findings further challenged the previously held hypothesis that cilia (short hair-like structures on a cell) were the primary agents allowing an embryo to correctly position its internal organs along the left-right axis. Instead, their research showed a single asymmetry mechanism linking ciliary, serotonergic (serotonin is the chemical substance involved in transmitting signals between neurons), and ion flow mechanisms. The data was strengthened by the operation of this mechanism through all three vertebrates. This is important because prior data was very

fragmented and different asymmetry-controlling systems appeared to be operating in frog/chick embryos vs. human/mouse/zebrafish embryos.

"In our previous research we showed that this developmental event happens earlier than expected in frogs by identifying an ion transporter that generates natural bioelectrical signals that ultimately control gene expression and the position of the heart and visceral organs," Levin said. "We have now identified and explored an additional component of this novel mechanism – a protein pump that generates voltage and pH gradients. For the first time, we have a glimpse of how three different vertebrates utilize such ion flows in concert with ciliary movement and the function of pre-nervous neurotransmitters."

The findings, to be published in the May 1 issue of Development (available online on April 18th) are key for understanding human development. According to Dr. Levin, this work shows a unified model for understanding embryonic development, and is therefore likely to provide important insight into human development. "Biased left-right asymmetry is both a fascinating and medically important phenomenon," said Levin. "Problems with left/right asymmetry are responsible for a wide-range of birth defects in humans including conditions that affect the heart, the digestive system, the lungs and the brain. Building on our earlier research, we are gaining a significant understanding of asymmetry and getting closer to understanding its impact on humans. This fascinating ion pump has additional roles during development that are a goldmine of novel cellular control mechanisms."

Dr. Levin's team looked at molecular genetic and physiological characterization of a novel, early, biophysical event that is crucial for correct asymmetry: the flow of hydrogen ion or H+ flux. A pharmacological screen implicated the H+-pump H+-V-ATPase in Xenopus (frog) embryo asymmetry, where it directs left- and right-sided gene expression. The cell cytoskeleton is responsible for the LR-

asymmetric localization of this pump during the first few cell cleavages in frog embryos. H+-flux across plasma membranes is thus asymmetric at the four- and eight-cell stages, and this asymmetry requires H+-V-ATPase activity. Artificially equalizing the asymmetry in H+ flux, by increasing or decreasing it on both sides equally, both randomized the location of the viscera without causing any other defects. To understand the mechanism of action of H+-V-ATPase, researchers isolated its two physiological functions, cytoplasmic pH and membrane voltage gradient (Vmem) regulation. Varying either pH or Vmem, independently of direct manipulation of H+-V-ATPase, caused disruptions of the normal LR pattern, suggesting important roles for both physiological parameters. V-ATPase inhibition also abolished the normal localization of serotonin at the 16-cell stage, suggesting that it helps to regulate the early flow of this important neurotransmitter. These data implicate H+-V-ATPase activity in patterning the left right axis of three different vertebrates, reveal mechanisms both upstream and downstream of its activity, and identify a novel role for this important ion transporter. Based on these observations, they proposed a detailed pH- and Vmem-dependent model of the early physiology of left/right patterning.

Michael Levin, PhD. is an Associate Member of the Staff in The Forsyth Institute Department of Cytokine Biology and the Director of the Forsyth Center for Regenerative and Developmental Biology, www.cellregeneration.org/. Through experimental approaches and mathematical modeling, Dr. Levin and his team examine the processes governing large-scale pattern formation and biological information storage during animal embryogenesis. The lab's investigations are directed toward understanding the mechanisms of signaling between cells and tissues that allows a living system to reliably generate and maintain a complex morphology. The Levin team studies these processes in the context of embryonic development and regeneration, with a particular focus on the biophysics of cell behavior.

Source: Forsyth Institute