# 'Lefty' protein tightens control of embryonic development

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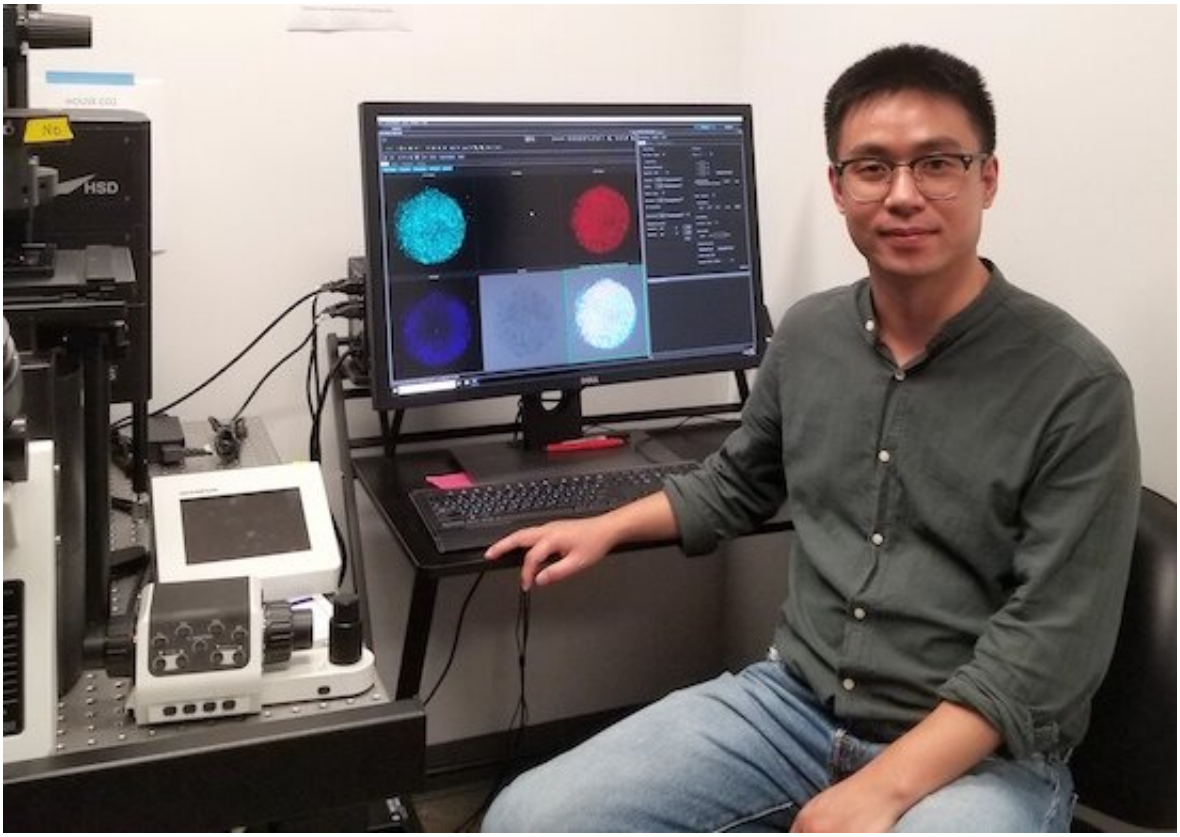


Rice postdoctoral researcher Lizhong Liu is lead author of a study that details the mechanism by which a protein known as Lefty pumps the brakes as human embryos begin to differentiate into bones, soft tissues and organs. Credit: Warmflash Lab

A protein known as Lefty pumps the brakes as human embryos begin to

differentiate into the bones, soft tissues and organs that make us.

This inhibitor protein is key during the early stages of life when the fates of embryonic stem cells are determined by the Nodal signaling pathway, according to Rice University bioscientists.

Experiments by the lab of Rice's Aryeh Warmflash and led by postdoctoral researcher Lizhong Liu have visualized for the first time the mechanism by which Nodal and Lefty interact to specify the future body plan in a mammalian embryo.

Their results appear in the open-access journal *Nature Communications.*

The study shows not only how Nodal signaling molecules, known as morphogens, are held in check by Lefty but also that Nodal proteins are passed directly from cell to cell, triggering transcription of new Nodals by the recipient. At the same time, a wave of Lefty transcription is transiently triggered in the cells when they first receive Nodal, regulating the speed of the wave.

"Basically, we show that rather than gradients of protein forming and instructing cells to become different cell types, the molecules involved do not diffuse at all," Warmflash said. "Instead, cells relay the signal so that each cell produces the signal and passes it to its neighbor, which causes the neighbor to produce it and so on. It's kind of like a game of telephone."

The unique experimental model developed by Warmflash and his team over many years allows them to see early stages of gastrulation, during which the initial stages of differentiation happen. The circular colonies of cells bear no resemblance to embryos and follow established ethics, but the cells communicate and react in a realistic way as they differentiate into three characteristic germ layers—the ectoderm,

mesoderm and endoderm—from the center toward the rim.

But actually visualizing the Nodal protein has, until now, been an issue. For the new study, Liu found a way to add fluorescent tags to the Nodal protein that didn't compromise gastrulation in any way.

"We needed to be sure that the fluorescent tag didn't affect the function," said Liu, who spent two years finding a solution. "Because the tag is so big—essentially half of the combined molecule with the Nodal protein—we didn't know if it was going to affect secretion (by cells) or diffusion."

Once assured the tag was benign, the lab began tracking Nodal's progression for up to 42 hours in various configurations of the colony and with or without Lefty's two human variants. They found that without Lefty's presence, Nodal diffusion toward the colony's center progressed much more quickly.

Liu said the ability to track individual proteins in a mammalian system could lead to discoveries about the mechanisms by which the morphogen and its inhibitor claim their territories.

Traditionally, patterns are thought to arise because some proteins diffuse faster than others, Liu said. Local Nodal activity could cause production of Lefty, which diffuses farther than Nodal and limits the signal to a territory of a particular size, a theory that he said has not been rigorously tested.

To the researchers' surprise, the direct observation of endogenous molecules showed no sign of Nodal diffusion. Instead, the wave that moves inward is the result of new Nodal proteins produced by each cell that then triggers its neighbor to do the same.

The lab was able to prove this is essential for the wave by creating cells that lack the Nodal protein. These cells could receive the signal but not pass it on to their neighbors.

The researchers also found that while Lefty didn't diffuse in their gastrulation experiments, it can move over much longer range in other contexts.

"We want to understand what determines the diffusivity of the Lefty proteins in a context-dependent way," Liu said. "Is it because the two variants of human Lefty show different diffusivity and expression patterns? It's challenging to answer this question, because they're very similar at both DNA and amino acid levels. We will have to figure out a way to distinguish them.

"Another thing we're interested in is to know how Nodal and Lefty cooperate with other co-factors to define the body axis," he said. "Where's the head, where's the tail, where are the left and right sides? We know Nodal works with co-receptors to do this in zebra fish, but in higher mammalian cells, the co-receptor might be fundamentally different."

**More information:** Lizhong Liu et al, Nodal is a short-range morphogen with activity that spreads through a relay mechanism in human gastruloids, *Nature Communications* (2022). DOI: 10.1038/s41467-022-28149-3

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