

DECEMBER 16, 2005

Researchers Discover Remarkable Developmental Pathway

Howard Hughes Medical Institute researchers have discovered an important regulatory pathway that enables frog embryos to develop normally even after being split in half—as happens in the development of identical twins.

The researchers said their findings suggest that efforts to apply embryonic stem cells therapeutically to regenerate damaged or diseased tissue may have to overcome similar self-regulatory mechanisms present in stem cells. Such mechanisms might otherwise drive stem cells to attempt to differentiate into embryos with many cell types, rather than restricting themselves to a desired single type of tissue.

"The key to making a perfect baby every time, these experiments tell us, lies in the ability to have a double gradient that will ensure a robust developmental system that produces the same structure time after time."

— Edward M. De Robertis

The researchers, graduate student Bruno Reversade and HHMI investigator Edward M. De Robertis, both at the University of California at Los Angeles, published their findings in the December 16, 2005, issue of the journal *Cell*.

The experiments were conceived in an attempt to learn more about the mechanisms underlying the establishment of a morphogenetic field. This field consists of a gradient of regulatory proteins that aids in organizing the differentiation of embryonic cells and gives an organism its overall shape. Although researchers had known that morphogenetic fields were responsible for the embryo's remarkable resiliency, very little was understood about how they function at the molecular level, said De Robertis.

For their studies, Reversade and De Robertis used early embryos of the African toad *Xenopus*. Widely used in embryological studies, *Xenopus* embryos are easy to grow and can be manipulated by tissue transplantation techniques. The researchers studied *Xenopus* embryos in the blastula stage,

which resembles a hollow sphere of a few thousand cells.

The scientists were seeking to understand more about the regulatory role of a family of proteins called bone morphogenetic proteins (BMPs). Certain BMPs are known to be key regulators of the dorsoventral (back-to-belly) patterning of embryos. In such patterning, dorsal cells differentiate into neural cells and ventral cells become epidermal cells.

“While BMPs were known to be important in this system, it had never been possible, for example, to turn an embryo completely into brain cells, or to destroy this morphogenetic system,” said De Robertis. “The embryo always tries to self-regulate and recover.”

In their experiments, the researchers split *Xenopus* embryos into dorsal and ventral halves and used techniques that enabled them to inhibit BMP signaling selectively in the halved embryos. They then observed what effects their manipulations had on embryonic development.

These experiments revealed that while the ventral half of the embryo required specific BMPs, “it was rather shocking to us that the dorsal part of the embryo developed fairly normally,” said De Robertis. The researchers' next series of experiments revealed that dorsal development required another member of the BMP family, called anti-dorsalizing morphogenetic protein (ADMP).

Their studies revealed that the two kinds of proteins in the two halves of the embryo were regulated in a “see-saw” fashion. For example, when the researchers decreased BMP signaling levels, ADMP levels would rise, and vice versa. Such compensatory ability is a key to self-regulation in the embryo, said De Robertis.

To their surprise, however, when they inhibited the activity of all the relevant regulators—BMP2, 4 and 7, and ADMP—the entire surface of the embryo became neural tissue. “This is a major transformation of a type you almost never see in embryos, said De Robertis. “It told us that BMPs play a crucial role in the establishment of a self-regulating morphogenetic field for dorsoventral patterning.” In addition, the researchers identified a number of other regulatory molecules that “fine tune” the morphogenetic field by selectively inhibiting BMPs.

One of the most dramatic results came from experiments in which the scientists grafted material from either dorsal or ventral BMP sources into the BMP-depleted embryos. The grafting restored the normal formation of the embryos.

“We think this finding is important in showing that the embryo is probably patterned by two gradients of BMP—one from the dorsal side and one from the ventral,” said De Robertis. “The key to making a perfect baby every time, these experiments tell us, lies in the ability to have a double gradient that will ensure a robust developmental system that produces the same structure time after time,” he said.

According to De Robertis, the discovery of such self-regulatory systems could have important implications for efforts to use stem cells to rejuvenate tissues lost to disease or trauma.

“When you try to differentiate stem cells in vitro, you tend to get a complex mix of different cell types,” he said. “This, we think, is because the cells are trying to self-regulate into making an embryo. And just as we found that the BMP system self-regulates in a see-saw fashion, other growth factor signaling systems in stem cells might be self-adjusting in this same way.” Thus, stem cell researchers might find it necessary to completely knock out self-regulatory systems, as De Robertis and Reversade did with the BMP system, to induce stem cells to produce specific tissues.

De Robertis and his colleagues plan to explore how other developmental regulatory pathways might integrate with the BMPs. The ultimate aim of such studies, he said, is to understand the intricate machinery of cellular signaling as an “integrated molecular circuit” that governs embryonic development.