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A large green and brown frog may help researchers define the pathway that leads to the development of the inner ear. **Dr. Jean-Pierre Saint-Jeannet**, assistant professor of developmental biology at the School, has worked for many years with the South African frog, *Xenopus laevis*, in his studies on neural crest cells formation. These cells are an important cell type of the body as they give rise to all bones and cartilage of the face, pigment cells, and most of the peripheral nervous system.

Current studies in his laboratory focus on Sox9 and Sox10, two members of a family of transcription factors involved in cell determination. When embryonic cells develop into the specialized cell types of the body, Sox proteins interact with the DNA and activate specific differentiation program. Like most transcription factors, they are, in essence, the switches that turn on genes, that encode a specific repertoire of proteins, signature of each cell types. It is thought that humans have about 1,000 different transcription factors.

"In recent years, a number of transcription factors that show restricted expression pattern in the developing inner ear, have been shown to play a major role in the specification and patterning of the auditory system," explains Saint-Jeannet. "We believe that Sox proteins are also part of that process."

Saint-Jeannet and his colleagues have shown that Sox9 and Sox10 are expressed very early in the progenitors that give rise to the inner ear. If the Sox9 gene is removed from the early frog embryo, the development of the ear is severely disturbed. The goal of these studies is to determine during which phase of the development of the inner ear Sox9 and Sox10 are essential. Saint-Jeannet thinks that it is very early in the process. In humans it has been shown that mutations in Sox9 and Sox10 genes result in severe pathologies characterized by a number of developmental defects, including deafness.

These studies, sponsored by the Deafness Research Foundation, hope to better define the processes involved in inner ear formation and therefore improve our understanding of the numerous pathologies associated with the auditory system.

Scientists find a gene that's key to cloning success

By Stephen Bradt

A team led by **K. John McLaughlin** and **Hans R. Schöler** at the School has found that the activity of a single gene is a powerful predictor of whether newly cloned mammalian embryos will survive and thrive, but the gene's sporadic expression in cloned mouse embryos casts fresh doubt on prospects for reproductive human cloning.

Despite the successful cloning of sheep, pigs and cats, mammalian cloning—in which an ordinary cell's nucleus is transferred to an egg whose nucleus has been removed—remains remarkably inefficient. Fewer than three in 100 cloned mouse embryos survive to birth.

McLaughlin and Schöler's groups showed that the activity of the gene Oct4 correlates strongly with the viability of cloned embryos but also found that in only one-tenth of cloned mouse embryos is the gene expressed at the right level in the right place at the right time. Without Oct4, embryos cannot survive; even if Oct4 expression is a tad high or low, an embryo will die.

"Cloning requires the precise reprogramming of the nucleus inserted into an enucleated egg," said Schöler, professor of animal biology and director of Penn's Center for Animal Transgenesis and Germ Cell Research. "This nucleus must abandon its former genetic program and adopt the genetic profile of an embryonic nucleus; failure to do so dooms the embryo."

To evaluate the accuracy of this genetic reprogramming, the Penn group analyzed Oct4 expression in cloned mouse embryos derived from cells that surround ovulated eggs in adult mice, cells that would not normally express Oct4. The result: Only 34 percent of embryonic cells correctly reprogrammed to express Oct4, and just 10 percent showed levels of Oct 4 expression conducive to further development.

Even as it suggests new hurdles for reproductive human cloning, the Penn work offers new support for the feasibility of therapeutic cloning using embryonic stem cells.

"The small number of clones that did adequately express Oct4 were capable of forming embryonic stem cell lines," Schöler said, "supporting existing evidence of the scientific feasibility of therapeutic cloning even as it shows the infeasibility of reproductive human cloning."

The Penn team chose Oct4 as a marker because its expression is tightly regulated throughout the mammalian life cycle. Oct4, which encodes a protein critical to normal embryonic development, is expressed only in the portion of the embryo that eventually gives rise to fetal tissues; in the adult, Oct4 is expressed only in germ cells.

Although improper expression of Oct4 can single-handedly obstruct embryonic development, Oct4 is likely not the only gene expressed incorrectly in cloned embryos.

"When we started the study, we thought Oct4 misexpression might account for a fraction of clone failures," Schöler said. "The big surprise was that Oct4 alone can explain most of the failures, although Oct4 is probably just one gene of many whose misexpression can cause cloned embryos to fail."

The research team was a combined effort of the laboratories of McLaughlin and Schöler, both faculty in the School's Department of Animal Biology, with major efforts from postdoctoral researchers **Michele Boiani** and **Sigrid Eckardt**, all part of the Center for Animal Transgenesis and Germ Cell Research at Penn's School of Veterinary Medicine. Their work is supported by the Marion Dilley and David George Jones Funds and the Commonwealth and General Assembly of Pennsylvania.