Frogs prove ideal models for studying developmental timing

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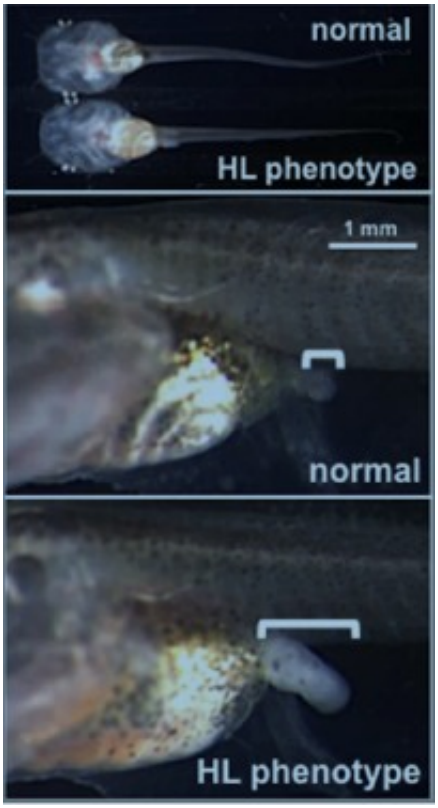

Hind limb phenotype in F1 offspring. Representative sibling offspring from a pair of TR! TALEN founders were imaged at feeding stage (upper panel). The developmental difference in hind limbs is shown in the lower panels. The hind limbs are bracketed.

University of Cincinnati endocrinology researchers were recently able to mutate the thyroid hormone receptor (THR) in one of two cells during

the first step of early egg division in tadpoles. As a result, they have successfully disrupted the developmental timing of the hind limbs, showing clear evidence for the importance of THR in the early development of vertebrates. The results of this study may also have the potential to shed light on the importance of hormones in early development in humans.

With new gene mutation technology developed in the last two years, UC researcher Daniel Buchholz, associate professor of biological sciences and graduate student Jinyoung Choi, along with scientists in the Department of Mathematical and Life Sciences at Hiroshima University, were able to successfully mutate the gene in the tadpole models. Together, they found the value of tadpoles as ideal models for studying the role of hormones in development because of the timely metamorphosis from tadpole to juvenile frog, and because that transition is completely dependent on hormones.

Choi and Buchholz recently published this research in the prestigious journal *Endocrinology*, titled Unliganded thyroid hormone receptor alpha regulates developmental timing via gene repression as revealed by gene disruption in Xenopus tropicali. Choi will also present their research at the 2015 ENDO Conference in March in San Diego, and Buchholz will present their work at the NASCE (North American Society of Comparative Endocrinology) biannual meeting in Toronto, June 21-25.

In earlier studies, Buchholz found that tadpoles don't metamorphose in the absence of hormones. They instead just become larger tadpoles.

This phenomenon was first discovered in 1916 when scientists were able to surgically remove the thyroid gland and found out that thyroid hormone is required for metamorphosis. Now, at the almost 100-year anniversary of this revelation, Buchholz and Choi are now able to study the other part of the story.

"Now we can manipulate the genes and the proteins that are the receptors to look even further into what these receptors do," says Buchholz. "This new technology has been cited in Science and Nature Magazines and could very well revolutionize the study of non-model organisms. Other scientists have been using this technology in other organisms, but we are one of the first to use the technology in tadpoles."

Since Hiroshima University was already using this new technique, they supplied Buchholz and Choi with the technology and sent the reagents here to make the mutation.

During the first step of development where the egg divides into two, Choi was able to mutate the THR in only one of the cells. While that cell makes up half the body, Choi was able to label what cell she manipulated, which gave rise to that half of the body. From that she could determine which side had the mutation and which side was normal. They then looked at what happened during development and consequently had a perfect control inside the same animal.

Choi and Buchholz discovered that THR alpha in frogs first controls developmental timing of the hind limbs. The normal development of the hind limb of their tadpole model shows a shorter stub on the left (middle image), while the mutated limb on the right (lower image) shows significant accelerated growth of the right limb, which was a surprise result for both Buchholz and Choi.

Buchholz explained that the study gets even more remarkable. Ironically, his post-doc advisor Yun-Bo Shi at the National Institutes of Child Health and Development (NICHD) — where Buchholz first looked at molecular biology in frogs from 2000 to 2006 — also studied this exact technology on tadpole models at the same time. While Buchholz, Choi nor Shi at NICHD knew about each others' studies until recently, they all found the same result after mutating only one hormone receptor.

In a sort of twin study, UC and NICHD replicated each others' studies and found identical results. As a consequence, both papers are published in *Endocrinology* at the same time and the publication will also produce a News and Views article on this topic because of the unique situation.

In mice, Buchholz points out that scientists have been able to knock out the gene for quite awhile because of the special structure of their reproduction system that can make embryonic stem cells. But in tadpoles, Choi and Buchholz were able to remove just one of the two (alpha and beta) thyroid hormone receptors. By knocking out only the alpha-receptor, Choi was better able to determine what each receptor controls in an organism.

"We already know what will happen if there is no thyroid hormone signal, as it will simply take away the hormone," says Buchholz. "In humans, no hormone at all creates cretinism where the person has short stature and mental retardation. So in that case it becomes quite severe."

In humans, Buchholz also explains that THR alpha controls heart rate, and THR beta controls thyroid hormone levels, and during development, also controls hearing. So being able to distinguish what one receptor does to the other is pretty important and has distinct consequences, especially when compared to just no hormone at all.

Frog metamorphosis has been compared to birth in humans because during frog metamorphosis there is a peak in blood levels of thyroid hormone dependence, and there is also a peak in thyroid hormone blood levels at the moment of birth in humans. Since humans and frogs are both vertebrates, Buchholz explains that there are many other similarities and the thyroid hormone receptors alpha and beta are expressed in similar cells types.

The cells can respond to similar agonists and antagonists, which are

chemicals that can block or induce thyroid hormone function. With this technology, we can test those kinds of chemicals and study what effects they have on the role of the receptors.

"Knowing that what the THRs do in frogs is very similar to what they do in people, we can hopefully better understand what is happening in people during the developmental stage, which is very difficult to study in humans," says Buchholz.

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