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New Approach to Gene Knockouts Reveals the "Master Planners" of the Skeleton

Howard Hughes Medical Institute researchers are moving closer to understanding how the global pattern of the skeleton of mammals is formed during development. In a demanding series of experiments, the researchers knocked out entire sets of two families of genes suspected of playing a central role in establishing the pattern of the skeleton in the mammalian embryo.

Their findings regarding the "paralogous" gene families known as *Hox10* and *Hox11* establish that the genes play important roles in orchestrating the construction of the ribs, spine and limb bones. Paralogous genes are sets of genes that have overlapping function. They arose during evolution through gene duplication.

The studies on *Hox10* and *Hox11* were published in the July 18, 2003, issue of the journal *Science* by HHMI investigator [Mario R. Capecchi](#) and colleague Deneen M. Wellik, who are both at the University of Utah.

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- **Mario R. Capecchi**

According to Capecchi, the findings should also spur other scientists studying mammalian development to test the effects of knocking out multiple members of the *Hox* gene families. Knocking out multiple genes will enable scientists to "peel away" the layers of redundant gene function to more closely discern the true developmental roles of specific members of the *Hox* gene families.

The 13 sets of *Hox* genes, each with multiple members, have long been known to be "transcriptional regulators" that control the multitude of genes

involved in embryonic development. However, said Capecchi, experiments in which one or another of the *Hox* genes were knocked out provided little information about the functions of individual *Hox* genes.

“It was confusing,” said Capecchi, referring to results from earlier gene knockouts of *Hox10* and *Hox11*. “When individual genes were knocked out, the resulting animals might have an extra rib or vertebrae or be missing one. And sometimes one structure would transform to look like another or just be misshapen. Even if you inactivated five out of the six genes, you still got very small effects. So, while it was clear these genes were working in the region of the ribs and spine, it wasn't clear what they were doing.”

So, Wellik and Capecchi attempted the difficult task of knocking out all of the *Hox10* or *Hox11* paralogous gene forms, or alleles. The experiments were particularly challenging because eliminating the genes profoundly affected the embryonic development and survival of the mice. Another complication was that many of the surviving animals were sterile. But when the scientists managed to produce knockout mice that survived to birth with the entire gene sets missing, the effects on development were dramatic.

“When we eliminated all the *Hox10* genes, we obtained animals that made ribs essentially all the way from the normal thoracic region down through the tail,” he said. “What's interesting is that this is the body plan of most fish as well as the early tetrapods such as the dinosaurs. However, this plan resulted in an inflexible body, so mammals basically adapted the *Hox* genes to get rid of some of those ribs to increase flexibility and speed.”

When the researchers knocked out the *Hox11* genes, the animals' lower, or sacral, vertebrae assumed the identity of lumbar vertebrae (those between the sacral and the rib-supporting thoracic vertebrae) and no sacral vertebrae developed in the animal.

The researchers also found that knocking out the *Hox10* or *Hox11* genes affected the length of specific limb bones, demonstrating a role for those genes in patterning of limbs.

“All these results tell us that these genes control global patterning of the skeletal structures, as opposed to forming the structures rib by rib, for example,” said Capecchi. “This understanding also suggests an evolutionary pathway by which vertebrates could evolve different patterns for different species.”

A major challenge for researchers studying the genetic control of development will be to detect where the panoply of *Hox* genes are expressed in the growing embryo, said Capecchi. “We've demonstrated that the expression patterns of these genes are fairly dynamic,” he said. “So, when researchers are looking for expression of specific *Hox* genes in a given tissue, they might not see them because the genes are expressed only during certain

periods of development.” Multiple knockout studies such as the ones done on *Hox10* and *Hox11* may also yield valuable clues into how the genes affect one another, he said.

Future experiments by the researchers, as well as their colleagues studying other *Hox* genes, may involve knocking out all genes in the individual paralogous *Hox* gene sets and attempting to discern the roles of those gene sets from observing the alterations in development of the body plan.

“However, my guess is that nature won't be that kind to us,” said Capecchi. “I suspect that sometimes development of a particular structure will involve using members of an entirely different paralogous family. So, our knockouts may have to be much broader than we now believe.”

Another major challenge, he said, will be determining which genes the *Hox* genes target to control development. “In the end, we have to figure out what it means in a molecular sense to make a rib or not to make a rib,” said Capecchi. However, he said, the research thus far has yielded important insights.

“Even among mammals, there are enormously different body shapes, from giraffes, to monkeys and humans, to mice. And the take-home lesson from research such as ours is that you can generate all these different body plans using moderately simple rules and the same set of genes, but just modulating them differently.”