

Spared Ribs

Genetic patterning of skeletal structures offers insights into evolution.

Scientists have known for some time that certain genes affect the way our shapes ultimately shape up—how we develop physically. Now, researchers have pinpointed two families of genes suspected of playing a central role in the “master plan” of how vertebrate skeletons are sculpted.

In a demanding series of experiments, Mario R. Capecchi, an HHMI investigator at the University of Utah School of Medicine, and postdoctoral fellow Deneen M. Wellik (now an assistant professor at the University of Michigan) discovered the roles of two groups of genes in orchestrating the construction of the ribs, spine, and limb bones. Their work appeared in the July 18, 2003, issue of *Science*.

The geneticists used “knockout mice,” breeds in which the genes under investigation have been removed or inactivated by genetic engineering. By examining what goes wrong in a mouse lacking the contributions of a particular gene, biologists can infer that gene’s nor-

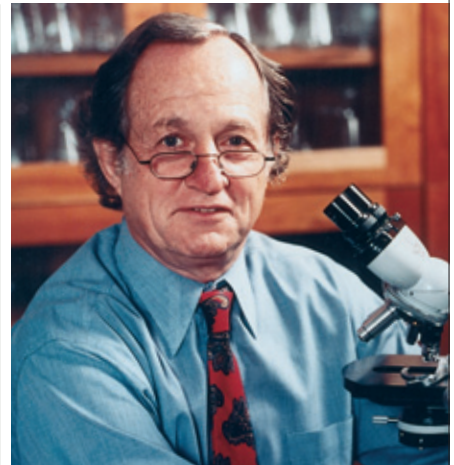
mal function. And because almost all human genes have counterparts in mice, knockout mice serve as valuable laboratory models for learning how genes work in humans.

Capecchi pioneered the techniques for knocking out mouse genes two decades ago and continues to refine them. In this case, he and Wellik focused on *Hox* genes, long known to be essential for controlling embryonic development in practically all animals. *Hox* genes encode molecular switches, Capecchi explains. They turn many other genes on and off, thereby orchestrating cascades of developmental changes in the body. One obstacle in understanding precisely what *Hox* genes do, however, is that there are so many—39 in mammals—and each one controls perhaps hundreds of target genes. These multiple *Hox* genes, termed paralogs, arose during evolution through gene duplication and share similar DNA sequences.

On top of this complexity, laments Wellik, is the issue of “functional redundancy,” the bane of all geneticists. Typically, two or more *Hox* genes act together as a work group. If only one of the genes is knocked out, the others can carry the load, and the mouse will hardly be affected. So to really understand what the genes are doing, one has to disable all the genes in a work group. That’s what Wellik did—in this case, to the paralogous *Hox10* and *Hox11* gene sets, which each contain three member genes.

Her undertaking might be described as heroic, although Wellik confesses in retrospect that “I’ve heard other adjectives, such as nuts, insane....” Capecchi wouldn’t go that far, although he does say that “I’m amazed she was willing to do it.”

Mice, like most animals, carry two copies of each gene, one from each parent. To produce mice with both copies of each of the three *Hox10* genes inactivated, Wellik first had to generate mice with just one of each pair disabled (termed “triple heterozygotes”) and then interbreed them. From those crosses, only rare pups were born with the sought-after trait of missing all six copies of the paral-



Mario Capecchi studies the skeleton’s “master plan.”

gous genes. Making the *Hox11* knockouts presented an even tougher challenge because the *Hox11* triple heterozygotes were essentially sterile: The males died shortly after sexual maturity, and the females couldn’t implant embryos. So Wellik had to generate offspring embryos using in vitro fertilization and then implant the embryos into surrogate mothers.

But the Herculean efforts paid off. When Wellik examined her prized mice, she saw some remarkable anomalies in the animals’ skeletons. Those missing all of their *Hox10* genes had misshapen limbs, confirming suspicions from prior research that *Hox10* controls skeletal patterning. But even more striking were their rib cages: Instead of having a normal one around the chest, the mice had ribs all the way down to their tails. The *Hox11* knockout mice, in addition to having malformed limbs, lacked all sacral vertebrae—the bones that normally attach to the pelvis. Remarkably, pointing back to the functional redundancy of these genes, mice containing just a single normal copy of any of the genes didn’t exhibit these drastic skeletal transformations.

It appears as if the primordial body plan was to have ribs along the full length of the spine, says Capecchi. He believes that the *Hox10* genes evolved, after water dwellers first ventured onto land, to become master regulators of spine development and shut off development of the extraneous ribs.

—PAUL MUHLRAD

Vertebrae Display In a normal mouse skeleton (top photo), the ribs end at T13, the 13th thoracic, or chest, vertebra. The lumbar (lower back) and sacral (pelvic) vertebrae form normally. But when *Hox10* genes are disabled (bottom photo), ribs grow from vertebrae all the way from the chest to the tail.

