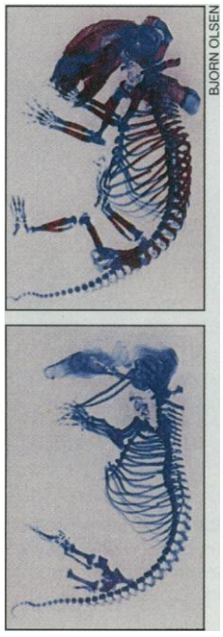
GENETICS

No Bones About a Genetic Switch for Bone Growth

"Here was the ugliest man who ever came to Troy, ... both shoulders humped together, curving over his caved-in chest, and bobbing above them his skull warped to a point. ..." Thus, Homer describes Thersites, a Greek soldier in the *Iliad* who taunts his own leaders, Odysseus and Achilles. Now, geneticists have come up with their own account of Thersites's symptoms, which appear to reflect an extreme case of a skeletal disease known as cleidocranial dysplasia (CCD). They have identified a gene—some call it a master gene for bone growth—that, when mutated, causes this disease.

In the 30 May issue of *Cell*, four papers tell the tale of the gene *CBFA-1*, drawing on evidence from human families, knockout mice, and cell cultures. Taken together, the data show that this gene turns precursor cells into osteoblasts, the cells that actually secrete bony matrix, and switches on at least one bone protein and probably many more. Although there are other key bone-forming genes, "this is the first one reported which, when inactivated, leads to the development of a complete organism—with no bone," says Rik Derynck, a cell and developmental biologist at the University of California, San Francisco.



Boneless. Normal mice *(top)* have bone and cartilage (red, blue); mutants *(above)* have only cartilage.

The identification of *CBFA-1* is "one of the top discoveries of the year," says Peter Lomedico, vice president for human genetics at Genome Therapeutics Corp. in Waltham, Massachusetts, a company active in bone research. The discovery "provides a powerful molecular insight to the regulation of osteoblast differentiation and bone formation," processes crucial not only to CCD but to more common diseases such as osteoporosis, he says.

Researchers had already uncovered a string of bone-forming proteins, the best known being the BMPs or "bone morphogenic proteins." But these molecules act early in development and are "anything *but* skeleton-specific," because they are expressed in all sorts of cells, says Patricia Ducy. A developmental geneticist in the laboratory of Gérard Karsenty at the M. D. Anderson Cancer Center of the University of Texas, Houston, she co-authored one of the papers. *CBFA-1*, in contrast, appears to exert its effects only in osteoblasts, the cells that actually deposit bone.

At the molecular level, very little was known about the osteoblasts, except that only these cells produce a bone-matrix protein called osteocalcin. In 1994, Karsenty identified multiple mouse genes that code for osteocalcin; Ducy followed up that work by searching for the gene that switched on an osteocalcin gene in the bone-forming cells.

In mid-1996, she found one—*CBFA-1*. To her "extreme surprise," it was already known—as the source of a protein important in the thymus gland, where the T cells of the immune system develop. But "the signal in bone was a hundred times stronger than in thymus," says cell biologist Bjorn Olsen of Harvard Medical and Dental Schools, another coauthor. Ducy spliced the new gene into mouse skin cells and found that they develop into osteoclasts and express osteocalcin.

Just as Ducy closed in on her discovery last fall, pediatric geneticist Stefan Mundlos of the University of Mainz in Germany, who was then in Olsen's laboratory, was using linkage analysis of CCD families to identify the gene involved in that syndrome. CCD patients rarely have the severe deformities of Thersites or "ugly" physiognomies, says Mundlos. But they often have a missing collarbone, allowing them to fold their shoulders together in front of their bodies—as in the Greek's "caved-in chest." Furthermore, their skull sutures don't close, so adults retain the soft fontanel normally found only in babies. Some patients also have extra teeth, up to 60 in all, which usually have to be extracted. In addition, growth in other bones is stunted and average height is about 164 centimeters for men and 154 centimeters in women, says Mundlos. His team found genetic deletions on chromosome 6 in many of these patients. The deletions led them to the gene, and they subsequently found that people with the disease have only one intact copy of *CBFA-1*; the other copy is mutated.

Meanwhile, Mike Owen at the Imperial Cancer Research Fund in London and postdoctoral fellows Florian Otto and Anders Thornell were exploring the role of *CBFA-1* in thymus development by creating mutant mice lacking one or both copies of the mouse version of the gene, *cbfa-1*. But they were baffled when the mice showed no evidence of immunological problems or defects in the thymus. Then, they realized that mice with a single copy of the gene were two-thirds the size of normal mice. And when they stained the skeletons of mice missing both copies, they hit the jackpot, recalls Owen.

These mice, which were born but survived only 10 minutes, gasping for breath, had no bone at all (see photo), only cartilage. Mice missing only one copy of the gene had no collarbone and other defects that exactly matched CCD in humans. At about the same time, molecular biologist Toshihisa Komori, in the research group of immunologist Tadamitsu Kishimoto at Osaka University Medical School in Japan, made other *cbfa-1* knockout mice—and got very similar findings.

All this adds up to a picture of *CBFA-1* as a crucial transcription factor—a gene that turns on other genes—that causes preosteoblasts to become osteoblasts and begin producing osteocalcin. This essential role leads Ducy and others to call *CBFA-1* a "master gene." Hundreds of genes are thought to be active in converting cartilage to bone, says Mundlos, but this one "is the first that could potentially control the final step of the transformation." Derynck agrees that *CBFA-1* is a key regulator, although he is wary of the term "master gene" because the other genes in the pathway are unknown.

The new one "is not a drug and never will be," warns Karsenty. Transcription factors make notoriously poor drugs because they act within cells, not between them, and so present delivery problems. Still, understanding this molecular switch is likely to benefit research on bone diseases, says Lomedico. For example, one theory of osteoporosis holds that aging reduces the body's ability to form new osteoblasts. Says Karsenty, "If you could recreate [*CBFA-1*'s] function later in life"—and cause precursor cells to become osteoblasts and secrete new bone matrix—"you could in principle increase bone formation and slow the progression of the disease."

The next goal is to find more genes that are turned on by *CBFA-1*, to see how bone is formed, gene by gene. All these questions "could not have been asked 6 months ago," says Olsen. Mundlos agrees, adding, "It's not the discoveries that explain everything that are the most exciting. It's the ones that open new doors."

–Steven Dickman

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