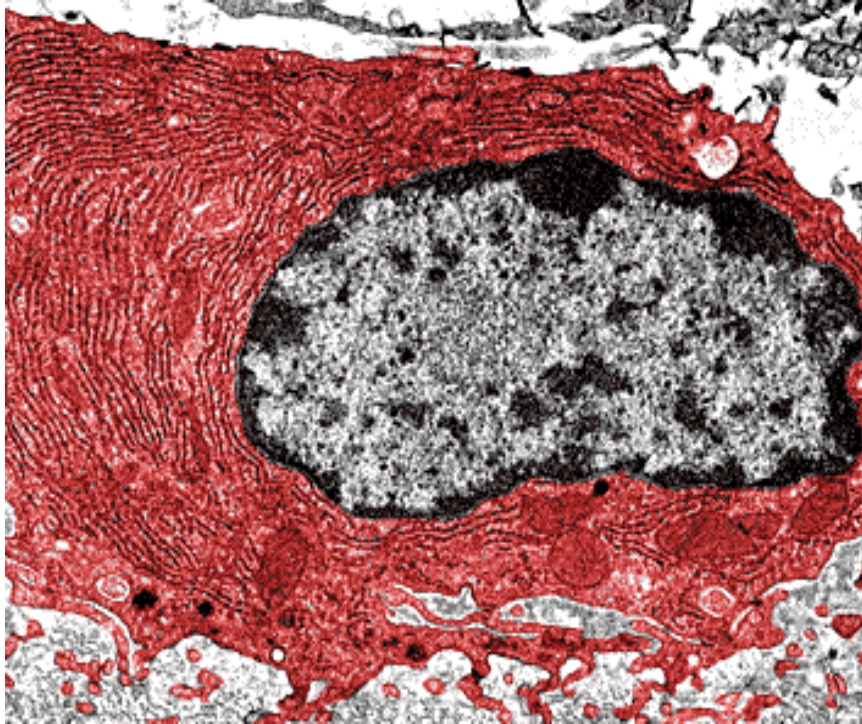


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## A New Way to Build Bone



**Image Title:** The osteoblast cytoplasm is colored red in this transmission electron micrograph of a mature osteoblast on the bone surface. - Monte Winslow, John Perrino

Howard Hughes Medical Institute (HHMI) researchers at Stanford University have found that they can increase bone mass in mice by tweaking the shape of a regulatory protein.

HHMI investigator Gerald Crabtree and HHMI predoctoral fellow Monte Winslow report that slightly increasing the activity of a protein called NFATc1 causes massive bone accumulation, suggesting that NFATc1 or other proteins that regulate its activity will make good targets for drugs to treat osteoporosis. They report their findings in a study published in the June 6, 2006, issue of *Developmental Cell*.

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- Gerald R. Crabtree

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In vertebrates, bone is constantly being formed and being broken down throughout life. Cells called osteoclasts continuously degrade bone, while cells called osteoblasts replenish it.

"Ideally, they are perfectly balanced," said Crabtree, the senior author of the study. "Over the course of a lifetime, if everything goes well, we'll maintain almost exactly identical bone mass." However, if the balance is upset, and more bone is destroyed than formed, osteoporosis results, increasing the risk of fractures.

The new study arose from the researchers' curiosity about reports that patients who were treated with the drug cyclosporine—often given to suppress the immune system before organ transplants—tend to lose bone mass. Those patients were also at increased risk of bone fractures, said first author Winslow, who led the study as an HHMI predoctoral fellow in Crabtree's lab. Winslow is now working as a postdoctoral fellow in the lab of HHMI investigator Tyler Jacks at the Massachusetts Institute of Technology.

Cyclosporine inhibits a signaling protein complex known as calcineurin, which chemically modifies the NFATc family of proteins. This modification changes its shape. With its new shape, NFATc can move into the nucleus of the cell, where it can trigger the activation of many genes. Although initially shown to regulate immune cell function, NFATc also functions in other cells to regulate heart development, blood vessel formation, neural development and function, and muscle development. Its function seems to depend on the time and place of its activation, like a context-sensitive key on a computer. In bone, it is NFATc1 that seems particularly important.

Since people with suppressed calcineurin/NFATc activity experience bone loss, Winslow, Crabtree, and their colleagues wanted to see whether this pathway would be important in bone development and function as well. They studied mutant mice in which the NFATc1 in osteoblasts had been modified so that it could move more easily to the nucleus and become a little more

active than usual.

Mice with the hyperactive NFATc in their osteoblasts had an immense increase in bone mass compared to normal mice, suggesting that the balance between bone formation and breakdown had tipped.

When the researchers examined the cells in these mice, they found that up-regulating NFATc signaling in osteoblasts increased the numbers of both types of bone cells. “It was clear that increased NFATc activity in osteoblasts influenced both osteoblasts and osteoclasts,” Winslow said.

The researchers found that mice with enhanced NFATc activity in their osteoblasts had many more of these bone-forming cells, which explained the increase in bone mass. They also found a possible explanation for why there were more bone-destroying osteoclasts. Osteoblasts with hyperactive NFATc expressed higher levels of inflammatory proteins called chemokines, which promote osteoclast development.

“Osteoblasts produce factors that recruit the progenitors of osteoclasts, and so when osteoblast numbers go up, osteoclast numbers go up,” Crabtree said. This link between osteoblast and osteoclast numbers explains in part how the two types of cells normally stay balanced in animals, he added.

Mice with abnormally active NFATc probably develop so much bone mass because this delicate balance between osteoblasts and osteoclasts has been altered, Crabtree suggested. In the mutant mice, “there's also a huge increase in osteoclasts, but they never catch up,” he said. “The balance has been tipped.”

This imbalance between bone formation and degradation could potentially be recreated by drug treatments for osteoporosis, Crabtree said. Very little NFATc1 must be activated to build extra bone, Winslow noted, which means that it may be possible to up-regulate the calcineurin/NFATc pathway to promote bone formation without disturbing other organ systems that use this same pathway.

“The results were dramatic, yet the molecular alteration is very, very minimal,” Crabtree said. NFATc1 in the mice that developed extra bone mass was only 10 percent more active than it is in normal mice.

The researchers are now screening chemical libraries for small molecules that could increase NFATc just enough to promote bone formation in people with osteoporosis, without causing undesirable side effects. "If you could find a small molecule that would flip 10 percent of the existing NFATc into the active form," Crabtree said, "you could favor the formation of osteoblasts and make stronger bones."