

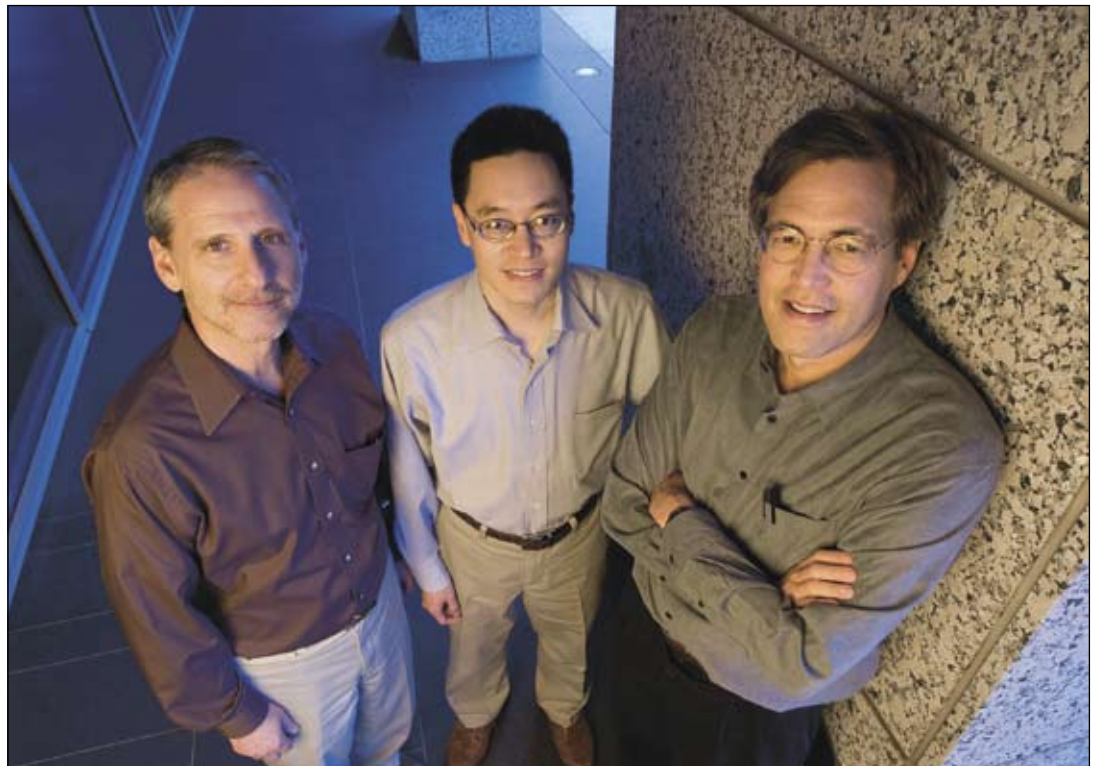
Growing and Mending Bones

G-Protein Signaling in Skeletal Development and Repair

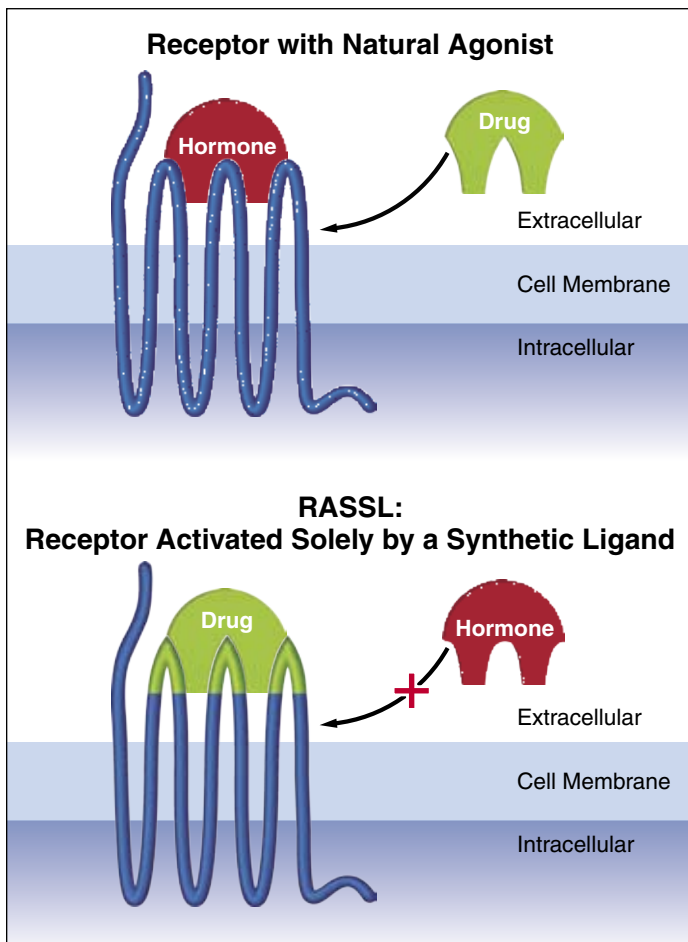
Musculoskeletal disorders are a growing health problem. Osteoporosis—the loss of bone with aging—affects over 10 million people in the U.S. and contributes to 1.5 million fractures each year. Arthritis or chronic joint symptoms are reported by over 70 million Americans each year, often impairing quality of life. In addition, bone fractures lead to more than 3 million visits to the emergency room each year in the U.S. Since the ability to treat and prevent these diseases is still rudimentary, elucidating the mechanisms that regulate bone formation is crucial for understanding the underlying pathology and for developing better treatments.

Dysfunction of the bone-forming cells, called osteoblasts, is thought to be a key mechanism in osteoporosis and poor bone healing. Cells from the osteoblast lineage participate in the regulation of bone development, acquisition of peak bone mass, maintenance and repair of the adult skeleton, and calcium homeostasis. Various cellular factors are necessary for osteoblast formation and function.

The intricate and delicate balance of hormone signaling is an intriguing feature of biology. Even small perturbations can lead to significant disease, often affecting multiple tissues and organs. Researchers at the Gladstone Institute of Cardiovascular Disease and their colleagues at UCSF and the San Francisco Veterans Administration Medical Center have uncovered a biochemical signaling pathway that leads to the formation of abnormally large bones in mice. The discovery may provide clues to childhood bone formation and osteoporosis as well as a path to improved osteoporosis treatments.



(l to r) Robert Nissenson, Ed Hsiao, Bruce Conklin



Building a RASSL. GPCRs normally respond to specific natural agonists, such as a hormone or drug. A RASSL is made by genetically modifying the GPCR so that it no longer binds to the hormone but will bind and respond to a small drug of choice. RASSLs can be made to elicit different responses.

osteodystrophy and McCune-Albright syndrome suggest that G_s signaling can influence bone growth.

Although activation of GPCRs, such as the parathyroid hormone receptor with parathyroid hormone (PTH), can increase bone mass, the exact in vivo roles of the different G-protein signaling pathways and how they interact with other aspects of skeletal biology have not been clearly elucidated.

Designer receptors, including receptors activated solely by synthetic ligands (RASSLs), provide a way to manipulate the timing and signaling of G-protein pathways experimentally. RASSLs have been a boon for studying the roles of G-protein signaling, cardiomyocyte function, neurological development and function, and other complex systems.

Small Mice, Big Bones, Intriguing Insights

The researchers used a strain of genetically engineered mouse in which a specially designed GPCR, designated Rs1, activates the G_s signaling pathway. They found that the mice with continuously active G_s signaling in osteoblasts developed abnormally large and misshapen bones by the age of 9 weeks.

“In fact, the bones were four to six times larger in cross section than normal—an astonishing effect,” said Robert Nissenson, UCSF professor of medicine and senior author on the study. “These results demonstrate that osteoblast lineage cells have a tremendous proliferation potential.”

The G_s pathway is clinically significant because in osteoblasts—the cells that create and maintain bone—it is normally activated by administration of PTH, which is commonly used to treat osteoporosis. Thus, the G_s pathway may be an important determinant of bone mass and lead to new strategies for enhancing bone repair and treating metabolic bone diseases.

“In humans, PTH has to be given as a daily injection and does not result in a very large increase in bone mass,” said Dr. Hsiao. “For treating osteoporosis, it would be desirable to optimize this process to improve the rate of bone formation.”

Understanding the age-dependent effects of GPCR-mediated skeletal growth is important. Intriguingly, when the G_s -activating receptor was turned off until the age of 4 to 6 weeks—when mice reach puberty—and then turned on, bone development was normal. In contrast, suppressing Rs1 expression after the bone malformations had already formed caused a partial reversal of the bone phenotype.

“This suggests that for this particular receptor, there’s a crucial window of time in terms of how and when its activation affects bone growth and development,” says Dr. Hsiao. “Susceptibility to fracture in old age is directly related to bone mass, which peaks at age 30 or so and then slowly declines. If this signaling system could somehow be manipulated in youth, it could be a way of reducing fracture risk later in life.”

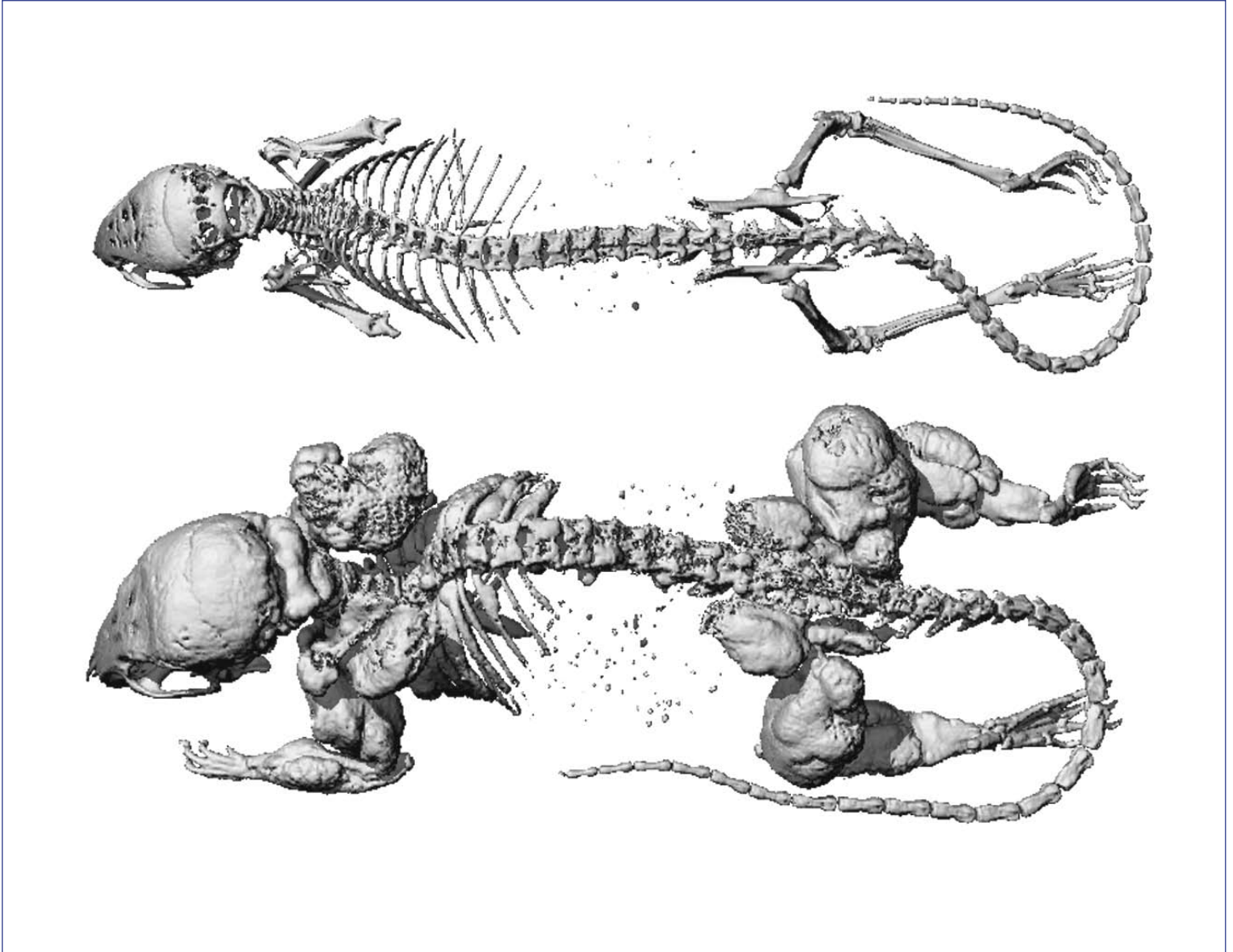
“Dissecting how these hormone signals control tissue development and function is crucial for understanding human diseases and developing future therapies,” said Edward Hsiao, the lead author of the study and a postdoctoral fellow in the laboratory of GICD investigator Bruce Conklin. “In our study, we genetically engineered a key receptor to gain control of bone growth.”

Engineering Receptors

The G protein-coupled receptor (GPCR) family includes over 400 hormone and olfactory receptors, making it the largest class of receptors in the human genome. GPCRs mediate a wide variety of biological processes and are the major targets for over 40% of modern pharmaceuticals. They are strongly implicated in development, but their precise roles in tissue differentiation are still being defined.

GPCRs signal through a select number of pathways, including one that activates G_s -alpha to increase intracellular cAMP. Studies of human genetic diseases such as Albright’s hereditary

Rs1-expressing mice show increased bone formation. MicroCT images of 9-week-old wildtype (top) and engineered (bottom) mice.



Dr. Hsiao cautions that the excess bone formed in the mice is an abnormal type called trabecular bone, which unlike normal skeletal bone is spongy, soft, and lacks a hard outer shell called the cortex. “This is not the ideal type of bone you’d want to grow to treat osteoporosis,” he says. “On the other hand, this shows that we are able to increase bone mass using cells already present in the animal. It’s possible that by tweaking other systems, we can stimulate the growth of normal skeletal bone.”

Mice expressing Rs1 in osteoblasts had a significant loss of bone marrow space, as well as decreased bone marrow fat and skeletal muscle. However, the joints were spared, suggesting an intrinsic mechanism for separating regions of cartilage and bone. Since fat, muscle, cartilage, and bone are all derived from the mesenchymal lineage, it is intriguing to hypothesize that G_S signaling induces preferential formation of bone-forming cells at the expense of the other tissues.

Understanding the mechanisms that underlie these observations may elucidate the role of bone in maintaining metabolic balance, explain how some medications affect bone mass, reveal how bone/cartilage interfaces are maintained, and provide insight into how abnormal bone formation occurs, such as calcification of heart valves.

“These results are very exciting to us,” said Dr. Conklin. “They promise to reveal new mechanisms for controlling bone formation that could find applications in regenerative medicine as well as improve the prevention and treatment of skeletal diseases.”

Hsiao EC, Boudignon BM, Chang WC, Bencsik M, Peng J, Nguyen TD, Manalac C, Halloran BP, Conklin BR, Nissenson RA (2008) Osteoblast expression of an engineered G_S -coupled receptor dramatically increases bone mass. *Proc. Natl. Acad. Sci. USA* 105:1209–1214.