BONE'S BALANCING ACT

Searching for cures, scientists are revealing the biological complexity of bones.

ILLUSTRATION BY PATRICK LÉGER
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bones are the body’s framework and support, our strongest tissues. Unlike the scaffold of a building, however, bones are anything but inert. They pulse with life and their maintenance requires a surprisingly delicate balancing act.

Throughout our adult lives, the body works day and night to clear away patches of weak bone and build up new and stronger bone. When that balance falters in either direction, it spells trouble. Osteoporosis, which literally means porous bones, occurs when bone breakdown outpaces bone buildup. Just bending or coughing can cause a fracture. Too much bone, on the other hand, can lead to rare bone diseases or even bone cancer.

A small army of bone researchers, including several HHMI investigators, is exploring the cellular pathways that strengthen weak bones and support healing in broken bones. Their efforts are yielding new drugs to treat osteoporosis, heal severe fractures, and treat rare hereditary bone diseases. They’re working on new precision therapies to stop bone cancer, as well.

The work is essential. By 2020, half of all Americans over age 50 will have weak bones and the low bone mass of osteoporosis, according to a 2004 Surgeon General’s Report. Roughly 4 in 10 white women age 50 or older in the United States will experience a hip, spine, or wrist fracture, most likely due to osteoporosis. And the problem isn’t limited to women. Up to one in four men over age 50 will break a bone due to the disease. The consequences are profound: one in four patients over 50 with a hip fracture will die within a year.

“Skeletal diseases are incredibly common,” says developmental biologist David Kingsley, an HHMI investigator at Stanford University School of Medicine, noting that patients with less common but devastating bone diseases desperately need effective treatments too.

Studying bone also sheds light on fascinating and fundamental biological questions, Kingsley adds. The development of bone reveals how “just a few cell types can be organized into highly specific shapes and sizes that underlie the things that animals do.” The forms of animal skeletons, past and present, offer clues about how their owners flew, ran, swam, and ate. Bone can do all that—but only if it stays in balance.

THE MAGIC IN BONE

Early in embryonic development, our bones are mostly cartilage, and it’s the cartilage-filled ends of bones that lengthen as we grow. As we mature and growth slows, bone gradually replaces that cartilage. The thick, hard outer layer of bone resembles reinforced concrete in its structure, deriving strength from fibrous proteins called collagen encrusted with crystals of a calcium-containing mineral. Even hard bone is plumbed with blood vessels and wired with nerves. And inside the outer layer sits marrow, a softer tissue packed with immature cells that can form blood, bone, and cartilage cells.

In 1992, Kingsley reported a pivotal discovery about how the body constructs bones. He examined a mutant line of mice with very short ears, a wide skull, and a reduced ability to heal fractured ribs in search of something that had been hinted at two decades earlier. An orthopedic surgeon named Marshall Urist had made an extract of rabbit bone and implanted it under the skin of a living rabbit. It produced an intact, marrow-filled bone under the rabbit’s skin. The discovery meant, Kingsley says, “that there is some magic in bone that could induce formation of new bone.”

He found that the short-eared mice had a mutation in a gene that produced that magic ingredient, called bone morphogenetic protein (BMP). Today 20 related BMPs are known, many of which induce the body to make bone or cartilage. BMP-2 is the active ingredient of a drug that grows new bone after spinal fusion surgery. Surgeons implant a biodegradable sponge soaked with the drug, called INFUSE Bone Graft by Medtronic. The procedure replaces an older, painful, and infection-prone method in which surgeons transplanted bone from the patient’s hip.

The same drug is used for other dental and orthopedic problems: to bolster bone that anchors crowns or teeth, to heal foot and ankle injuries, and to repair gunshot wounds to the jaw. It can help knit shin bones shattered in motorcycle accidents, for example, or replace sections of cancerous bone that have been removed in children, according to bone cell biologist Hari Reddi, of University of California, Davis, who purified the first BMPs in the 1980s.

However, the drug must be implanted with the sponge, rather than given as a pill or injected into the bloodstream, because it doesn’t easily circulate to where it’s needed, according to Reddi.
Clinicians would like better alternatives, and they need treatments that preserve and repair bone when it gradually deteriorates throughout the body, as it does in osteoporosis.

BUILDING BONE BULK
Even when bone is healthy, it’s always growing, dying, changing. To build and maintain healthy bone, we need weight-bearing exercise such as tennis, hiking, and walking; a healthy diet with calcium-rich food such as milk, cheese, and certain vegetables; and vitamin D, from dairy products and sunlight, or a supplement. This is true during childhood and adolescence when the body builds 85 percent of adult bone mass, and it’s true during adulthood to prevent bones from thinning and weakening.

The only reliable detection method for osteoporosis before a fracture is a bone density scan. Such tests have shown that a full 44 million Americans have low bone mass and 10 million, most of them women, have osteoporosis.

<i>n</i> men, the disease is linked to low testosterone levels, smoking and alcohol use, and lack of physical activity. In women, it is linked closely to menopause, when estrogen levels in the body drop.

<i>n</i> the 1980s and 1990s, doctors recommended that postmenopausal women prevent osteoporosis and fractures by taking estrogen supplements, which jam two cellular pathways used for bone resorption—one involves compounds called cytokines and a second is called the Rank ligand pathway. But in 2002, researchers running a long-term trial called the Women’s Health Initiative reported that estrogen plus progestin supplements raised the risk for breast cancer and stroke; two years later, estrogen alone was found to also increase the risk for stroke. Estrogen use plummeted.

Fortunately, by then researchers had begun uncovering cellular signaling pathways that maintain bone’s thickness and strength. Three types of bone cells balance breakdown and repair: osteoclasts, which clear away patches of weak or defective bone; osteoblasts, which build it; and osteocytes, which are entombed in solid bone, sensing and directing the others. For all these cells, “the quest is to find out how signaling works and start designing therapies around that,” says bone biologist Alex Robling of Indiana University School of Medicine.

Today, several targeted therapies are available to prevent and treat osteoporosis by blocking bone breakdown. They include four members of a class of drugs called bisphosphonates—Fosamax, Actonel, Boniva, and Reclast—and Denosumab, which blocks the Rank ligand pathway. These bone-preserving drugs are also used to treat osteogenesis imperfecta, which causes children to produce weak, defective bone.

But while bisphosphonates and Denosumab are effective at preventing bone resorption, they also cripple the bone-degrading cells that are supposed to clean out weak or damaged bone. Over time this can make bones even more brittle, increasing the risk of fractures and, rarely, cause the jawbone to decay. Researchers are now investigating ways to get around this dangerous side effect, such as giving patients drug holidays after a few years.

Bone endocrinologist and geneticist Gerard Karsenty of Columbia University Medical Center likens osteoporosis to a house fire and antiresorptive drugs to water. “If you come with water you are going to stop the destruction, but you still need to rebuild,” he says. Just one drug exists to rebuild lost bone—Forteo (teriparatide), a synthetic form of human parathyroid hormone. Although the drug looks safe in humans so far, long-term treatment with high doses of it caused bone cancer in rats; as a result, doctors stop giving it to patients after two years. New drugs that build bone would be helpful. “That’s where the need is,” says endocrinologist and bone expert Sundeep Khosla of the Mayo Clinic in Rochester, Minnesota.

LEARNING FROM OVERGROWTH
Researchers have identified at least three ways to build bone. Two involve a cellular signaling pathway called Wnt. In 2001,
an international consortium led by HHMI investigator Matthew Warman, a pediatrician and geneticist at Children’s Hospital in Boston, showed that a Wnt pathway gene called LRP5 is mutated in a disease called osteoporosis-pseudoglioma syndrome, which causes people to develop brittle bones.

A year later, HHMI investigator Richard Lifton of Yale University School of Medicine reported a different LRP5 mutation that makes the LRP5 protein overactive, leading patients to make too much bone. His team showed that the mutation causes high bone mass by preventing the natural antagonist Dkk and, by inference, the related protein sclerostin from inhibiting LRP5 function. Warman and Lifton later found other LRP5 mutations in patients with syndromes characterized by high bone density.

Since loss of LRP5 causes brittle bones and people with overactive LRP5 build extra bone, drugs that boost LRP5 activity could boost bone growth without keeping damaged bone from being recycled. This is why Warman says that "LRP pathways in bone are very exciting targets."

Warman has studied bone building in patients who make too much bone to better understand—and ultimately enhance—bone building. What happens in these patients resembles an extreme version of what happens during weight-bearing exercise like hiking or weight lifting, which spurs the body to bolster bone. Warman’s team engineered mice to produce the overactive version of Lrp5 but only in mature osteocytes in hard bone. Osteocytes are believed to sense mechanical stress and release compounds that recruit osteoblasts to lay down more bone. Warman’s collaborator, Robling, at Indiana University, anesthetized the mice and then mimicked the effects of weight-bearing exercise by repeatedly bending their forelimbs. Mice with overactive Lrp5 in their osteocytes produced three times more bone than normal mice put through the same exercises.

"That tells us that the high bone mass mutation works in very mature bone cells," Warman says. "And, if we can think of a way to make an LRP5 receptor in mature bone cells think that it has a high bone mass mutation, then you and I can have more bone." At least three companies have looked for and found a compound that tricks the LRP5 receptor in just this way. For example, Amgen is running phase 2 clinical trials on its version of an antibody to sclerostin. Eli Lilly and Company has developed a chemical compound that keeps sclerostin from blocking LRP5.

Warman is eager to develop bone-building drugs to help some of his youngest patients—children with osteogenesis imperfecta. This hereditary disease can kill before birth or make children so susceptible to fractures that they must spend their lives in a wheelchair, Warman says.

As Warman’s team tries to build bone by targeting LRP5 in bone cells, Karsenty’s team is trying to build it by blocking production of a compound called serotonin in the gut. In 2008, his team found to their surprise that LRP5 blocks gut cells from producing serotonin, which normally signals bone-building cells to stop multiplying. Last year, they reported in Nature Medicine that a drug that blocks serotonin synthesis in the gut builds bone in mice. The results seem to conflict with Warman’s, but “it’s possible that both groups are in part correct, and more needs to be done to sort out that whole story,” Khosla says.

While most researchers seeking bone-building drugs have targeted Wnt signaling, including LRP5, HHMI investigator Gerald Crabtree, at Stanford University, has discovered a second pathway that seems to help the body build bone. A few years ago, Monte Winslow, an HHMI predoctoral fellow in Crabtree’s lab, was investigating why the anti-rejection drug cyclosporine causes bone loss. They knew that cyclosporine indirectly changes the shape of a protein called NFATc that typically sits in the cell’s cytoplasm but moves into the nucleus to activate genes.

When they engineered mice with a mutant version of NFATc that stays in the nucleus just 10 percent longer, the result was “the boniest mouse anyone ever produced,” Crabtree says. Unlike a normal mouse, which feels “soft and cuddly,” Crabtree says, these mice “felt like a bag of bones.” Now, he and biochemist and HHMI investigator Stuart Schreiber of Harvard University are hunting for chemical compounds that tweak normal NFATc to act like the nucleus-loving version. Such compounds will shed

"WE’RE VERY EXCITED BECAUSE WE’VE GOT WHAT WE THINK IS A MORE AUTHENTIC MODEL OF OSTEOSARCOMA,” LEE SAYS.
light on how the NFATc pathway leads to bone growth and, with luck, may lead to drugs that boost bone growth in a new way.

**IMMATURE BONE AND CANCER**

Sometimes bones lose their balance by overgrowing and becoming cancerous. Brendan Lee, a pediatric geneticist and HHMI investigator at Baylor College of Medicine in Houston, pursues therapies for osteosarcoma, the most common type of cancer that originates in the bones, from which about 60 percent of patients recover. To do so, he draws on insights gained by treating patients with hereditary bone, cartilage, and joint diseases at the Skeletal Dysplasia Clinic at Texas Children’s Hospital.

Among them are children with spondylocostal dysostosis, a rare disease in which babies are born with fused and misshapen vertebrae and a rib cage several sizes too small. Children who survive often can’t breathe without a ventilator and sometimes need a hole inserted in their trachea, or major rib and chest surgery, just to breathe. “It tugs at your heartstrings,” Lee says. “Their brain is okay, but they’re kind of trapped in a body.”

Lee noticed that many patients with spondylocostal dysostosis weren’t growing. What’s more, their bones were “washed-out” or darker on x-rays, which suggested they had low bone mass—an observation that Lee corroborated with bone density scans. Lee knew that genes in a signaling pathway known as Notch were mutated in this disease and that Notch signaling helped immature stem cells in the bone marrow decide which of two types of blood cells to become. He suspected that Notch might help another type of bone marrow stem cell decide whether to become a bone- or cartilage-forming cell.

Sure enough, his lab group discovered, it did. Mice with overactive Notch in their bone marrow stem cells developed far too much bone in their skull, ribs, and leg bones, the team reported in *Nature Medicine* in 2008. But it was immature bone, not the strong, layered bone that supports the healthy adult skeleton. Lots of immature bone forms in human osteosarcoma, too. That “helped us make the leap to bone cancer,” Lee says.

To see if Notch signaling was also altered in bone cancer, Lee’s group tested lab-grown human bone cancer cells, including tissue cultured directly from patients’ bone tumors. As anticipated, Notch signaling was overactive, suggesting that the pathway contributed to human osteosarcoma. And compounds that block (continued on page 48)
Notch signaling dramatically slowed the growth of human tumors implanted in immune-deficient mice, the group reported in Human Molecular Genetics in 2009.

Since then, the researchers have engineered a line of mice with an intact immune system that would be better than immune-deficient mice at predicting how potential drug compounds might affect tumors in people, Lee says. Notch is activated continually in these mice, and the animals develop bone cancer, Lee’s team reported last October at the American Society for Bone and Mineral Research. “We’re very excited because we’ve got what we think is a more authentic model of osteosarcoma,” Lee says.

Now they’re testing whether blocking Notch genetically in mice will prevent bone cancer. If so, then compounds that block Notch signaling could also stop the disease. And if that works in mice, Lee plans to test them on osteosarcoma patients.

As with other bone diseases, treating bone cancer is also a matter of regaining balance. Lee thinks it’s possible: “If we could inhibit Notch in osteosarcoma, that would be spectacular.” New drugs for bone cancers, childhood skeletal diseases, fracture healing, and a major disease of aging may all come from these pathway explorations.

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A Role for Inflammation
Cholesterol build-up causes inflammation too, which is a risk factor for atherosclerosis. That inflammation pathway offers another target for drug developers.

When cholesterol accumulates along artery walls, macrophages—immune cells that recognize foreign material—are the first cells to encounter the clumps. The reaction of the macrophage to the cholesterol can either help clear the artery or make problems worse.

“A macrophage is a scavenger for extracellular garbage,” says HHMI investigator Peter Tontonoz of the University of California, Los Angeles. “And when there are cholesterol deposits, they’re recognized by the macrophage as junk that it wants to clear.” Normally, this is a good thing—macrophages help remove LDL from the artery wall. But when a macrophage is overwhelmed with too much cholesterol to process, it turns into a foam cell—so named because the LDL in its interior looks like foamy bubbles.

Foam cells are the first sign of an atherosclerotic plaque. The foamy macrophage produces inflammatory molecules and recruits other immune cells to the site, setting up an inflammatory response, a hallmark of coronary artery disease. “The reason the plaque eventually gets so big and complicated is that the macrophage talks to and recruits other cell types,” says Tontonoz.

But what scientists have struggled to understand is why the macrophage recruits inflammatory molecules when it fills with cholesterol. When the macrophage eats other foreign material, it clears them with no inflammation.

Tontonoz has an answer: a protein called LXR. Originally identified by HHMI investigator David Mangelsdorf, of UT Southwestern, LXR switches between an inactive form, in the presence of low cholesterol, and an active form, in the presence of high cholesterol. In its active form, LXR causes the cell to pump cholesterol out and stop taking cholesterol in.

There are different versions of LXR in different cell types, including macrophages. Mangelsdorf and Tontonoz published a 2003 paper showing that LXR also has anti-inflammatory effects. Tontonoz has since discovered that mice without LXR are more susceptible to a host of diseases, including listeria and tuberculosis. Other studies have shown that drugs increasing the activity of LXR in macrophages have the potential to stop the formation of a foam cell—by pumping cholesterol out—and to decrease arterial inflammation. The combination could stop atherosclerosis.

As Tontonoz has explored the pathway of LXR, he’s also discovered how it arrests cholesterol input, and it’s a familiar mechanism: degradation. In a July 2009 paper in Science, Tontonoz reported that one of the proteins that LXR turns on is a protein called Idol. Idol in macrophages has the same job as Hobbs’s PCSK9 in the liver—degradation of LDL receptors. So Idol, like PCSK9, could be a target for new pharmaceuticals. Already, compounds activating LXR are in the pharmaceutical pipeline.

Pieces of the Puzzle
For every 10 milligrams per deciliter of blood that you decrease your LDL, you have a 10 percent decrease in coronary heart disease risk, says Hobbs. Statins have been an effective way to achieve this LDL reduction, but for some patients, they’re not effective enough to stop heart disease. The network of proteins and genes that regulate cholesterol in the body is complex and far-reaching. Statins affect only one part of this system.

The next cholesterol drug—be it a compound that blocks PCSK9, degrades HMG-CoA reductase, or turns on LXR—will likely be used in concert with statins to come at the problem from two angles.

You can’t predict which aspect of the field will lead to the next breakthrough, says Goldstein. “You have to wait and see. But the important thing is to keep looking at this from new angles.”

As scientists forge ahead in probing those new angles and revealing each part of the cholesterol puzzle, they get closer to that next breakthrough, and the promises of the next drug come into focus.