Boning Up: Newly Isolated Proteins Heal Bad Breaks

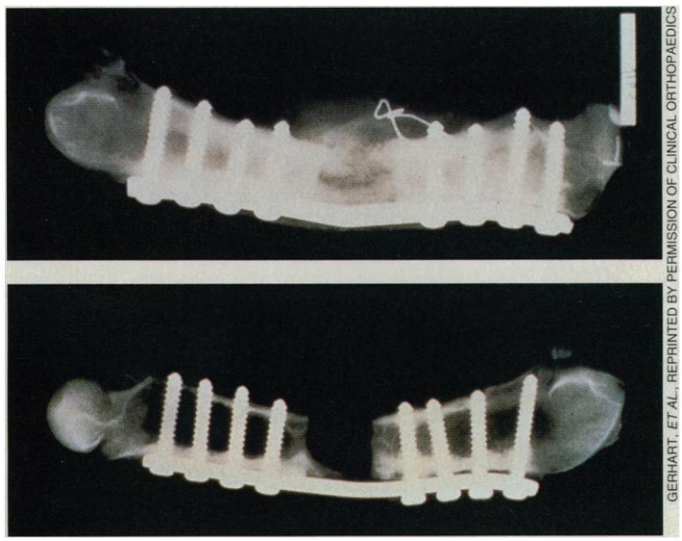
Among all the mature tissues and organs of the adult body, only one can return to the womb. That tissue is bone. When broken, bone remakes itself by triggering processes more familiar to an embryo than to an adult. The repair starts when a class of undifferentiated cells known as bone cell progenitors travel to the damaged site and begin a metamorphosis. Some of these cells produce a calcified scaffolding around the break; others change into nascent bone cells and move into this new framework.

Researchers would love to know how bone cells become born again. And now they are on the verge not only of defining this activity, but of putting it to use in the clinic, thanks to a family of proteins purified in the 1980s. Known as osteogenic proteins (OPs) or bone morphogenic proteins (BMPs) —depending on which of the two labs in a patent dispute you listen to—this family has shown the ability in clinical trials to replace missing bone fragments in severe fractures and to repair tooth interiors; it also shows promise to increase growth in children with kidney disease.

Brigid Hogan, a developmental biologist at the Howard Hughes Medical Institution and Vanderbilt University School of Medicine, says that "there is no doubt that these compounds are important. The range of activities they display makes them very interesting, both in terms of clinical potential and basic biology." For basic researchers, the proteins, known as morphogens for their ability to transform undifferentiated cells to specialized ones, may reveal long-sought secrets of cell development.

Much of the clinical research on these proteins has been done by two Massachusetts-based companies: Creative BioMolecules in Hopkinton, which uses the OP moniker, and Genetics Institute in Cambridge, which favors the name BMP. Both firms began the final stage of clinical trials for bone replacement in 1992, and approval for clinical use may come from the Food and Drug Administration late next year. The companies have similar patents, and though lawyers are negotiating, a court may determine the rights to this potentially lucrative market.

A growth field. The search for morphogenic proteins took off in 1965, when Marshall Urist, director of the bone research laboratory at the University of California, Los Angeles, School of Medicine, showed that crude bone protein extracts would induce new bone formation in vivo. Urist found that the extracts prompted regrowth in several stages. First, progenitor cells called mesenchymal stem cells migrated to the region where the extract was applied. Next, these cells differentiated into cells called chondrocytes and osteoblasts. The chondrocytes secreted collagen and calcium phosphate to form the bone matrix; the osteoblasts filled in the framework, giving rise to new bone. But Urist was unable to isolate the ingredient within the extracts that was responsible for bone morphogenesis (although he did give it a name: BMP).



GERHART, *ET AL.*, REPRINTED BY PERMISSION OF CLINICAL ORTHOPAEDICS

Knitting a sheep. A broken sheep femur, treated with a bone morphogenic protein, shows healing in 3 months *(top)*, while an untreated femur doesn't come together as well *(bottom)*.

One reason Urist couldn't nail down the active protein was that he lacked a reproducible, quantitative bioassay for measuring bone growth. He and other investigators struggled with this problem for nearly 20 years, until 1983, when biochemists T. Kuber Sampath and Hari Reddi at the National Institute of Dental Research developed an in vivo bone induction assay. "It was tedious and certainly not elegant," says Sampath modestly, but, he adds, "it worked." The assay, still used today, involves implanting a pellet with the protein mixture of interest beneath the skin of a male rat. Within a few days, if the protein is effective, mesenchymal stem cells migrate to the site and bone formation begins. The implant is removed 12 days later and the effect of the protein is measured according to two indexes: the activity of alkaline phosphatase—an enzyme that swings into action during bone formation—and calcium content.

Soon after the assay was developed, its two progenitors were lured away from the dental institute by the two biotech firms, and the development race was on. Genetics Institute hired Reddi—he has since moved on to Johns Hopkins—as a consultant, and Sampath joined Creative BioMolecules. The companies reached the finish line virtually simultaneously in 1987. Both purified enough morphogen to determine a partial protein sequence, which contained a specific distribution of the amino acid cysteine. That pattern is characteristic of proteins belonging to the transforming growth factor-β (TGF-β) superfamily of regulatory proteins. This clue allowed the groups to fill in the blanks in the incomplete protein sequence with sequences from other members of the TGF-β superfamily, and then to develop gene probes.

Playing the name game. Using these gene probes as bait, the two groups went fishing among a library of human genome fragments. Sampath and his colleagues at Creative BioMolecules reeled in a gene they called OP-1 at almost the exact same time as the Genetics Institute team identified genes —and then proteins—corresponding to what they called BMP-2, BMP-3 and BMP-4. (They also isolated a protein called BMP-1, but it didn't turn out to be a bone morphogen.) Subsequently, the Genetics Institute team fished out three more BMPs, including BMP-7, which turned out to be identical to OP-1. In the meantime, Hermann Oppermann, Engin Özkaynak, and others at Creative BioMolecules isolated OP-2, which corresponds to BMP-8, and then OP-3. The fishing continued, and as of today, the companies have pulled out complementary sets of genes and proteins.

At this point, it takes a scorecard—and a lawyer—to keep the players straight. The two companies are in an odd situation: Beginning in 1990, the Patent Office granted both companies patents for both sets and "has essentially decided to let the courts work out the problem of who owns the rights to which proteins, which genes, and which uses," says Stuart Weisbrod, chief biotechnology analyst for Merrill Lynch. The two firms, not surprisingly, are protecting their patent positions by refusing to compromise on the naming issue.

By whatever name, and whatever the out-

come of the patent fight, the morphogens are slated to become important tools for tissue repair. Closest to the clinic are drugs aimed at healing what orthopedists call segmental bone defects. These are fractures so bad that nonhealing gaps arise between segments of bone. Currently, bone grafts are used to fill the gaps, but that usually takes two to three grafting operations, and even then one-third of the defects fail to heal.

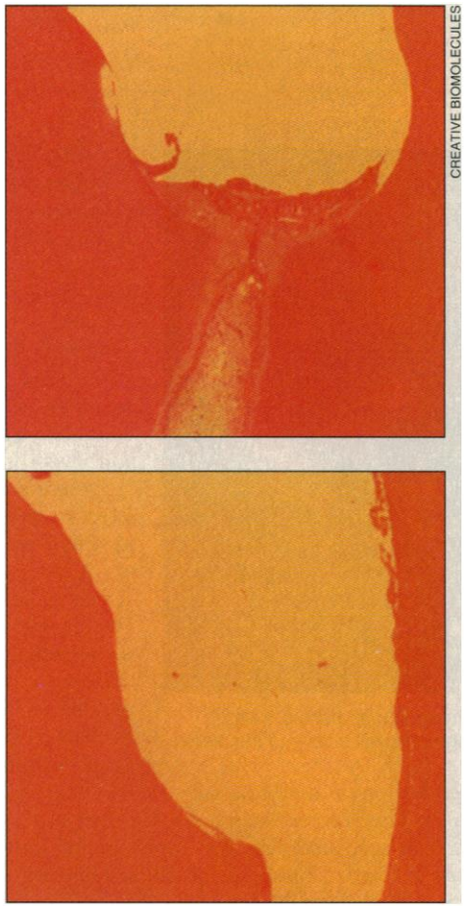
Researchers at both biotech companies decided that this situation could be improved using morphogens. When bone grafts work, it's likely that they happen to contain enough morphogen to induce new growth. So researchers at the Genetics Institute, using BMP-2, and at Creative BioMolecules, using OP-1, applied a highly concentrated mixture of morphogen to bad breaks. The mixture, combined with a matrix composed partly of demineralized collagen, is placed in the injured area and molded to resemble the neighboring bone.

In animal tests, bone formation in the defects was evident within a week. Implants containing the matrix but no morphogen showed no bone growth. After 9 weeks, new bone completely spanned the morphogen-treated areas, and in a year the new bone was indistinguishable from its surroundings. And in several dozen people, independent clinical trials of both proteins have shown that more than 95% of injuries heal when treated with the mixture.

The good break of morphogen treatment may also prevent people from hearing the hideous words: "root canal surgery." Normally, unchecked tooth decay will create a hole in the dentine, the hard material beneath the enamel. This creates a nonhealing opening—dentine does not repair itself—for bacteria to reach the blood-rich pulp in the heart of a tooth. That's when patients hear the unwelcome words. But if infection has not already set in—the situation in about 40% of all root canal surgeries performed today—OP-1 may make it possible to repair the dentine layer. After drilling out the decayed enamel and dentine layers, the dentist packs in a matrix-morphogen paste. The matrix seals the hole, preventing further damage, while the morphogen induces new dentine formation. Creative BioMolecules began clinical trials on patients in 1993, and expects to complete them in 3 or 4 years.

Producers and receptors. Such potent clinical results raise the question that has developmental biologists excited: How do the morphogens work? Though details are still sketchy, investigators are starting to learn where the proteins are produced and what some of their molecular targets might be.

Oppermann's group at Creative BioMolecules has found, for example, that the major production site for OP-1 messenger RNA (mRNA)—and presumably the protein—is not bone, but the kidneys. That organ also produces the only other known human morphogen, erythropoietin, which triggers blood cell growth. The team also found that OP-1 mRNA levels drop with age. This could explain why the bones of children with kidney disease fail to grow, and why transplantation with an adult kidney—the standard procedure—fails to correct this problem.



Filling a tooth. Dentine, the hard material beneath tooth enamel, grows back when treated with a bone morphogen *(top)*, but not when untreated *(bottom)*.

It also suggests that OP-1 could be an important adjunct to kidney transplantation therapy. "This would be a very important advance if this work pans out," says Douglas M. Ford, a pediatric kidney transplant specialist at the Denver Children's Hospital. "Superhigh levels of growth hormone can help these kids catch up in growth rates, but they can also damage the transplanted kidneys." Investigators are currently trying to determine whether systemic administration of OP-1 carries any similar risks.

Other researchers are attempting to determine the targets for the proteins, looking hard for OP/BMP receptors. Michael Hoffmann, a molecular biologist at the University of Wisconsin's McArdle Laboratory for Cancer Research, has found a gene that encodes a BMP-2 receptor; he plans to use mRNA probes to determine its distribution in various tissues. John Wozney, director of bone research at the Genetics Institute, and his colleagues have identified receptors that bind several BMPs, though he cautions that matching them up with the right proteins won't be easy. "There's going to be some cross-reactivity among these proteins and receptors because there's significant cross-reactivity in their action within the body," he says. In human beings, he continues, "it's going to be very difficult to sort out the biology of these compounds."

In other species, the sorting has been a bit easier. In fact, bone morphogens have already been shown to have a a multitude of developmental roles. BMP-2, for example, is homologous to decapentaplegic protein (dpp) from *Drosophila*. Several groups, notably those of Hoffmann at the University of Wisconsin and William M. Gelbert at Harvard, have found that in the developing fly, dpp specifies the fate of dorsal cells in the early embryo, directs gut development later on, and plays a critical role in segment development in the adult. And in what Hoffmann calls "an interesting demonstration of evolutionary conservation," he showed that BMP-2—the human protein—can rescue a lethal dpp-minus *Drosophila* mutant.

These finding raise hopes that morphogens will be highly relevant to developmental biology's central riddle: how one cell becomes a differentiated organism with billions of specialized cells. "These BMPs belong to a very, very ancient family, one that has been highly conserved across a tremendous amount of evolution, so we hope that they will lead us to answers about those most basic steps in embryogenesis," says Hogan. Researchers will be boning up on these basics for years to come.

–Joseph Alper

Additional Reading

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