bond metal implants, fill up defects or cracks in bone, or even serve as an implant material itself.

To create these new materials, which Stupp calls organoapatites, his lab grows crystals of hydroxyapatite—the same calcium phosphate mineral that adds hardness to natural bone and teeth—in a solution of polymeric long-chain organic molecules. As the crystals form, they capture the polymers, creating a material in which the polymers are threaded throughout the ordered crystal lattice of the apatite, much as organic molecules and proteins are threaded through the mineral lattice of natural bone. The polymer network toughens the replacement material and helps control the size of the crystals. Laboratory-grown hydroxyapatite crystals are usually far larger than the nanometer-sized ones found in bone, but, says Stupp, "the crystals of our artificial bone look like those of natural bone."

That could be a major advantage. Stupp's research has shown that, unlike normal hydroxyapatite, which is sometimes sprayed on metal implants to encourage tissue adhesion, organoapatites are naturally degraded by osteoblasts, the bone-forming cells of normal bone. "This material is recognized by the cells as dead bone," he says. "I think the crystal size is important to the ability of the cells to recognize and break down the apatite." In its place, the osteoblasts deposit natural bone—a better material than any substitute.

Stupp foresees the possibility of endowing the artificial bone with other properties by manipulating the incorporated polymers. For example, says Stupp, "You can use growth factors or drugs as the organic factors you thread through the lattice," turning the organoapatite into a slow-release drug-delivery system. His lab has already made an organoapatite that combines a biocompatible polymer found in soft contact lenses—for strength and flexibility—with an anti-inflammatory drug and a series of amino acids that promotes tissue regeneration. The idea, says Stupp, would be to ward off any immune attack on the bone substitute—while hastening its natural demise by encouraging new bone formation.

With only very preliminary animal testing done on a few types of organoapatites, Stupp admits it's too early to say whether this novel class of materials will fulfill the promise he envisions. Moreover, Stupp faces stiff competition from other investigators and biotech firms who have their own candidate bone substitutes. Comments Myron Spector, director of orthopedic research at Brigham and Women's Hospital in Boston, "I believe organoapatite is a particularly innovative approach [to a bone substitute]. But it's difficult to say how valuable it will prove to be in a clinical setting."

–J.T.

BIOTECHNOLOGY

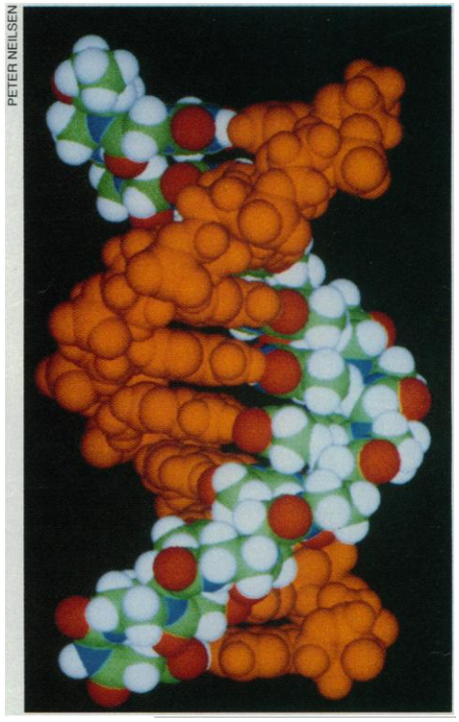
Can DNA Mimics Improve On the Real Thing?

You wouldn't think DNA and RNA needed to be replaced or updated. After all, those two nucleic acids have done a pretty good job for billions of years in their traditional roles of coding and passing on genetic information. But with the coming of modern biomedicine and its vision of using bits of DNA and RNA to tag or combat harmful genes, the natural compounds don't always fit the bill anymore. They break down quickly in the body, and they don't bind to their targets as eagerly as some researchers would like. Those faults make the possibility of improving on nature irresistibly attractive to some biotech companies.

At the moment the highest hopes of these biotechnicians lie in a class of manmade compounds, invented a mere 2 years ago, that look like and act like DNA—but are completely different under the skin. The new molecules, brainchildren of chemists Michael Egholm, Peter Nielsen, Ole Buchardt, and Rolf Berg at the University of Copenhagen in Denmark, string the four chemical bases that serve as coding characters in DNA and RNA along a chemical backbone borrowed from a completely different class of molecules: proteins. These new compounds, known as peptide nucleic acids (PNA), are not only more stable in cells than their natural counterparts, but also bind natural DNA and RNA 50 to 100 times more tightly than the natural nucleic acids cling to each other (*Science*, 6 December 1991, p. 1497).

This discovery has attracted interest from basic scientists, who are intrigued by the notion that, as Jack Cohen of Georgetown University puts it, "man is beginning to come up with analogs of things nature made with evolution." And it has spurred a flurry of activity at biotech companies, among them Denmark's PNA Diagnostics, which is on the verge of marketing the first PNA-based test kits, and Isis Pharmaceuticals of Carlsbad, California, which is starting animal tests of PNA therapies. And researchers have been studying PNA at several other companies, including Glaxo in Research Triangle Park, North Carolina.

As the development of PNAs moves forward, however, Cohen and many of his academic colleagues are feeling qualms. They worry that these doppelgängers could reveal nasty traits that might preclude their use as drugs. Indeed, biochemists working with PNAs admit that the properties that make them so promising—tight binding and longevity—could also worsen side effects. "These [compounds] might be of immense therapeutic value," says biologist Malcolm Pluskal of Millipore in Bedford, Massachusetts. "But from an ethical standpoint I think it's important to look at these concerns." Isis research scientist John Keily also tempers enthusiasm with caution. "There will be some nonspecific effects, so you have a legitimate concern," he says. "It's just too new to know how serious the concerns are."



PETER NIELSEN

Risky embrace? A peptide nucleic acid *(orange)* binds to a DNA strand far more tightly than can the complementary DNA sequence.

The biotech executives who have jumped into PNA development are willing to run that risk on the chance they will be able to find ways to avoid the dangers and exploit PNA's attractive qualities. The most important of them is that, like natural DNA and RNA, it can seek and bind another strand with the complementary sequence of nucleotide bases. That's the idea behind the DNA-based diagnostic probes that are often used to seek out mutated genes or genes of pathogens such as the AIDS virus. It's also the basis of an emerging form of genetic therapy known as antisense. The idea behind antisense is to block the action of unwanted genes—either mutations or viral genes—with nucleic acid strands carrying the opposite

coding sequence. Usually antisense schemes target the messenger RNA that transfers DNA's genetic information into proteins. The antisense strand can bind to the messenger RNA and prevent it from being translated into protein.

Soon after the antisense strategy was conceived, in the 1970s, researchers realized that in the cell, segments of DNA and RNA degrade too quickly to attack the rogue messenger RNA with the requisite efficacy. As a result, investigators started tinkering with nucleic acids, hoping to render them more stable. By replacing a few atoms along RNA backbones, researchers created compounds that showed enough stability to allow the first clinical trials of antisense, such as one now being staged by a company called Hybridon, based in Massachusetts, on a group of 24 HIV-positive patients in France.

Egholm says he and his colleagues wanted to try something more radical than the minor substitutions tried so far—so they attached the coding bases to an entirely new backbone. In DNA and RNA each coding base is linked to an identical chemical unit consisting of a sugar and a phosphate group, which hook up like links in a chain. The Danish team did away with the phosphate-sugar groups and instead attached each base to a peptide group—one of the family of units that link up to form proteins. "If we had told people about it at that time, they would have laughed at us," says Egholm. "It was believed that God created the best backbone in the world and nothing else would work."

Egholm himself was surprised, he says, when the peptide backbone of a protein—a chemical structure known for its stability—turned out to be chemically compatible with the four nucleotide bases. He was even more surprised when test tube experiments showed that the resulting hybrid could bind to natural DNAs and RNAs carrying complementary sequences. People had thought the basic structure of the natural phosphate backbone was necessary to give a molecule the right geometry to bind to a complementary sequence, says Egholm.

And these new molecules didn't just match the binding ability of their natural counterparts; they held their targets many times tighter than segments of RNA or DNA do. Egholm says he believes the extra binding power stems from the fact that the peptide backbone, which is electrically neutral, eliminates the repulsion created by negative charges in the backbones of DNA and RNA. Those electrical properties might help explain another surprising result, says Egholm: PNA's ability to attack genes by "invading" the DNA's normal double helix—something DNA or RNA segments can't do. At the same time, the compounds fulfilled the original hopes by resisting attack by the enzymes that normally chew up DNA and RNA. As a bonus, say chemists, peptide chemistry is relatively simple, so making a desired sequence of PNA may end up costing much less than making an equivalent segment of RNA or DNA.

So promising was PNA, says Egholm, that the group filed a patent application—and they've already sold marketing rights to three companies. "When we first learned of PNA there were many reasons to be excited about it," says Stanley Crooke, CEO of Isis. Millipore, a chemical and biopharmaceutical company, hopes to get in on a possible PNA boom by becoming a supplier of PNA building blocks, as well as ready-made PNA chains, to other biotech companies and to basic researchers. To satisfy what Millipore hopes will be a large demand, research scientist James Coull says Millipore chemists are working to perfect the synthesis methods.

"It was believed that God created the best [nucleic acid] backbone in the world and nothing else would work."

—Michael Egholm

Millipore will be assured a market if other companies' hopes for PNA are fulfilled. At PNA Diagnostics, "what we are trying to do —and succeeding—is developing new ways to use PNA in diagnostics," says Chris Stanley, a researcher with the company. PNA's tighter binding should make it more powerful than natural nucleic acids at identifying or tagging target genetic sequences in diagnostic tests, says Stanley. "If you have a sample with, say, HIV, you have such a small quantity of the virus," he says. "The better your affinity, the better you are at catching the [viral sequences]." Within a year Stanley and his colleagues expect to perfect a test with unprecedented specificity and sensitivity for identifying the mutations that cause cystic fibrosis. They are also developing tests for infectious diseases, such as salmonella, based on PNA strands with a sequence complementary to some telltale part of the bacterial genetic code.

Do PNAs make antisense? Isis, meanwhile, is trying to turn PNA molecules into antisense drugs, an area the company had already been exploring with other DNA variants. "Any therapy where we would consider antisense DNA, we would also consider PNA," says Keily. Indeed, says Isis CEO Crooke, the company hopes PNAs will outdo their natural counterparts—and not only because of their stability. The greater binding power of PNAs means they could be given in smaller doses, and their unique ability to invade double-stranded DNA itself should allow them to bypass RNA and go straight to the heart of genetic disorders. Because one DNA sequence produces many RNA molecules, says inventor Egholm, "DNA should be a more sensitive target."

So enthusiastic is Isis that it is already developing antisense PNAs to combat several genetic disorders, which company sources won't divulge. But Isis is banking on overcoming some major drawbacks. The biggest problem, say Egholm, is that, at least in test-tube cultures, PNA doesn't penetrate cell membranes the way RNA segments can. So far the researchers have had to inject it into cells—a problem that would rule out using PNAs in their current form as a therapy. "This is a serious hurdle," admits Isis's Keily. However, he says, it's hard to be sure how serious the problem is from in vitro tests, so Isis researchers plan to study how the antisense PNAs fare in animal tests beginning within the next two months. He adds that even if PNAs in their current form don't work ideally, researchers may be able to tinker with the PNA backbone to improve the molecules' uptake by cells.

If they do get some form of PNA into cells, however, researchers will still have to contend with the threat of side effects. "Since the molecule is artificial we don't have any way to predict the toxicity," says Keily. Because PNA binds so strongly, notes National Institutes of Health biochemist Arthur Krieg, who edits a journal about antisense technology, it may lack the specificity of its natural counterparts and end up binding not just to target sequences but also to other strands of DNA, RNA, or even proteins, incapacitating the cell in unforeseen ways. And while the ability of PNA to attack DNA itself is exciting, it's also unnerving, Krieg says. Investigators still don't really understand how this happens or what sort of hybrid PNA-DNA structure results. "PNAs are so new no one has done an x-ray structure."

Indeed, Krieg says he isn't even sure that advantage of stronger binding is worth the risk. "People pitch [PNA] by saying hybridization is one of the major problems [with ordinary antisense technology]. It's not." Michael Sherman, a researcher at Pharmagenics in New Jersey, also questions the added value of a better grip. "What you really want to know is how much better it binds to what you want than what you don't want."

Egholm defends PNAs by saying that they attack their targets as specifically as natural nucleic acids do. He and his colleagues have tested that specificity by mixing PNA and RNA segments that have exactly complementary sequences, heating them to measure their binding strength, then repeating the measurement for PNAs and RNAs that have

one or two intentional mismatches. They they find that mismatches hinder the binding of PNAs as much as (or possibly more than) they do that of the natural molecules.

But even if PNA turns out not to be the best DNA analog for biomedical purposes, says Georgetown's Cohen, its discovery has opened the eyes of the research community to the possibility of radically different synthetic versions of the DNA and RNA. Egholm says he and his colleagues are now considering other variations. "PNA took us away from the natural backbones," he says.

It also shattered his notions about the uniqueness of life's genetic machinery, he says. "Why did nature settle on DNA as the universal genetic material?" he asks. With radically different possible alternatives, it may be we got DNA in our chromosomes by blind chance. In fact, in spite of the success that nature seems to have over a couple of billion years with DNA, it might have had even more with PNA.

–Faye Flam

ASTROPHYSICS

Pattern Emerges in Cosmic Ray Mystery

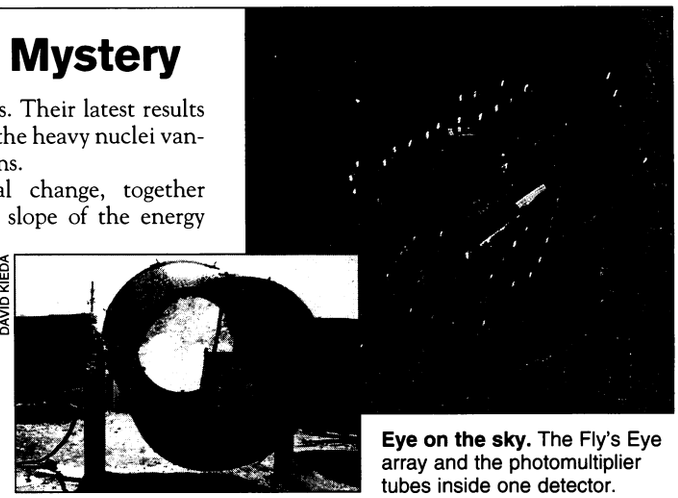
Anyone who thinks that physics is in danger of running out of mysteries should contemplate high-energy cosmic rays, particles that arrive from space at energies millions of times higher than ever achieved in an earthly particle accelerator. There are plenty of candidates for the sources of cosmic rays that arrive with relatively modest energies: the sun, the shock wave at the edge of the solar system, or supernovae within our galaxy. But at energies thousands and even millions of times higher—above a quadrillion electron volts (10^{15} ev)—it has been anyone's guess what manner of heavenly accelerators are launching these particles toward Earth.

Now a collaboration of physicists working with the University of Utah's Fly's Eye cosmic ray detector reports a series of clues that may narrow the search. At energies of about 10^{19} ev, they report in the 22 November issue of *Physical Review Letters*, one kind of cosmic ray source that generates mainly heavy nuclei seems to peter out and a new source, specializing in protons, takes over. That top-end source, the researchers say, seems to lie outside the galaxy—but not too far afield. That, at least, is the implication of the most energetic cosmic ray ever detected, a single particle that the Fly's Eye registered at an extraordinary 3×10^{20} ev—a wallop, says University of Utah physicist Pierre Sokolsky, that is equivalent to "a brick falling on your toe."

The Fly's Eye detector is a swarm of phototubes spread over two hillsides at the Dugway Proving Grounds, an hour's drive from Salt Lake City. The devices look upward into the night sky for the bluish fluorescence from the nitrogen ionized when a cosmic ray plows into the atmosphere and triggers a shower of secondary particles. The altitude of the fluorescence provides a hint of composition—heavier nuclei should trigger air showers at higher altitudes—and its intensity gives a measure of energy.

High-energy events are so rare that the Fly's Eye had to be run for more than a decade before the collaboration could say much about them (*Science*, 8 January, p. 177). By last January, however, the researchers knew enough to say that at 10^{17} ev, several steps below the highest energies, most cosmic rays seemed to be heavy nuclei such as iron. Now, they find that the pattern changes dramatically at higher energies. Their latest results suggest that by 10^{19} ev the heavy nuclei vanish, leaving only protons.

That compositional change, together with a change in the slope of the energy spectrum at the same energy, seems to mark the upper limit of one source, which the Fly's Eye researchers speculate lies within the galaxy. An energy of about 10^{19} ev is where you would expect galactic cosmic rays to become less abundant, says Tom Gaisser of the Bartol Research Institute at the University of Delaware and a Fly's Eye collaborator, because at that point "the particles will be so energetic that they can no longer be contained" by the galaxy's magnetic fields. Moreover, lighter nuclei would have an easier time escaping the magnetic field than heavier ones. Thus it would make sense, if the source is galactic, that most of the cosmic rays reaching Earth would be heavy nuclei.



DAVID KIEDA

UNIVERSITY OF UTAH

Eye on the sky. The Fly's Eye array and the photomultiplier tubes inside one detector.

The evidence of a galactic origin for these cosmic rays is necessarily indirect, because the same magnetic fields that can trap cosmic rays also bend their paths, making it impossible to trace their origins. At the highest energies, however, where iron nuclei give way to protons, the bending should be small enough that the trails of the protons should still point roughly toward their origins. A galactic source would be readily apparent, as Sokolsky explains: "If these particles were coming from the galactic disk, where most of the matter in the galaxy is located, we should begin to see striking anisotropies in arrival directions." But the ultra-high-energy protons seem to come from all directions, suggesting that they originate beyond the galaxy.

Not too far beyond the galaxy, however—at least in the case of the falling-brick event, recorded by the Fly's Eye on 15 October 1991, and discovered by Hong Yue Dai, a postdoc on the experiment. That event had three times the energy of any other cosmic ray ever detected, and at that energy, say theorists, a proton couldn't have made it very far before interacting with the photons of the cosmic microwave background radiation that pervades the universe. According to Sokolsky, at 3×10^{20} ev, the particle would only travel perhaps 10 megaparsecs, the distance of the local supercluster of galaxies, before it lost energy.

So where did the blockbuster ray come from? Because of its extraordinary energy, the particle "should point back to its source," says Sokolsky. But, he adds, "the interesting point is there's nothing there. No obvious hot galaxies, radio galaxies, any of the standard models for extragalactic particles."

David Schramm, a University of Chicago astrophysicist, suggests one exotic explanation: The particle was accelerated by the decay of an object left over from the earliest moments of the universe. "It may be the result of the decay of some grand unified thing," says Schramm, "maybe even a topological defect" such as a cosmic string, a kind of hypothetical fracture in the fabric of space time formed during the earliest moments of the Big Bang. "When those remnants decay," he adds, "they give you very, very energetic particles. If that was the case, then these particles would be a probe of extraordinarily fundamental conditions."

Schramm's notion is intriguing, but, as is so often true with high-energy cosmic rays, the evidence is too thin for anything more than speculation. "It's only one event, and you need more," says Sokolsky. "It's an exciting thing, but you don't know what to do with it." Aside, that is, from fueling cosmic mysteries.

–Gary Taubes