South American mammals like the marmoset and guinea pig convert little or none of the element to methylated forms. He suggests in an article in press at *Environmental Health Perspectives* that these species might have evolved an alternative way to tolerate inorganic arsenic in the blood—perhaps by binding it to proteins.

Intriguingly, people may have the same geographic split in their ability to detoxify arsenic. Aposhian's team has been studying a group of indigenous villagers in Chile, who for thousands of years apparently have been drinking water laced with dangerous levels of arsenic, but who have no signs of cancer. Aposhian hopes to isolate some of the group's arsenic-metabolizing enzymes from lymphocyte samples. It's "fascinating stuff," says the National Institute of Environmental Health Sciences' Michael Waalkes, who says some populations may have a genetic variant that causes them to metabolize arsenic differently. That could explain why people exposed to arsenic in Chile and other geographic regions, such as Mexico, seem to be less susceptible to arsenic-related cancers than are Taiwanese and Indians, Waalkes says.

A possible link between arsenic methylation and cancer fits squarely with a booming research area: the role of methylation in switching on or off cancer-related genes. When cells add methyl groups to arsenic, they deplete a compound called SAM that's needed to methylate DNA and tag genes that should be turned off. If the pool of SAM is depleted by arsenic, that might hinder the cell's ability to control gene expression, says Waalkes. His lab has done experiments showing that rat liver cells treated with arsenic are "hypomethylated"—that is, the genome doesn't have as many methyl groups as it normally would. He's also shown that an oncogene, *c-myc*, switches on in these cells.

But arsenic might also have a deleterious effect in some cells by boosting methylation, says Marc Mass, a toxicologist at EPA's research lab in Research Triangle Park. His group has found in experiments on human lung cells that arsenite spurs the activity of an enzyme that attaches methyl groups to the *p53* tumor suppressor gene. The methyl groups are added to a region of the gene where they would slow its transcription—and thus increase the risk of cancer, says Mass.

Clearly, the mystery is far from solved. And that leaves scientists in the uncomfortable position of assessing EPA's proposal to reduce maximum arsenic levels in drinking water without being able to point to a proven mechanism of arsenic's carcinogenicity. EPA wants to reduce those levels from 50 micrograms per liter to as few as 2 micrograms per liter in 2001—a plan that could cost utilities up to $1.5 billion a year.

–Jocelyn Kaiser

PALEONTOLOGY

Missing Link Ties Birds, Dinosaurs

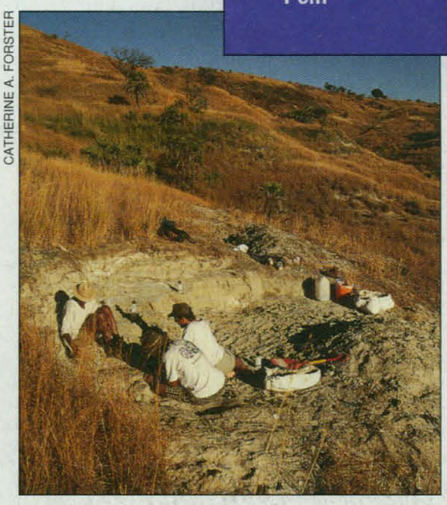
Paleontologist Catherine Forster and colleagues were working in their lab one weekend in 1995, chipping the skeleton of an ancient bird from a block of sandstone, when they noticed that the bird's half-buried second toe seemed unusually large. Forster joked that perhaps this specimen would help settle the long-running battle over whether dinosaurs gave rise to birds by having a long sickle claw like some dinosaurs. Half an hour later, the whole toe was exposed—and, amazingly, the raven-sized bird had a "wicked-looking" sickle claw, fit for a Velociraptor or other dromaeosaurid dinosaur. "We knew then that this was a really primitive bird, walking in the gray area between bird and dinosaur," says Forster, of the State University of New York, Stony Brook.

On page 1915, Forster and her colleagues describe this new species of primitive bird from Madagascar, called *Rahona ostromi* (*Rahona*, for menacing cloud in Malagasy, and *ostromi* in honor of Yale University paleontologist John Ostrom). *R. ostromi*, which lived 65 million to 70 million years ago, had feathered wings like a modern bird, but a long bony tail and a sickle claw like a meat-eating theropod dinosaur. Although it lived 80 million years after the first known bird, *Archaeopteryx*, *R. ostromi* may be one of the most primitive birds known and joins a gallery of recently discovered creatures that seem part bird and part dinosaur, researchers say. "It's a great discovery," says *Archaeopteryx* expert Peter Wellnhofer of the Bavarian State Collection of Paleontology and Historical Geology in Munich, Germany. "This fossil is very strong support for the theropod ancestry of birds." But this find won't end the fight over bird origins; researchers skeptical of a dinosaur ancestry say that Forster's team may have mistakenly combined bird and dinosaur bones.

In 1995, in their second field season in the sandstone hills of Madagascar, Forster and paleontologist Scott Sampson of the New York College of Osteopathic Medicine in Old Westbury, on a dig led by David Krause, also of Stony Brook, dug up a long, slender lower wing bone with quill knobs for feather attachment. It lay just above, although not attached to, several hind limb bones that fit together, as well as a long, bony tail like that of *Archaeopteryx*. The researchers knew they had something special, so they chopped out a 27-kilogram block of stone containing the bird and shipped the whole thing home to New York.

After preparing the specimen, they found that the bird's pelvic and pubic bones resemble those of *Archaeopteryx* and other early birds—making *R. ostromi* surprisingly primitive for its time in the late Cretaceous, when modern birds had already taken flight. What's more, the bird's last six dorsal vertebrae have an extra articular face, as seen in theropods but not in modern birds. These features, plus that sickle claw, make *R. ostromi* look even more like a theropod than *Archaeopteryx* does, says Forster.

FORSTER *ET AL.*



CATHERINE A. FORSTER

Bird of passage. A slashing claw (*above*) on an ancient bird dug up in Madagascar (*left*) suggests that it was in transition from dinosaurs to birds.

The new specimen joins a collection of strange-looking birds and dinosaurs, such as *Confuciusornis* and *Protoarchaeopteryx* from China, *Mononykus* from Mongolia, and *Unenlagia* from Argentina, whose combinations of features are hard to explain if birds evolved from some predinosaurian reptile, argues University of Chicago paleontologist Paul Sereno. "In the past 5 years, we've discovered so many wonderful intermediate forms that are close to the transition from dinosaurs to birds," he says. The best way to explain these specimen's half-bird, half-dinosaur appearance, he says, is that birds evolved from dinosaurs. Animals such as *R. ostromi* then retained many primitive dinosaurian traits for millions of years, making it "a living fossil in its own time," says Wellnhofer.

Researchers like John Ruben of Oregon

State University in Corvallis, who argues against a dinosaur origin of birds, agree that *R. ostromi* looks like a dinosaur—because, they say, its hind limbs actually come from a small dinosaur. "I think it's a chimera—a little dinosaur hindquarter, with a bird's forelimbs," Ruben says. Agrees University of Kansas paleo-ornithologist Larry Martin, "It's another dinosaur trying to hit it big as a bird." Martin thinks that the hind limb belonged to a dinosaur and that the wing bones could have been those of another ancient bird found at the same site, *Vorona berivotrensis*; the only known skeleton of that bird is missing its wings. "They owe us a close explanation why this can't be that bird," says Martin.

As discussed in the paper, Forster can't rule out that the wing bones and hind limb come from two different animals. But she contends that the hind limbs are clearly bird legs, possessing avian traits such as an opposable big toe and a small fibula, or lower leg bone. "They make a pretty good case that there are subtle avian characters in the hind limb," agrees University of Pennsylvania paleontologist Peter Dodson.

He adds that even if the bones all come from a bird, "the overall impression is that it's dinosaurian. It's exciting because if it's a single animal, it's sitting on the fence, somewhere between birds and dinosaurs." But whether *R. ostromi* is what it appears to be probably won't be settled until another specimen—complete with wings, tail, and slashing claws—rises from the sandstone of Madagascar.

–Ann Gibbons

PROTEIN DESIGN

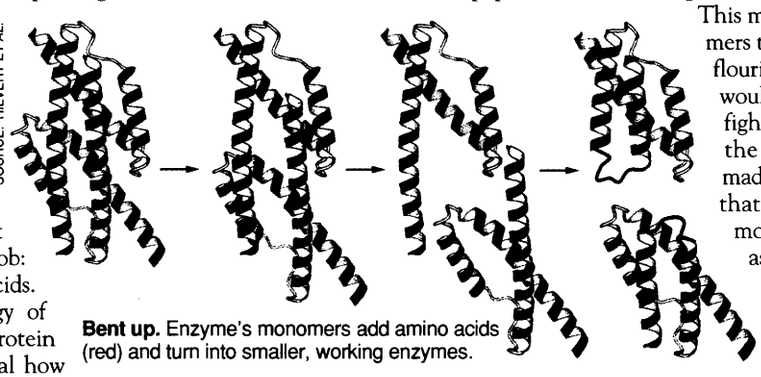
The Bare Bones of Catalysis

Nature has given us millions of enzymes, the chemical workhorses that speed up reactions inside living organisms. So you'd think that bioengineers who use enzymes in test tubes or industrial vats could simply choose the best one for the task at hand. But the sad truth is that good enzymes are hard to find. So biochemists have been harnessing an artificial version of evolution to refine natural enzymes, making new variants that work faster, longer, and at higher temperatures. Now researchers have used test tube evolution to create a redesigned enzyme that still performs the function of its natural counterpart.

By tearing apart an enzyme and pushing its fragments through a round of mutation and selection to recover the original function, chemist Donald Hilvert of the Swiss Federal Institute of Technology in Zürich and his colleagues report on page 1958 that they have come up with a new, smaller version that is equally adept at the original job: helping to assemble amino acids. Researchers hope this strategy of stripping an enzyme or other protein to its bare essentials will reveal how particular kinks and folds dictate how that protein works. It might also lead to tiny molecules that retain a therapeutic protein's function while lasting longer in the body. This molecular miniaturization is "on the frontier of protein design," says David Eisenberg of the University of California, Los Angeles.

One of the first successful downsizings came in 1996, when chemist Andrew Braisted and protein engineer James Wells of Genentech in South San Francisco, California, chopped away at one binding site of protein A, a bacterial protein that binds to a class of antibodies called G-type immunoglobulins. When one of three helices that help form this binding site is truncated, they found, the molecule and the antibody don't get together. To try to patch up that relationship, the researchers turned to evolution. They randomly fiddled with nucleotides at specific locations within the gene encoding the two full helices, creating about 100 million new versions. Next they inserted these mutated genes into viruslike particles called phages, which expressed the protein on their surface. Any new proteins with truncated helices that worked like the original would bind to an antibody stuck to the bottom of a plastic well.

Braisted and Wells rinsed away phages that had not bound and collected ones that did. They mutated the genes again and repeated the selection process. After three rounds of mutation and selection, the duo had evolved a new truncated peptide that bound to the antibody with nearly the same affinity as the original protein. "It's like taking an animal, amputating one of its legs, and evolving it so it can walk again," says Michael Hecht, a chemist at Princeton.



SOURCE: HILVERT *ET AL.*

Bent up. Enzyme's monomers add amino acids (red) and turn into smaller, working enzymes.

Now Hilvert and his colleagues—Gavin MacBeath at Harvard University and Peter Kast at the Swiss Federal Institute of Technology, all working at The Scripps Research Institute in La Jolla, California—have taken this approach a step further by applying it to an enzyme. Their target was chorismate mutase (CM), an enzyme that helps bacteria and higher plants make certain amino acids. Like protein A, CM sports helices linked by short strings of amino acids, called turns. However, CM is a dimer: two identical monomers, each consisting of three helices, locked in a tight embrace. Hilvert's group set out to part them and force a single monomer to develop the dimer's ability to catalyze a chemical reaction needed to make the amino acids.

Splitting the dimer required the researchers to bend one of the helices into a "U" by inserting a new turn. Because it is nearly impossible to predict how a turn will alter the structure or function of a helix, Hilvert's team decided to let natural selection pick a winner. They created a "library" of DNA sequences encoding millions of versions of the enzyme, each one with a different turn, and slipped these genes into a strain of *Escherichia coli* that can't make CM. Next, they added a selection pressure: The bacteria were grown on food lacking the amino acids that CM helps make. This meant that bacteria with monomers that work like the dimer would flourish, while ill-equipped bacteria would perish. "We let the organisms fight it out," says Hilvert. When the team sampled the proteins made by the survivors, they found that 0.05% of the variants were monomers that could work as well as the original dimeric CM.

"It's an impressive accomplishment," says Frances Arnold, a chemical engineer at the California Institute of Technology in Pasadena who has applied evolution to protein design (*Science*, 19 August 1994, p. 1032). Re-creating an enzyme's function in a molecule with a different structure, Arnold says, "is a very difficult design problem—and he let nature tell him what the answers are."

Redesigning proteins this way may have practical payoffs. For instance, says Eisenberg, therapeutic or industrial proteins cranked out by engineered bacteria sometimes clump into insoluble lumps. "Maybe you could make an alteration that could keep [the protein] as a monomer" that would not stick together as dimers and larger aggregates, he says. And because test tube evolution is a strategy with "very few design constraints," says Hilvert, "it's possible that we'll find surprises."

–Erik Stokstad