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.

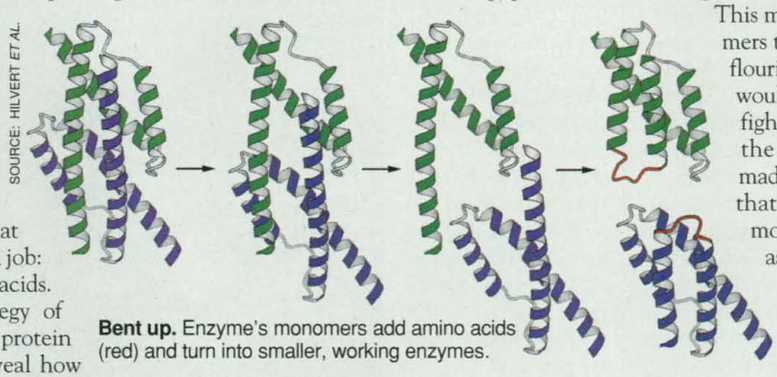
–Ann Gibbons

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.



SOURCE: HILVERT *ET AL.*

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

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.

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