tain point in the southern sky at some 600 kilometers per second. The cause of these peculiar motions—presumably a huge concentration of Dark Matter—was quickly dubbed the Great Attractor.

Now, said Dekel, he and his colleagues Edmund Bertschinger of the Massachusetts Institute of Technology and Sandra Faber of the Lick Observatory in California have re-analyzed that same velocity data with a new mathematical algorithm that allows them to map the mass concentrations in our cosmic neighborhood with unprecedented detail. Our galaxy is located at the center of that map (see figure), near the edge of a wide valley of very low density, while off to one side, about 200 million light-years away, rises the mountainous Great Attractor. The dense Virgo cluster, which was long thought to be the dominant concentration of mass in our vicinity, turns out to be nothing more than a shoulder on the mountain.

The second, complementary mapping technique was discussed in Brighton by members of two independent observing teams. Both have taken carefully selected samples of the galaxies from the most complete survey of the local universe available—the catalog of galaxies identified in 1983 by NASA's Infrared Astronomy Satellite (IRAS)—and then systematically measured the selected galaxies' redshifts due to cosmic expansion. Since each redshift is approximately proportional to the galaxy's distance, the result is a map of the galaxy distributions in three dimensions.

These galaxies represent only a tiny fraction of the mass contained in the invisible Dark Matter, of course. But presumably their distribution is roughly the same as the Dark Matter's. And indeed, the redshift surveys yield density maps that are qualitatively similar to the one discussed by Dekel. When all these new maps are taken together, moreover, they yield a new estimate of the average mass density—and one that, for the first time, begins to approach the theoretical expectations. Within the uncertainties, the new average density appears to be at least half the critical density—already a fivefold increase over the previous figures—and may very well be equal to the critical density.

As intriguing as the results are, of course, the observers are quick to say that the uncertainties are still very large. But then, as University of California, Berkeley, astronomer Marc Davis pointed out, both mapping approaches are only in their infancy, although they are rapidly improving. "This is so complicated," he says, "we'll be playing this game for years. But we've started to demonstrate the power of the method."

■ M. MITCHELL WALDROP

On the Road to Mandelate...Racemase

The three-dimensional structure of the enzyme mandelate racemase provides a surprising clue to enzyme evolution

EVERY ORGANISM DEPENDS ON ITS ENZYMES to perform the everyday functions of life, obtaining energy from food, for example. A simple bacterial cell has a couple thousand of these essential protein catalysts; a human being has tens of thousands. How did all these enzymes, each with its own specific set of chemical capabilities, evolve?

Now a serendipitous—and surprising—discovery by a team of enzymologists and structural chemists may help answer that question. The researchers have found that two distinct enzymes, known to catalyze different chemical reactions, have essentially the same three-dimensional shape. That kind of structural similarity has never been seen before. And it is more than just a biochemical oddity.

When an enzyme catalyzes a reaction, it must first bind the reacting chemicals, called "substrates" in enzymology jargon. The binding is exquisitely specific, and one theory of enzyme evolution focuses on that specificity, postulating that an enzyme evolves by undergoing mutations that enable it to perform a new reaction on the same old substrates. But the current discovery suggests, says team member Gregory Petsko, an x-ray crystallographer at Rosenstiel Center at Brandeis University, that in at least some cases the reverse may be true; that evolution may work by selecting a preexisting enzyme for its chemical capabilities and then altering its specificity so that it can bring about its usual chemical reaction on a new substrate. "It looks to us like it's the chemistry that counts," says Petsko.

That idea is "right on the money," agrees chemist Bruce Erickson of the University of North Carolina at Chapel Hill, who likes the work so much that he recently arranged for Petsko to give a seminar at UNC. "It's more difficult to change chemistry than specificity."

Not only are the findings providing new insights into evolution, but they may also have practical applications for enzyme design. Researchers are very interested these days in using the techniques of genetic engineering to mutate enzyme proteins to improve their efficiency or otherwise alter the reactions they catalyze. But the mutations do not always have predictable effects on enzyme activity, notes another team member, George Kenyon of the University of California, San Francisco. He suggests that a better understanding of natural enzyme evolution might help researchers design their mutations more rationally.

Kenyon, Petsko, and their colleagues did not set out to trace enzyme evolution, however. Kenyon is a biochemist who for the past 20 years has been studying an enzymatic pathway that enables some strains of the common soil bacterium *Pseudomonas putida* to get their nutrition from a chemical called mandelate, which is produced by decomposing plant matter. The pathway consists of five enzymes that work together to convert mandelate to benzoate. The benzoate is then broken down by another set of enzymes (in the beta-ketoadipate pathway) to compounds that can be used to generate adenosine triphosphate, the cell's major source of chemical energy.

Kenyon knew from earlier work that the five enzymes of the mandelate pathway are physically clustered within the bacterium. He was interested, he recalls, in crystallizing all of the enzymes in the pathway and determining their three-dimensional structures. He wanted to see whether the surfaces of the proteins have affinities for one another that could explain the clustering. So Kenyon joined forces with enzymologists John Gerlt and John Kozarich, both of the University of Maryland, and crystallographer Petsko.

Gerlt was intrigued by the project because of the opportunity to learn more about mandelate racemase, the first enzyme in the pathway. This enzyme rearranges the order of the atoms attached to one of the carbon atoms in mandelate by removing a hydrogen ion from one side and attaching it to the opposite side. "It looked like a very simple model for understanding how enzymes make and break carbon-hydrogen bonds," Gerlt says. "This is a fundamental, but poorly understood, part of enzyme-catalyzed reactions."

Gerlt agreed to clone and sequence the

mandelate racemase gene, with the idea that once the cloned gene was in hand it could be used to make enough of the enzyme protein for Petsko to crystallize it and determine its three-dimensional structure. Once the structure had been solved, the scientists anticipated that the combination of structural and sequence data would allow them to work out the details of the enzyme's activity at an atomic level.

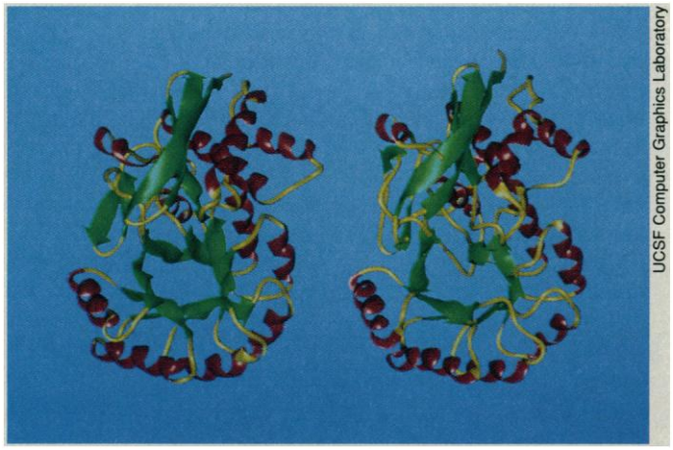
Petsko, then on the faculty at the Massachusetts Institute of Technology, assigned the project to his graduate student, David Neidhart. As Neidhart was working out the final details of the enzyme's structure, he sought the advice of fellow graduate student Gregory Farber, who was very familiar with proteins of the general structural class of mandelate racemase. When Farber looked at the model of mandelate racemase on Neidhart's computer screen, he asked a question that gave Neidhart quite a jolt.

Was he sure, Farber wanted to know, that the enzyme he had crystallized was in fact mandelate racemase? It looked just like muconate lactonizing enzyme, a member of the beta-ketoadipate pathway—whose structure had already been solved by Adrian Goldman at Yale. "I was in a virtual panic thinking that my graduate career was going to be ruined because I had solved a structure that had already been solved," says Neidhart, who is now at Abbott Laboratories in Abbott Park, Illinois. He called Gerlt, who had access to the sequences of both enzymes, and asked him to read the one for muconate lactonizing enzyme.

With great relief, Neidhart learned that it and mandelate racemase are indeed different; their sequences are only 26% similar. Yet their structures are virtually superimposable. Neidhart's graduate career was secure. He had not only solved the structure of the right enzyme, but the discovery of the structural similarity between mandelate racemase and muconate lactonizing enzyme had also raised some new and provocative questions.

It's not unusual for enzymes that perform similar functions to have structures that resemble one another, but the reactions catalyzed by the two enzymes don't appear at all similar. In contrast to mandelate racemase, which converts one stereoisomer of mandelate to another by breaking a carbon-hydrogen bond and then reforming it in a different configuration, muconate lactonizing enzyme forms a ring out of a linear molecule. "There's not a soul on earth," says Kenyon, "who could have predicted a relationship between these two enzymes."

On closer inspection, however, the scientists realized that the resemblance between the enzymes makes both evolutionary and chemical sense. The mandelate pathway occurs in less than 5% of *Pseudomonas* strains, so the research team speculates that the pathway enzymes probably evolved sometime after *Pseudomonas* bacteria established themselves and didn't have much time to diverge significantly from their predecessors. The beta-ketoadipate pathway, meanwhile, is apparently long-standing, found in all *Pseudomonas* strains. All this makes the team think that mandelate racemase may have been directly derived from muconate lactonizing enzyme.



Spitting images. *Mandelate racemase (left) and muconate lactonizing enzymes have virtually identical shapes.*

But why the connection between those two particular enzymes? That's where the team members turned to their knowledge of chemistry for an explanation. The reactions catalyzed by the two enzymes, while different overall, have similar mechanisms. "Each reaction has more than one step," notes Petsko, and both proceed through the formation of the same class of unstable intermediate. And in both cases, the catalytic activity of the enzymes depends on their ability to stabilize the intermediates.

This suggests, Petsko says, that the ability of muconate lactonizing enzyme to stabilize the reaction intermediate was the primary reason for its selection to perform the racemase reaction. "In the beginning, a variant of the muconate lactonizing enzyme probably had some low level of activity with mandelate as substrate," he hypothesizes. "After all, if the first enzyme could only bind mandelate, but did nothing, the selective advantage would never become apparent."

But then the gene for muconate lactonizing enzyme would have had to have undergone mutations that would enable it to use mandelate more effectively as a substrate. "First, nature selects for the chemical activity, and later the enzyme's active site is modified to handle the substrate better," Petsko proposes. The mutations could not seriously disturb the three-dimensional structure of the evolving enzyme, however, without destroying its ability to catalyze the reaction by stabilizing the intermediate.

Is the evolution of mandelate racemase unique, or do most enzymes evolve this way? "Once we recognized the similarity between mandelate racemase and muconate lactonizing enzyme, we realized there was a lot of evidence for similarities between other enzymes in the pathway and previously existing enzymes," says Gerlt. The team has gone on to identify possible ancestors for almost all of the enzymes in the mandelate pathway. They have also embarked on a project to recapitulate the evolution of mandelate racemase.

To do this, Gerlt and Kozarich have set out to see if they can convert muconate lactonizing enzyme into mandelate racemase. One way they will do this is by mutating specific portions of the gene for muconate lactonizing enzyme and then testing the resulting proteins for mandelate racemase activity.

The researchers will also attempt to emulate nature's own system for altering enzymes. In this scheme, the team will remove the endogenous mandelate racemase gene from a strain of *Pseudomonas*, a modification that should make it impossible for the bacteria to use mandelate as a nutrient. The researchers will then look for mutants that have regained the ability to thrive on mandelate and determine which existing enzyme had to change in order to give the bacteria that ability. If they're right about mandelate racemase evolution, they will find that the muconate lactonizing enzymes will have changed in some of the mutants. Then, they can analyze the altered enzyme to assess exactly what changes were necessary. "Rather then our deciding, we think we should let nature do it. Then we can look at the changes and see what nature did to make the changes occur," Kenyon says.

The researchers concede that they have a long way to go before they understand enzyme evolution. "Almost everything about this project has been a surprise—which makes us realize that we don't yet think about things the way nature does," says Petsko. ■ MICHELLE HOFFMAN

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