directly with Orc2, one of the proteins in the ORC complex.

Even if it is true that Dbf4 brings Cdc7 to the origin, Diffley still doesn't have an answer to his original question: What are the proteins that change the footprint at the origin early in G1? Dbf4 and Cdc7 don't quite fit the bill, because the footprint changes before the *DBF4* gene is turned on.

One set of interesting candidates is a group of proteins known as the Mcm or Cdc46 family. These proteins seem to be necessary for DNA replication, says Li, and they "are in the nucleus when Diffley sees this change in footprint." The possibility of Mcm/Cdc46 involvement "is certainly an exciting one," says Diffley, but he cautions that "there is virtually no convincing evidence" yet that this is the case.

Whether the Mcm/Cdc46 proteins play a role or not, the emerging model suggests a two-step prologue to DNA replication, beginning in early G1. First, the complex of proteins bound to the origin changes, perhaps by adding Mcm/Cdc46 proteins; this sets the stage for the second step, which may be the arrival of Dbf4 and Cdc7. After they act, the stage-setting proteins depart, leaving ORC alone on the DNA. Replication can't be initiated again until the cell passes through another mitosis, allowing the permissive proteins to hop on again. "I think of it as a gun," says Li. "You have to cock it, and then you have to trigger it, and you can't recock it until you are in mitosis again."

While researchers hope the work on Cdc7 and Dbf4 has put them on the right track toward understanding the control of replication initiation, they caution that the problem is far from solved. Berkeley's Rine notes that studies of genetic mutants isolated in Sclafani's and Johnston's labs suggest that there might be another step between Cdc7's action and initiation of replication that may or may not take place at the origin. Direct biochemical evidence placing Cdc7 at the origin, he says, would be reassuring.

If Cdc7 is indeed the trigger that acts at the origin to kick off replication, it must be doing so by phosphorylating a key protein there. "The $64,000 question right now is what is the physiologically significant substrate for Cdc7," says Diffley. It could be a subunit of ORC; in fact, Denver's Sclafani has preliminary evidence that Orc2—the same subunit Hardy found linked to Cdc7—receives a phosphate from the Cdc7 kinase. Of course there are other candidates, including the Mcm proteins and the other ORC components, as well as still-unidentified proteins that may also be at the scene. If it turns out that Cdc7 phosphorylates any protein at the origin of replication, the connections of the "not-so-famous" kinase will have won it its moment of glory.

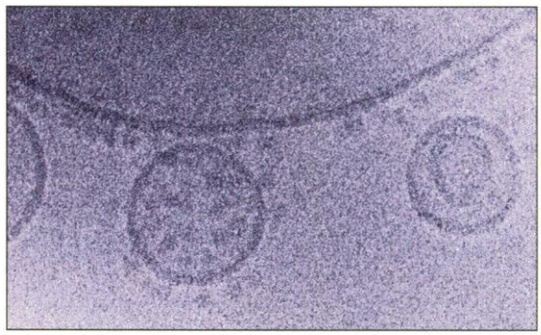
–Marcia Barinaga

BIOCHEMISTRY

New Enzyme Structure Reveals Cell's Rotary Engine

CAMBRIDGE U.K.—Almost all life on Earth is powered by energy from the sun. Biochemists have known for 40 years that solar energy is converted in plants and ultimately in animals to the compound adenosine 5'-triphosphate (ATP), a cellular "fuel" that powers chemical synthesis, communication, and motion. And the key to capturing the sun's power is the enzyme ATP synthase. "This enzyme is found in almost all forms of life," says biochemist Harvey Penefsky of the Health Sciences Center of the State University of New York at Syracuse, adding that it "catalyzes the last step in the cell's energy conservation mechanism."

ATP synthase may be significant, but it's also a puzzle: For decades biochemists have been theorizing about just how the enzyme manages to grab hold of the necessary chemical precursors and churn out ATP at the incredibly high rate needed to fuel the rapid growth of plants. Those decades of puzzlement may be ending, however, since this week researchers took a big step toward solving the mystery with the publication of the three-dimensional structure of the catalytic region of ATP synthase in *Nature*. A team at the Medical Research Council's Laboratory of Molecular Biology in Cambridge, U.K., led by biochemist John Walker, used x-ray crystallography to unravel the structure which, with a molecular mass of 371,000, is the largest asymmetric molecular structure ever to be resolved in full atomic detail.



RODERICK A. CAPALDI

Turning heads. Lollipop-like ATP synthase molecules projecting from the surface of bacterial membranes.

"This is a major accomplishment and a big stimulus for the field," says biochemist Alan Senior at the University of Rochester Medical Center, who studies ATP synthase in the bacterium *Escherichia coli*. "This [structure] is going to be applicable to plants, all animal, and all bacterial ATP synthesis—we can assume the mechanism will be the same." The structure provides firm evidence for a model of the workings of ATP synthase developed around 1980 by Paul Boyer, then head of the Molecular Biology Institute at the University of California, Los Angeles. The model predicts that the globular catalytic region of ATP synthase spins like a top. In the course of one revolution, its three active regions change shape three times, helping them catch the precursors of ATP, catalyze the reaction, and release the new molecule—like a rotating production line.

Boyer, now retired, is gratified to see his ideas borne out. "This is obviously a landmark paper," he says, "it's going to have a huge impact." Biochemist Roderick Capaldi of the Institute of Molecular Biology at the University of Oregon sees support in the new results for his deductions about how movement within the ATP synthase molecule influences the enzyme's activity. He says "the Walker model makes such sense...I don't think there's a possibility that it's wrong."

While only plants and specialized bacteria can capture the energy of sunlight directly through photosynthesis, thereby storing up ATP, other cell types are able to make ATP by a process called oxidative phosphorylation, which consumes fats, carbohydrates—such as glucose—and oxygen. Whatever the energy source, the process takes place at specialized membranes such as the inner membrane of the mitochondrion, the cellular organelle where energy production and storage takes place. Energy from sunlight, or oxidation of foodstuffs, creates a build-up of positively charged hydrogen ions, or protons, on one side of the membrane. The resulting difference in proton concentration on the two sides of the membrane is known as the "proton motive force," because it makes the protons "want" to move back across the membrane to even out the disparity in concentration.

ATP synthase threads through the membranes and provides the channels through which protons can flow back across the membrane under the influence of the proton motive force; it is this proton flux that drives ATP production. The enzyme molecule has three parts. The first, designated F_o, threads through the membrane. The second component is a short stalk that connects F_o to the third element, a globular molecule known as F_1 that is less than 10 nanometers

across. Using an electron microscope, it is possible to see the stalks with their F_1 heads like rows of lollipops lining the inner mitochondrial membrane.

The F_1 part of ATP synthase is the catalyst that creates ATP molecules by combining ADP [adenosine 5'-diphosphate] and phosphate. In some situations, such as in anaerobic bacteria, it catalyzes the opposite reaction—splitting ATP back into ADP and a phosphate. For their crystallography study, Walker's team chose to look at F_1, and a portion of the stalk, separated from the rest of the molecule. In this independent form, F_1 is called adenosine 5'-triphosphatase, or ATPase for short. They chose ATPase because alone it has the advantage of being water soluble, making it easier to crystallize; the regular array of molecules in a crystal is essential to determining structure by x-ray crystallography.

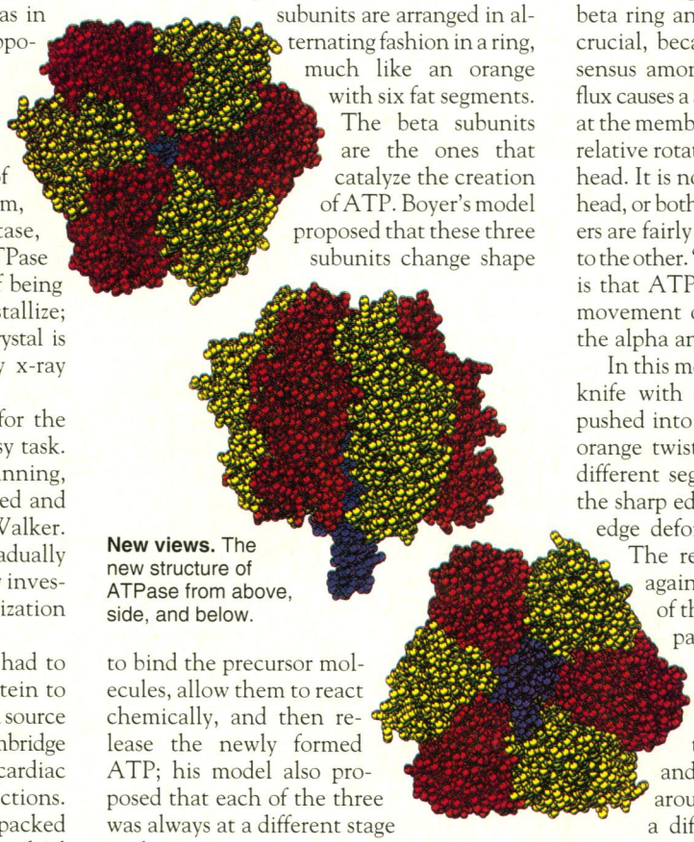
Even with this help, the search for the structure of the enzyme wasn't an easy task. "We had crystals from the very beginning, but they were small and poorly formed and diffracted x-rays very weakly," says Walker. "Over the next 7 years or so we gradually improved the quality of the crystals by investigating a very wide range of crystallization conditions."

The first problem Walker's team had to solve was obtaining enough pure protein to grow crystals. The trick here is to find a source of tissue rich in the protein, and the Cambridge team used beef hearts, because the cardiac muscle burns ATP to fuel its contractions. As a result, the cells of the heart are packed with mitochondria: The mitochondrial membranes consist of nearly 10% ATPase. From three hearts, Walker could extract up to 100 milligrams of the enzyme in 2 days—a remarkable amount by the standards of protein chemistry. "We must have prepared a total of 30 grams of enzyme over the years," he says, adding that for future experiments he hopes to grow the protein in bacteria.

The next challenge was growing perfect crystals of the enzyme that were up to 1 millimeter across—the size needed for crystallography. The tricky part at this stage was that every protein favors slightly different conditions—such as solvent and pH—for crystallization, and pinning down just the right conditions for growing the crystal is still more an art than a science. In addition, ATPase changes shape during catalysis, and it was important to get all the molecules in the crystal to adopt the same shape. A key finding was that "heavy" water, made with the hydrogen isotope deuterium, was the best solvent, possibly because it stabilized forces between the different parts of the enzyme, helping to make flawless crystals. Added to the solvent was a mixture of ADP and a nonhydrolyzable analog of ATP, which kept all the enzyme molecules in the same shape.

From earlier biochemical studies, the researchers knew that the globular head of ATPase consists of six subunit proteins, three each of two types, known as alpha and beta subunits. Electron microscopy and low-resolution x-ray studies showed that the six subunits are arranged in alternating fashion in a ring, much like an orange with six fat segments. The beta subunits are the ones that catalyze the creation of ATP. Boyer's model proposed that these three subunits change shape to bind the precursor molecules, allow them to react chemically, and then release the newly formed ATP; his model also proposed that each of the three was always at a different stage in the process.

SOURCE: M.R.C.



New views. The new structure of ATPase from above, side, and below.

This model had been disputed by some researchers, such as Mario Amzel and Peter Pedersen of Johns Hopkins University in Baltimore, who said that in their low-resolution x-ray analysis all the beta subunits had the same shape—the molecule was symmetrical. But researchers are confident that Walker's structure has cleared up such doubts. "[The new structure] certainly does show asymmetry between the three beta chains," says bioenergeticist Stuart Ferguson of Oxford University, who says the result was "quite widely anticipated, but this really gives you detailed insight." On Walker's evidence, "the suggestions of a symmetric arrangement are out," says Boyer. But Amzel maintains that he and Pedersen were looking at a different freeze-frame in their rat enzyme. "There are states of the molecule more symmetrical than the one presented [by Walker]."

While Walker's structure supports the idea that ATP is produced as each beta subunit undergoes a sequence of deformations, there is still the question of how this reaction is powered by the protons flowing through the F_o part of ATP synthase. The key to this problem is the connecting stalk, and the new x-ray structure offers guidance here too, since it contains half of the gamma subunit, the first part of the stalk (although none of the remaining six stalk subunits).

The new structure shows that the gamma subunit is a slightly curved, elongated protein running through the center of the alpha/beta ring and out at the base. The curve is crucial, because it supports a growing consensus among biochemists that the proton flux causes a shape change in the stalk, starting at the membrane end, which translates into a relative rotation between the stalk and the F_1 head. It is not known whether it is the stalk, head, or both that actually move, but researchers are fairly certain that one rotates relative to the other. "What the structure seems to show is that ATP synthesis is driven by physical movement of the gamma chain relative to the alpha and beta units," says Ferguson.

In this model, the gamma subunit is like a knife with a curved sharp edge which is pushed into the center of the orange. If the orange twists around relative to the knife, different segments come into contact with the sharp edge of the blade in turn, and this edge deforms the shape of the segment. The relative rotation of the F_1 head against the gamma stalk brings each of the beta subunits in turn against a particular part of the gamma unit, and the nature of the contact between the two deforms part of the beta subunit into a shape that makes it pick up an ADP and a phosphate. Twisted further around, the beta subunit moves into a different shape which makes the two compounds react; another third of a revolution and the shape changes to eject the newly formed ATP. Capaldi already had experimental evidence that the relative movement of the stalk and the head played an important role: He found that cross-linking the alpha/beta ring to the stalk—stopping the rotation—blocked the enzyme's activity; breaking the links restored it again.

Capaldi and other researchers are looking forward to the work they can do with the new map of ATPase. "The next phase for everyone is investigating the [changes in shape]—what we need to see now is different states," he says. Walker is now doing just that, as well as working on crystals of the stalk and of F_o. Senior envisages that ultimately we could "learn more about energy transfer, which could be used to improve crops and fermentation," he says. Having puzzled for years over a molecule whose structure they could not see, Walker's work has given researchers new vision. Says Capaldi: "My heart beats twice as fast as it did last week—this is very exciting."

–Claire O'Brien

Claire O'Brien is a science writer based in Cambridge, U.K.