CELL BIOLOGY

Yeast Enzyme Finds Fame in Link to DNA Replication

Everyone knows that becoming famous is often a matter of having the right connections. Imagine Laurel without Hardy. Or, in another sphere, consider the yeast enzyme Cdc7, which has long labored in obscurity because it lacked the connections that would make cell biologists sit up and take notice. Now Cdc7 may be about to achieve its Warholian 15 minutes of fame. Work from several groups, one of which reports its findings on page 1243, places the enzyme at the site of a key event in the cycle of cell growth and division: the initiation of DNA replication.

Until now, Cdc7 has been overshadowed by its famous cousin, Cdc28 (also known as Cdc2). Both were discovered in yeast, and both are kinases, enzymes that add phosphate groups to other proteins. But Cdc28 has received far more press, as researchers uncovered its role in controlling several points in the cell cycle.

The role of Cdc7, in contrast, remained a mystery. Yeast geneticist Lee Hartwell of the University of Washington identified the *CDC7* gene in the late 1970s and found that it is required for yeast cells to copy their DNA before dividing. But no one knew where Cdc7 acted in the cell, or how direct its involvement was in triggering DNA synthesis. "We always call Cdc7 the not-so-famous kinase," says yeast geneticist Robert Sclafani of the University of Colorado in Denver, who cloned the *CDC7* gene in 1985.

Among the recent findings that brought this not-so-famous enzyme into the spotlight are those of Simon Dowell, Piotr Romanowski, and John Diffley, of the Imperial Cancer Research Fund in Hertfordshire, England. They report in this issue that Dbf4, a protein that binds to Cdc7, is found at the sites in the genome where DNA replication begins. This result suggests Dbf4 may act to bring Cdc7 to the sites, called "origins of replication," where it may then turn on DNA synthesis. "People... never knew exactly how to connect [Dbf4 and Cdc7] to the initiation of DNA replication," says Joachim Li, who studies DNA replication at the University of California, San Francisco. "We are placing [them] at the center of action now."

The stage was set for the present work 2 years ago when Stephen Bell, a postdoc in Bruce Stillman's lab at Cold Spring Harbor Laboratory on Long Island, identified a group of six proteins that bind to origins of replication; he named them ORC, for origin recognition complex.

While ORC's presence at the origins suggested it is involved in turning on DNA replication, it was clear from Diffley's work that there had to be more to the story than just the six ORC proteins. Publishing in the same issue of *Nature* where the Stillman group's results appeared, Diffley and his postdoc Julie Cocker reported that, in living yeast cells, ORC apparently sits on the origin throughout the cell cycle. But DNA synthesis is turned on only at a specific point in that cycle: the start of the DNA synthesis, or "S" phase. Clearly, something must happen to trigger that event. One possibility, says Stillman, is that "ORC may be in part a landing pad for other proteins [that regulate replication]. The question is what are those proteins?"

Diffley thought he had a clue. His group had described the extent of ORC contact with the origin site, using a method called "footprinting," in which DNA is enzymatically destroyed except in regions protected because they are covered by proteins. They found that the footprint grows after cell division, as the cell enters the first growth phase—G1—which precedes S. "It looks as though, in G1, there is an additional complex present at the replication origin, in addition to ORC," Diffley says.

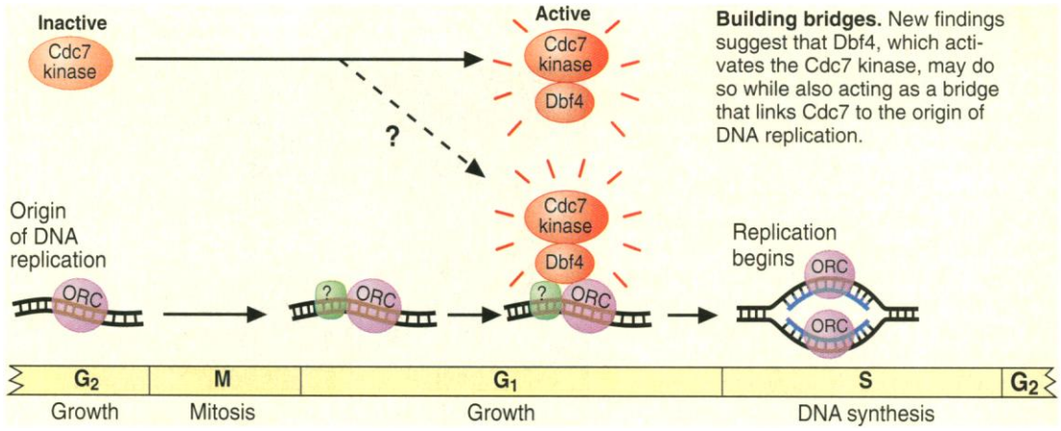
The obvious next step was to find that complex. Diffley, Dowell, and Romanowski set out to find candidate proteins using a technique designed to identify genes whose protein products bind directly to the origin or to proteins already at the origin. The gene they pulled out in this way was the yeast gene *DBF4*; further work suggested that the Dbf4 protein binds to ORC as it sits on the origin.

And that's where Cdc7's connection to fame comes in. Two years ago, Lee Johnston and his co-workers at Britain's National Institute for Medical Research in Mill Hill, showed that the *DBF4* gene is turned on late in G1, before cells begin copying their DNA—and just about the time the Cdc7 kinase becomes active. Earlier work by the Johnston group, which discovered Dbf4 in the early 1980s, had already shown that it is required for DNA replication just as Cdc7 is: Mutations in either gene prevent yeast cells from copying their DNA.

Further evidence for a link between Dbf4 and Cdc7 comes from a team that includes Denver's Sclafani and John Rosamond of the University of Manchester, England, who found last year that Dbf4 turns on Cdc7's kinase activity. These findings suggest that Dbf4 is the normal activator of the Cdc7 kinase during the cell's reproduction cycle. Indeed, says Sclafani, genetic experiments in his lab suggest activating Cdc7 may be the only important role for Dbf4 in the cell.

Because Dbf4 seems to bind Cdc7 to activate it, its presence at the origin site implies that Cdc7 is there as well. In the current work, Diffley's group shows that Dbf4 has separate binding sites for Cdc7 and for ORC, suggesting, says Diffley, that Dbf4 "acts as a bridge" between Cdc7 and ORC. "The model that comes out of that," says Denver's Sclafani, "is that Dbf4's role is to sequester Cdc7 to the ORC, and that Cdc7 does its job there."

That idea is buttressed by several pieces of unpublished data. Studies of genetic mutants in Jasper Rine's laboratory at the University of California, Berkeley, suggest that Cdc7 interacts with ORC. And Chris Hardy, at Washington University in St. Louis, has unpublished results similar to Diffley's, as well as preliminary evidence that Cdc7 interacts



Building bridges. New findings suggest that Dbf4, which activates the Cdc7 kinase, may do so while also acting as a bridge that links Cdc7 to the origin of DNA replication.

ILLUSTRATION: K. STULIFF

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.

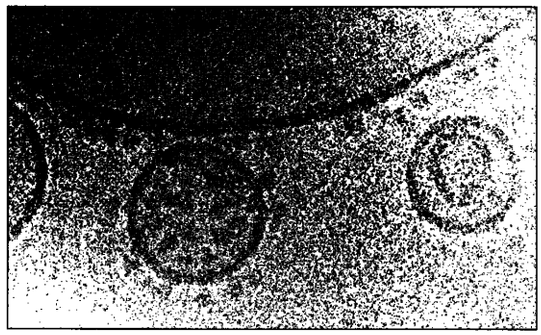
–Marcia Barinaga

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