A New Twist to the Cell Cycle

Researchers have found that the timed destruction of key proteins plays a critical role in regulating the cell cycle

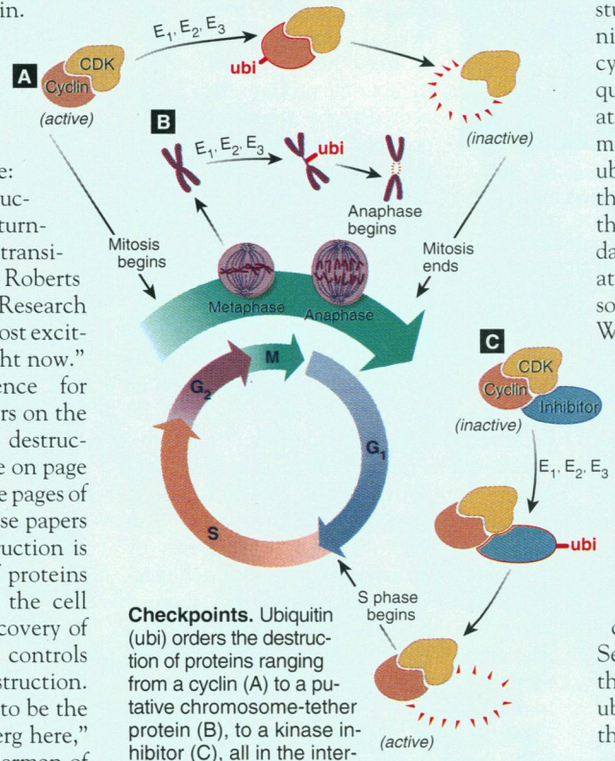
Every high school biology student learns about the cell cycle, the meticulously orchestrated series of events that culminates in cell division. Despite its textbook familiarity, however, the cell cycle retains its ability to stump the experts, fascinating and surprising cell biologists who strive to understand the intricate biochemical machinery that keeps it running. The latest surprise is a deluge of new evidence tying the cell cycle to another well-known cellular pathway that was previously thought to be unrelated. That other pathway is one for protein degradation, triggered by a molecule named ubiquitin.

Researchers once expected the cell cycle to be regulated mainly by the well-timed production of regulatory proteins. But today one of the hottest topics in the cell-cycle field deals with exactly the opposite: its regulation by sudden protein destruction, or proteolysis. "Proteolysis is turning up at each and every cell-cycle transition," says cell-cycle researcher Jim Roberts of the Fred Hutchinson Cancer Research Center in Seattle. "It is one of the most exciting areas [of cell-cycle research] right now."

Among the strongest evidence for Roberts's claim is the flood of papers on the role of ubiquitin-triggered protein destruction in the cell cycle, including one on page 682, that have been pouring into the pages of journals in the past year or so. These papers tell a story in which protein destruction is responsible for turning on and off proteins that play key roles in regulating the cell cycle. They also chronicle the discovery of the biochemical machinery that controls these precise acts of protein destruction. "We are just seeing what promises to be the tip of a very big and exciting iceberg here," says cell-cycle researcher Joan Ruderman of Harvard Medical School.

The significance of the work goes beyond understanding the internal workings of the cell cycle. If protein destruction is key to cell-cycle control, and therefore to cell division, it means the mutations that affect the machinery of destruction might lead to the uncontrolled cell growth of cancer. Researchers have already begun looking at various tumor cell lines to see whether abnormal protein destruction contributes to their excess growth, although they don't have a cancer link yet.

Although research into the role of protein degradation in controlling the cell cycle has surged recently, the first hints that it was important came in the early 1980s. Tim Hunt of the University of Cambridge, U.K., and his co-workers discovered a protein whose levels build during mitosis—the stage of the cell cycle in which the cell partitions its chromosomes and divides in two—and then drop precipitously. They named it cyclin. Subsequently, several groups showed that cyclin activates a regulatory enzyme known as a kinase that adds phosphate groups to other proteins. The activity of this cyclin-dependent kinase (CDK) triggers mitosis.



Checkpoints. Ubiquitin (ubi) orders the destruction of proteins ranging from a cyclin (A) to a putative chromosome-tether protein (B), to a kinase inhibitor (C), all in the interest of moving the cell cycle forward.

ILLUSTRATION: K. SUTLIFF

Part of the way through mitosis, after the CDK has done its job, cyclin levels plummet, and the kinase stops functioning, allowing the cell to finish mitosis. That finding, says cell-cycle researcher Kim Nasmyth of the Research Institute of Molecular Pathology in Vienna, Austria, was the first suggestion that "cell cycle control might revolve around changes in proteolysis."

In 1989, Andrew Murray and Marc Kirschner, then at the University of California, San Francisco, provided further evidence for the idea that protein degradation is at the heart of cell-cycle control. They found they could render cyclin indestructible by removing one end of the molecule; cells carrying this mutant cyclin got stuck in mitosis. That meant that inactivation of the cyclin-dependent kinase was necessary for the cell to complete mitosis.

That experiment also showed that the part of the cyclin they had removed must contain "some recognition component ... important for its degradation," Kirschner says. Following up on that clue, Kirschner student Michael Glotzer identified a specific nine-amino-acid sequence as necessary for cyclin's destruction. It turns out that the sequence has its effect because it directs the attachment to cyclin of ubiquitin, thus marking cyclin for degradation. Until then, ubiquitin had a rather boring reputation as the cell's garbage disposer, because most of the proteins it tagged for destruction were damaged or malformed. "Ubiquitin-mediated proteolysis was a process in search of some biological relevance," says Murray. With Glotzer's finding, ubiquitin found that relevance in spades: It was needed for a crucial step in cell-cycle control.

The cyclin Glotzer and colleagues were studying is only one of many cyclins that activate kinases at various times in the cell cycle. And it isn't the only one regulated by ubiquitin. For instance, there is another cyclin that activates a kinase that in turn triggers the beginning of a stage of the cell cycle called S phase, when the DNA is copied. Several labs working on yeast have shown in the past year that this cyclin is tagged with ubiquitin and abruptly degraded as soon as the cell begins to copy its DNA.

Turning on as well as off

Even more recently, evidence has begun to accumulate showing that protein destruction is just as important for turning CDKs on as it is for turning them off. In the past 2 years, researchers have identified multiple inhibitory proteins that bind to the CDKs, preventing them from acting. In work described in *Cell* last October, Nasmyth's group provided genetic evidence showing that in yeast one of these inhibitory proteins, known as $p40^{Sic1}$ (Sic1), must be degraded in order for its CDK partner to be activated and DNA replication to begin.

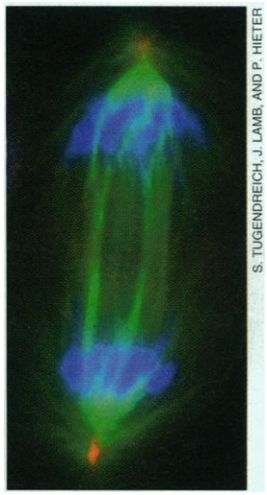
That genetic evidence also suggested that Sic1 is directly degraded by the ubiquitin pathway, but it didn't directly show that to be the case. So it is complemented by results reported in this issue by Michele Pagano and his colleagues at Mitotix Inc., a Cambridge, Massachusetts, biotech company investigating the cell cycle for possible new therapeutic approaches to disease. Pagano's group was working with a mammalian CDK inhibitor called p27. In a series of experiments that included the biochemical blocking of the machinery that degrades ubiquitinated proteins in cells, the Mitotix team showed that p27 is degraded via the ubiquitin pathway.

Pagano's and Nasmyth's findings establish inhibitor destruction by ubiquitin as an important theme in cell-cycle control, Murray says. "You can turn cyclin-CDK complexes off by destroying the cyclin. Now, apparently, in a different situation you can turn them on by destroying their inhibitor."

And ubiquitin's new-found fame doesn't stop with kinase regulation. Other research suggests it controls the destruction of yet other proteins whose demise is necessary for the cell cycle to move forward. Two years ago, Murray and postdoc Sandra Holloway found they could prevent anaphase, the stage of mitosis when the chromosome pairs separate, by blocking the addition of ubiquitin to proteins during that time. In contrast, they found that anaphase proceeded just fine when they specifically blocked cyclin destruction.

That result led them to conclude that there is another noncyclin protein that must be destroyed before the chromosome pairs, known as sister chromatids, can separate. Nasmyth's lab also has evidence that a noncyclin protein is required for anaphase. Murray suggests the mystery protein may be a "tether" that holds sister chromatids together.

With ubiquitin-mediated destruction popping up as a specific control step at various points in the cell cycle, the next big question for the cell biologists pursuing the cell cycle is: What factors determine which proteins are destroyed and when they are destroyed?



S. TUGENDREICH, J. LAMB, AND P. HIETER

Pinned down. The red staining shows that the protein Cdc16, a member of the complex that puts ubiquitin on proteins, localizes to the mitotic spindle.

Probing the ubiquitinating machinery

In search of the answer, researchers turned their attention to the biochemical machinery that adds ubiquitin to proteins. In 1983, Avram Hershko's group at the Technion-Israel Institute of Technology in Haifa showed that ubiquitin is added to proteins by a biochemical "bucket brigade," which passes ubiquitin from an enzyme called E1 to a second enzyme called E2, and finally, aided by a third enzyme called E3, to the target protein. While there seems to be only one E1, a variety of E2s and E3s participate in ubiquitinating different proteins.

Even though there are multiple forms of E2, this factor doesn't seem to have the temporal specificity needed to degrade some proteins only at certain times in the cell cycle. Last year, a collaborative team composed of Ruderman's and Hershko's groups showed that the E2s that add ubiquitin to cyclin in clam eggs are active throughout the cell cycle. That general finding was confirmed in frog eggs by work from Marc Kirschner's group, now at Harvard.

Earlier this year, Hershko's and Ruderman's groups provided evidence pointing to a novel E3 as possibly having the needed temporal specificity. Unlike other E3s, which seemed to be single proteins, this E3 appeared to be a large particle—possibly a complex of many proteins. In the February issue of *The Molecular Biology of the Cell*, the team reported that the new E3 becomes active for a short period in the middle of mitosis and is then rapidly inactivated.

With E3 emerging as a possible candidate for temporal control of the cell cycle, much attention is focusing on its makeup. Three of the proteins in the E3 complex were recently identified in a fortunate convergence of work in yeast, frogs, and mammals reported in the 21 April issue of *Cell*. Stefan Irniger of Nasmyth's group in Vienna supplied one piece of the puzzle by genetically identifying three yeast genes required for cyclin destruction. All three—known as *CDC16*, *CDC23*, and *CSE1*—were known to be necessary for cells to complete mitosis, but their specific functions remained a mystery.

Meanwhile, Stuart Tugendreich and John Lamb in Philip Hieter's lab at Johns Hopkins University were investigating the protein products of two of those genes, Cdc16 and Cdc23, as well as a third protein, Cdc27, to see whether they are involved in attaching the chromosomes to the mitotic spindle, a network of protein fibers that pulls the sister chromatids apart at anaphase. Lamb had found that the three proteins form a complex in yeast cells, and Tugendreich had used antibodies to the human forms of Cdc16 and Cdc27 to tag the complex's cellular location. "The antibodies localized to the mitotic spindle, which was very exciting," says Hieter. But rather than attaching the chromosomes to the spindle, the complex seemed to serve a regulatory role: When Tugendreich injected the antibodies into dividing cells to block the action of the complex, mitosis stalled before anaphase, at the same point where *cdc16*, *cdc23*, and *cdc27* mutants stall in yeast. That suggested the complex is involved in triggering anaphase.

How it might do that was unclear, but the clue soon came, Hieter says, when Nasmyth told his group about Irniger's unpublished finding that two of his cyclin-degradation mutations were in the *CDC16* and *CDC23* genes. "That linked the Cdc16/23/27 complex of proteins somehow to cyclin degradation," says Hieter, "and an important issue became how direct this link would turn out to be."

It turned out to be very direct. Last December, Tugendreich learned from Randall King and Jan-Michael Peters in Kirschner's group that they had isolated an E3 complex from frog eggs that was the same size as the Cdc16/23/27 complex from yeast and mammalian cells. Could the Cdc16/23/27 complex be E3? To ask that question, King and Peters checked to see whether Tugendreich's antibodies recognized the E3 complex. They did. That meant, says Kirschner, that Cdc16 and -27, and probably -23, "are directly involved as components of this E3 complex."

That finding connected the work of all three labs and verified the identity of three out of a total of eight proteins in the E3 ubiquitination complex that initiates the protein degradation steps needed for cells to complete mitosis. Even the Hieter team's results placing the complex on the mitotic spindle took on a new significance with the complex's identity as E3. Researchers have known that something in the cell checks to see if all the chromosomes are arranged on the spindle before giving the signal to start anaphase, and E3 is a good candidate for that job: Not only is it in the right place at the right time, but as it controls the protein degradation that triggers anaphase, it is well positioned to delay the step if the spindle isn't ready.

"The genetics and the biochemistry all came together and produced a story which neither of them could really have produced on their own," says Nasmyth. "This particle ... is a completely new player on the cell-cycle scene." Its potential role in destroying not only cyclins and CDK inhibitors, he says, but other cell-cycle regulatory proteins as well, suggests that "it plays as important a role in the cell cycle as the CDK itself."

So as researchers flesh out the role of protein destruction in regulating the cell cycle, ubiquitin prepares to take its place in the textbook chapters on the cell cycle, right beside cyclins and the kinases they regulate. And researchers studying the cell cycle can only wonder where the next surprise in their ever-astonishing field will come from.

–Marcia Barinaga