The Cell Cycle: Spinning Farther Afield

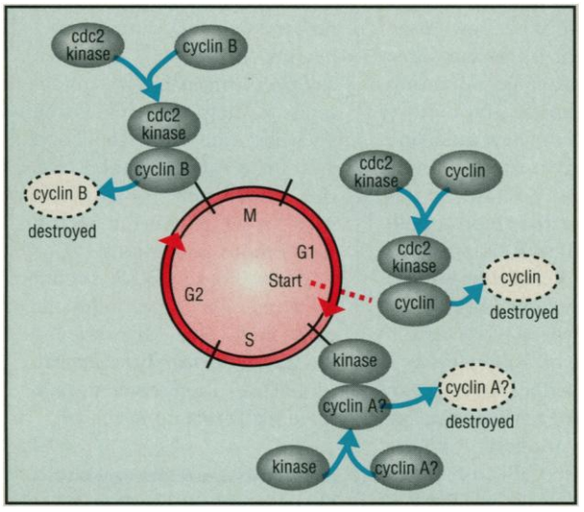
The remarkable expansion of cell cycle research in the past 2 years has caused it to intersect with research on oncogenes and tumor suppressor genes

ONCE UPON A TIME, SAY ABOUT 3 YEARS AGO, cell cycle research was a relatively narrow field. Though it focuses on a fundamental biological process—the exquisitely timed series of events that enables cells to divide—it was largely the province of biochemists and geneticists concerned with yeast cells and what cell cycle expert Joan Ruderman of Harvard Medical School once called "the little things that people laugh at": things like clam, sea urchin, and frog eggs. No longer. As a recent meeting at Cold Spring Harbor Laboratory* showed, cell cycle research is expanding in all directions.

It is beginning to connect with some of the hottest areas in cancer biology, including research on growth factors and the cancer-causing oncogenes, as well as on the tumor suppressor genes whose loss or inactivation is also thought to be important for cancer development. Indeed, one of the most exciting findings presented at Cold Spring Harbor had to do with the possibility that the protein encoded by one of the tumor suppressor genes—the retinoblastoma gene—is a link between the cell cycle machinery and gene expression (see box on page 1492).

These new insights into processes that underlie the uncontrolled growth of cancer cells reflect the great strides that researchers have made in figuring out how the cycle of normal cells is regulated. The research began to accelerate a couple of years ago, when several lines of evidence coalesced to give cell cycle researchers their first good look at the biochemical engine that drives cell division (*Science*, 21 July 1989, p. 252). Initially, that engine seemed relatively simple: a collaboration between two proteins. One, the working partner, was an enzyme, a kinase that regulates other proteins' behavior by attaching phosphate groups to them; the other was a protein called cyclin, which turns the kinase on at the right time to bring about the changes needed for cell division.

But at the Cold Spring Harbor meeting, it became clear that this simple model will have to be replaced by a much more complicated picture that involves many more kinases and many more cyclins. About a dozen cyclins and perhaps as many as ten different kinases have already been discovered—and this may be only the beginning. "The cyclins are like God," notes Cold Spring Harbor's Bruce Futcher. "Their ways are many and mysterious."



Tricycle. *Cyclin-activated kinases may be needed at three control points in the cell cycle.*

What do all those cyclins and kinases do? Well, for one thing, they regulate at least two, and possibly three, key points in the cell cycle, not just one as previously thought. That discovery is helping to resolve some puzzles that have been bedeviling cell cycle researchers since they first discovered the cyclins and their associated kinases. In almost all organisms, they found, cyclin activates its kinase partner—usually called the *cdc2* kinase after the mutation that led to its identification—at mitosis, the time when the nuclear membrane dissolves and the duplicated chromosomes are pulled apart into the daughter cells.

The budding yeast *Saccharomyces cerevisiae* appeared to be different, however. The first cyclins isolated from the organism did not closely resemble the mitotic cyclins of other species. Moreover, it seemed that the cyclin-activated kinase of budding yeast was working earlier in the cell cycle than the kinases of other organisms. The cell cycle has four phases: G1, in which the cells begin to grow; followed successively by S, the period when DNA synthesis occurs as the cell replicates its chromosomes; a second growth phase (G2); and finally mitosis (M). Genetic experiments suggested that the *cdc2* kinase of budding yeast functioned not at mitosis, but late in G1, at a point called "Start," defined as the time when cells become committed to divide. In practical terms, cells need the continued presence of growth stimulatory factors to grow before Start, but afterward will proceed to mitosis even without the factors.

These differences between the yeast cyclins and the mitotic cyclins of other organisms raised a big question: Might budding yeast be somehow different in the way it controls its cell cycle?

The answer appears to be no. Yes, yeast has a control point for a cyclin-activated kinase at Start, but it also has another one at mitosis, as other organisms do. In April two groups, one including Kim Nasmyth of the Research Institute of Molecular Pathology in Vienna, Futcher of Cold Spring Harbor, and their colleagues, and the other including Steven Reed and his collaborators at the Research Institute of Scripps Clinic in La Jolla, California, reported that they had isolated new cyclins from budding yeast—and these had all the earmarks of the classical mitotic cyclins of other organisms.

And in a reciprocal development, three independent groups, led by Andrew Arnold of Harvard's Massachusetts General Hospital, David Beach of Cold Spring Harbor Laboratory, and Charles Sherr of St. Jude Children's Research Hospital in Memphis, have identified what may be the mammalian equivalents of the yeast Start cyclins. "They're the first mammalian genes found to be active in G1," Sherr says. "Without them the cells don't progress to S." Nevertheless, he cautions that more work will be needed to prove that the genes encode true

*The meeting, the fifty-sixth annual Cold Spring Harbor Symposium on Quantitative Biology, ran from 29 May to 3 June.

mammalian Start cyclins. But if they do, it would mean that the cell cycle of mammalian cells, like that of the budding yeast, is under cyclin-*cdc2* kinase control at both Start and mitosis.

What's especially intriguing about the new mammalian cyclins, however, is the possibility that their abnormal activity may contribute to cancer development. That possibility was pointed up by the very way the new proteins were discovered. Although the Beach group found their cyclin while screening for human genes that could replace the activity of the Start genes in yeast, the other two groups made their discoveries unexpectedly—in the course of work on oncogenes and on growth factors.

Arnold and his colleagues had implicated a gene, located on band q13 of human chromosome 11, as the possible site of the defect causing the increased growth of a benign tumor of the parathyroid gland. When they cloned and sequenced the gene, they found that the protein it encodes bears a family resemblance to the cyclins. Subsequent work in collaboration with Harvard's Ruderman showed that the protein indeed has cyclin activity. In fact, it turned out to be the same cyclin picked up by Beach.

Meanwhile, Sherr and his colleagues were looking for the cellular genes that mediate the stimulatory effects of colony-stimulating factor 1 on the growth of mouse macrophages. And, like Arnold, Sherr's group suddenly found themselves studying cyclins. They came up with three genes encoding cyclin-like proteins, one of which is the mouse equivalent of the human cyclin gene identified by the Arnold and Beach groups.

There is reason to believe that this gene may be involved in more than just benign parathyroid tumors. Abnormalities in the region it maps to on chromosome 11q13 have been linked to breast cancer as well as a human leukemia. Two oncogenes have already been found in the region, and another one, the *bcl-1* oncogene, which is thought to contribute to the development of a B cell leukemia, has been mapped there, but has not yet been cloned. Could the new cyclin gene be *bcl-1* or another oncogene? No one knows yet, but as Sherr points out, a gene that acts at Start, when cells become committed to mitosis, independent of growth factors, would be a logical site for cancer-causing mutations.

Also of note, says Bruce Stillman of Cold Spring Harbor, who organized the meeting with Beach and lab head James Watson, was Sherr's finding that his new cyclins may be expressed in a cell-specific manner, one in macrophages, for example, and another in T cells. "This raises the possibility for many more cyclins in the future," Stillman says.

The second puzzle addressed at the meeting concerns the mysterious cyclin A. The first cyclins identified fell into two structural classes, designated simply A and B. The mitotic cyclins proved to be of the B type. But no one could find a function for cyclin A. It did not work at mitosis, nor was it one of the Start cyclins. Naturally, cell cycle researchers want to know what it does.

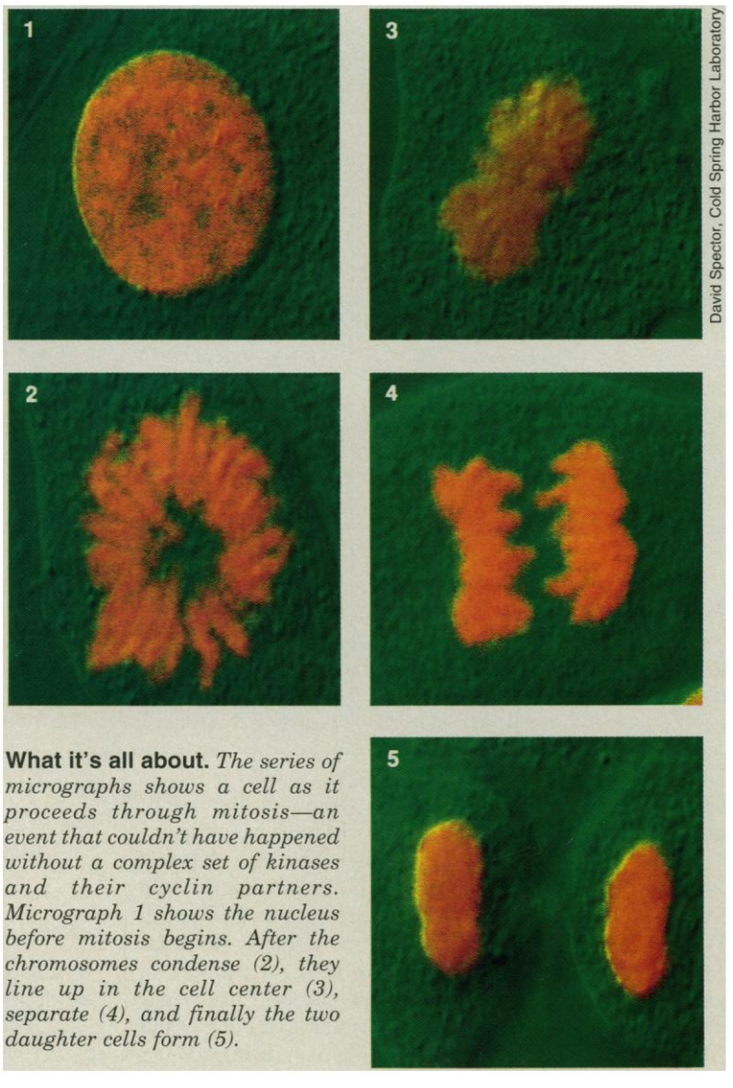
The answer, according to data presented at the Cold Spring Harbor meeting by Giulio Draetta of the European Molecular Biology Laboratory in Heidelberg, Germany, and James Roberts of the Fred Hutchinson Cancer Center in Seattle, is that it may act at still another control point in the cell cycle, namely at the beginning of S phase when DNA synthesis is initiated. It appears to be working there by stimulating a kinase that tacks phosphate groups onto one of the proteins that helps get DNA replication under way. Stillman and his colleagues have shown the protein is phosphorylated in the cell at the beginning of S phase, and that one of the enzymes that does this is a *cdc2* kinase.

But this kinase may not be cyclin A's only partner under physiological conditions. Cells contain more than one kinase capable of being activated by cyclins, and two researchers, John Newport of the University of California, San Diego, and Michel Philippe of the Centre National de la Recherche Scientifique Unité Associée 256 at the University of Rennes, France, described new results suggesting that one of the others, which was identified by Philippe, participates in the initiation of DNA synthesis.

Cyclin A is not needed to initiate DNA synthesis in all cells, however. Christian Lehner of the Max Planck Institute in Tübingen, Germany, found that knocking out the cyclin A gene does not affect the entry of the cells of early fruit fly embryos into S phase.

As complex as the cell cycle picture now appears, it's a sure bet that it will become even more complicated in the near future. "The last 6 months have been filled with adding lots of new cyclins to the literature," says Ed Harlow, who recently moved from Cold Spring Harbor to the Massachusetts General Hospital Cancer Center in Charlestown. "And now we're in the process of adding new *cdc2*-related kinases." Harlow's own group has cloned genes for eight new kinases. And Tony Hunter of the Salk Institute in La Jolla said that he and his Salk colleague Jonathan Pines have cloned five. Other participants in the Cold Spring Harbor meeting mentioned new kinases as well.

Most of the new enzymes haven't been characterized yet, and some of the isolates picked up by the different researchers are likely to be the



David Spector, Cold Spring Harbor Laboratory

What it's all about. *The series of micrographs shows a cell as it proceeds through mitosis—an event that couldn't have happened without a complex set of kinases and their cyclin partners. Micrograph 1 shows the nucleus before mitosis begins. After the chromosomes condense (2), they line up in the cell center (3), separate (4), and finally the two daughter cells form (5).*

How the Retinoblastoma Gene May Inhibit Cell Growth

The retinoblastoma gene is one of the most venerable of the tumor suppressor genes: It was cloned back in 1987. But, to their frustration, cancer researchers have found that having the gene in hand hasn't helped them figure out how it exerts its inhibitory effects on cell growth. And that's been a major disappointment. Loss or inactivation of the gene has been implicated in common malignancies, including breast cancer and lung cancer, as well as in the rare eye cancer that gave the gene its name. The hope was that understanding what the gene does might help design therapies for suppressing cancer growth—but those hopes have been thwarted by the difficulty in working out the gene's mechanism of action.

Now, data from several groups presented at Cold Spring Harbor suggest that researchers may at last be getting a handle on retinoblastoma (Rb) gene function. The new results show that the protein encoded by the gene is connected on the one hand to the machinery of the cell cycle—and on the other to the apparatus that regulates gene activity. The discovery of this interconnection suggests that when the cell cycle machinery is activated, it transmits a signal to the Rb protein, in turn bringing about the altered pattern of gene expression needed to start mitosis.

There had been hints all along that the Rb protein might be linked to the cell cycle. The number of phosphate groups attached to the protein in cells varies depending on what stage of the cell cycle the cells are in. In resting cells and in G1, when cells begin to grow, Rb lacks phosphate groups. But Rb is phosphorylated later in the cycle, about the time cells begin replicating their DNA to get ready for mitosis and during mitosis itself. This difference has considerable functional significance: The unphosphorylated form has the growth suppressive activity.

One thing that makes those observations interesting is the recent discovery that specific kinases—enzymes that add phosphate groups to proteins—are critical components of the engine that drives cells to mitosis (also see p. 1490). Might one or more of the cell cycle kinases be adding the phosphates to the Rb protein, thereby releasing the brakes that Rb puts on cell growth?

The new findings presented at Cold Spring Harbor suggest the answer may be yes, although they don't yet add up to a watertight case. Researchers, including Wen-Hwa Lee of the University of California, San Diego, Ed Harlow of Massachusetts General Hospital Cancer Center in Charlestown, and Tony Hunter and Jonathan Pines of the Salk Institute in La Jolla, California, have shown that the Rb protein can bind to components of the cell cycle machinery. Moreover, cell cycle kinases, usually referred to as *cdc2* kinases, can phosphorylate the protein. "That suggests that the *cdc2* kinase is throwing a master switch," says Harlow.

While those findings give some clues to how the action of the Rb protein is controlled, they don't shed any light on how it inhibits cell growth before being shut off by phosphorylation. But several other groups have shown that the Rb protein also binds to proteins known to regulate gene transcription into messenger RNA, the first step in gene expression. "People have presumed that Rb would act as a negative regulator of one or more transcription factors," says Hunter, "and there are beginning to be glimmerings of something like that happening."

At the meeting, Joseph Nevins of Duke University Medical School and David Livingston of the Dana-Farber Cancer Institute in Boston reported that Rb binds to a transcription factor known as E2F (they also report their results in the 14 June *Cell*), and Rene Bernards of the Massachusetts General Cancer Center described his group's findings showing that the Rb protein also binds to the protein product of the *myc* oncogene, which is part of a protein complex that regulates gene transcription. In addition, Lasantha Bandara and Nicholas La Thangue, of the MRC National Institute for Medical Research in Mill Hill, England, report in the 6 June issue of *Nature* that the Rb protein binds a transcription factor they called DRTF1, although it's likely the same as E2F.

Particularly intriguing, according to many meeting participants, was the Nevins group's finding that E2F binds only to unphosphorylated Rb protein, the form that predominates during G1 and is therefore believed to possess growth suppressive activity. That helps tie the protein's interactions with transcription factors into cell cycle control. "It's one of those times when everything falls down and simplifies," says Harlow. "It makes great sense."

The idea is that binding of the Rb protein to transcription factors such as E2F inactivates them. But when Rb is phosphorylated by the cell cycle kinases, it releases the transcription factors, allowing them to activate the gene transcription necessary for progression through the cell cycle. So if the Rb protein is absent because the Rb gene is deleted or defective, the transcription factors might be constantly turned on, leading to the uncontrolled cell growth of cancer.

Nevins' results provide support for that idea as well. Cancer researchers learned a few years ago that the oncogenic proteins of certain animal tumor viruses work by binding to the Rb protein and preventing it from exerting its growth suppressive action. The E1A protein of adenovirus is one of these oncogenic proteins, and the Duke researchers have found that EIA, by complexing the Rb protein, takes it away from E2F, thereby allowing the transcription factor to work again. Bandara and La Thangue report similar findings in their *Nature* paper.

So all this is consistent with the mechanism of action proposed for Rb. Everyone cautioned, however, that the new findings are just the beginning and a great deal more research will be required to establish that Rb works as postulated. ■ **J.M.**

same enzymes. Still, it will take some doing to sort out which kinases interact with which cyclins and when.

Then there's a surprising observation reported by Beach and also by Steve Osmani of Baylor College of Medicine in Houston that *cdc2* kinase activation, while necessary for mitosis, is not sufficient as previously thought. Researchers want to know what's happening there, as well as what regulates the synthesis and activities of the various cyclins and kinases and the identity of the kinase targets.

This year's meeting filled the 360-seat Grace Auditorium to overflowing. If Cold Spring Harbor wants to hold another one in a year or two, and the number of cyclins keeps growing along with the number of cell cycle researchers, the organizers may have to consider moving it to a Long Island neighbor—Shea Stadium. ■ JEAN MARX

ADDITIONAL READINGS:

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