Cell Cycle Research: Down to the Nitty Gritty

Having adopted a "universal model" for the cell cycle, researchers are turning an eagle eye on the molecular details

Vienna—ONE OF THE MOST EXCITING AND fastest moving areas of cell biology in the past few years has been the study of how the cell cycle—the exquisitely timed sequence that enables cells to divide and return to the resting state—is controlled. Converging lines of evidence from many labs have led to a remarkable advance: adoption of a "universal model" specifying that cell division follows the same biochemical pathway in all eukaryotic organisms, from yeast to human beings.

Adoption of a universal model hasn't resolved all the questions relating to cell division. At a recent conference in Vienna, attended by more than 500 scientists, it was clear that work in the field is shifting from grand unifying paradigms to molecular details.* Many of those details center on an enzyme called cdc2 kinase that acts as a kind of master control unit, orchestrating the action of many other enzymes when cells divide. Researchers would like to know what role the kinase plays in the start of DNA replication and how the enzyme itself is controlled. And, although cdc2 kinase seemed to get the lion's share of the attention at the Vienna meeting, supporting players in the cellular drama also got their due.

Cdc2 kinase is one element of a protein complex called "maturation promoting factor," or MPF; the other subunit is cyclin, a protein found in multiple forms that is probably needed for activation of the kinase. The identification of cdc2 kinase was a watershed in understanding the cell cycle, since this enzyme is thought to play a role in most of the major events of the cycle, including condensation of chromosomes, reorganization of the cell's structure, and the breakdown of the membranes surrounding the nucleus.

Recent work presented at the Vienna meeting reinforces the omnipresence of cdc2 in the cycle. It has been established for some time that the enzyme is an initiator of the M phase of the cycle: the segregation of chromosomes into daughter cells during mitosis. But the evidence was not as clear that cdc2 also controls the onset of the earlier S phase, in which the DNA is replicated. In one of the most interesting of the Vienna presentations, Paul Nurse of the Imperial Cancer Research Fund's Cell Cycle Group at Oxford University presented data suggesting that the kinase does indeed trigger the S phase as well. "What we have are two major control points," Nurse said, "at S phase and at M phase. And both involve the cdc2 protein kinase."

Nurse reported that his team has isolated mutant strains of the yeast *Schizosaccharomyces pombe*, altered at the cdc2 gene, which undergo the S phase but fail to go on to divide. In fact, these organisms suffer from a malady that one of Vienna's most famous former residents, Sigmund Freud, might have diagnosed as "repetition compulsion": Under appropriate conditions, they halt the cycle in the middle—and undergo a second S phase.

From this evidence and other data Nurse postulates that cdc2 might be in two forms, an M form and an S form: "The transition through S phase converts the S form into the M form, and the transition through M phase converts it back. Mutations that allow you to go into S phase without going through mitosis short-circuit this dependency between the two processes."

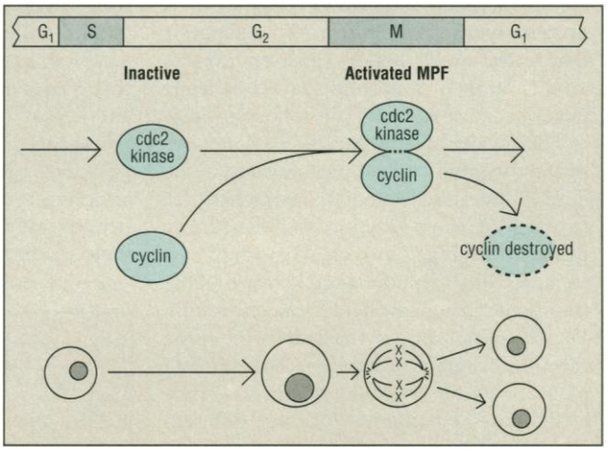
Indeed, Nurse thinks that the enzyme's flip-flop between its two forms is what drives the cell cycle, allowing cdc2 to serve as a universal regulator of cell division in eukaryotic cells. The conversion from one form to another, he suggests, could be triggered by post-translational modifications in the proteins, or association with other proteins, possibly including cyclins.

Nurse has plenty of company in trying to figure out the molecular switches that turn cdc2 kinase on and off at the appropriate time in the cell cycle. Several labs in the United States and Europe are turning up evidence that at least one important aspect of the control of cdc2's activity is the addition and removal of phosphate groups from various amino acids in the enzyme's protein chain. For example, Erich Nigg of the Institute for Experimental Cancer Research in Epalinges, Switzerland, reported recent work with cdc2 kinase from chickens that enabled his team to correlate phosphorylation (addition of phosphate groups) at different sites on the enzyme with the corresponding cell cycle stages. Nigg and his colleagues cloned mutant and wild type versions of the chicken cdc2 gene and incorporated them into the genome of HeLa cells, a human cell culture line, by way of transfection with bacterial plasmid DNA. They found that phosphorylation occurs at four sites, including one (on the amino acid tyrosine at position 15 in the chain) that had already been identified as a key site for activation of the kinase at the beginning of mitosis.

In an intriguing finding that provides a link to Nurse's work, Nigg's group discovered that the pattern of phosphorylation is different during the S and M phases. For example, both tyrosine (at position 15) and threonine (at position 14) are abruptly dephosphorylated at the beginning of mitosis. In contrast, as the S phase progresses, phosphorylation of a serine at position 277 decreases markedly.

"The timing of these phosphorylations is very appealing," Nigg said. "If cdc2 kinase is the workhorse that pulls the cell cycle along, it is clear that knowing exactly where phosphates are added to or taken from particular sites is essential to understanding how the enzyme is regulated."

Nigg's results also bear on the question of what molecules cdc2 kinase acts on when the enzyme is turned on. He presented evidence that cdc2 kinase can phosphorylate a class of proteins called lamins, which are major constituents of the lamina, a structure underlying the inner of the two membranes that surround the cell nucleus. When the lamin molecules are dephosphorylated,



Wheels within wheels. *Both the S phase of the cell cycle (in which the DNA is replicated) and the M phase (in which the daughter cells divide) seem to be triggered by the action of an enzyme called cdc2 kinase.*

*"The Control of Proliferation in Normal and Malignant Cells," hosted by the Research Institute of Molecular Pathology, held 3–5 May at the Austria Center.

they break up into their subunits, which allows the chromosomes to detach themselves from the nuclear membrane and line up on the mitotic spindle. The recent results make it seem likely that along with its other roles, cdc2 kinase is an essential element in this step, too.

Although cdc2 kinase and cyclin seem to play the central role in cell cycle regulation, growing evidence points to supporting roles for other players. Some of them don't send the cycle moving forward—they act as checkpoints to interrupt it if it has gone off the tracks. Ted Weinert, from the University of Arizona in Tucson, presented work with genes in the yeast *Saccharomyces cerevisiae* that appear to control one key "checkpoint" of the mitotic cycle.

In eukaryotic cells in which the DNA is damaged or incompletely replicated, the cycle comes to a halt and the cells do not proceed to mitosis. This checkpoint ensures the viability of the daughter cells, as well as the fidelity of the genome passed along to them. In earlier work, Weinert and his colleagues had found that in *S. cerevisiae* a gene called RAD9 is essential for this failsafe mechanism.

Weinert has now identified five additional genes that are involved in this damage-induced halt. He and his group are investigating how these genes act to stop the cell cycle in its tracks. He postulates that they are activated by a signal—as yet unidentified—sent out by the damaged DNA. At least in the case of the RAD9 gene, the action of the inhibitor does not depend on production of new protein—since the cell cycle is arrested even in the presence of a protein synthesis inhibitor. This suggests that activation of RAD9's function involves an alteration of a protein that has already been made, possibly by phosphorylation.

The details of how specific enzymes are phosphorylated or dephosphorylated is the kind of question cell cycle researchers are turning to now—having established the grand unifying "universal model." In Vienna, Nurse found a graphic way of illustrating the change the field is going through. During his talk, he had the front cover of the meeting program, featuring a line drawing of Vienna's St. Stephen's Cathedral, projected onto the screen. "All these different strands are coming together to a point, which is the universal model," he said. "And sitting at the top of the tower are cdc2 and cyclin." Then he flipped the image upside down, so the tower was at the bottom. "And this is a symbol of where the field is going. From the unified model into all the complexities." ■ MICHAEL BALTER

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The Stately Cycles of Ancient Climate

Drill holes sunk in New Jersey reveal a 200-million-year-old record of climate cycles with periods of up to 5 million years

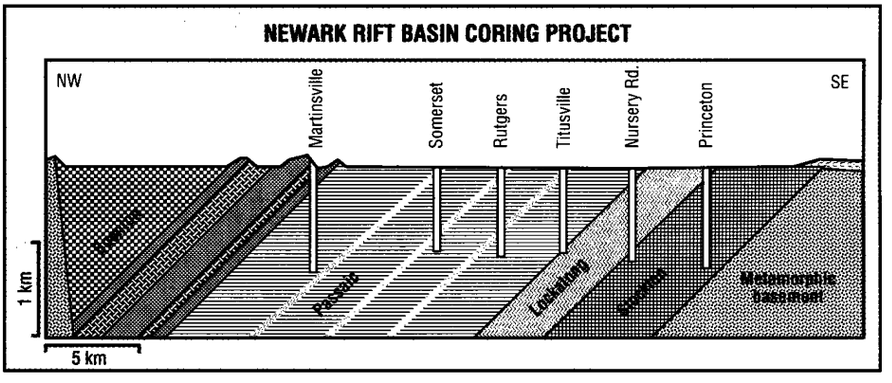
THE EARTH'S CLIMATE VARIES YEAR BY YEAR, millennium by millennium, eon by eon. Ice sheets grow and retreat, deserts bloom, and grasslands turn to dust. Some of these shifts are steady and irreversible, resulting from the uplift of mountains or the drifting of continents. But other climate changes have been periodic, varying in cycles of up to 100,000 years. Now, researchers examining core samples of rock drilled from such unlikely New Jersey sites as the campus of Rutgers University and the outskirts of Princeton have found that 200 million years ago the earth was in the grip of even longer climatic cycles, lasting as long as several million years.

The discovery underscores the importance of a fundamental pacemaker of climate change. Over the past 15 years, paleoclimatologists have recognized that many of the recent oscillations of climate have an astronomical source. They are driven by cyclic variations in the tilt and orientation of the earth's spin axis and in the shape of its orbit around the sun. These astronomical cycles, which take place on many different time scales, affect the distribution of sunlight around the globe and how it changes from season to season. The end result is a climate that varies in cycles of 20,000, 40,000, and—most dramatically of late—100,000 years, that last cycle having paced the repeated ice ages of the past million years. But although the astronomical cycles also have periods even longer than 100,000 years, there has been little evidence of a matching signal in recent climate history.

A 30-million-year climate record, preserved in sedimentary rock that accumulated in New Jersey 200 million years ago and more, has now confirmed that orbital variations can set up strong climate oscillations with a period of 400,000 years. The record also hints at even longer climate cycles, one lasting 2 million years and another 4 to 6 million years. These ancient climate cycles may serve as a point of comparison for researchers trying to understand the climate of the past few million years, when shorter oscillations have stood out.

Paul Olsen and Dennis Kent, a paleontologist and a paleomagnetician at Lamont-Doherty Geological Observatory, took advantage of some favorable geology to extract their climate record. The Newark Basin, where the researchers did their drilling under the auspices of the revived Continental Scientific Drilling Program, is a paleoclimatologist's dream. The basin formed more than 200 million years ago as the crust of the supercontinent Pangaea was slowly pulled apart in the early stages of the formation of the Atlantic Ocean. The resulting rift valley, like the Great Rift Valley in Africa today, trapped a series of lakes, including one that was the size of Lake Tanganyika under present-day New Jersey.

The site then lay near the equator, where monsoon rains draining into the basin turned the lake into a huge rain gauge. When the rains were heavy, the lake brimmed. But as Earth's climate varied under the influence of orbital cycles, the monsoon periodically faltered and lake levels fell.



An economical approach. *Tilted strata made it possible to retrieve a complete climate record from these ancient lake beds by drilling six shallow holes instead of one deep one.*