Research News

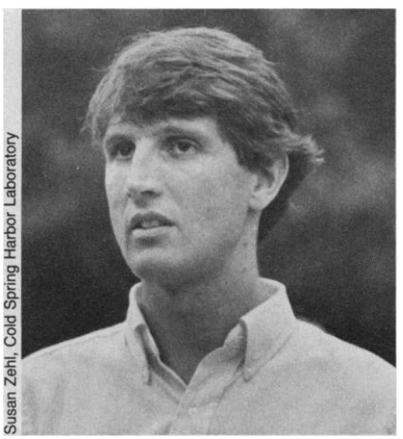
The Cell Cycle Coming Under Control

Two disparate lines of research merge, revealing that the biochemical machinery for controlling cell division is the same in species ranging from yeast to man

WITHIN THE PAST YEAR, researchers have made rapid strides toward understanding one of the critical events in the life cycle of the cell—namely, cell division. And as they have identified the biochemical machinery that controls this event, they have made a welcome discovery: the basic mechanisms are apparently the same in species ranging from yeast to man. "That's the wonderful thing," says David Beach of Cold Spring Harbor Laboratory. "Cell cycle control is now much simpler than it was 2 years ago. It's the grand unification theory of the moment."

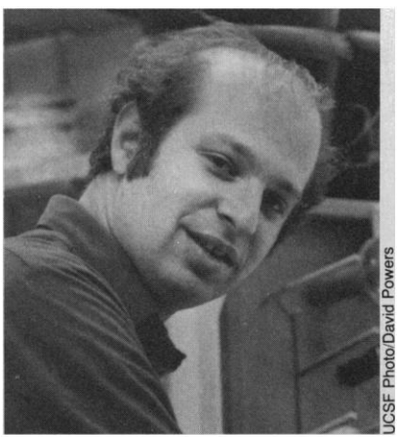
This increasingly detailed picture of cell cycle control has emerged from the confluence of two previously disparate lines of research. In one set of laboratories around the world, geneticists used the classical methods of their discipline to identify the genes controlling cell division in the simple yeasts. Meanwhile, the cell biologists and biochemists were applying their traditional methods to isolate the proteins regulating cell division in clam, sea urchin, and frog eggs. In the end, everyone found that they were studying the same proteins.

As Joan Ruderman of Duke University puts it, sea urchins and clams "are little things that people laugh at. But the pathway that we have got onto here seems to be universal."



Susan Zehl, Cold Spring Harbor Laboratory

David Beach. *One of the yeast geneticists whose work helped to uncover the molecular machinery that tells cells that it is time to divide.*



UCSF Photo/David Powers

Marc Kirschner. *A cell biologist whose studies of maturation promoting factor in* Xenopus *eggs recently intersected with the yeast results.*

As biological mechanisms go, the pathway discovered by the yeast geneticists and cell biologists is relatively simple, involving just two major proteins. One of these is a particular type of enzyme, a kinase which adds phosphate groups to other proteins. Such phosphate additions and removals are commonly used by cells to modify protein activities. Although researchers do not fully understand how the kinase they have identified works, they do know that its activation is the signal that sets in motion the cellular changes necessary for division.

And this is where cyclin, the other major protein of the cell cycle, comes in. Cyclin concentrations fluctuate, going up just before cells divide and turning on the kinase, although the cyclin may do this indirectly. The cyclin concentrations then drop precipitously and the kinase activity also subsides, allowing the cells to complete the division cycle. Resynthesis of cyclin can then trigger another round of division.

Although simple, this picture was nearly 20 years in the making. The going was slow for much of this time. But then suddenly in the past year or two, the rate of advance surged—at least partly because of the readiness of the various groups to communicate and collaborate with one another. "We're a pretty friendly lot," comments Tim Hunt of the University of Cambridge, England. "It's competitive, but on the whole information has been flowing freely."

The first glimmerings of the cell cycle story emerged back in 1971 when Yoshio Masui and Clement Markert of Yale University and, independently, L. D. Smith and Richard Ecker of Argonne National Laboratory discovered that immature eggs from the frog (*Xenopus laevis*) produce a factor that causes them to undergo meiosis and mature, preparing them for fertilization. This factor is known as "maturation promoting factor" or MPF.

Meiosis is a special type of cell division, occurring only in the germ cells that give rise to eggs and sperm. But researchers soon found that the maturation factor could also induce mitosis—the division of ordinary somatic cells.

Not only that, key experiments done in the early 1980s by John Gerhart and Michael Wu of the University of California, Berkeley, and Marc Kirschner of the University of California, San Francisco, showed that MPF activity in frog eggs fluctuates during the cell cycle; it rises as cells enter meiosis or mitosis and then drops sharply after they divide. Activation of the factor appeared to serve as sort of an internal clock that tells cells when to divide. "All the aspects of the cell cycle one is familiar with can be caused by the addition and withdrawal of MPF," Kirschner says.

Correction

An article in our 30 June issue ("'Dangerous' liaisons in cell biology," p. 1539), which discussed the commentary in the 2 June issue of the journal *Cell* by Max L. Birnstiel and Meinrad Busslinger regarding the transgenic mice experiments of Corrado Spadafora and coworkers, stated that Vienna's Institute of Molecular Pathology (IMP), for which Birnstiel and Busslinger work, was seeking patents on extensions of the Spadafora experiments. On further investigation, we have learned that this is not correct. At present no applications have been applied for or granted to IMP or its parent companies Genentech and Boehringer Ingelheim. We wish to correct the record, apologize to Dr. Birnstiel and Dr. Busslinger for this misstatement, and alert our readers to their letter, which is printed in our Letters section on page 243.

Despite the intriguing results obtained on MPF during these years, researchers were frustrated by their inability to purify the regulatory substance and determine its structure. They knew that it was a protein but had little other information about its biochemical nature. That would not come until much later.

Meanwhile, in the early 1980s, Hunt was embarking on a series of experiments that was to provide a major piece of the puzzle. His initial plan was to study the control of protein synthesis in sea urchin eggs.

While the Cambridge researcher was at the Marine Biology Laboratory in Woods Hole, Massachussetts, he met Eric Rosenthal, then a graduate student in Ruderman's laboratory, who told him about experiments in which that group had identified three new proteins that appear in clam eggs shortly after they are fertilized.

Hunt and Rosenthal decided to try similar experiments with sea urchin eggs but were not able to detect the synthesis of any new proteins when they fertilized the egg with sperm. The development of sea urchin eggs can be triggered artificially, as well as by sperm, however, and when the researchers did this they spotted a new protein that was first made within 10 minutes of fertilization. This protein came and went with each egg cell division.

As luck would have it, the very next evening, Hunt ran into Gerhart at a wine-and-cheese party, and Gerhart told him about the MPF experiments. Hunt realized that the sea urchin protein behaved just like the maturation factor. "And then the penny began to drop," the Cambridge researcher says. "The obvious hypothesis was that this protein in sea urchins that came and went with the cell cycle controlled the cell cycle." Hunt and his colleagues consequently named the protein "cyclin." The clam egg proteins also proved to be cyclins.

A few years later, Katherine Swenson of the Ruderman group showed that a messenger RNA that directs the synthesis of a clam cyclin triggers meiosis when injected into *Xenopus* oocytes. This suggested that the protein might have the regulatory role proposed for it, but at this point the relation of cyclin to MPF was still unclear. Did cyclin activate MPF? Or might it be MPF?

While all this work was going on, the yeast geneticists were doing their part, identifying a series of mutations that interrupt the cell cycle at various points in their chosen organisms. One of these proved particularly interesting. This was the *cdc2* mutation (where *cdc* stands for cell division cycle) identified in the fission yeast (*Schizosaccharomyces pombe*) by Paul Nurse and his colleagues at the Imperial Cancer Research Fund's Cell Cycle Control Laboratory in Oxford, England. (Fission yeast is so called because the cells multiply by dividing in half, not by budding the way the better known yeast *Saccharomyces cerevisiae* does.)

The gene affected by the *cdc2* mutation turned out to encode a kinase, and many of the cell's important regulatory proteins are kinases. The activity of the *cdc2* kinase, which is also known as the p34 kinase because it has a molecular weight of 34,000, is needed specifically to send fission yeast cells into mitosis.

Work by Beach and Nurse, among others, established that comparable kinases are widely distributed in nature. The *cdc2* gene is equivalent to the previously discovered *CDC28* gene of budding yeast. And sea

The Oncogene Connection

So far none of the cancer-causing oncogenes have been found among the genes that control cell division. But recent discoveries indicate that the proteins encoded by these two groups of genes may be able to talk to one another and influence each other's activities. If so, the research may produce an improved view of oncogene action.

"Nobody really knows when oncogenes act in the cell cycle," says James Maller of the University of Colorado School of Medicine in Denver, "but it may be that they prevent cells from returning to normal after they divide. Many of the structural properties of [cancerous] transformed cells resemble those of mitotic cells."

The activation of a protein known as maturation promoting factor (MPF) is the immediate trigger for cell division in higher organisms. Researchers have recently identified a kinase, an enzyme that adds phosphate groups to proteins, as one component of the maturation factor (also see story on p. 252).

Now, Maller's group, in collaboration with that of David Shalloway of Pennsylvania State University in University Park, has found that the MPF kinase phosphorylates the protein product of the *src* "proto-oncogene." (Proto-oncogenes regulate normal cell growth, but can, if they malfunction, make cells cancerous.)

Harold Varmus, J. Michael Bishop, and their colleagues at the University of California, San Francisco, have made a similar observation about the *src* protein. "The big issue here," Varmus says, "is whether or not this phosphorylation is important. At this point the issue is pretty much open."

Earlier work by Shalloway and his colleagues suggests, however, that the phosphorylation may help to bring about the characteristic cell changes of mitosis. They found that certain specific amino acid residues in the *src* protein briefly acquire phosphate groups at mitosis—just when that kinase is active. Moreover, the current work shows that the MPF kinase phosphorylates those same amino acids.

Meanwhile, David Beach and his colleagues at Cold Spring Harbor Laboratory have evidence indicating that the *src* protein, itself a kinase, phosphorylates the MPF kinase protein. Neither the Maller nor Varmus group has been able to confirm this with the native protein, however, and the issue remains to be resolved. If the *src* protein does phosphorylate the MPF kinase in living cells, it could mean that the two enzymes engage in mutual communication and control during mitosis.

And just as important, it would mean that a natural target for the *src* enzyme has finally been identified. The oncogene was discovered more than 10 years ago, and researchers have been trying unsuccessfully ever since to find out what its targets are.

The *src* oncogene is not the only one that may interconnect with the MPF kinase. George Vande Woude of Bionetics Research Inc. in Frederick, Maryland, and his colleagues have evidence suggesting that the protein encoded by the *mos* proto-oncogene is a normal activator of maturation promoting factor during the meiotic divisions of frog and mouse eggs. Vande Woude does not yet know whether it has a similar role in the mitotic divisions of ordinary cells, but he points out, "the evidence is that what happens in meiosis and mitosis is the same."

Also unclear is the relation between the *mos* product and the second component of maturation promoting factor, the protein cyclin, which may also participate in the factor activation. The *mos* protein may act through cyclin, but that remains to be seen.

In any event, researchers now have evidence indicating that the normal products of at least two oncogenes interact with the machinery that immediately controls cell division. Possibly then the cancerous changes that the genes produce in cells when they malfunction may be the result of the cells inappropriately expressing mitotic features when they should be resting. ■ **J.L.M.**

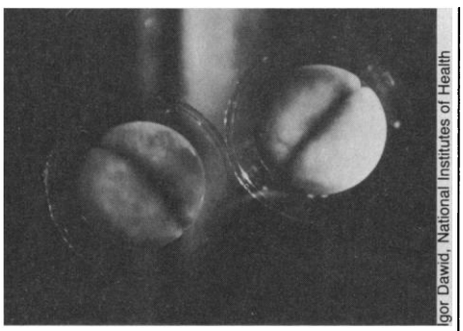
urchins, clams, *Xenopus*, and even human cells have their own *cdc2* genes. The human p34 kinase is 63% identical to the yeast enzyme, Nurse says, which is a very high degree of structural conservation for species so widely separated in evolution.

Moreover, the yeast and human kinases are functionally equivalent. "It's absolutely astonishing," Nurse remarks. "Over 1000 million years these proteins are absolutely exchangeable. You can study the control proteins in yeast and they have relevance for human beings."

During this time, the yeast geneticists and cell biologists were aware of each others' work. They all knew that the behavior of the p34 kinase suggested that it might in fact be the still elusive MPF. "We talked about it and speculated about it," Kirschner says, "but there was no direct evidence then." That did not materialize until 1988, which proved to be a watershed year for cell cycle research. Developments began to come thick and fast as the disparate lines of research began to merge.

One of the critical developments occurred when Fred Lohka, Marianne Hayes, and James Maller of the University of Colorado Medical School in Denver finally isolated maturation promoting factor. "We've purified a lot of things," Maller says, "and that one was the most difficult we ever did. People thought we were crazy to try because so many others had failed." The key element in the eventual success, Maller explains, was a new method developed by Lohka for assaying MPF activity in a cell-free system that made it much easier for the investigators to follow their progress.

The Denver workers found that the maturation factor contains two proteins, with molecular weights of 34,000—certainly auspicious in view of the yeast p34 kinase results—and 45,000. Then the Maller group, in collaboration with Nurse and his colleagues, showed that an antibody to the yeast kinase also recognizes the 34-kilodalton MPF component, thereby providing direct evidence that the two proteins are identical. Beach, with William Dunphy and John Newport of the University of California, San Diego, using a totally different approach, came to the same conclusion.



Xenopus eggs. *Providing clues to cell division.*

By the end of 1988 then, the p34 kinase was shown to be one component of maturation promoting factor. But what was the other component? And where does cyclin fit in?

Evidence from Hunt, Maller, and their colleagues now indicates that cyclin is the second MPF component. Cyclin is also consistently found complexed with the *cdc2* kinase, Beach says.

And it is certain that cyclin is somehow necessary for activation of the kinase. Most recently, for example, Andrew Murray of the University of California, San Francisco, and Kirschner showed that cyclin synthesis is sufficient by itself to activate maturation promoting factor and send frog eggs into mitosis. Conversely, cyclin destruction is required to shut off MPF and allow the completion of cell division. If the degradation is blocked, frog egg cells get stuck in mid-mitosis, Kirschner and Murray find.

So the simple picture that comes out of all this shows that cyclin is made constantly in the cell and when its concentration builds up to sufficient levels, the p34 kinase of MPF becomes active and cell division begins. At this point, the cyclin is abruptly degraded, mitosis comes to completion, and the whole cycle starts all over again.

Of course, nothing in cell biology is likely to be that simple, and the cell cycle story is no exception. The results described so far were obtained with yeasts and rapidly dividing, newly fertilized eggs.

But cell cycle control may be somewhat different in older embryos, even though cyclin and the MPF kinase are still key players. "Now what you have to digest is that there is not one way, but two ways, to control the cell cycle," says Patrick O'Farrell of the University of California, San Francisco.

Bruce Edgar of O'Farrell's group has shown that *string*, a gene needed for normal fruit-fly development, is the equivalent of the *cdc25* gene, another gene that researchers have identified as participating in cell cycle control in fission yeast.

Early embryonic development is controlled by proteins made under the direction of maternal messenger RNAs, which are stored in the egg. Later on, these messengers are destroyed and the embryo's own genes kick on and take over. The *string* gene is one of the first of the fruit-fly embryonic genes to become active.

When this happens, O'Farrell says, the timing of mitosis is no longer controlled by cyclin accumulation but is instead regulated by synthesis of the *string* gene product. Cyclin is still required for cell division but is no longer rate-limiting.

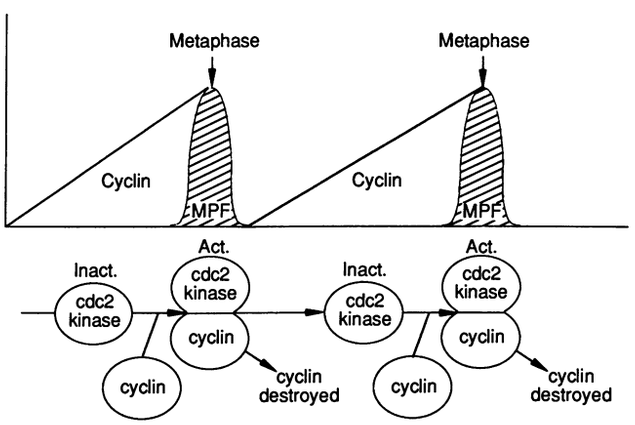
The switch may reflect the changing circumstances of embryonic cells during development. "The egg is gigantic—about 1 million times bigger than a normal cell. You have this enormous cytoplasm and this little impotent nucleus trying to take care of it," O'Farrell points out.

The newly fertilized egg may then rely on its stored maternal RNA to produce the relatively simple cell cycle control pattern in which fluctuations in cyclin content determine when cells divide. Once the nuclei of the embryonic cells can take control, however, the timing of mitosis often varies from cell to cell and it is at this point that an individual cell's *string* gene product becomes the dominant force.

The switch in mechanisms for timing mitosis may not be unique to the fruit fly. "If the [*string*] gene is conserved from yeast to flies, it's presumably for a purpose—and I think it will perform a similar role in other organisms," O'Farrell says.

Although researchers have identified the central players in cell cycle control, a great many questions remain to be answered. There is as yet very little information about the protein targets of the MPF kinase, although some interesting possibilities have recently been uncovered.

Beach and his colleagues identified the p34 kinase as a component of the enzyme designated as the "M phase–specific" or



Controlling the cell cycle. *Cyclin concentrations increase until the* cdc2 *kinase component of maturation promoting factor (MPF) is turned on at metaphase, the midpoint of mitosis. The cyclin is then degraded and MPF activity drops as the cell divides. Then another cycle starts.* [*Adapted from G. Draetta* et al., Cell ***56***, *829 (1989)*]

"growth-associated" histone H1 kinase, which was first discovered some 15 years ago. Histones help to maintain chromatin structure and the phosphorylation of histone H1 by the p34 kinase might bring about the coiling and condensation of the chromosomes that occur as mitosis begins.

But many things beside chromosome condensation happen in a cell going into mitosis, and it is unlikely that histone phosphorylation could explain all of them. Two additional new candidates for a p34 kinase target are also intriguing, however.

One is the *src* "proto-oncogene" (also see box on p. 253). and the other is the enzyme that begins protein synthesis by transcribing genes into messenger RNA. "We think it means that there might be some tie between cell cycle regulation and transcription regulation," says Jeffry Corden of the Howard Hughes Medical Institute at Johns Hopkins University School of Medicine. Corden and Lars Cisek, who is also at Johns Hopkins, discovered that the gene-transcribing enzyme, RNA polymerase II, is phosphorylated by the p34 kinase.

Another major puzzle concerns the activation of the p34 kinase. Indications are that this requires the removal of a phosphate from the kinase, but how this happens is far from clear. Also unclear is what signals the degradation of cyclin at the appropriate time in the cell cycle.

Researchers clearly have to do a great deal of work before they understand cell cycle control in all its ramifications. Nevertheless, the collaboration between the yeast geneticists and the cell biologists has proved to be a fruitful one. ■ JEAN L. MARX

Good News for Volcano Watchers

Something is stirring beneath Mammoth Mountain, but hardly anyone seems to care. The lack of excitement over a swarm of tiny earthquakes beneath this dormant but hardly extinct volcano on the edge of Long Valley, just east of Yosemite National Park, is in part a result of some recent—and very welcome—news. Volcanologists have discovered that even startling restlessness need not connote imminent disaster.

The good tidings, which should relieve the resort dwellers of Long Valley, emerged from a historical study of 138 calderas, the broad depressions like Long Valley that are the scars of huge volcanic eruptions. In trying to determine how often caldera rumblings turn catastrophic, U.S. Geological Survey volcanologists Christopher Newhall and Daniel Dzurisin drew on the geologic, geophysical, and geographic literature as well as historical records from Roman philosophers, monks, explorers, traders, and airline pilots.

D. J. Roddy, USGS Photo Library

A restless land. *Mammoth Mountain (foreground) shares the restlessness of Long Valley.*

The lesson from history was quite different than those from geology. "Most episodes of unrest end without an eruption," says Newhall. Indeed, "Eruptions are the exception rather than the rule. And unrest is almost universal among young calderas. They are so dynamic and in such a delicate equilibrium, really small disturbances can lead to unrest."

So how do you tell the difference between harmless unrest and precursors of disaster? That was what Newhall was wondering when Mount St. Helens began spouting ash in March of 1980, months before Long Valley's unrest became so obvious that May. "When I arrived at Mount St. Helens in 1980," he says, "I saw a gap in our efforts to predict volcanic behavior. There was day-by-day monitoring, and the geologic record of eruptions was well known. What we didn't have was a good look at historical records around the world looking for analogs to this volcano's behavior." In addition to minor ash eruptions, Mount St. Helens was steadily growing a bulge on its north slope. "If geologists had had a comprehensive literature search, they could have said that the only known outcome of such cases was a lateral blast." And that is just what happened, killing 60 people when it caught volcanologists by surprise.

To Newhall the lesson was that "it's impossible during a crisis to put together such a [literature] search." So in the case of the Long Valley unrest, he and Dzurisin took their time, 5 years of it. They found only one sure connection between caldera unrest and subsequent behavior. If a large part of the caldera floor began to bulge rapidly, at a rate of several meters per day, an eruption always followed within 3 days. A few kinds of less striking unrest usually, but not always, led to eruptions within hours or days.

There were also circumstances that did not lead to eruptions. Eruptions occurred during half of the episodes of unrest at all well-studied calderas, but six long-quiet calderas of the silicic type, including Long Valley, have had 28 episodes of unrest during the past century without an eruption.

That long-quiet calderas can be restless without erupting is good news to the inhabitants of Long Valley's Mammoth Lakes. The swarm of imperceptible earthquakes began there in early May and peaked with hundreds per day in late June. Was it a sign of magma or magma-heated water moving beneath the volcano? Since the 1980 series of moderate earthquakes, there have even been indications that magma could be heading for the surface from a slowly filling magma chamber more than 5 kilometers deep. Did all this geological rumbling portend an imminent eruption, the scientists and citizens of Mammoth Lakes naturally wondered? Newhall and Dzurisin's historical hindsight now allows such questions to be contemplated more calmly.

When Long Valley started acting up 8 years ago, "there wasn't the awareness of the pervasiveness of unrest," says Newhall. "I'm sure the Long Valley unrest was perceived as a more unusual situation than it is now." Then again, no one seems anxious to see another. ■ RICHARD A. KERR

ADDITIONAL READING

C. G. Newhall and D. Dzurisin, *Historical Unrest at Large Calderas of the World*, U.S. Geological Survey Bulletin 1855, (Government Printing Office, Washington, DC, 1988).