CELL BIOLOGY

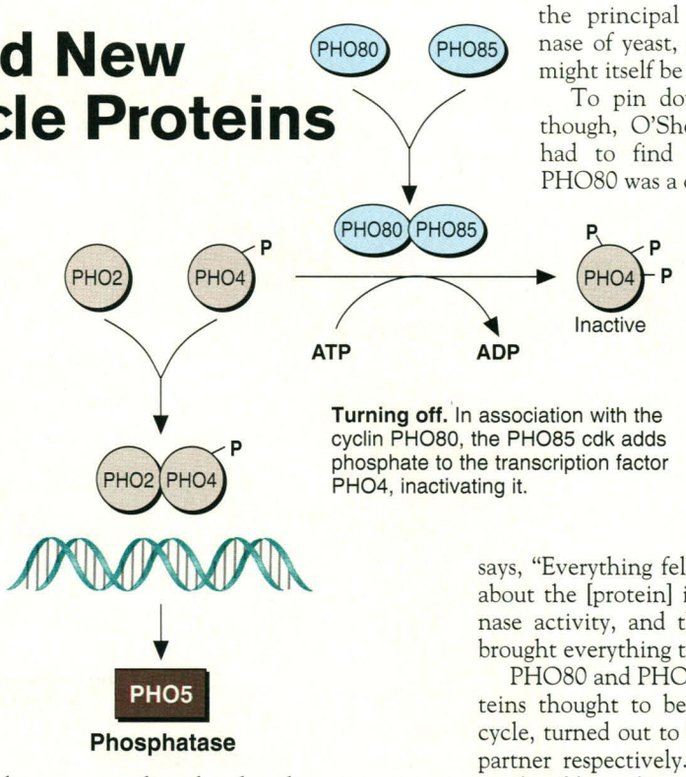
Researchers Find New Role for Cell Cycle Proteins

Over the past half-dozen years, a dance of two proteins has captivated cell biologists. The dancers are the cyclins and the kinase enzyme partners they interact with, and the dance is compelling because it sets the pace for even grander choreography: the intricate cell cycle, which pushes cells to divide. But that's not the only kind of dance in the repertoire of these proteins, according to a team led by biochemist Erin O'Shea of the University of California, San Francisco (UCSF). They may set the pace for other parts of a cell's biochemical machinery as well.

On page 1153, O'Shea and colleagues Arie Kaffman and Ira Herskowitz, also at UCSF, and Robert Tjian of the University of California, Berkeley, report that a cyclin and its associated cyclin-dependent kinase (cdk) are key players in a pathway that regulates the synthesis of a phosphatase enzyme of yeast. The phosphatase provides yeast cells with a critical nutrient—inorganic phosphate—but is not an obvious part of the cell cycle machinery. "What's interesting is that until this work, the only clear role of the cyclins and cyclin-dependent kinases was in cell cycle control," says Fred Cross of Rockefeller University, who studies the cell cycle in yeast. Another cell cycle expert, Andrew Murray of UCSF, agrees that the finding is "very important. Even though they [the cyclins and cdks] were discovered because of their role in the cell cycle, their role in biology is likely to be more general."

The results may point to an even broader integrative function for the cyclins and cdks: linking seemingly unrelated events in cell metabolism with the cell cycle. "Clearly the cell has to integrate nutritional input with the cell cycle machinery," notes Brenda Andrews of the University of Toronto. She and her colleagues, along with David Morgan's group at UCSF have still-unpublished findings that, together with O'Shea's, support the possibility of such integration.

These are links O'Shea never expected to find when she embarked on the yeast phosphatase studies. She was, she says, originally interested in using the gene for the enzyme and its related regulatory genes as a model for understanding control of gene expression. The phosphatase gene system was particularly well-suited to this purpose, O'Shea explains, because genetic studies in the 1970s by Yasuji Oshima and Akio Toh-e of Osaka University in Japan, had identified its main components, including several genes whose protein products either activate or suppress the gene encoding the phosphatase.



Turning off. In association with the cyclin PHO80, the PHO85 cdk adds phosphate to the transcription factor PHO4, inactivating it.

As the Japanese and other workers cloned and sequenced those genes, they began to get a handle on the genes' functions. For example, two activators, *PHO2* and *PHO4*, encode transcription factors: proteins that bind to the phosphatase gene's regulatory sites and turn it on. But that didn't exhaust the system. There was also the *PHO80* gene, which collaborates with another gene, *PHO85*, to turn off the phosphatase gene. How PHO80 does that was still unclear by last summer, when the O'Shea team began its work.

There was one clue to its function, however: Oshima's genetic studies had indicated that PHO80 somehow acts through the transcription factor PHO4. O'Shea decided to follow up on this observation by finding out whether the two proteins interact directly. And, indeed, she found that they precipitate together from yeast cell extracts, a strong indication that they are closely associated in the cell. The O'Shea team also noticed that the precipitates contained kinase enzyme activity, meaning they could add phosphate groups to proteins—including PHO4. That kinase activity turned out to belong to none other than PHO85, the protein known to collaborate with PH080 in turning off the phosphatase gene.

That's where matters lay when O'Shea ran into Aaron Neiman, then a graduate student in the Herskowitz lab, who observed that her kinase data were very reminiscent of what they had been seeing with cdks. That observation, together with Toh-e's earlier finding that the protein sequence of *PHO85* is 50% identical to CDC28, the principal cyclin-dependent kinase of yeast, suggested that PHO85 might itself be a cdk.

To pin down the identification, though, O'Shea and her colleagues had to find an associated cyclin. PHO80 was a candidate because it, as well as PHO85, is needed to turn off the phosphatase gene, but it was not supposed to resemble any cyclins. When Neiman reexamined the PHO80 sequence, however, he found similarities to two relatively unknown yeast cyclins, called HCS26 and OrfD. Then, O'Shea says, "Everything fell into place. We knew about the [protein] interaction and the kinase activity, and the sequence similarity brought everything together."

PHO80 and PHO85, two regulatory proteins thought to be unrelated to the cell cycle, turned out to be a cyclin and its cdk partner respectively. And that solved the puzzle of how the two inhibit phosphatase synthesis. Like any cyclin, PHO80 activates its cdk, PHO85, by combining with it. PHO85 then phosphorylates PHO4, thus blocking its transcription factor activity.

But PHO85's kinase activity is apparently not restricted to the phosphatase gene regulatory system. A cdk's cyclin partner determines the enzyme's specificity, and this particular cdk seems to associate with two other cyclins besides PHO8O. F. Hernan Espinoza of the Morgan lab has shown that it also interacts with HSC26, and Andrews and her colleagues have found that it binds OrfD. Since both of these cyclins have been implicated in cell cycle regulation, PHO85 might therefore link cell-cycle events to the cell's nutritional need for phosphate. The finding that PHO85 has three separate cyclin partners may also aid in determining how the cyclins confer specificity on the kinases.

Now that researchers have had a glimpse of a broader biochemical repertory for cyclins and cdks in yeast cells, they are wondering whether these proteins will show similar versatility in mammalian cells. The suspicion is that they will, since mammalian cells appear to have more cyclins and cdks than they need merely for cell cycle control. But, as Murray notes, "it's one thing to say we don't know the function and quite another to find one, as O'Shea has." Still, Morgan is optimistic. "There are tons of cdks in higher eukaryotes," he notes. "No doubt this [cyclin-cdk interaction] is going to be a common regulatory mechanism."

–Jean Marx