

# Whittling Thousands to a Few

*The key to identifying yeast proteins of interest is to get a handle on them.*

KINASES CALL A LOT OF THE SHOTS INSIDE CELLS. THESE ESSENTIAL ENZYMES regulate a vast array of processes, such as whether cells grow normally or become cancers. But the functions of many of the more than 120 kinases that exist in yeast are mysteries—and no simple method exists to determine each one’s modus operandi. Now, chemical and genetic engineering has allowed a team of researchers at the University of California, San Francisco, led by HHMI investigators Erin K. O’Shea (now at Harvard University) and Kevan M. Shokat, to pinpoint the protein substrates acted on by a yeast kinase called Pho85. “To understand the function of a particular kinase,

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KEVAN SHOKAT

we need to know what kinds of proteins it controls,” says O’Shea.

The researchers expect their two-step method will prove useful for many kinases, especially because no one else has come up with a systematic approach to identifying a kinase’s constellation of substrates.

Kinases activate proteins by grabbing the cellular energy-supplying molecule

ATP (adenosine triphosphate), shearing off a phosphate, and adding it to the protein. Using radioactive ATP as a source of phosphate is one way to mark targeted proteins so that they can be identified amid a sea of others. But all 120 kinases exist in test tube extracts of yeast, and each of those can affix radioactive phosphate to proteins.

To get around this problem, O’Shea and Shokat developed the first step of their method—a molecular trick that allows only the kinase of interest to grab the radioactive ATP. They added a chemical tab to radioactive ATP and engineered Pho85 to have a slot to match the chemical tab, making them fit together much like pieces of a jigsaw puzzle. So only the Pho85 is capable of transferring a radioactive phosphate to its proteins.

## THE BIG PICTURE

### A Family of Overachievers

**PROTEIN KINASES** belong to a very large family of enzymes. The human genome contains about 500 protein kinase genes, or about 2 percent of the total. Kinases play a major role in controlling many key activities of the cell and are particularly prominent in transmitting signals within a cell and in coordinating the steps of cellular replication.>>**A PROTEIN KINASE MODIFIES** other proteins by chemically adding phosphate groups to them—a process called phosphorylation. The process usually results in a change in the target protein’s activity, its location within the cell, or its interactions with other proteins. Each kinase can be a multi-tasker, having several substrates and acting as a substrate for other kinases.>>**PRECISE CONTROL** of protein phosphorylation is critical to normal cell behavior. Uncontrolled kinase activity is a frequent cause of disease. For example, in cancer, kinase-mediated regulation of many aspects of cell growth, movement, and death is disrupted. For these reasons, compounds that inhibit the activity of protein kinases are being studied as therapeutic agents. In fact, some kinase inhibitors are already available for treating patients, including the two anticancer drugs Gleevec (imatinib mesylate) and Iressa (gefitinib).



O'Shea: Christopher Jones Illustration: Joel Lardner

But how to find those proteins that Pho85 controls in yeast cells? Step two: The team designed yeast proteins to have unique molecular “handles.” These antigenlike handles can be snagged with an antibody that recognizes them specifically and pulled out of the extract by a method called tandem affinity purification (TAP). Within a yeast cell, only one type of protein out of the multitude present is marked with a TAP handle.

The researchers then created 4,250 yeast strains, all identical except for the unique TAP-handled protein each strain makes. This bevy of strains represents a large percentage of all the proteins a yeast cell makes—and that a kinase can act

upon. The importance of making such strains, says O’Shea, was to keep the kinase in an environment close to its natural habitat, thereby reducing the possibility that the kinase would behave in any way different from normal.

After adding Pho85 to a mash of pooled yeast strains, the team pulled out all the proteins displaying a TAP handle. The ones that showed up with a dab of radioactivity—from Pho85—could be investigated further. O’Shea and Shokat found 24 proteins that came through the steps radioactive,



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spotlighting two dozen possible Pho85 substrates. As an added bonus, one of the proteins had been previously reported to be under Pho85’s control, affirming the value of their method.

Besides Pho85, Shokat has made more than 75 additional slotted kinases, all but three of which work well with the modified radioactive ATP. “We think the trick will be generalizable to almost every kinase,” says Shokat. If so, this technique will help researchers move many kinases off the mystery list. ■ - MARY BECKMAN