X-RAY CRYSTALLOGRAPHY

Taking a First Look at a Tyrosine Phosphatase

Everybody knows that a picture is worth almost any number of words. And this principle definitely holds true in cell biology, where researchers who want to understand fully the proteins they study yearn to see clear, three-dimensional pictures of those proteins. Particularly high on cell biologists' wish lists are images of the major regulatory enzymes that control cell growth—not only because these enzymes are central to the cell's normal activities, but also because they may contribute to the abnormal growth of cancer if they go awry. By showing how such proteins are shaped, the images can provide clues to their functions and possibly help researchers design drugs for use in cancer therapy that either block or mimic the proteins' activities.

Now, David Barford, Andrew Flint, and Nicholas Tonks of Cold Spring Harbor Laboratory have weighed in with the first crystal structure of one type of major growth regulatory enzyme, a member of the protein tyrosine phosphatase family (see p. 1397). Another team, including crystallographer Mark Saper and biochemist Jack Dixon of the University of Michigan Medical School in Ann Arbor will shortly add a second portrait to the tyrosine phosphatase gallery. (Their work is still unpublished but was presented at a recent Keystone meeting.)

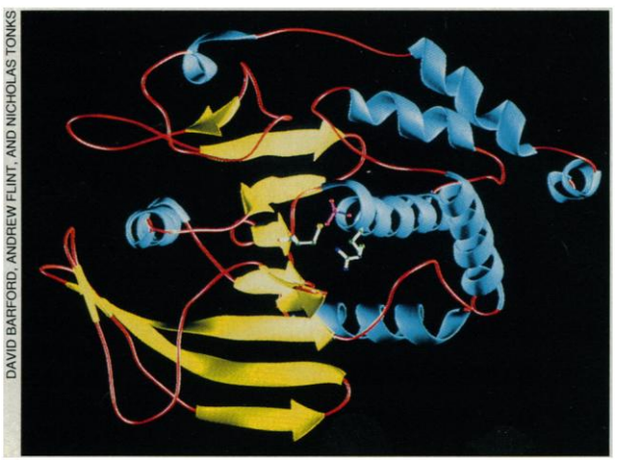
Researchers who study growth regulatory enzymes are thrilled to see the tyrosine phosphatase structure. "It's a major, major contribution, and it's come so fast," says protein chemist Susan Taylor of the University of California, San Diego, who notes that it's only been 6 years or so since Tonks, then a postdoc in Edmond Fischer's lab at the University of Washington in Seattle, discovered the first tyrosine phosphatase.

The significance of these enzymes stems from their ability to remove phosphate groups from the amino acid tyrosine in their target proteins. This action makes them the opposite numbers of another key set of enzymes: the tyrosine kinases, which add phosphate groups to the same amino acid. Such phosphate additions and removals are an extremely common method of regulating proteins and thereby controlling key cellular activities. Many tyrosine kinases, for example, help transmit the signals for cell division from the cell's membrane to the interior. And, since uncontrolled growth is a feature of cancer, it isn't surprising that the genes for several kinases have turned out to be cancer-causing oncogenes. The tyrosine phosphatases, in contrast, would be expected to inhibit cell growth, but there is also evidence that in some pathways they may stimulate it.

Whatever their precise function, the tyrosine phosphatases are essential for normal cell growth, and hence have become the subject of intense investigation. The new crystallographic structure will aid those efforts greatly, since it reveals the exact shape of the tyrosine phosphatase's active site: the part of the enzyme that binds to the targets and catalyzes the phosphate removal. "In terms of understanding how the phosphates come off, it is a breakthrough," Taylor says.

The Cold Spring Harbor group performed their x-ray crystallography study on a truncated form of protein tyrosine phosphatase 1B, the same enzyme Tonks originally isolated with Fischer, who with Washington colleague Edwin Krebs won the 1992 Nobel Prize for medicine for their work on protein phosphorylation. The structure reveals that the protein folds to form a rough sphere about 50 Ångstroms in diameter. The enzyme's active site can be clearly seen, says Tonks, as a cleft in the surface of the molecule.

The structure shows that 27 amino acids, found to be conserved in all the eukaryotic tyrosine phosphatases shown to have catalytic activity, are located in and around the active site. Some of these amino acids maintain the active site's shape, while other have been identified by researchers, including Dixon, as being directly involved in binding the phosphorylated tyrosines of the target proteins or in removing the phosphate. The amino acid that actually breaks the bond between the phosphate and its tyrosine is located at the bottom of the cleft. Its location, combined with the shape of the cleft, accounts for the enzyme's specificity for phosphorylated tyrosines, says Tonks.



DAVID BARFORD, ANDREW FLINT, AND NICHOLAS TONKS

Centrally located. Here the tyrosine phosphatase active site *(near center)* binds tungstate instead of the normal phosphate.

Two other amino acids in proteins, serine and threonine, can also be phosphorylated, but serine and threonine are smaller than tyrosine, and therefore don't fit far enough into the cleft to contact the catalytic amino acid at the bottom. "It's a really elegant way of getting specificity—a cleft just right for the one, but not the other two," says Tonks, who notes that a similar arrangement has been seen with SH2 domains, which also recognize phosphorylated tyrosines.

The Cold Spring Harbor workers think the tyrosine phosphatase 1B structure will turn out to be a prototype for other tyrosine phosphatases—and early indications from the Michigan group's work suggest that they are right. Dixon and his colleagues analyzed a very different tyrosine phosphatase, one made by bacteria of the genus *Yersinia*, which cause plague—the "Black Death." This protein is something of an outlier in the tyrosine phosphatase family; its amino-acid sequence is only 25% identical to that of tyrosine phosphatase 1B. Still, Dixon says, "it's evident that the two structures are very similar to one another in how they are folded." He cautions, however, that subtle differences might have been missed because "we haven't compared the intricate details of the structures."

Although this first look at a tyrosine phosphatase structure is very gratifying, it doesn't reveal everything about the enzymes. Ben Neel, a tyrosine phosphatase expert at Boston's Beth Israel Hospital, notes that the structure so far provides little information about how the enzymes are regulated. But even now, he expects it to be very helpful to researchers. Knowing how the active site is shaped, Neel says, will "definitely serve as a useful starting point for developing specific inhibitors."

Such inhibitors are wanted, partly because they will help researchers pin down what the tyrosine phosphatases do in the cell, but also because they have potential for treating diseases, including cancer and diabetes. The insulin receptor is a tyrosine kinase, and compounds that prevent removal of the phosphates it adds to proteins might potentiate insulin's effects. Tyrosine phosphatase inhibitors have "a lot of potential impact in the pharmaceutical industry," Dixon says. And that's only one reason the newly acquired pictures may be worth more than many thousands of mere words.

–Jean Marx