Biologists Turn on to "Off-Enzymes"

The number of tyrosine phosphatases sky-rockets as researchers flock to study an enzyme family that may play a key role in cell growth control and carcinogenesis

OVER THE PAST YEAR OR TWO A NEW GROWTH area has sprung up for cell biologists: the study of a previously unheralded set of enzymes called the protein tyrosine phosphatases. These enzymes are stirring excitement because they may be the long-sought counterforce to a much better known group of enzymes called the tyrosine kinases. The hunt for the tyrosine phosphatases took nearly 10 years, but the effort was considered well worth it because the genes encoding the enzymes might play a role in carcinogenesis, possibly acting as tumor suppressor genes.

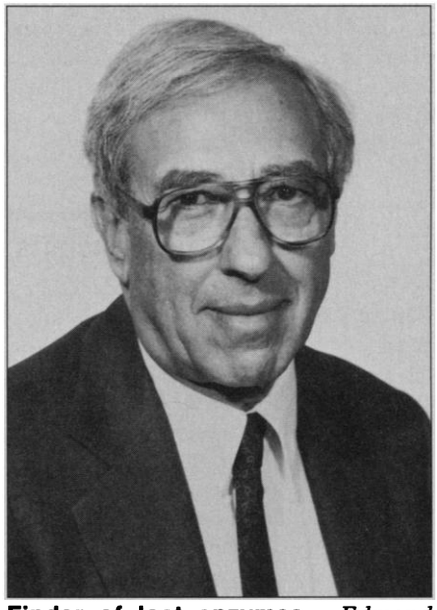
The idea that there might be tyrosine phosphatases dates back to the discovery of the tyrosine kinases, one of the most exciting developments in cell biology of the late 1970s. The kinases proved to be major stimulators of cell growth—an activity that depends on their ability to attach phosphate groups to proteins on one specific amino acid, namely tyrosine. The tyrosine kinases held the limelight in growth control research during the 1980s as investigators identified more than 40 cellular genes encoding the enzymes. Not only were they important for normal growth control—some proved to be receptors through which growth factors transmit their effects, for example—but the tyrosine kinase genes also had a proven potential to mutate to produce oncogenes that can trigger cancerous changes in cells.

But all along, researchers knew that if the enzymes that put phosphates on the tyrosine residues of proteins are so important, they must be counterbalanced by tyrosine phosphatases—the enzymes that remove the phosphates. Otherwise the system could get stuck in the "on" position, causing cells to grow out of control. "Obviously a system that relies on tyrosine kinase needs a phosphatase," says Tony Hunter of the Salk Institute, one of the pioneers of tyrosine kinase research.

During most of the 1980s, however, researchers were unable to purify the tyrosine phosphatases and their study lagged way behind that of the kinases. The breakthrough didn't come until about 3 years ago, when a group at the University of Washington finally got a pure phosphatase isolate, opening the floodgates to the current surge of work. Now, researchers can finally begin addressing the questions they want answered.

One thing that they have already learned is that the tyrosine phosphatases are not just housekeeping enzymes that exist to clean up after the tyrosine kinases. Indeed, the phosphatases constitute a novel class of receptors that make up an independent signalling pathway in the cell. "This is what brought excitement to the field," says Edmond Fischer of the University of Washington, a member of the group that isolated the first phosphatase and made the receptor connection. "Otherwise people might have said that the enzyme was just another phosphatase."

That excitement will surely grow if the tyrosine phosphatases prove to be growth inhibitors, as predicted. The genes for the enzymes might then play a role in cancer development, presumably as tumor suppressors rather than oncogenes, which would mean that loss or inactivation of the genes would be carcinogenic. Moreover, if the tyrosine phosphatases have tumor suppressive action, then it might be possible to use them or drugs that mimic their action to devise new cancer therapies. That possibility still belongs only to the realm of speculation, however.



Finder of lost enzymes. *Edmond Fischer's group at the University of Washington purified the first tyrosine phosphatase.*

Why did the tyrosine phosphatases take so long to isolate? The main reason, says Nicholas Tonks of Cold Spring Harbor, who undertook the task of isolating one after he joined Fischer's group in Washington as a postdoc in 1985, is that the cell is loaded with phosphatases with many different specificities. Researchers didn't have a good way to pick out just the ones that remove phosphates from tyrosine residues. What was needed, Tonks says, was an artificial substrate that would specifically bind tyrosine phosphatases and could be made in sufficiently large quantities to be used in a purification technique.

It took Tonks several months to produce such a substrate, a phosphorylated derivative of the protein lysozyme. But once he did, he says, the goal was at hand: a pure tyrosine phosphatase preparation. "From there," Tonks says, "it all took off."

Harry Charbonneau and Kenneth Walsh, also of the University of Washington, determined the amino acid sequence of the tyrosine phosphatase. When the Washington group compared that sequence to others in the protein sequence banks, they got a couple of surprises. For one thing, their enzyme did not resemble any of the other known phosphatases. In contrast, the protein kinases show sequence similarities, indicating that they evolved from a common ancestor. "We expected the same for the phosphatases," Fischer says. "But no!"

The second surprise came when the researchers found the only protein in the databases that the tyrosine phosphatase did resemble: the "leukocyte common antigen" (also known as CD45), which occurs on the surfaces of white blood cells. Although the function of CD45 was unknown at the time, its sequence showed that it has the characteristic structure of a receptor, with three major domains: an external segment of about 400 amino acids, a membrane-spanning segment consisting of 22 amino acids, and a large intracellular

segment of about 700 amino acids. The internal segment contains two structurally related sequences, each about 300 amino acids long, and it was these sequences that turned out to be similar to the tyrosine phosphatase sequence.

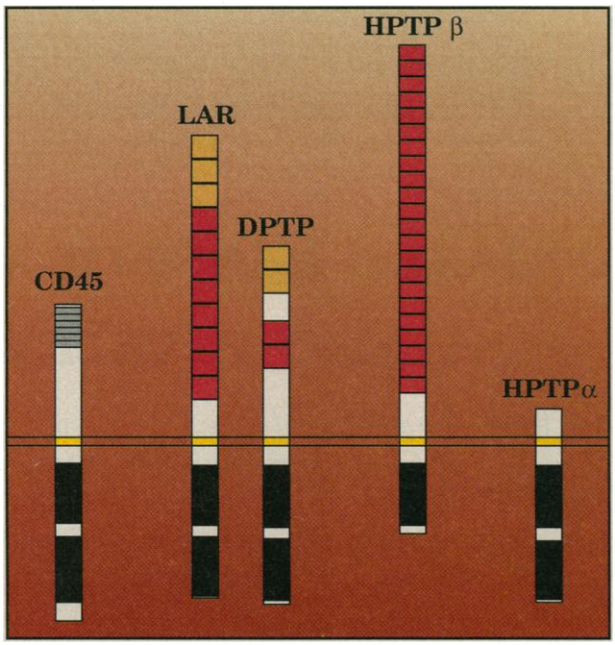
The similarity between CD45 and the tyrosine phosphatase suggested that the enzymes might have a greater role than researchers originally envisioned. In the first place, the resemblance indicated that CD45 might have tyrosine phosphatase activity, a hypothesis subsequently confirmed by the Washington group. And that notion, in turn, placed tyrosine phosphatases in the cell's signalling pathways. The implication, says Fischer, is that "the tyrosine phosphatases don't just oppose the tyrosine kinases." They can respond on their own to incoming signals and therefore control cell activities independently.

One of the first consequences of this discovery was an influx of new workers into the tyrosine phosphatase field. "For a long time I was extremely interested [in tyrosine phosphatases], but I was lazy," says one of the newcomers, kinase expert Josef Schlessinger of New York University Medical Center. "When I learned about the receptor work, I knew it was time to join in."

So far, both the newer and older participants have been working primarily on identifying more tyrosine phosphatases. Once the first sequence was in hand, it was possible to use the information to make nucleic acid probes for fishing out tyrosine phosphatase genes, which is much less tedious than purifying proteins. "There's been a rampage of PCR and an orgy of low-stringency screening," says Fischer, referring to the two methods being used to search for new tyrosine phosphatase genes. And the screening has paid off. The current total stands at about twenty, isolated by various labs, Tonks says. Most of them appear to encode membrane receptors, although three so far, including the tyrosine phosphatase purified by Tonks and Fischer, are located inside the cell, in the cytoplasm.

What the final total of tyrosine phosphatases will be is still unclear. Schlessinger predicts that there is a tyrosine phosphatase for every tyrosine kinase—in which case cells would have to have more than forty.

While tyrosine phosphatase identification is going full steam, efforts to understand the physiological functions of the enzymes are still in the early stages, with little known about what they actually do. Jack Dixon and his colleagues at Purdue University found one very unexpected role, however: They showed that a tyrosine phosphatase gene is the cause of the extreme virulence of the plague bacterium *Yersinia pseudotuberculosis*. That's surprising, Dixon says, because bacteria are not supposed to have tyrosine kinases and would thus seem to have no need for a tyrosine phosphatase. He suggests that the bacteria may have picked up the phosphatase gene from an infected animal, much as viruses have picked up oncogenes from the organisms they infect.



Receptor body plans. *The intracellular segments of the tyrosine phosphatases are similar, with most having two phosphatase domains* (solid bars). *But the external segments can vary dramatically, reflecting their status as "signal detectors."*

How the tyrosine phosphatase gene increases *Yersinia* virulence is, like much else about the enzymes, unclear. Recent work by Dixon's group and that of Stanley Falkow at Stanford University School of Medicine indicates, however, that the tyrosine phosphatase molecules made by the bacteria somehow get into the macrophages of infected animals where they dephosphorylate several proteins. As a result, the activities of the macrophages, which are needed to initiate several immune responses, may be inhibited. And that in turn "may allow the bacteria to replicate in the presence of a crippled immune system," Dixon proposes.

The Dixon group has also found recent evidence that vaccinia virus, the cause of chicken pox, has a tyrosine phosphatase gene, and it remains to be seen how widespread the enzymes are among pathogens.

There is currently little direct evidence that tyrosine phosphatases have the expected growth inhibitory effects, however. "Right now it's an area of tremendous promise rather than achievement," says Ian Trowbridge of the Salk Institute, whose own earlier work on CD45 served as an entry to the tyrosine phosphatase research.

But researchers at least have a few hints to encourage their belief that the phosphatases might inhibit cell growth. One of these hints emerged from a structural analysis of the enzymes. Haruo Saito, Michel Streuli, and Neil Kreuger of the Dana-Farber Cancer Institute in Boston found that the external segments of some of the tyrosine phosphatase receptors they have isolated are structurally similar to certain cell surface molecules known to mediate interactions between cells during embryonic development. This resemblance suggests that the tyrosine phosphatase receptors might mediate another kind of cell-to-cell interaction: contact inhibition, the cessation of growth that normally occurs when cells make contact.

Saito and his colleagues propose that when the external segments of the receptors on one cell interact with those on another, the internal phosphatases may be activated, setting in motion a chain of events that inhibits cell growth. In that event, mutations that inactivate the tyrosine phosphatase genes could lead to loss of contact inhibition—which is one of the hallmarks of cells that have undergone cancerous transformation.

Researchers are also beginning to ask whether the tyrosine phosphatases can oppose the effects of oncogene action. These experiments are turning out to be somewhat difficult to do, however. They usually involve transferring a gene for one of the enzymes into cells to see how that affects what happens when an active oncogene is also added, and having excess tyrosine phosphatase production as a result of the gene transfer is turning out to be hazardous to the health of recipient cells.

Nevertheless, David Hill and his colleagues at Applied bioTechnology, Inc., in Cambridge, Massachusetts, managed to produce a cell line carrying a transferred tyrosine phosphatase gene. After establishing that the cells were indeed making the enzyme, the researchers put in the *neu* gene, which is one of the tyrosine kinase oncogenes. The result: although the *neu* gene caused the cancerous transformation of control cells, it did not transform the cells with the added tyrosine phosphatase. "We can make a suggestive argument," Hill says, "for the [enzyme] being able to suppress a tyrosine

kinase oncogene such as *neu*, presumably because it dephosphorylates the *neu* target." So in one case a tyrosine phosphatase has been shown to have anti-oncogenic effects.

Although the idea that tyrosine phosphatases might inhibit cell growth is one of the principal reasons people are interested in them, it's also clear, paradoxically, that in some circumstances the enzymes may be needed for cell proliferation. Matthew Thomas and his colleagues at Washington University School of Medicine developed a line of mutant T cells that no longer have a functional CD45 protein. Those cells were unable to proliferate, as they normally do, in response to antigen stimulation. "The cells lost the ability to signal through the antigen receptor," Thomas says. "Instead of being a negative regulator, [the phosphatase] was required for function." The effects of the tyrosine phosphatases—whether growth inhibitory or growth stimulatory—may depend, Hill suggests, on the type of cell in which the enzymes are active.

Researchers obviously still have a great deal to learn about how the tyrosine phosphatases operate. High priorities for future work include efforts to identify the external signals that activate the receptor proteins and turn on the phosphatases. And equally important will be the identification of the targets on which the phosphatases work.

Finding those targets will be no mean feat. Cell biologists began looking for the proteins phosphorylated by the kinase encoded by the *src* oncogene more than 10 years ago, and only in the past couple of years have they begun to understand fully just what the kinase does.

Although the researchers have their work cut out for them, they do not seem to be daunted by the prospective difficulties. "We're having fun," says Dixon. And the research does offer the opportunity to get a much firmer grasp on the intricate network of reactions that control cell division. If the 1980s was the decade of the tyrosine kinases, then the 1990s may become the decade of the tyrosine phosphatases.

■ JEAN MARX

ADDITIONAL READING

H. Charbonneau, N. K. Tonks, K. A. Walsh, and E. H. Fischer, "The leukocyte common antigen (CD45): A putative receptor-linked protein tyrosine phosphatase," *Proc. Natl. Acad. Sci. USA* **85**, 7182 (1988).

K. Guan and J. E. Dixon, "Protein tyrosine phosphatase activity of an essential virulence determinant in *Yersinia*," *Science* **249**, 553 (1990).

T. Hunter, "Protein-tyrosine phosphatases: The other side of the coin," *Cell* **58**, 1013 (1989).

G. A. Koretzky, J. Picus, M. L. Thomas, and A. Weiss, "Tyrosine phosphatase CD45 is essential for coupling T-cell antigen receptor to the phosphatidyl inositol pathway," *Nature* **346**, 66 (1990).

N. X. Kreuger, M. Streuli, and H. Saito, "Structural diversity and evolution of human receptor-like tyrosine phosphatases," *EMBO Journal* **9**, 3241 (1990).

D. L. Jones

A more accessible oceanic plateau? *The dark basaltic rock at the base of these cliffs may have been an oceanic plateau now sandwiched into southwestern Canada.*

Did a Burst of Volcanism Overheat Ancient Earth?

Marine geologists are tying a surge in volcanic activity about 120 million years ago to a uniquely warm climate

ONE HUNDRED MILLION YEARS AGO ALLIGATORS and crocodiles were thriving at the latitude of present-day Labrador. Ever since the fossil evidence of far northern tropical warmth began turning up, scientists have wondered what could have made it so warm, warmer than the greenhouse world of the next century.

Now marine geologists are seeing signs that the answer to this climate enigma may lie on the sea floor. They are becoming convinced that an extraordinary burst of submarine volcanic eruptions struck the Pacific basin about 120 million years ago, pouring vast amounts of gas-laden lava over the ocean floor. The primary evidence for this volcanic spasm is a collection of massive undersea lava plateaus that formed nearly simultaneously. The immense volume of magma brought to the surface to form these structures, a growing number of geologists believe, would have released so much carbon dioxide that the resulting greenhouse effect would help account for the warmest global climate in 500 million years. Once the surge of volcanism began to subside about 100 million years ago, climatic deterioration would have set in until the intermittent ice ages of the past few million years took hold.

One researcher in particular, marine geophysicist Roger Larson of the University of Rhode Island, has compiled considerable evidence for a pulse of volcanism in the mid-Cretaceous Period. He sees at least a 50% increase in the production of ocean crust—more than enough to produce the balmy climate of that time and perhaps an array of other geological phenomena as well (see box). And he's winning over some skeptics. Marine geologist Edward Winterer of Scripps Institution of Oceanography, who had long doubted claims that ocean crust production had surged in the mid-Cretaceous, is one such convert. "What's happened in recent years," he says, "is that we've become more aware of the enormous volume of material emplaced in oceanic plateaus."

Although plateaus cover only about 3% of the present sea floor, Larson's compilation of their volumes and ages suggests that at least in the mid-Cretaceous they loomed large in global volcanism. The standout of them all is the Ontong Java Plateau. Now more than 2 kilometers beneath the sea off the Solomon Islands, Ontong Java covers 1.5 million square kilometers. That rivals the extent of any of the great outpourings of lava that have spilled across the continents, such as the continental flood basalts of the Deccan Traps in India. But there is even more to Ontong Java than meets the eye. While the Deccan Traps' layer of lava is 1 kilometer thick, this plateau of new crust extends downward 36 kilometers, making a volume of 50 million cubic kilometers. That's equivalent to a cube of rock 370 kilometers on a side. And all this new crust may have spewed out in just a few million years—an