RESEARCH PROFILE

Nicholas Tonks

Persistence comes naturally to Nick Tonks, FRS, who has the distinction of having laid the foundations for the identification and functional characterization of a "superfamily" of 107 regulatory enzymes called protein tyrosine phosphatases, or PTPs. The experiment that led to his first breakthrough on PTPs was performed in the late 1980s, while he was working with Dr. Edmond Fischer, a revered mentor and future Nobel laureate, who predicted that his approach "will never work."

"Right," Tonks acknowledges today with a hearty laugh. "So I stuck to my guns and got on with the project!" He had already learned that one had to fight for the ideas one believed in, a lesson that has served him repeatedly. Right now, his CSHL lab is honing novel biochemical methods that could form the basis for new classes of therapeutics in diabetes and cancer.

PTP1B: more than a 'housekeeper'

It was Dr. Fischer — Tonks' postdoctoral advisor at the University of Washington, Seattle — who, with co-Nobelist Edwin Krebs, provided the first demonstration of the regulation of a protein by phosphorylation, in the early 1950s.

Protein phosphorylation, which occurs at thousands of sites in most cells, acts like a switch to regulate proteins involved in essential biochemical processes. It is facilitated by a large class of enzymes called **protein kinases**, which transfer a phosphate group from the donor molecule ATP to an amino acid residue — usually a serine or threonine, and less often a tyrosine — on the protein being acted upon, called the substrate. The process, importantly, is reversible; a class of enzymes called **protein phosphatases** recognizes phosphorylated residues and functions to remove the phosphates that kinases have added. It is the under-appreciated protein phosphatases that have been the focus of Tonks' research career, since his days as an undergraduate.

While working with "Eddy" Fischer, Tonks focused on PTPs, phosphatases that specialize in removing phosphates from tyrosine residues. Why tyrosine? Phosphorylation has different implications depending on the identity of the amino acid that is phosphorylated. Tyrosine phosphorylation has been implicated in growth and metabolic regulation and its disruption leads to major diseases such as cancer and diabetes. The cellular receptor for insulin, for example, was known to be a tyrosine kinase that became phosphorylated and activated when insulin bound to it. A PTP, then, would halt the insulin signal in a cell, by removing the critical phosphates that had activated the signal in the first place.

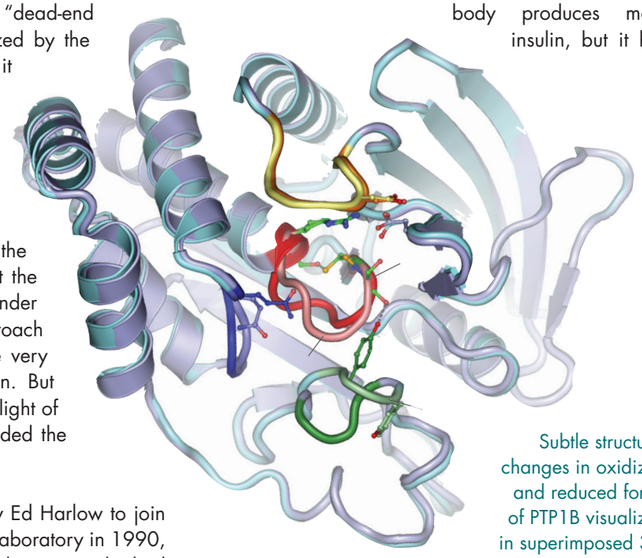
At the start of his career Tonks sought to purify a PTP, something that had not been done before. Then he could sequence it and try to understand its mechanism of action, how it was regulated, and its function. The key step was developing a "dead-end substrate" that would be recognized by the PTP and trapped by it, so that it could be used to extract the PTP from the complex mixture of proteins in a tissue sample. Tonks had discovered how to produce such substrates while doing undergraduate research at Oxford under Sir Philip Randle, and honed the technique as a doctoral student at the University of Dundee in Scotland under Sir Philip Cohen. It was this approach that Dr. Fischer thought would be very challenging, hence his admonition. But Tonks made it work, and, to the delight of all, it led to two papers that provided the foundation for the PTP field.

By the time Tonks was recruited by Ed Harlow to join the faculty at Cold Spring Harbor Laboratory in 1990, there was already evidence that the PTP Tonks had purified, which he named PTP1B, was more than a "housekeeper" for tyrosine kinases, "cleaning up their mess," as Tonks characterizes the then-prevailing wisdom about the entire class of phosphatases. In fact, he and Dr. Fischer had demonstrated the existence of transmembrane "receptor PTPs," which, like receptor kinases, could themselves bind to ligands and directly control the response of cells to environmental stimuli.

The search for a PTP1B inhibitor

In 1999 a team from McGill University generated a line of mice lacking the gene that encodes the PTP1B enzyme. The animals appeared normal, except "they could be fed a high-fat diet, the equivalent of hamburgers and french fries, and they did not get fat," Tonks says. When his team separately showed how PTP1B "recognizes the insulin receptor as a substrate," it became possible to think of a diabetes drug based on inhibition of PTP1B.

"Diabetes is preceded by a resistance to insulin," Tonks explains. Normally, the hormone binds to its receptor — a tyrosine kinase — initiating signaling that directs cells to absorb glucose from the blood and to store it, lowering blood sugar levels. In type 2 diabetes, insulin binds to the receptor but fails to signal properly. The body produces more insulin, but it has



Subtle structural changes in oxidized and reduced forms of PTP1B visualized in superimposed 3-D ribbon diagrams.

no effect. The problem is not a lack of insulin, but a failure of its signal.

PTP1B's role in insulin signaling is normally to antagonize it, via dephosphorylation of the receptor. But, "if you can *suppress* the activity of PTP1B in an insulin-resistant state, you can *facilitate* insulin signaling," Tonks suggests. Based on this concept, he and others — including chemists at major pharmaceutical firms — spent years trying to come up with small-molecule inhibitors of PTP1B. They succeeded; but there were technical problems with delivery of the molecules, deemed insuperable. Big Pharma dropped the idea.

Tonks with postdoctoral researcher Li Li. "I like people who bring their own ideas and passions to the work we do," he says.

True to form, Tonks was not ready to quit. He and colleagues have continued to work on the problem, seeking other solutions. "I firmly believe that the function of academia in these situations is to think outside the box and discover and validate new approaches that we can then present to partners in industry for development into therapeutics," he says.

Several concurrent projects in Tonks' lab show great promise. With postdoc Navasona Krishnan, Tonks has "defined an entirely new mechanism for the inhibition of PTP1B." They are working with an inhibitor that binds to an alternative, allosteric site. This molecule, a natural product, is currently in preclinical testing, and is one of two approaches to diabetes now being pursued. [For the other, see: "Harnessing oxidation"]

Potentially, inhibitors of PTP1B have another important application, in HER2-positive cancers, such as breast cancer. The HER2 oncoprotein — the target for the drug Herceptin — is a tyrosine kinase. Published experiments have shown that mice engineered to express HER2 but to lack the PTP1B gene have "attenuated tumorigenesis and the tumors don't metastasize." This intriguing result suggests that PTP1B plays a *positive* role in transmitting the signal from HER2 and "that if you inhibit PTP1B you could have a new strategy for treating cancers that express HER2," Tonks says. He and Associate Professor Senthil Muthuswamy are currently testing natural-product inhibitors in Muthuswamy's mammary epithelial cell models of breast cancer. Discussions are under way to take this strategy into the clinic in 2012.

"I've been trying to do this kind of thing since the mid-1990s," Tonks says. "And now, for the first time, we have the possibility of getting an inhibitor of that enzyme I purified 25 years ago to treat major human disease. The idea that one's research can lead to treatments for real patients — well, there is no other way to put it. It's just a huge motivating factor." **Peter Tarr**

Harnessing oxidation: an alternative diabetes strategy

Tonks and colleagues have also been working to harness new knowledge they've obtained about how oxidation changes the structure of PTP1B. In excess, oxidation damages living tissue. But controlled production of limited quantities of oxidizing compounds such as hydrogen sulfide, in defined subcellular locations, "makes possible an exquisite level of regulation we didn't know about before," says Tonks. This is true in insulin signaling, where oxidation of PTP1B removes the inhibitory effects of the PTP and enables the signal to be transmitted effectively. Stony Brook University grad student Aftabul Haque last year generated antibodies that selectively recognized the oxidized, inactive form of PTP1B, stabilization of which resulted in enhanced and prolonged insulin signaling in cells. The lab now seeks a small molecule drug that can produce the same effect.