BIOCHEMISTRY

Src Structure Crystallizes 20 Years of Oncogene Research

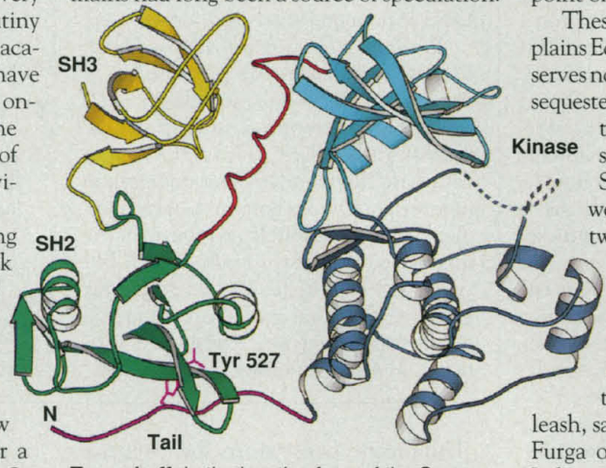
Just over 20 years ago, researchers discovered the first cancer-causing gene, or oncogene, in cells. In Nobel Prize–winning work, they realized that a normal cellular gene, when modified by a virus or mutations, can suddenly trigger wild cell growth—and cancer. The first such Jekyll-and-Hyde gene was called *src* for the sarcomas it caused in chickens, and the notion of "the enemy within," as Nobel laureates Michael Bishop and Harold Varmus called it, was so powerful that almost every aspect of *src* has been under intense scrutiny ever since. For 2 decades, researchers in academe and at pharmaceutical companies have sought the controls for the Src protein's on-off switch. Now, the first descriptions of the crystal structure of this protein and one of its close relatives offer dramatic new evidence on how Src is regulated.

In last week's issue of *Nature*, Wenqing Xu, Stephen Harrison, and Michael Eck of Children's Hospital and Harvard Medical School in Boston describe the structure of inactive Src to a resolution of 1.7 angstroms; in a companion paper, Frank Sicheri, Ismail Moarefi, and John Kuriyan at Rockefeller University in New York City report a similar structure for a related protein called Hck. Experts on Src and its relatives are thrilled by the structures. Biochemist Sara Courtneidge of Sugen Inc. in Redwood City, California, who has built her career around the protein, describes the Src structure as "very beautiful. ... It's exciting and satisfying to see it finally."

One reason the structure is so satisfying is because it supports previous research on the function of various parts of the protein. But it also reveals some surprising new twists, showing how in the benign form of Src, the active site of the protein is kept closed by the regulatory segments. The structure also paves the way for rational design of drugs that might keep Src and its relatives harmless, and thus be useful in cancer therapy. "The structures will be useful for refining inhibitors to make them potent and specific," predicts another longtime Src researcher, Tony Hunter of the Salk Institute in La Jolla, California. And because both Src and Hck, a blood-cell protein, have quite similar structures, the results may be applicable to the whole Src family, which includes proteins involved in everything from immune responses to bone development.

The Src protein is a tyrosine kinase, an enzyme that attaches phosphate groups to the amino acid tyrosine in certain proteins, thereby triggering a functional change in the recipients. For their structure, Xu, Harrison, and Eck worked with a large fragment containing 85% of Src's 536 amino acids, including four key substructures. These business ends of the molecule are the kinase domain that does the catalytic work, plus three regulatory regions: the so-called Src homology (SH) domains, SH2 and SH3, and the carboxyl tail of the protein.

The interactions among these four domains had long been a source of speculation.



SOURCE: XU *ET AL.*

Turned off. In the inactive form of the Src protein, the SH3 domain (yellow) binds to a helix (red) and helps to force part of the kinase's active site (dashed line) closed.

The tail region, in particular, has proved critical to Src's oncogenic abilities, for the loss of a single tyrosine amino acid here can keep the protein active all the time and trigger uncontrolled cell growth.

The new structures now reveal for the first time how these regions interact. Both proteins appear to be held in a benign, inactive state by a "belt and braces" architecture that keeps their catalytic sites locked up. Src's tail, with its crucial tyrosine, is the belt, bending back and winding around the bottom of the SH2 domain. That aspect of the structure was no surprise, as it had previously been predicted by retrovirologist Hidesaburo Hanafusa of Rockefeller University.

But in an unexpected finding, the structure reveals that the SH2–carboxyl tail complex doesn't directly block the catalytic site—in defiance of "almost every model that was ever drawn for Src," says biochemist Joan Brugge of ARIAD Pharmaceuticals in Cambridge, Massachusetts, who helped discover the Src protein. Instead, SH2 works with SH3 to form a brace that keeps the active site of the catalytic domain locked in a viselike grip. And in perhaps the biggest surprise, the SH3 domain plays a crucial role in preventing the catalytic site from functioning.

The SH3 domain had been something of a mystery, because it was known to bind to a particular kind of helical protein structure called a polyproline helix, but no one had ever predicted such a helix in the Src protein. Now, both structures show that Src proteins do indeed have one such helix. The contortions of the SH2 domain and the tail force this helix against the catalytic domain, where the SH3 domain latches onto it. This deforms the catalytic domain and is responsible, perhaps more than anything else, for keeping the kinase inactive and harmless. So, contrary to all expectation, "the SH3 domain is closest to the point of action," says Kuriyan.

These interactions serve dual purposes, explains Eck. "This very remarkable arrangement serves not only to turn off the kinase, but also to sequester the SH2 and SH3 domains" so that they cannot bind to other molecules, he says. The bonds holding the SH2 and SH3 domains in place are relatively weak, however. So, in a normal cell, the two domains can be readily released by other proteins that bind to them more strongly, thereby allowing the Src kinase to be switched on. Thus, the structure allows precise and economic regulation that keeps the potentially dangerous protein on a short leash, says molecular biologist Giulio Superti-Furga of the European Molecular Biology Laboratory in Heidelberg, Germany.

From these structures, it's possible to make an informed guess about what happens to make the oncogenic Src spin out of control, says Kuriyan. When the crucial tyrosine in the tail is missing, it no longer binds to the SH2 domain. As a result, the polyproline helix is not pushed near the catalytic domain, and the binding of SH3 does not interfere with the catalytic site—leaving the protein free to phosphorylate wildly.

The next major goal is the structure of a Src protein with its regulatory domains in an active conformation, says Sugen's Courtneidge. That could help answer questions such as whether altering any one of the three regulatory interactions can cause the whole protein to pop open, or whether the kinase can be activated if only one interaction is disrupted. And the structure of an active Src might help to understand the regulation of many kinases with SH2 and SH3 domains, says Superti-Furga. For now, biochemists and molecular biologists can at last interpret their mutational studies in the light of a real structure, he says: "It's harvest time for us now."

–Carol Featherstone

Carol Featherstone is a writer in Cambridge, U.K.