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## Researchers Determine How Drug Blocks Leukemia-Triggering Enzyme

By exploring how a new anticancer drug inhibits a runaway protein switch that causes chronic myelogenous leukemia (CML), researchers have discovered evidence that alterations in the shape of the protein can be exploited to flip the switch off in a precise manner.

If proven a general phenomenon, such precise control over the activity of these switches, called kinases, could give pharmaceutical companies and basic researchers new tools for manipulating the signaling pathways that control cell growth and a host of other functions.

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— John Kuriyan

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In an article in the September 15, 2000, issue of the journal *Science*, Howard Hughes Medical Institute investigator John Kuriyan and colleagues at The Rockefeller University, Memorial Sloan-Kettering Cancer Center and the State University of New York at Stony Brook report that their x-ray crystallography studies have revealed how the anticancer drug STI-571 inhibits the Abelson tyrosine kinase (Abl).

In CML, the Abl protein, which is normally a well-behaved cellular switch, becomes overactivated by a chromosomal mixup that occurs during blood cell development. The genes *Abl* and *Bcr*, which are found on different chromosomes, become fused together, resulting in a hybrid Bcr-Abl enzyme that causes the overproliferation of white blood cells.

"The regulation of the Abl kinase has been a major puzzle that we've focused on for many years," said Kuriyan. "In particular, we were interested in the fact that although *Abl* is very similar to the well-known *Src* family of

oncogenes that also produce kinases, the drug STI-571 inhibits Abl but not the Src kinases." Kinases are enzymes that switch on proteins in cellular signaling pathways by adding phosphate groups to those proteins. STI-571 was developed by Novartis Pharmaceuticals Corporation and is currently being tested as a treatment for CML in phase II clinical trials.

"This puzzle of STI-571's extreme affinity and specificity is of broader interest because protein kinases are crucial elements in signal transduction pathways that control cell growth, cell death and other processes," said Kuriyan. "Thus, understanding how kinases are turned on and off is a matter of extreme interest."

To understand the molecular basis of STI-571's specificity for Abl, the scientists used x-ray crystallography to obtain a high-resolution crystal structure of STI-571 bound to Abl's catalytic domain. In x-ray crystallography studies, researchers bombard protein crystals with x-rays and then deduce the structure of the protein by analyzing the diffraction pattern produced by the x-rays.

Analysis of the combined structure of STI-571-Abl and the results of additional biochemical experiments were surprising, Kuriyan said, because they revealed that STI-571 bound only to inactivated Abl and did not recognize the activated form of the protein. The studies showed that STI-571 targeted the Abl protein only when a key Abl structure, called an activation loop, was shut down. "Basically, we demonstrated that STI-571 is binding to Abl in its off position, but not when the activation loop is in its on position," said Kuriyan. Thus, he emphasized, the drug's preference for Abl over Src can be explained by the difference in the shapes of inactivated Abl and Src.

The discovery of differences between the inactive conformations of Abl and Src offers hope that inactive forms of other kinases may also prove to be distinctive, said Kuriyan. "The human genome contains hundreds of protein kinases, of which the Abl and Src proteins are just two examples," he said. "And each of these kinases is a crucial switching element but in a different signaling circuit, with different substrates and different signals that turn them on or off.

"However, since all of the kinases catalyze essentially the same chemical reaction, the worry has been that it would be extremely difficult to discover specific small molecules that could turn off one kinase but not others.

"This latest finding is certainly heartening in the promise that it holds for development of specific protein-kinase inhibitors," said Kuriyan, although he cautioned that "CML may be special in that the cause is so singularly related to the activation of a specific kinase."

Nevertheless, he said, understanding the structural differences between kinases does offer hope for engineering kinase-specific compounds. In the case of Abl, for example, knowing the three-dimensional shape of the recognition site may spur development of novel drugs that inhibit Abl activity by blocking that site. These drugs may prove an attractive alternative to

STI-571, should it be rendered ineffective by drug-resistance or other physiological factors.

Kuriyan also sees the work by his group and others as offering promising routes for additional basic research. "The details of the molecular gymnastics that these proteins undergo as they turn on and off are proving to be really rewarding and fascinating to study as basic science; and it's doubly rewarding to see this work intersect with drug development efforts," he said.