

FEBRUARY 04, 2005

Structural Studies Improve Understanding of Key Drug Targets

Researchers have developed a new structural understanding of how the two key subunits of one of the cell's most important enzymes—protein kinase A—work together. Protein kinase A and approximately 600 “cousin” kinases are among the cell's most important switching components because they control the activity of other proteins by attaching phosphate groups to them.

As central cellular control molecules, kinases are prime targets for drugs to treat an array of diseases. Protein kinase A (PKA) alone regulates a variety of processes including growth, development, memory, metabolism, gene activation and lipid breakdown.

"Now that we have these kinds of detailed structures, we can begin to computationally dissect these molecules and understand how they function."

— Susan S. Taylor

The researchers believe their latest findings offer insights that will extend far beyond the workings of PKA, and will serve as a general model for understanding how kinases function. Such functional insights, they said, could offer new ideas for developing drugs to treat disease.

The researchers, led by Susan S. Taylor, a Howard Hughes Medical Institute Investigator at the University of California at San Diego, published their findings in the February 4, 2005, issue of the journal *Science*.

“Protein phosphorylation is probably the most important mechanism of regulation in eukaryotic cells,” said Taylor. “The kinases are absolutely one of the most important gene families that serve as switches to turn pathways on and turn them off. They constitute one of the largest gene families, accounting for about two percent of mammalian genes,” she said.

According to Taylor, who with her colleagues first deduced the structure of PKA in 1991, the enzyme is a particularly good exemplar for studying kinases because the protein is easily purified and manipulated. “Since then,

PKA has provided the template for the entire field of kinase studies,” said Taylor.

Like other kinases, PKA's elegant design and precise function has led some to compare it to the molecular equivalent of a fine Swiss watch. The enzyme consists of a dual-lobed catalytic subunit that performs two central functions: The small lobe accepts the molecular phosphate source—the energy-rich adenosine triphosphate. And the larger lobe docks with the target protein that is to be phosphorylated. That phosphorylation takes place in a key region of the enzyme, called the active site, in a cleft that opens during activation.

PKA also includes a regulatory subunit with domains that bind to the chemical messenger molecule—cyclic AMP (cAMP)—which triggers PKA into action. That regulatory subunit features a flexible extension that inhibits the catalytic subunit by docking with the active site—maintaining PKA in a dormant state until it is triggered by cAMP.

Cyclic AMP is an ancient messenger—evolutionary conserved from bacteria to humans—that serves as a universal signaling molecule. Its primary responsibility is to sense changes in the environment outside the cell and communicate those changes to structures in the interior of the cell to trigger a response.

In addition to the two subunits, PKA includes molecular features on its surface that enable it to integrate into the tightly regulated signaling machinery of the cell. Basically, cAMP activates PKA by plugging into the regulatory subunit, triggering it to release the catalytic subunit. The catalytic subunit opens the active-site cleft, which proceeds to phosphorylate the target protein. The regulatory subunits also bind to scaffold proteins thereby creating signaling units in close proximity to substrates.

While the function of many of these components was understood from other biochemical and structural studies, a major gap existed in understanding PKA, said Taylor. For example, scientists were still lacking a detailed structural view of the critical interface between the catalytic and regulatory subunits.

In the *Science* paper, the researchers report new information about this interface that improves the structural understanding of how the catalytic and regulatory subunits interact at the molecular level. At the outset of the studies, the researchers constructed a special version of the two bound subunits that was stable enough to be crystallized and subjected to structural analysis using x-ray crystallography. In this widely used technique, x-rays are directed through crystals of the protein or protein complex under study. The structure of the protein is then deduced by researchers who use computers to analyze the patterns of x-rays that are diffracted by atoms in the protein.

The resulting structure of the PKA subunits, said the scientists, reveals more precisely how the inhibitor sequence on the regulatory subunit docks to the active site, and how cAMP binding leads to activation. The structure also reveals that the large lobe of the catalytic subunit acts as a stable scaffold for

such functions as binding the regulatory subunit. In contrast, the new structure reveals that the regulatory subunit undergoes major conformational changes during cAMP binding and activation.

The insights from the new structure also reveal which individual amino acid units, or residues, in this lobe are common among kinases, and which ones could contribute to the diversity that enables kinases to insert themselves into the vast array of signaling mechanisms in the cell.

“What's unusual, about the protein kinases is that they are a very sophisticated enzyme family,” said Taylor. “They not only carry out catalysis, but they bind to many other proteins, and they do so in a very dynamic way. They use their surface to recognize other proteins—in a sense using different sets of surfaces as complex molecular ‘language.’ When they are in an inhibited state, they interact with one set of surfaces (molecules); and when they are active, they interact with a completely different set of molecules. They can bring together a whole signaling complex of proteins by adding phosphates and interacting with other proteins.

“So, the rules we are discovering for how the regulatory subunit binds to the catalytic subunit of PKA are now enabling us to ask what are some of the general rules for kinases,” she said.

Such studies could lead to a novel class of drugs to inhibit specific kinases to treat diseases, said Taylor. “Most of the highly specific inhibitors, such as Gleevec, interfere with the ATP binding site of the kinase,” she said. “But this kind of understanding of PKA presents a real opportunity to identify other kinds of molecules that could specifically target other sites on these enzymes.

“The kinases are a really exciting family of enzymes for biology because their role in regulation is so important,” said Taylor. “And now that we have these kinds of detailed structures, we can begin to computationally dissect these molecules and understand how they function. And from there, we can extend our understanding to how they build signaling networks with other proteins; as well as how they function internally—for example, how the binding of a molecule at one end of the protein can be sensed at the other end.”