A new kind of deregulation: UCSD/SDSC researchers find clues to cellular signal system

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A NEW KIND OF DEREGULATION: UCSD/SDSC RESEARCHERS FIND CLUES TO CELLULAR SIGNAL SYSTEM

In living organisms, processes including cell growth and division, tissue differentiation in embryos, memory and thought, and most of the complex instructions for cellular changes from birth to death are regulated by a family of enzymes called protein kinases.

Faulty regulation in these kinases is linked to several diseases, including diabetes and some cancers.

Now, scientists from UCSD and the San Diego Supercomputer Center have taken two major steps in understanding major elements of these enzymes and their activities.

In articles appearing today in the scientific journals Cell and Science, the researchers offer a three-dimensional description of the processes and how they offer templates for the rational design of drugs capable of regulating this intricate signaling system for clinical applications. The group also identified a novel and unexpected targeting signal that plays a key role in this cascade of events.

Combined, the work points to ways to attack a variety of human ailments ranging from birth defects to spinal cord injuries.

"All these protein kinases have to be kept off when you don't need them," said Susan Taylor, UCSD chemist and senior fellow at SDSC, and principal investigator of the two studies. "So a major way to regulate them is to inhibit their activity--turn them off--until needed, or to move them to a different part of the cell.

"Now that you have the basic structure and the function of one of these kinases and its inhibitor are known in detail, we can seriously begin to design some better analogs and inhibitors," she said.

Any highly evolved cell--plant, animal, or human--can be thought of as a massive dispatch station or telephone switchboard. Instead of voice, paper or electronic signals, however,

this system passes molecular messages back and forth in relays that circulate the signals from one cellular structure to another--from the nucleus to various places in the cytoplasm to (or through) the outer cell membrane and back.

One of the principal signaling systems is a constant dance of phosphorylation and dephosphorylation, that add highly charged phosphate groups to (or deleting them from) signaling molecules. The presence or absence of the phosphate groups changes the structure and function of the modified molecules--often other kinase molecules.

Parts of this system are explained for the first time in the two articles appearing today.

"What we have found are fundamental steps in the path along which kinases are activated, targeted to some part of the cell, and deactivated," said Lynn Ten Eyck, co-author of the Science paper who co-directs with Taylor the Computational Center for Macromolecular Structure. CCMS is an NSF-funded project at SDSC, under which the computation for the work reported was carried out.

When a signal, such as a hormone, binds to the outside of a cell, the cell must translate that signal into a biological response that causes the protein kinases to get busy inside. The "first messenger" to the cell is the hormone, and its binding signals the synthesis of a "second messenger," a small molecule inside the cell.

The kinase family contains several hundred distinct members, ranging from the simple protein kinase A (PKA), studied by the researchers, to more and more complex or specific kinases. For PKA, the first messenger is frequently the hormone adrenaline. The second messenger, inside the cell, is cyclic adenosine monophosphate (cyclic AMP or cAMP), which is present in all cells.

In its inactive state, the protein kinases studied consists of two catalytic subunits and two regulatory subunits (R). The separation of R from C, which is the key activation step, is triggered by the binding of cAMP to the R subunits. When cAMP molecules bind to the R subunits of PKA, the enzyme splits into three pieces--the two R subunits and cAMP molecules stay together, and the two C subunits are each freed to interact with other proteins.

In their study described in Science, Taylor's group examined a mutant but functionally reliable form of a single R subunit of PKA. Their studies revealed the detailed nature of the three- dimensional structure of this R subunit and the two sites (A and B) where cAMP binds to R--the "cAMP binding domain."

The single R units were crystallized and their structure studied by X-ray crystallography (both a multiwire areas detector at UCSD and a synchrotron at Stanford were used) in combination with computational refinement and modeling that required the aid of a large supercomputer (C90) at SDSC. Subsequent scientific visualization of the structure showed the specific topography of each cAMP binding domain as an arrangement of amino acids resembling a "jelly roll," according to Ying Su, a graduate student in Taylor's group and the Science paper's first author. The work constitutes her Ph.D. dissertation.

The subtlety here is in the order of binding. The first cAMP molecule binds to the B site, and this changes the shape or conformation of the R unit, setting the stage for easier binding of a second cAMP molecule, this time to the A site. This is called "cooperative binding," and although it was known to occur, the reasons for it, which have to do with the structure of the R unit, were only revealed by this research.

The other authors of the Science article include Taylor, Ten Eyck, Wolfgang Dostmann (of the Institute for Pharmacology at the University of Munich), Friedrich Herberg (of the University of the Ruhr at Bochum), Kyle Durick (a postdoctoral researcher in Taylor's group), Nguyen-hu Xuong (of the UCSD Departments of Biology, Chemistry, and Physics; inventor of the multiwire device), and K.I. Varughese (a senior researcher in Xuong's laboratory).

Turning back to an examination of the catalytic unit of PKA, Taylor and colleagues have also published a major paper in this week's Cell, a prestigious biological journal. The original solution of the C unit by the Taylor group in 1991 (Science,, vol. 263, p. 407) was of a complex consisting of the C unit and part of a well-known inhibitor of C's activity, called protein kinase inhibitor (PKI). By using fluorescent tags for parts of this inhibitor, the group demonstrated in laboratory experiments that a very small sequence of amino acids embedded in PKI constitutes a

signal that instructs the C subunit to leave the cell nucleus and move out into the cytoplasm, where it is available for binding to an R subunit.

This nuclear export signal (NES) is the first such signal identified. Scientists have discovered a number of nuclear localization signals (NLS), which binds to and escorts molecules containing an NLS into the cell nucleus. But there has been debate about whether a clear signal exists telling compounds to leave the nucleus. The Cell paper's first author is Wei Wen (another graduate student in the Taylor group), who also showed that a similar signal is found in other proteins, such as HIV-Rev, a protein that is essential for biosynthesis of the active HIV virus.

Other authors of the Cell paper include Taylor, Judy Meinkoth (Department of Pharmacology, University of Pennsylvania), and Roger Y. Tsien (Department of Chemistry and Biochemistry, UCSD, and Department of Pharmacology and Howard Hughes Medical Institute, UCSD School of Medicine).

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