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Technique Eavesdrops on the "Inner Life" of Proteins

Howard Hughes Medical Institute researchers have developed an analytical technique that can be used to reveal the amino acids that are critical links in the internal communications network of proteins.

These networks, which are embedded within intricate globular protein structures, are the routes by which different parts of protein molecules communicate important information, such as whether receptors on the protein's surface have been activated. This tight coordination of activity is critical to proteins' roles as key regulators of cell function.

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— David J. Mangelsdorf

According to the researchers, their tool—called statistical coupling analysis (SCA)—may help guide development of new classes of drugs that can specifically affect a single activity of a complex protein without globally altering its function, which could lead to unwanted side effects.

The researchers, led by Howard Hughes Medical Institute investigators David Mangelsdorf and Rama Ranganathan, published their findings in the February 6, 2004, issue of the journal *Cell*. The laboratories of both researchers are at the University of Texas Southwestern Medical Center at Dallas.

“The basic question in many signaling proteins is the way that these molecules are able to somehow communicate a molecular binding event at one site on the protein to a different molecular interaction site located at some distance,” said Ranganathan. In many proteins, the binding of a ligand to a receptor protein affects the protein structure in an entirely separate region, enabling that protein to trigger activity in a downstream protein that is part of a common cellular signaling pathway.

“So it is the essence of life, if you will, for such proteins to have structural mechanisms that can convey such information,” said Ranganathan. “In many ways, understanding these mechanisms remains one of the core problems of structural biology today,” he said.

The internal communication systems are known as allosteric networks. The networks transmit signals across the protein structure through changes in the shape of individual amino acids, the small building blocks that make up the protein.

While techniques to determine overall protein structure, such as x-ray crystallography, are crucial to understanding protein function, they often cannot reveal functional networks of interacting amino acids, said Ranganathan, whose laboratory concentrates on studying the signaling mechanisms of proteins. And efforts to reveal such networks by selectively altering individual amino acids through mutation are far too laborious to trace complex networks, he said.

Due to the limitations of laboratory approaches, Ranganathan and his colleagues decided to “eavesdrop” on the experiments that nature had already conducted in evolving large families of proteins with slightly different functions. Statistically analyzing how the patterns of amino acids are altered in such proteins could reveal which ones participate in the allosteric networks that coordinate the various functions of the protein.

“The basic idea is that while we couldn't do all the mutagenesis experiments to reveal allosteric networks, maybe evolution has done the experiments for us in carrying out systematic random mutagenesis while selecting for this function,” said Ranganathan. “So, if we can get a look at the record evolution has left us in all these sequences of related members of a protein family, we could extract these positional energetic relationships between amino acids.”

The researchers chose as their model the retinoid X receptor (RXR), a protein that is activated by the binding of a retinoid hormone and transmits that activating signal through its structure to trigger activation of genes in the cell nucleus. “This protein turned out to be a very nice system to study, since it has several distinct molecular surfaces whose activities have to be integrated in order to produce a final output, which is the control of gene expression,” said Mangelsdorf, whose laboratory studies the function of nuclear hormone receptors.

A prime example of such functional integration in RXR arises from the fact that RXR usually links with a similar protein to form a structure called a heterodimer. This double protein exhibits an unexplained phenomenon called a “phantom ligand effect,” in which activation of one partner in the heterodimer somehow reverberates through the protein complex to activate the other partner.

In applying SCA to reveal the allosteric network in the RXR protein, the researchers statistically compared the amino acid sequences of the ligand-binding domains of 560 members of the superfamily of receptor

proteins related to RXR. The analysis was designed to determine whether evolutionary variation of any single amino acid was statistically coupled to variation in another. Such coupling would suggest that the two amino acids had co-evolved and were likely part of an allosteric network necessary for the protein's function.

Their analysis of the RXR protein superfamily revealed a collection of amino acids that seemed to snake through the protein, forming a largely contiguous pathway connecting the protein's functional surfaces. Importantly, said the researchers, the network seemed to reach across the two components of the heterodimer and could explain the phantom ligand effect. The researchers also performed experiments in which they altered individual amino acids in the network, finding that these mutations could affect the communication of ligand binding in the partner proteins.

The researchers caution that only protein families with structural characteristics and an evolutionary history that permit such sequence comparisons are amenable to SCA. However, they said that a considerable number of such protein families do appear to exist.

The mapping of networks does not reveal the mechanisms by which they communicate information from one part of a protein to another—an understanding that will require considerable further study. “At this point, the statistical coupling analysis is a hypothesis generator that will enable researchers to guide experiments,” said Mangelsdorf.

The researchers believe that SCA could offer considerable advantages to researchers whose aim is to develop drugs that selectively affect particular functions of complex proteins, to avoid undesirable side effects. “It's known that different drugs that interact with the same receptor molecule can have very different effects—acting as agonists or antagonists, or somewhere in between,” said Mangelsdorf. “We believe that using SCA analysis to map allosteric networks will give researchers the ability to make mutations that affect the activity of one ligand but not another. This ability will offer a potential screening strategy for drugs that affect one response pathway over another.”