IN SEARCH OF BETTER IMMUNOSUPPRESSANTS

Cyclosporin-cyclophilin-calcineurin complex has been analyzed structurally

Researchers have unraveled the complex structure of a ternary complex formed by the drug cyclosporin A, the binding protein cyclophilin, and the enzyme calcineurin [Proc. Natl. Acad. Sci. USA, published online Sept. 6, http://www.pnas.org/cgi/content/abstract/192206699].

The study was carried out by professor of pharmacology and molecular science Jun O. Liu of Johns Hopkins University School of Medicine, professor of biochemistry and biophysics Hengming Ke of the University of North Carolina, Chapel Hill, and coworkers. The work could lead to better agents for immune suppression and a more in-depth understanding of the binding interactions and molecular mechanisms that underlie immunity.

The mechanism of action of immunosuppressive drugs like cyclosporin A and FK506—administered by transplant patients to prevent tissue rejection—has been a topic of longstanding study by a number of research teams.

A crucial breakthrough in the field was the discovery that binary complexes of cyclosporin A and FK506 that form in the body go on to form ternary complexes with a common endogenous target. That work was done by Liu (then a postdoc), chemistry and chemical biology professor Stuart L. Schreiber of Harvard University, Irving Weissman of the pathology department at Stanford University School of Medicine, and coworkers [Cell, 66, 807 (1991)].

The endogenous molecular target is the enzyme calcineurin, a phosphatase enzyme in the signaling pathway leading to immune-cell activation. The two ternary complexes that form are as follows:

- One forms between cyclosporin A, the immunophilin (immunosuppressant binding protein) cyclophilin, and calcineurin.
- Another complex forms between FK506, the immunophilin FK binding protein (FKBP), and the common calcineurin target.

Calcineurin is "the only case of a common protein target shared by two structurally distinct protein-ligand complexes," Liu explains. "Nature is teaching us an important lesson—that there is more than one solution to the problem of molecular recognition of the same protein target."

In 1995, the crystal structure of one of these ternary complexes (FK506-FKBP-calcineurin) was determined by crystallographer James P. Griffith and coworkers at Vertex Pharmaceuticals, Cambridge, Mass. [Cell, 82, 507 (1995)], and then independently by J. Ernest Villafranca and coworkers at Agouron Pharmaceuticals, San Diego [Nature, 378, 641 (1995)]. Both groups found that the binary FK506-FKBP complex binds at some distance from the calcineurin active site but is still able to hinder the approach of substrates to that site.

Liu, Ke, and coworkers have now closed the circle, in a sense, by obtaining the crystal structure of the other calcineurin-based ternary complex—the one formed by cyclosporin A, cyclophilin, and calcineurin.

The structure shows that cyclosporin-cyclophilin and FK506-FKBP—despite the lack of any significant structural similarity—bind to the same site in calcineurin. About four-fifths of the calcineurin residues involved in interacting with the former complex are also involved in binding to the latter—although some of the common residues interact with the two drug-immunophilin complexes in a strikingly different manner.

"It is daunting to imagine how these two complexes have come to existence during evolution, considering that cyclosporin A is produced by fungi while FK506 is of bacterial origin," Liu says. Liu, Ke, and coworkers hypothesize that the common binding site on calcineurin is likely also involved in the binding of calcineurin to endogenous substrates—helping to explain the enzyme's relatively narrow substrate specificity.

The Liu-Ke structure "confirms the broad picture of calcineurin inhibition" provided by earlier structure studies, comments Manuel A. Nava, senior vice president at Essential Therapeutics, Waltham, Mass., a coauthor of the 1995 Cell paper. For example, it once again highlights the plasticity of the calcineurin binding site, he says. However, the ability to design improved immunosuppressants will also depend on future studies on ways to reverse the nephrotoxicity and other serious side effects triggered by immunosuppressant-induced calcineurin inhibition, Nava notes.—STU BORMAN

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