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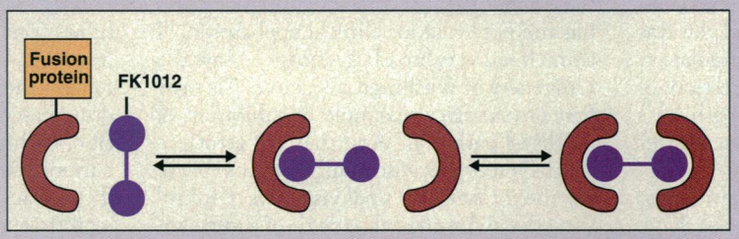
Making Molecular Matches in the Cell

Like most of us, many membrane-bound receptors and other cellular proteins get by only with a little help from their friends. These proteins must coalesce into groups of two, three, or more similar molecules before they exhibit any biologic activity. Investigators would love to have a method for bringing such proteins together at will inside the cell. It would help them understand how signals are sent from the cell surface to the nucleus, determine the function of new proteins, and even perhaps create "gene switches" for controlling gene expression in transgenic animals and humans undergoing gene therapy.

Now biologist Gerald Crabtree of the Howard Hughes Medical Institute at Stanford University, chemist Stuart Schreiber of Harvard University, and their co-workers have succeeded in producing just such a tool. Through deft modifications of a powerful immunosuppressive drug, they have synthesized "molecular matchmakers" that can aggregate genetically engineered proteins within cells (see page 1019 of this issue). Though the technique has so far been demonstrated only in cell culture, it is drawing rave reviews. "I think it's absolutely spectacular. The potential is extraordinary because this is a way to get inside the cell and regulate protein-protein interactions," says Richard Klausner, chief of the cell biology and metabolism branch at the National Institute of Child Health and Development.



A marriage made in cells. The two-headed FK1012 molecule brings two fusion proteins together by binding to their immunophilin domains.

Like many advances these days, this one comes from a cross-disciplinary team—a collaboration of chemists and biologists. Their first order of business was to search for molecules that could be manipulated to bring proteins of interest together. After looking at hundreds of candidate matchmakers, they homed in on a drug known as FK506, a fungus-derived organic substance that is used to suppress the immune system in transplant patients.

FK506 begins its immunosuppressive action by binding to a protein known as an immunophilin. Then this immunophilin-drug complex binds to and disrupts the function of another protein, called calcineurin. Calcineurin plays a major role in propagating a key immune system activation signal: the one that passes from the T cell receptor to the nucleus of T cells and turns on a number of genes. By blocking the T cell receptor's signal, FK506 is a very effective inhibitor of the immune system, but it has a major drawback: Cells outside the immune system also depend on calcineurin, so the drug is toxic to organs such as the kidney.

To turn FK506 into a matchmaker, however, required some major reconstruction. The Harvard and Stanford workers synthesized a two-headed derivative of the drug by covalently bonding two FK506 molecules. Not only did this create a molecule, called FK1012, that could bind two immunophilins at the same time, but since the covalent bond was placed where calcineurin would normally attach, the new drug has no apparent immunosuppressive or toxic actions.

That wasn't the end of the quest. What was needed was a way to use immunophilins, and FK1012, to hook more interesting proteins together. That took a neat bit of genetic engineering. By splicing together the DNA sequence for immunophilins with other DNA sequences, Crabtree, Schreiber, and their co-workers modified T cells to produce an artificial version of their T cell receptor, a so-call fusion protein. This manmade receptor was composed primarily of one of the receptor's intracellular chains—the ζ chain—and a few immunophilin domains.

When they add FK1012 to such T cells, says Schreiber, the drug clumps the immunophilins together and "brings the zeta chain along for the ride." And that alone is enough to start part of the signal cascade that activates a T cell. Indeed, treatment with FK1012 turned on a reporter gene that had been added to the T cells. Investigators could even silence this artificial signal with another FK506-derivative, a monomer that breaks up the immunophilin clustering by displacing FK1012.

Work on the T cell receptor system was gratifying, since it offered clear proof that the researchers had found a way to hook cellular proteins together. Indeed, immunophilin-FK1012 matchmaking should prove useful for almost any intracellular protein that needs to cluster in order to function. For instance, says Crabtree, "if someone clones the gene for a new receptor, this should quickly allow us to learn what it does." Investigators would alter cells to combine the novel receptor with immunophilins, then use FK1012 to draw the fusion proteins together and activate the receptor's signaling pathway.

Their collaborative group is already working with the Fas receptor, which, when activated, produces programmed cell death within hours. Though biologists don't know the external stimuli that trigger Fas, using FK1012 to convene synthetic immunophilin-Fas receptors now enables them to activate it at will. That could be a powerful tool in developmental biology. In transgenic animals expressing engineered Fas receptors, "one might kill a cell type at a particular time in development," notes cardiologist Edgar Haber of the Harvard School of Public Health, and observe the result in an embryo.

Transgenic researchers should find many other uses for the new tool. Just as Crabtree and Schreiber could turn their reporter gene on and off, it should be possible to do the same for any gene and thus create animals with inducible phenotypes, something investigators have desired for years. "It allows for an entirely new approach to regulating gene expression in cell culture and in vivo," says Haber, who believes the approach might help him create superior transgenic models of atherosclerosis.

This research may also have profound implications for human gene therapy. It might one day allow individuals, through a simple oral drug, to tell their bodies when to produce needed proteins. For example, instead of injecting insulin, a diabetic might simply swallow a drug like FK1012 that would turn modified insulin genes on. A number of gene therapy companies have already talked to Crabtree and Schreiber and to their universities, which hold the patents on the research. At least one company, GeneMedicine in Houston, has been developing its own system, based on synthetic steroids, of drug-controlled gene therapy. "The 'gene switch' is really the next wave of technology," says physician Fred Ledley, one of the company's founders. But for the moment, admits Schreiber, such grand visions are merely a "dream"; in the short term, FK1012 will help proteins meet their match largely for the enjoyment of basic researchers.

–John Travis