STRUCTURAL BIOLOGY

# Protein Matchmaker May Lead New Gene Therapy to the Altar

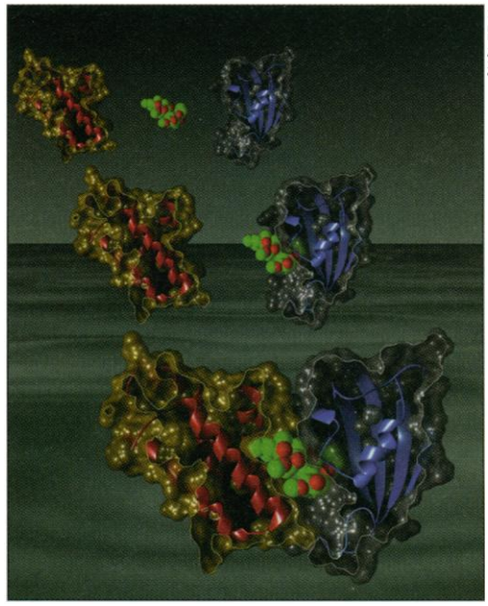
An ancient molecular weapon, devised by a lowly bacterium to fight off fungi and other competitors for scarce nutrients, could become one of the newest additions to the arsenal of drug design and gene therapy. A billion or so years ago, the ancestors of the soil microbe *Streptomyces hygroscopicus* evolved the ability to synthesize a molecule that could short-circuit the growth of neighboring organisms. In 1975, scientists isolated this multiringed compound, rapamycin, and found in addition to its antifungal activity that it was a potent immune suppressor. And now they have learned the surprising structure behind this function, which could make rapamycin and related compounds templates for a generation of new medicines.

On page 239, a team led by Jon Clardy at Cornell University in New York and Stuart Schreiber of the Howard Hughes Medical Institute at Harvard University in Massachusetts show, using x-ray crystallography, that rapamycin plays the role of a molecular marriage broker, linking two proteins that normally ignore each other into a complex, called a heterodimer, that actively interferes with the proliferation of the immune system's T lymphocytes. With a little molecular tinkering, researchers say, that structure might perform other functions as well. "The findings are very exciting," says Francis Dumont, an immunologist at the Merck Research Laboratories in Rahway, New Jersey.

For example, says Gerald Crabtree, an immunologist at Stanford University in California, portions of the two proteins linked by rapamycin, FKBP12 and FRAP, could be grafted onto other proteins. This would create molecular partners that, when linked by rapamycin or similar molecules, would perform specific functions, such as turning off or on cell signaling pathways or specific genes. Indeed, scientists at ARIAD Pharmaceuticals in Cambridge, Massachusetts, recently reported that a modified version of rapamycin could be used to control the production of human growth factor, a result that could lead to new strategies for gene therapy.

Researchers following rapamycin's tracks in the cell first learned that it bound to FKBP12 several years ago. Ordinarily, the FKBP family of proteins appears to assist in the assembly and transport of other proteins in the cell. But when bound to rapamycin, FKBP12 was found to play a rather different role by interfering with the cell cycle. In this way it blocked the proliferation of T lymphocytes—which explained rapamycin's immunosuppressant ability—as well as the growth of yeast and some other cell types. Experiments with T cells and yeast revealed that rapamycin's interference with the cell cycle seemed to involve gumming up an enzyme required for protein synthesis, known as p70 ribosomal protein S6 kinase.

It soon became clear, however, that the FKBP-rapamycin complex wasn't doing this job alone, because in test tube systems it didn't inhibit the kinase, implying that it was getting help from some other factor. In yeast, this turned out to be a series of proteins called TOR ("target of rapamycin"). In 1994, a mammalian homolog of TOR—named FRAP—was isolated by several laboratories, including Schreiber's group at Harvard University. Under normal conditions FRAP turns the kinase on, but when it is bound to the FKBP-rapamycin complex the switch is turned off.



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**Molecular marriage broker.** Rapamycin *(green)* brings two proteins together.

The new x-ray structure, which shows rapamycin linking the two proteins by fitting neatly into binding sites on each, may provide a clue to this effect, say Clardy and other researchers. Clardy thinks that the binding site on FRAP may correspond to a regulatory domain—perhaps targeted by natural regulatory molecules—that allows kinase activity to be switched on and off according to the organism's needs. "This is a nice juicy spot for a biological interaction," says Crabtree. By jamming the regulatory domain on FRAP, rapamycin may switch off the protein's activation of the kinase. If so, the identification of the FRAP binding site may ultimately prove fruitful for understanding how the protein normally regulates T cell proliferation and for designing new immunosuppressant drugs.

In all these effects, FKBP12, the other part of the heterodimer, actually seems to be a bystander. Indeed, because that protein is not normally involved in regulation of the kinase, it appears that rapamycin is playing matchmaker to two proteins that normally have little to do with each other. "It's clear that rapamycin shanghaied FKBP12 to interact with FRAP," says Clardy. One reason to doubt a natural relationship between them, he says, is the x-ray data showing that the proteins make relatively little contact with each other in the resulting heterodimer: "If they came together naturally there would be a fair amount of protein-protein interaction."

But the evidence that rapamycin can force a marriage between disparate proteins is exactly what is causing the most excitement at the moment. In a vivid demonstration of where this ability might lead, a team led by Michael Gilman at ARIAD has found that they can control the expression of a human gene using the rapamycin system. In results presented in May at a gene therapy meeting in Hilton Head, South Carolina, the ARIAD group genetically engineered two key elements of a DNA transcription factor, which turns on gene expression—a DNA-binding segment and an activation protein—and attached the binding segment to FKBP12 and the activation protein to FRAP. They then separately introduced these two protein complexes into a human cell line, along with an inactivated version of the human growth hormone gene.

When the cells were then implanted into mice, the ARIAD team was able to use a modified version of rapamycin to bring the two complexes together—and turn on production of human growth hormone, which was detectable in the mice's blood serum. Moreover, they could control the amount of growth hormone produced simply by varying the rapamycin dose the mice received. Clardy—who serves as a consultant to ARIAD—says that this demonstration is particularly exciting because "you can get therapeutically useful levels of expression, and you can keep that going for as long as you dose with rapamycin." And that's pretty advanced work for a billion-year-old fungicide.

–Michael Balter