Playing Tag With Membrane Proteins

Lipid tags are needed for the activity of many membrane-bound proteins, possibly because they help direct the proteins to the right cellular locations

OVER THE PAST DECADE, CELL BIOLOGISTS have learned that approximately one-fifth of all human cancers—including three big killers, lung, colon, and pancreatic cancer—are in part brought on by mutations in an oncogene that encodes a key protein in cell signaling. Imagine then the amount of attention both the oncogene, which is known as *ras*, and its protein product have received. Yet despite the many years of work, researchers still haven't worked out exactly how the Ras protein contributes to cancer. But suddenly there may be hope.

Recent findings in one of the hottest new areas of cell biology—the study of how proteins are modified by the attachment of fatty molecules known as isoprenoids—may lead not only to a better understanding of Ras function but also to a point of attack for interfering with that function and possibly helping to control cancer cell growth.

Although the isoprenoid modifications were first detected about 2 years ago, only now are their full extent and importance to the cell becoming apparent, as was made clear last month at the fall symposium* of the American Society for Biochemistry and Molecular Biology. Researchers in several labs have found that Ras and many fellow proteins—possibly as many as 100—undergo isoprenoid addition, in a reaction known as prenylation. Indeed, the once "esoteric" field of research has been moving so rapidly that at the symposium, the first ever devoted solely to the topic, Joe Goldstein of the University of Texas Southwestern Medical School in Dallas predicted that the 1990s would be the "decade of prenylation just as the 1980s is remembered as the decade of phosphorylation." He was referring to the transfer of phosphate groups between proteins and other molecules, which over the past 10 years has gained increasing attention as researchers have come to realize that it plays a vital role in normal cell signaling as well as in the activities of many of the proteins implicated in cancer development.

The isoprenoids used to modify the proteins originate from the same biochemical pathway that produces cholesterol, and so the interest of Goldstein and his long-time collaborator Michael Brown, also of Texas Southwestern, comes naturally, as an extension of their studies of cholesterol metabolism and inherited atherosclerosis (for which they shared the 1985 Nobel Prize). But they and their colleagues in the world of cell biology now have several reasons to be excited. For one, the proteins modified by prenylation are crucial in many signaling pathways within the cell. The Ras protein, for example, is apparently a component of a pathway transmitting growth-control signals, and the mutant proteins encoded by the *ras* oncogene are thought to be carcinogenic because they stimulate cell growth in an uncontrolled way.

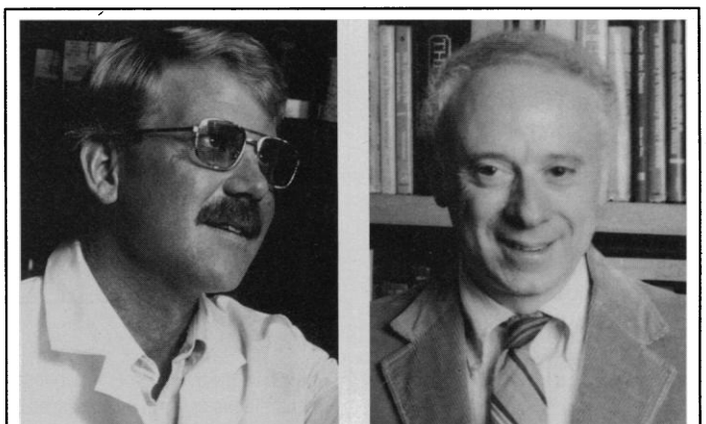
What's more, the prenylation work is also helping to clarify one of the major issues in cell biology: how proteins get targeted to their final destinations in the cell. In Ras's case, the protein, which is synthesized in the cytoplasm, somehow has to know that it is needed over at the cell's outer membrane and not at some other membrane within the cell. Scientists have long wondered what provides such a protein with its road map. Now an answer appears to be that many membrane proteins get a prenylated "tag" that helps to do the trick in the same way that a postal address on a letter directs it to the proper destination.

Researchers first started to appreciate the role of prenylation in general while trying to solve a puzzle about Ras targeting. The problem? Ras's function as a signaling protein is dependent on its being positioned at the cell membrane, but no one could figure out how the membrane binding was achieved.

It's not that there aren't lots of proteins that are adept at attaching to cell membranes. However, most of these contain a "transmembrane sequence," consisting of about 25 of the more lipid-soluble amino acids, which serves to lock the protein into its target membrane. Otherwise the protein couldn't penetrate the membrane's lipid layer. And that was what was so mysterious about Ras: It's a membrane protein without a transmembrane sequence.

The conceptual breakthrough came 2 years ago when three teams independently solved the problem. William Schafer, Jasper Rine, Sung-Hou Kim, and their colleagues at the University of California at Berkeley, and, independently, John Hancock and Chris Marshall at the Institute for Cancer Research in London, along with Anthony Magee and his group at the National Institute for Medical Research, also in London, showed that a lipid—specifically an isoprenoid—was appended onto Ras after it was synthesized. So Ras's lipid-inserting region was not built-in but was added on later. Meanwhile, Patrick Casey, then a postdoc in Alfred Gilman's lab at the University of Texas Southwestern Medical School, along with Channing Der and Janice Buss of the La Jolla Cancer Research Foundation in California, identified the isoprenoid as farnesyl, a lipid containing 15 carbon atoms.

The results immediately suggested a good therapeutic strategy for inhibiting the carcinogenic effect of Ras, says Casey, who is now at Duke University School of Medi-



Prenylation pursuers. *Patrick Casey* (left) *and Joe Goldstein are studying how isoprenoid lipids are added to proteins.*

*"Biological Significance of Lipid Modification of Proteins," American Society for Biochemistry and Molecular Biology, Fall Symposium, 11 to 14 October, Keystone, Colorado.

cine. "Since farnesyl addition to Ras is essential for expression of the oncogenic activity of Ras, the hope was to interfere with that process and stop Ras from inducing cancerous transformation in cells."

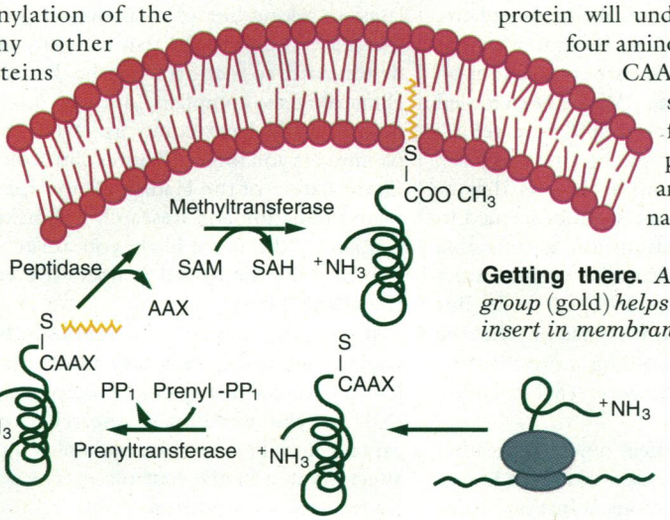
Which brings this tale to May of this year when postdoc Yuval Reiss and his colleagues in the Goldstein-Brown lab identified an enzyme, called farnesyl transferase, that attaches the farnesyl group to the Ras protein. If they could stop the transferase from doing that, they hoped, they would be on their way to sabotaging the oncogenic activity of Ras.

Of course, a question of enormous import remains: Will it be possible to inhibit Ras prenylation without interfering with the prenylation of the many other proteins that also undergo the reaction? There are encouraging signs. In the past year, enzymologists in the lab of Hans Rilling at the University of Utah and, independently, Christopher Farnsworth, Michael Gelb, and John Glomset at the Howard Hughes Medical Institute at the University of Washington in Seattle discovered that a majority of proteins are prenylated not by the 15-carbon farnesyl isoprenoid but by the 20-carbon geranylgeranyl isoprenoid. Since Casey's group showed that a different enzyme puts the geranylgeranyl group on proteins, the hope was that it would at least be possible to inhibit the farnesyl transferase without affecting geranylgeranyl addition.

But this hopeful picture has recently gotten more complicated. The transferase enzymes are composed of two protein subunits, designated alpha and beta, and last summer Miguel Seabra, a graduate student in the Goldstein-Brown lab, Reiss, and Casey showed that the alpha subunit of both transferases is the same. So a drug that works by inhibiting the alpha subunit may result in a very nonspecific shutdown of prenylation in general, explains Casey.

Still, all may not be lost because the beta subunit is not shared by the two transferases. Indeed, it's this subunit that determines whether the transferase will add farnesyl or geranylgeranyl groups to proteins, and the beta subunit might thus be a target for specific drug therapy. Development of such drugs may be facilitated, the researchers say, by results obtained during the past 4 months that are helping to decipher the rules by which the beta subunit recognizes its target protein. "Before then, the rules were completely confused," says Casey. "Now that we have the relevant enzymes, we know which enzymes prefer which protein. We can now predict fairly well which isoprenoid will be attached to which protein."

The key to the type of prenylation a protein will undergo lies in its last four amino acids, the so-called CAAX box, which consists of a cysteine(C), followed by two aliphatic (A) or linear amino acids, and finally ending in any of several different amino acids (X). If X is serine, methionine or glutamine, the protein will be farnesylated. But if the final amino acid of the CAAX box is leucine, then the isoprenoid used will be a geranylgeranyl group. Geranylgeranyl addition can also be signaled by two other motifs, a CC or CXC sequence on the end of the protein, according to work done by Gelb, Glomset, and their colleagues, and, independently, by William Maltese's group at the Weis Center for Research in Pennsylvania and by Der's team in collaboration with Michael Sinensky at the Eleanor Roosevelt Institute for Cancer Research in Denver, Colorado. The specificity of recognition by the prenylating enzymes for the CAAX box and related motifs is so high that it has renewed hopes of finding therapeutic agents that can specifically block farnesylation, say, without affecting geranylgeranyl addition.



SOURCE: PATRICK CASEY ILLUSTRATION: J. CHERRY

Getting there. *Addition of a prenyl group* (gold) *helps proteins such as Ras insert in membranes.*

Another major issue the researchers are now addressing concerns the significance of isoprenoid addition for normal cell functioning. "Ever since we learned that two isoprenoids can be used, we have been wondering whether they have specific purposes," says Casey. Could it be, for example, that the different prenylation tags are analogous to different postal addresses?

The answer to that question, Casey says, is usually yes. Proteins like Ras, which are attached to the outer cell membrane, are generally modified by a farnesyl group. In contrast, proteins that are typically found on membranes on structures within the cell are more likely to be modified with a geranylgeranyl group. But those broad distinctions don't always hold, and the exceptions suggest, Casey points out, that prenylation by itself doesn't determine a protein's cellular localization.

In one intriguing experiment, for example, Adrienne Cox, a postdoc in Der's lab, swapped prenyl groups, giving a nononcogenic form of the Ras protein a geranylgeranyl group instead of the usual farnesyl. In work that many researchers say has provided one of the first demonstrations that the isoprene group does make a difference, Cox has shown that the prenyl group may alter the site at which the protein becomes attached to the membrane. Specifically, she found that the altered Ras protein could barely perform its usual growth-promoting function. "When we substituted a geranylgeranyl group on normal Ras, we may have directed it to a site on the membrane where it was out of reach of the events that have to activate it, so it couldn't perform its normal function, and cell growth was stopped," says Cox.

But if prenylation can direct the site of attachment on a particular membrane, what accounts for the choice of membrane in the first place? One answer, says John Hancock, now of the Royal Free Hospital Medical School in London, may be additional amino acid sequences within the protein. He and Marshall have found that a series of six iterations of the positively charged amino acid lysine, just adjacent to the CAAX box in a Ras protein, are essential to lead the protein to the plasma membrane. He suggests that the lysine-rich region helps prenylated proteins find their way to the correct location on the cell membrane by interacting with other proteins there.

Even as some aspects of protein targeting are being worked out, biologists are starting to ask new questions. What advantage does lipid modification offer over the built-in membrane-spanning regions carried by most other cell membrane proteins? Seabra and others speculate that prenylation may turn out to have an important regulatory function, since some prenylated proteins may cycle on and off the membranes with which they are associated and having an isoprenoid tag, rather than a built-in membrane-targeting sequence, may facilitate the cycling. Of course, working out the details of prenylation's function and mechanism will occupy biochemists to the next decade.

■ MICHELLE HOFFMAN