Covalent modification of proteins facilitates a tremendous expansion of their functional potential. Some modifications serve to enlarge the chemical diversity of proteins beyond that provided by the 20 standard amino acids utilized in protein synthesis, providing the new shapes and reactive groups that allow new types of binding and catalytic interactions. Other modifications, often reversible, serve to modulate protein function. Among the large number of reactions that modify intracellular eukaryotic proteins are three sequential enzymatic steps that recognize proteins synthesized with a C-terminal CAAX tetrapeptide motif, where C is a cysteine residue, A is generally an aliphatic residue, and X can be a variety of residues (1–3). Such proteins are initially lipidated in a reaction that adds either a 15-carbon farnesyl or a 20-carbon geranylgeranyl group to the sulfur atom of the cysteine residue. The third step is a potentially reversible methyl esterification reaction of the newly exposed C-terminal cysteinyl carboxyl group by a membrane-bound enzyme of the endoplasmic reticulum (Figure 1). Many proteins are modified in this way, including some nuclear lamins, a cGMP phosphodiesterase, and several small G-proteins involved in cell signaling, such as the Ras and Rho proteins. The importance of Ras, especially the activated oncogenic forms, has led to a large interest in this pathway in terms of the possibility of controlling human cancers.

These reactions can provide the modified protein increased membrane attachment with the newly formed hydrophobic C-terminus (4), directed binding to signaling partners via the isoprenyl and methyl groups (5, 6), and protection against proteolytic degradation (7, 8). In this issue of the JCI, Bergo et al. provide new insight into these functions and have identified new targets for the development of anticancer drugs (9). In this study, they developed transgenic mice (and fibroblast cell lines derived from these mice) where the expression of the Icmt gene encoding isoprenylcysteine carboxyl methyltransferase (Icmt) can be controlled by the Cre-loxP system. There are two striking results from this study. First, the authors show that loss of the methyltransferase can have different effects in different methyl-accepting proteins. For example, they show that Ras proteins are stabilized but the RhoA protein is destabilized. Second, they show that the decreased levels of RhoA in the absence of methylation results in increased levels of p21Cip1 and the inhibition of the cell cycle. The authors thus present a case for anticancer therapeutic approaches based on inhibiting Icmt.

**Fighting cancer by disrupting C-terminal methylation of signaling proteins**

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Protein methylation at the C-terminus of mammalian isoprenylated proteins has been implicated in membrane attachment, protein-protein interactions, and protein stability. A new paper describes surprising results: in the absence of methylation some target proteins have increased stability, whereas others have decreased stability. The decreased stability of the RhoA protein is correlated with an increased resistance to Ras-dependent transformation and suggests the basis for the development of a new approach to antitumor therapy (see the related article beginning on page 539).

Stabilization of isoprenylated proteins by methyl ester formation

Unmethylated isoprenylated cysteine groups at the C-terminus of a protein can be particularly susceptible to proteolytic attack. For example, in yeast strains lacking the STE14 gene encoding the ortholog of the mammalian Icmt gene, a soluble form of the Ras protein accumulates and has the gel mobility of the non-isoprenylated precursor protein rather than the unmethylated protein (7, 10). Unmethylated intracellular yeast a mating factor is subject to a fivefold higher rate of proteolytic degradation (11). In mammalian cells, inhibition of methylation in a macrophage cell line results in the decrease of the half-life of the RhoA small G-protein from 31 to 12 hours, and of the Cdc42 small G-protein from 15 to 11 hours (8).

Biochemists search for simplicity in understanding metabolic reactions, and it was thus reassuring to see that Bergo et al. found that the level of RhoA was reduced 90 to 95% in Icmt-deficient fibroblasts (9), clearly confirming the results of Backlund (8) in a distinct cell culture system. In fact the complete absence of Icmt in the fibroblasts led to an even greater reduction in the half-life of RhoA from 22 to 2.8 hours. However, nature often reveals her more complex side, and Bergo et al. found that another isoprenylated signaling protein displayed just the opposite behavior! They measured an increase in the half-life of K-Ras from 13.9 hours to 32.5 hours (9). Clearly, there is now no direct relationship between proteolytic stability and the presence of the carboxyl terminal methyl ester.

Loss of methyl esterification by the Icmt enzyme is associated with cell growth inhibition

Bergo et al. found that inhibition of the methyltransferase caused reduced growth and inhibited K-Ras-induced oncogenic transformation (9). While this result is exciting, it is perhaps not totally unexpected, given that K-Ras is a substrate for isoprenylation and methylation. What was surprising, however, was the authors’ finding that the effect was apparently not directly associated with the inhibition of K-Ras function itself; Ras-dependent growth factor–induced activation of Erk and Akt was unaffected. It turns out that much of the inhibition of transformation can be laid at the door of unmethylated RhoA, where the reduction in protein level is correlated with an upregulation of the p21Cip form that binds cyclins and stops the cell cycle. Furthermore, the effect of Icmt deficiency is not limited to K-Ras–induced transformation: transformation induced by an activated (V599E) form of B-Raf was also attenuated by the loss of Icmt.

Signaling from the endoplasmic reticulum

The finding by Bergo et al. (9) that K-Ras can signal activation of downstream events — even though they show that K-Ras is not associated with the plasma membrane — is quite intriguing. What could account for this observation? It is possible that a small amount of K-Ras is associated with the plasma membrane and is enough to signal downstream events. It is also possible that a Ras-independent pathway functions to catalyze growth factor–induced activation of Erk and Akt in the absence of Icmt. Finally, Bergo et al. (9) suggest the possibility that K-Ras can signal from intracellular locations such as endoplasmic reticulum and Golgi. This idea is particularly interesting, given the recent report that Ras targeted to the intracellular membranes can activate the Erk pathway (12).

Cancer therapeutics

Inhibition of posttranslational modification of signaling proteins provides a promising approach to anticancer therapy. Enzymes catalyzing the three consecutive biochemical reactions on CAAX motif proteins are all potential targets of drug action. Much of the recent work has focused on small-molecule inhibitors of the protein farnesyltransferase that catalyzes the addition of a C-15 isoprenyl group to the cysteine side chain of many CAAX motif-containing proteins (13). Many of these...
compounds are competitive inhibitors of farnesyltransferase with respect to its substrates farnesyl pyrophosphate and the CAAX motif (1, 14). In addition, compounds that inhibit farnesyltransferase by chelating zinc, a tightly associated metal that is involved in catalysis, have been identified (15). In preclinical studies, these compounds could inhibit the growth of tumors in mice and even cause regression of Ha-Ras–activated tumors (14). Several inhibitors are currently being evaluated in clinical trials, and beneficial effects have been reported with hematological and solid malignancies (16). However, it has been disappointing that these inhibitors have so far failed to exhibit efficacy against pancreatic tumors where oncogenic forms of Ras are found at high frequency (16). This failure has been attributed to the action of the apparently redundant geranylgeranyltransferase I on K-Ras.

The mixed success with farnesyltransferase inhibitors has led investigators to explore the possibility of targeting the protease Rce1, which removes the AAX residues, as well as the Icmt enzyme. Since only a single enzyme catalyzes each of these reactions (17, 18), this approach may be more effective. It has been shown, for example, that Rce1 inactivation reduces Ras-induced transformation (19). The results of Bergo et al. (9), it now appears worthwhile to resued the search for more effective inhibitors of the Icmt-encoded methyltransferase that may mimic the antiproliferative effect of the knockout of this enzyme in mice. Because the Icmt enzyme is one of a relatively small class of methyltransferases characterized by multiple membrane-spanning segments, it may be possible to develop a new class of inhibitors that take advantage of this particular structure (3).

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