Protein Prenylation: A Mediator of Protein-Protein Interactions

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Over the last 5 years it has become apparent that prenylation of proteins on COOH-terminal cysteine residues can play an important role in protein function. About 0.5% of the proteins in animal cells is thought to be prenylated, and defective prenylation has been implicated in the etiology of human choroideremia, an X-linked disease characterized by retinal degeneration (*1*). Prenylation substantially increases protein hydrophobicity, and this effect is enhanced by carboxyl methylation of the prenylated cysteines. Although prenylation is likely to facilitate protein interaction with membrane lipids, new evidence suggests that it may also mediate protein-protein interactions.

Prenylated proteins in mammalian cells fall into at least four size classes: 66 to 72 kD, 53 to 55 kD, 41 to 46 kD, and 21 to 28 kD (*2*). There are two types of prenyl groups, C_{15} *trans,trans*-farnesyl and the more abundant C_{20} all-*trans*-geranylgeranyl (Fig. 1). The majority of proteins in the larger size ranges, such as the nuclear lamins, appear to be farnesylated. Proteins in the 21 to 28 kD class, which include members of the Ras superfamily of small guanine nucleotide binding proteins, are mainly geranylgeranylated, although $p21^{ras}$ itself is farnesylated. In addition to the guanine nucleotide binding proteins, a growing number of other proteins involved in signal transduction (for example, rhodopsin kinase and the α and β subunits of cyclic guanosine monophosphate phosphodiesterase) appear to carry this modification.

Three sequence motifs for prenylation have been identified (*3*). The CaaX motif, which is found at the COOH-terminus of nuclear lamins, $p21^{ras}$, and many Ras-related proteins, directs a triplet of coupled modifications—prenylation, proteolysis of the last three (aaX) amino acids, and carboxyl methylation of the COOH-terminal prenylated cysteine (Fig. 1). A major determinant of whether a protein is farnesylated or geranylgeranylated is the X position. If X is serine, alanine, or methionine, the protein is farnesylated; if X is another residue, especially leucine, the protein is geranylgeranylated (*3*). The second motif for prenylation is CXC, which, in the Ras-related protein Rab3A, leads to geranylgeranylation on both cysteine residues and methyl esterification (*3*). The third motif, CC, is also found in members of the Rab family, where it appears to direct only geranylgeranylation and not carboxyl methylation (*3*).

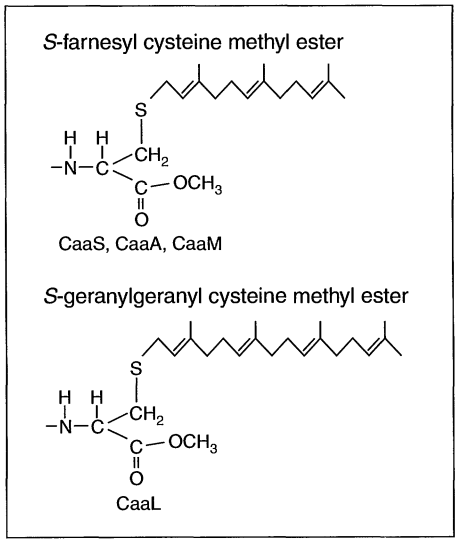

Fig. 1. Structures of prenyl groups. These thioether-linked prenyl groups are found at the COOH-terminal cysteine of proteins whose primary translation products terminate in a CaaX motif (where "C" is cysteine, "a" is an aliphatic amino acid, and "X" is any amino acid). When the X position is serine, alanine, or methionine, the protein is farnesylated; when X is leucine, the protein is geranylgeranylated.

The enzymes that catalyze prenylation are now well characterized. Farnesyl transferase (FT) and geranylgeranyl transferase type I (GGTI), which prenylate CaaX-containing proteins, share a common prenyl pyrophosphate binding α subunit but have distinct β subunits (*4*). Because the farnesyl modification on $p21^{ras}$ oncoproteins appears to be necessary for transforming activity, there has been considerable interest in developing inhibitors of FT as anticancer agents (*5*). The geranylgeranyl transferase active on Rab CC proteins (GGTII) consists of a B component containing subunits related to those of FT and GGTI and an unrelated A component that is deficient in cells from choroideremia patients (*1*). This exciting result suggests that a Rab protein is required for the normal development or function of the visual system.

What is the function of protein prenylation? Many prenylated proteins are associated with intracellular membranes, and multiple experiments have shown that mutation of cysteine prenylation sites blocks membrane localization (*3*). Prenylation markedly increases protein hydrophobicity, with the longer chain length of geranylgeranyl having a greater effect than farnesyl (*6*). It is not yet clear why some proteins are farnesylated and others geranylgeranylated. In the case of $p21^{H\text{-}ras}$, the distinction is important, as replacement of a geranylgeranyl group for the native farnesyl group can make $p21^{ras}$ an inhibitor of cell growth (*7*).

Increased hydrophobicity is one potential mechanism that may explain the affinity of prenylated proteins for membranes. However, there are a number of reasons for thinking that lipid association may be only part of the way in which prenylated proteins bind to membranes. First, proteins with the same prenyl group can localize to different intracellular membranes—Rap1 and Rab5, for example, are found in the Golgi apparatus and early endosomes, respectively (*8*). Second, when the CaaX motifs from Ras proteins are fused to heterologous proteins, the chimeric proteins are correctly prenylated and carboxyl methylated but remain cytosolic; plasma membrane association occurs only when additional COOH-terminal sequences are included in the chimeras (*9*). Third, the branched structure of the prenyl group suggests that it might not readily insert into the lipid leaflet of cellular membranes.

One solution to these problems is to propose that there are receptor molecules in the membrane that interact with the prenyl group and with other regions of the protein, such as the palmitoylated residues or polybasic domains of $p21^{ras}$ (*9*). This putative receptor could be a protein, in which case it could be argued that prenylation and carboxyl methylation are required not only for interactions with lipids but also for interactions with proteins. Indeed, there is increasing evidence to support this hypothesis. One example is the demonstration that the interaction between the α subunit and the β-γ subunits of transducin is much more favored if the γ subunit is farnesylated and carboxyl methylated (*10*).

Further examples are provided by studies on the proteins that regulate small guanine nucleotide binding proteins. The latter proteins cycle between an active guanosine triphosphate (GTP)–bound form and an inactive guanosine diphosphate (GDP)–bound form. In the GDP-bound state, the proteins interact with exchange factors that regulate GDP release, whereas in the GTP-bound state they interact with effectors and GTPase-activating proteins (GAPs); for example, $p21^{ras}$·GTP is presumed to interact with an effector for cell proliferation (*11*). Are these interactions dependent on prenylation? Stimulation of GDP release by an activity referred to as guanine nucleotide dissociation stimulator converts these proteins to their GTP-bound form. A GDS active on several members of the Ras superfamily, including $p21^{K\text{-}ras4B}$, $p21^{rhoA}$, and $p21^{rap}$, requires a

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prenylated COOH-terminus on the guanine nucleotide binding protein for activity (*12*). This requirement is seen in the absence of membranes, suggesting that prenylation is needed for the protein-protein interaction. Since carboxyl methylation only occurs on prenylated proteins, it cannot be ruled out that it is the methylated COOH-terminus that is important in the protein-protein interaction.

Are prenylation and carboxyl methylation required for the function of a guanine nucleotide binding protein once it is in the GTP-bound state? Prenylation does not seem to be required for the interaction of $p21^{ras}$ with GAPs, but blocking farnesylation of oncogenic $p21^{ras}$ inhibits transformation, suggesting that farnesylation is critical for effector function (*3*). A direct biochemical link between prenylation and $p21^{ras}$ function is the demonstration that activation by $p21^{ras}$ of a membrane-free adenylyl cyclase complex in *Saccharomyces cerevisiae* is much more effective with farnesylated $p21^{ras}$ than with the nonfarnesylated form (*13*). Similarly, in membrane-free extracts from *Xenopus* oocytes, prenylation of $p21^{ras}$ is necessary for activation of the mitogen-activated protein kinase pathway (*13*). These results suggest that signaling by $p21^{ras}$·GTP is dependent on the farnesylated COOH-terminus and that this dependence may reflect an interaction with a protein effector rather than with membranes. Indeed, the effector may even be cytosolic.

Other interactions of the Ras superfamily proteins also require prenylation. Takai and co-workers identified GDP dissociation inhibitors (GDIs) for Rab3A and for $p21^{rho/rac}$. These proteins form 1:1 stoichiometric complexes with their respective guanine nucleotide binding proteins only if the latter are posttranslationally modified (*14*). A striking property of GDIs is that they release their cognate guanine nucleotide binding proteins from membranes (*15*), presumably by burying the prenyl and carboxyl methyl groups in a hydrophobic pocket. The choroideremia susceptibility gene product has sequence homology with GDIs, suggesting that it may allow the geranylgeranylated substrate of GGTII to be released into an aqueous cytosolic environment.

The ability of GDIs to release proteins from membranes suggests that prenylated proteins can cycle between membranes and cytosol in a regulatable manner. This idea fits well with the extensive evidence that members of the Rab family are involved in intracellular vesicle trafficking (*16*). But do other prenylated proteins cycle between cytosol and membrane? An exciting possibility comes from the observation that activation of the superoxide-generating system in neutrophils requires the rho-related protein $p21^{rac}$ as well as membranes and two cytosolic components, p47 and p67 (*17*). After activation, p47 and p67 translocate to the membrane, and it is conceivable that this translocation occurs in association with release of $p21^{rac}$ from its cytosolic complex with rho GDI.

Cycling between cytosol and membrane is not confined to guanine nucleotide binding prenylated proteins; rhodopsin kinase translocates from the cytosol to the retinal membranes after photon stimulation (*18*). This translocation is absolutely dependent on the farnesylation of rhodopsin kinase at its CVLS motif. The studies with Rab and rho GDIs suggest that the cytosolic rhodopsin kinase may form a 1:1 stoichiometric complex with a GDI-like molecule. Interestingly, a mutant form of rhodopsin kinase that is modified by geranylgeranyl has been found to localize constitutively at the membrane, showing that the translocation system requires a specific prenyl modification (*18*).

Thus, protein prenylation may be required both for interactions of proteins with membrane lipids and for protein-protein interactions. In concert with GDI-like proteins, the prenyl modification may function generally in the cycling of proteins between cytosol and membranes. For guanine nucleotide binding proteins, the association with GDI-like molecules may be regulated by exchange of GDP for GTP (Fig. 2).

Recent evidence suggests that carboxyl methylation may also be involved in signaling and in protein cycling between cytosol and membranes. Neutrophils contain a cytosolic pool of prenylated but not carboxyl methylated Ras-related proteins. Stimulation with FMLP, a chemoattractant for neutrophils, activates superoxide generation, aggregation, and granule release and leads to membrane association and carboxyl methylation of Ras-related proteins, including $p21^{rac2}$ (*19*). Prevention of carboxyl methylation by *N*-acetyl-*S*-*trans*,*trans*-farnesylcysteine blocks the superoxide response to FMLP. This carboxyl methylation of Ras-related proteins appears to be a consequence of GTP binding, as carboxyl methylation can be stimulated by GTP in cell-free systems (*19*). Thus it appears that carboxyl methylation in response to guanine nucleotide exchange is an additional factor regulating the association of prenylated proteins with their partners in signal transduction.

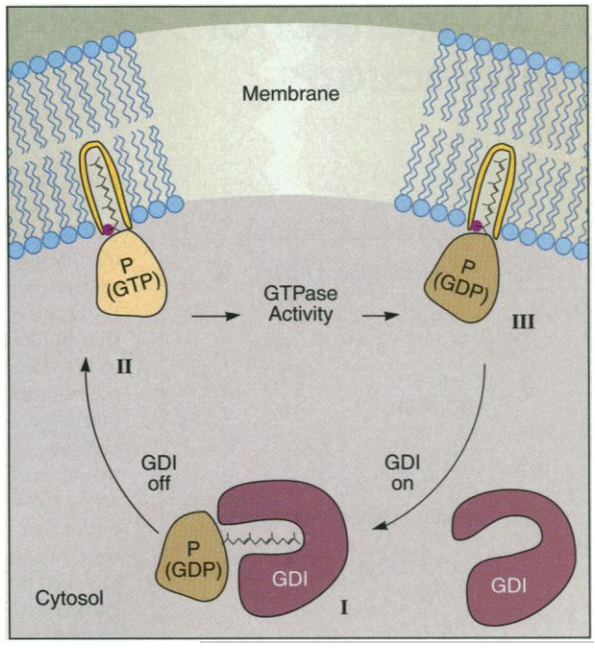

Fig. 2. Model for membrane cycling of a prenylated protein. This model postulates that there is a membrane receptor (depicted here as an integral membrane protein) for the prenylated protein (P). A membrane-bound guanine necleotide binding protein in state II can interact with exchange factors to promote exchange of GDP for GTP. It can then interact with effectors and GAPs, which results in GTP hydrolysis and conversion to state III. Once in state III, the protein is released to the cytosol (state I) by GDI. The membrane-bound forms of the protein are shown in carboxyl methylated form (pink dot), as suggested by (*19*).

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