PROTEIN PRENYLATION:
GENES, ENZYMES, TARGETS,
AND FUNCTIONS

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INTRODUCTION

The regulation, functional activation, and intracellular targeting of biological macromolecules are often mediated through the covalent attachment of specific chemical moieties. The posttranslational modification of specific proteins by phosphorylation, glycosylation, acetylation, fatty acylation, and methylation has been studied extensively, and a variety of critical biological functions have been ascribed to these particular side groups. In contrast to these familiar types of chemical modification, protein prenylation is a process
which, until recently, was poorly understood. This article reviews the current understanding of the biological role for prenylation in the function of specific eukaryotic proteins, focusing on genetic studies of specific prenylated proteins and the enzymes that catalyze protein prenylation. In addition, we discuss possible models for how prenyl groups mediate specific protein functions, such as intracellular protein targeting. We also speculate on the possible relevance of protein prenylation to the medical problems of oncogenesis and hypercholesterolemia.

Prenylation refers to the covalent modification of a molecule by the attachment of a lipophilic isoprenoid group (reviewed in 26, 29, 46). Isoprenoids are a diverse family of lipophilic molecules based on a repeating five-carbon structure. Farnesyl diphosphate, a 15-carbon molecule, and geranylgeranyl diphosphate, a 20-carbon molecule, are the isoprenoid compounds most relevant to protein prenylation. Other biologically important isoprenoid molecules include cholesterol and other steroids, carotenoids, terpenes, and dolichols. A variety of small molecules are prenylated, including quinones (coenzyme Q), porphyrins (heme a and chlorophyll), amino acids (dimethylallyl tryptophan), and purines (cytokinins). In addition, some transfer RNA molecules are prenylated at a specific adenine residue. All isoprenoids are synthesized from the six-carbon precursor mevalonate through a common biosynthetic pathway (47).

Many specific proteins are posttranslationally modified with prenyl groups. Most, if not all, prenylated proteins are modified by the attachment of either a 15-carbon farnesyl or a 20-carbon geranylgeranyl group (117) in a thioether linkage to a cysteine residue. In most organisms, geranylgeranylation is a more common modification than farnesylation, although the relative proportions of farnesylcysteine and geranylgeranylcysteine in total cellular protein vary somewhat between organisms and cell types (37). It is possible that other types of prenyl modification of proteins may exist; for example, a mammalian protein appears to be covalently modified with a prenylated adenine moiety (39). Prenylated proteins are found in a variety of cellular compartments, including the nucleus, the cytosol, and membrane-bound organelles (85, 129).

GENES ENCODING PRENYLATED PROTEINS

Most of the prenylated proteins whose identities have been determined belong to one of of four protein families: small GTP-binding proteins, lipopeptide pheromones, nuclear lamins, and trimeric G-proteins. The importance of prenylation for the functions of these proteins is discussed below.

The Ras Superfamily

Most of the known prenylated proteins are members of the Ras superfamily of low-molecular-weight guanine nucleotide-binding proteins. These proteins
can be divided into four smaller protein families: the Ras, Rho, Rap, and Rab/Ypt families (23). These proteins participate in a variety of cellular functions, including control of cell growth and differentiation, cytokinesis, and membrane trafficking. All small GTP-binding proteins contain cysteine residues at or near the carboxyl-terminus that appear to serve as targets for posttranslational prenylation. At least three distinct types of prenyl-dependent processing are involved in the maturation of these proteins, each of which affects a distinct subset of Ras-related proteins with a distinct carboxyl-terminal motif.

THE CAAX MOTIF The Ras proteins are plasma membrane-localized molecules that regulate cell differentiation and proliferation in mammalian and yeast cells. Ras proteins contain a carboxyl-terminal sequence called a CaaX motif, with a cysteine followed by two aliphatic residues and a carboxyl-terminal “X” residue, which can be C, S, M, Q, or A (135). Ras proteins undergo farnesylation of the cysteine residue (21, 54), proteolytic removal of the three amino acids distal to the cysteine, and methylation of the carboxyl-terminus (35, 50). Some Ras proteins are also palmitoylated at a cysteine residue near the farnesylated cysteine (19). Ras proteins that lack this second cysteine residue are not palmitoylated but contain instead a region of basic amino acids in the hypervariable domain near the carboxyl-terminus (54). Ras is synthesized on soluble cytoplasmic ribosomes (50, 130), and is localized to the plasma membrane independently of the secretory pathway.

The localization and functional activity of Ras proteins depends on the presence of the farnesyl group. Genetic or pharmacological inhibition of protein prenylation blocks the membrane association and biological activity of both human and yeast Ras proteins (54, 124). However, since RAS2 protein from Saccharomyces cerevisiae lacking the modifiable cysteine retains some functional activity when overexpressed in vivo (34), it appears that the function of prenylation is primarily to direct the polypeptide to the correct intracellular location. Proteolysis and methylation appear to play lesser roles in mediating the association of Ras proteins with membranes. Whereas human Ras proteins lacking these modifications exhibit a reduced affinity for membranes (52), mutant K-ras alleles that prevent proteolysis and methylation do not block the membrane association or the transforming activity of the K-ras protein in vivo (71). Similarly, yeast mutations that cause a defect in carboxymethylation do not block Ras localization or functional activity (63, 90). In addition to the farnesyl group, either a palmitoylation site or a polybasic region is required for efficient localization to the plasma membrane and is important for the transforming activity of oncogenic mutant proteins (55).

THE CAAL MOTIF A second group of small GTP-binding proteins contain a carboxyl-terminal motif similar to a CaaX box, called a CaaL motif, with a
leucine residue in the carboxyl-terminal position. This group includes many proteins implicated in cytokinesis, such as the Rap and Rho protein families. Many of these proteins are known to be geranylgeranylated, including G25K (143), RhoA (70), RaI, Rac (74), Rap1a (18), and Rap1b (72). Several of these proteins are also known to undergo carboxyl-terminal proteolysis and carboxymethylation (18, 70, 72). Geranylgeranylation of these proteins is widely thought to mediate association with intracellular membranes; however, only in the cases of Rap1 and Rho has this hypothesis been supported experimentally.

Rap1a and Rap1b are a pair of closely related genes that are members of the Ras family of small GTP-binding proteins (108). Overexpression of Rap1a (also known as K-rev-1) results in suppression of the transforming activity of activated Ras alleles (76). Unlike Ras proteins, which are localized to the plasma membrane, Rap1 proteins are associated with the Golgi complex (13). The posttranslational processing at the carboxyl-terminus of the Rap1b protein has been investigated in detail. The carboxyl-terminus, which has the sequence K-A-R-K-S-S-C-Q-L-L, undergoes four modifications: (a) geranylgeranylation of the cysteine residue; (b) proteolytic removal of the three terminal amino acids; (c) methylation of the new carboxyl-terminus, and (d) phosphorylation of the distal serine residue by A-kinase (72). A prenylated carboxyl-terminus is required for the association of Rap1b with membranes. Prenylation and phosphorylation are both required for interaction of Rap1b with a factor known as GDS, which stimulates GDP/GTP exchange (56), since prenylated, phosphorylated peptides corresponding to the Rap1b carboxyl-terminus can compete with intact Rap1b for interaction with GDS, whereas other peptides lacking either modification can not (131).

The Rho proteins are also localized to the Golgi (91), and also appear to require geranylgeranylation for association with membranes and with exchange factors. Mature RhoA protein binds to membranes, and associates with two GDP/GTP exchange factors, the stimulatory factor GDS and the inhibitory factor GDI. In contrast, bacterially expressed RhoA protein, which lacks carboxyl-terminal processing, is defective in all three of these interactions (59). Prenylation may be generally involved in mediating interactions between other small GTP-binding proteins and exchange factors (7).

OTHER MOTIFS A third group of small GTP-binding proteins contain the carboxyl-terminal sequences C-C, C-X-C, or C-C-X-X. These proteins include most of the Rab/Ypt proteins, which are involved in regulating intracellular trafficking, and are present both in the cytoplasm and associated with distinct membrane compartments (9). Several of these proteins, including mammalian Rab1b and Rab3a and Ypt1, Ypt3, and Ypt5 of Schizosaccharomyces pombe, have been shown to be geranylgeranylated in vivo (73, 102); genetic
and biochemical evidence indicates that others, including the mammalian Rab1b, Rab2, and Rab5 (75), and YPT1 of S. cerevisiae (119), are geranylgeranylated as well. Using a variety of approaches, including mutation of the terminal cysteine residues (97), depletion of intracellular isoprenoid pools (73, 75), expression in bacterial cells (7), and analysis of geranylgeranyltransferase mutants (119), geranylgeranylation has been shown to be essential for association of these proteins with membranes.

Although proteins containing C-C, C-X-C, and C-C-X-X motifs are all geranylgeranylated, some evidence indicates that the modifications directed by these sequences may differ in important respects. Mammalian Rab3A, which contains the C-X-C motif, is geranylgeranylated at both carboxyl-terminal cysteine residues and is carboxymethylated (38). This pattern of modification may be common to all C-X-C proteins; another protein containing this sequence motif, the YPT5 protein of S. pombe, is also geranylgeranylated and carboxymethylated (102). In contrast, proteins with carboxyl-terminal C-C motifs may be modified differently. The YPT1 protein of S. cerevisiae, with a C-C motif, is palmitoylated, and this palmitoylation is dependent on the presence of the carboxyl-terminal cysteines (97). However, no methylation of the C-C proteins YPT1 and YPT3 of S. pombe is detected under the labeling conditions used to detect methylation of YPT5 (102). Thus, C-C proteins may be geranylgeranylated and palmitoylated, but not methylated. The sites of these modifications are undetermined. Since, in vitro, the yeast and human methyltransferases appear to methylate essentially any prenylcysteine with a free carboxyl (63, 132), the apparent lack of a methylated carboxyl-terminus suggests that the carboxyl-terminal cysteine of C-C proteins may not be prenylated. Thus, a reasonable hypothesis compatible with these data is that the carboxyl-terminal cysteine is palmitoylated, and that the adjacent cysteine is geranylgeranylated. A novel carboxyl-terminal sequence, C-C-P, is found in Rah, a mammalian small GTP-binding protein related to Rho proteins (99). It is tempting to speculate that this may represent a new prenylation motif.

**Lipopeptide Pheromones**

Haploid fungal cells secrete mating pheromones that trigger cell fusion with cells of the opposite mating type to form diploid zygotes. Several fungal mating pheromones are lipopeptides, consisting of an oligopeptide to which a prenyl group is covalently attached in a thioether linkage to a carboxyl-terminal cysteine residue. The most extensively characterized is the mating pheromone a-factor of yeast. Yeast contain two a-factor genes, MFA1 and MFA2, which encode polypeptide precursors of 36 and 38 amino acids, respectively, each with a carboxyl-terminal CaaX sequence (15, 93). The a-factor precursor undergoes extensive processing independently of the classic secretory pathway.
before being secreted as the mature 12-amino acid lipopeptide (134). Analysis of the structure of mature a-factor reveals that four modifications occur in the processing of the precursor to yield active a-factor: (a) proteolytic removal of a 21-amino acid pro sequence to yield the mature amino terminus of the peptide, (b) attachment of the farnesyl group to a cysteine residue three amino acids from the carboxyl-terminus, (c) proteolytic removal of the three amino acids distal to that cysteine, and (d) methylation of the newly generated carboxyl-terminus (5).

The four steps in a-factor maturation occur in a defined order. In vitro studies of a-factor precursor processing have demonstrated that farnesylation is a precondition for carboxyl-terminal proteolysis (8) and methylation (61). Furthermore, cells depleted for mevalonate in vivo accumulate an a-factor species with the electrophoretic mobility characteristic of the a-factor primary precursor (124, 133), suggesting that farnesylation is a precondition for amino-terminal proteolysis. Thus, farnesylation is the obligatory first step in a-factor precursor processing, followed by proteolysis and methylation at the carboxyl-terminus, and proteolysis at the amino terminus. It is not known whether amino-terminal proteolysis and carboxyl-terminal proteolysis occur in a fixed order. Farnesylation and/or proteolysis is also apparently required for the secretion of a-factor, since unfarnesylated, unproteolyzed a-factor precursors accumulate in the cytosol (124), whereas unmethylated a-factor is readily secreted (90). The farnesyl group is also important for the ability of a-factor to induce growth arrest and morphological alteration of yeast cells of the α mating type (5).

Interesting variations on this process are seen in the processing of other pheromones. Rhodotorucine A, a lipopeptide pheromone from the basidiomycetous jelly fungus *Rhodosporidium toruloides*, is an 11-amino acid peptide with a carboxyl-terminal cysteine covalently bound to a farnesyl group in a thioether linkage (69). In contrast to a-factor, the rhodotorucine A carboxyl-terminal cysteine is not methylated. Three genes, *RHA1, RHA2* and *RHA3*, encode rhodotorucine A precursors consisting of three to five tandem copies of the rhodotorucine A peptide (2). These peptide sequences are separated by a four-amino acid spacer peptide, (C)-T-V-A/S-K. The rhodotorucine A precursor is apparently cleaved to produce multiple pheromone peptides, a process reminiscent of α-factor processing in *S. cerevisiae* and neuropeptide processing in mammals. Maturation of rhodotorucine A may involve cleavage of the large precursor to produce multiple peptides containing canonical CaaX sequences, which are then processed in a manner similar to a-factor precursor. Alternatively, farnesylation of internal cysteine residues could occur prior to proteolytic maturation. In either case, rhodotorucine A provides a precedent for prenylation of internal cysteine residues (see also *Proteases* below).
The processing of the tremerogen A-10 pheromone, produced by the jelly fungus *Tremella mesenterica*, is another variation on the maturation process of a-factor. Tremerogen A-10 is synthesized as a large precursor of unknown primary sequence. The secreted pheromone is a farnesylated, carboxymethylated, 10-amino acid lipopeptide with an apparent molecular weight of 1.5 kd. Interestingly, the farnesyl group contains an alcohol group at position 30 (122). Compactin, an inhibitor of isoprenoid biosynthesis, inhibits maturation of tremerogen A-10 precursor, resulting in the accumulation of a 28-kd species that is presumably unfarnesylated. However, tunicamycin, an inhibitor of glycosylation, and monensin, an inhibitor of Golgi transit, also inhibit processing, and result in the accumulation of lower molecular weight intermediates, implying that the precursor is glycosylated and processed in the Golgi (96). Tremerogen A-10 is therefore a novel case of a farnesylated polypeptide that, unlike a-factor and the small GTP-binding proteins, is also processed in the secretory pathway. Since farnesylation is an early processing step that may occur in the cytoplasm, prenylation may mediate entry of tremerogen A-10 into the endoplasmic reticulum.

In addition to these examples, a wide variety of evolutionarily divergent fungal species secrete prenylated pheromones. For example, h+ cells of the fission yeast *S. pombe* secrete a nine-amino acid methylated lipopeptide known as M-factor, which is encoded by two genes, *MFm1* and *MFm2*. The precursors encoded by these genes contain CaaX sequences, and appear to undergo the same posttranslational modifications as a-factor (32). A number of mating factors from jelly fungi are prenylated peptides; some, such as tremerogen a-13 from *T. mesenterica*, contain a thioether-linked farnesyl group, whereas others, such as A-9291-I from *T. brasiliensis*, contain an oxidized farnesyl group (67, 69, 122). In addition, the mating type of the basidiomycetous smut fungus *Ustilago maydis* is determined in part by which of two genes, *mfal* or *mfa2*, is present at the A mating-type locus; both *mfal* and *mfa2* encode short polypeptides that contain canonical CaaX sequences (14). Thus, prenylated pheromones may be ubiquitous among the fungi. It is unclear whether prenylated peptide hormones are found in other organisms.

### Nuclear Lamins

Lamins are nuclear proteins that are a major component of the karyoskeleton. Lamins are thought to form intermediate filaments that are associated with the inner surface of the nuclear membrane and mediate nuclear breakdown and reformation during mitosis. Lamin proteins fall into three types, A-type, B-type, and C-type. B-type lamins are always associated with the nuclear membrane, whereas A- and C-type lamins are found in a soluble form during mitosis (42). Both A- and B-type lamins undergo posttranslational processing
involving prenylation, and both contain a carboxyl-terminal CaaX sequence. In B-type lamins, posttranslational processing resembles the processing of Ras proteins. The cysteine residue of the CaaX sequence is farnesylated (139), and the three amino acids distal to that cysteine are removed (137). There is also evidence that lamin B is carboxymethylated, although the methylation appears to be transient, and actually may play a role in regulating the disassembly of the nucleus during mitosis (25). As in the cases of Ras and α-factor, prenylation appears to be the first step in processing of lamin B, and a precondition for other processing steps (11, 137). Since the presence of the CaaX sequence is necessary for nuclear membrane targeting of lamin B molecules (58), prenylation and perhaps proteolysis and methylation are also necessary for the targeting of the lamin proteins to the nuclear membrane. Depletion of nonsterol isoprenoid pools in animal cells causes cells to arrest specifically at the G1/S transition in the cell cycle (111). It has been proposed (46) that this requirement could in fact reflect a requirement for lamins at this stage of the cell cycle.

The processing of lamin A is more complex. The lamin A precursor is initially modified with a farnesyl group to produce an intermediate known as prelamin A. Subsequently, however, the 18 carboxyl-terminal amino acids, including the farnesylated CaaX sequence, are removed by an endoprotease; thus, the mature form of lamin A that is assembled into the nuclear lamina is not prenylated (11). Mevalonate-starved cells accumulate unfarnesylated lamin A precursor in nuclear inclusions, and this precursor is rapidly processed and assembled into the lamina upon addition of mevalonate. On the basis of this observation, it has been suggested that farnesylation of lamin precursors occurs not in the cytosol, but in the nucleus (82). Interestingly, the carboxyl-terminal region of the lamin A precursor is apparently not required for assembly of lamin A into the lamina. This has led to the speculation that the prenylated lipopeptide released in lamin A processing may itself have a biological function (82).

G-proteins

G-proteins are heterotrimeric GTP-binding proteins that mediate signal transduction between transmembrane receptors and intracellular second messengers. The γ subunits of trimeric G-proteins contain either CaaX or CaaL consensus sequences. Several of these γ subunits are known to be prenylated. The γ subunit of bovine transducin, the G-protein involved in visual signal transduction, contains a farnesyl group covalently linked to the carboxyl-terminal cysteine residue (44, 80). Transducin γ is also modified by proteolysis and carboxymethylation. In addition, STE18, the γ subunit of the G-protein involved in yeast mating, requires a prenyl modification, probably farnesyl, for membrane localization and functional activity (41). A number of uniden-
tified Gγ subunits from bovine brain and from rat tissue culture cells are geranylated (84, 100, 142). In the Gγ subunits of bovine brain, the prenylated cysteine is apparently carboxymethylated (142).

The farnesylation of bovine transducin is of particular interest because several other proteins involved in visual signal transduction in the rod outer segment (ROS) membrane are prenylated. For example, the cGMP phosphodiesterase, which responds to activated transducin by degrading the second-messenger cGMP, is a heterotetramer composed of an α, β, and two γ subunits. The α subunit of this enzyme is farnesylated and carboxymethylated, whereas the β subunit is geranylated, but apparently not methylated (4). In addition, rhodopsin kinase, which is involved in the attenuation of rhodopsin activation, is also farnesylated and carboxymethylated (66). Rhodopsin itself contains a novel type of prenyl modification: the covalent attachment of 11-cis-retinal, a molecule derived from carotenoids, to a lysine residue of the opsin protein. Rhodopsin is also palmitoylated, a modification common to many prenylated proteins (104). The involvement of several prenylated proteins in a single signal transduction pathway suggests that this modification may mediate colocalization of and interaction between these proteins.

At least two Gα subunits contain carboxyl-terminal sequences that resemble the CaaX motif. However, neither of these proteins is prenylated in vivo (84, 123). Since mutant versions of these α subunits containing the sequence C-V-L-S in place of the normal sequence C-G-L-F are prenylated (68), it has been suggested that the glycine residue prevents prenylation of these proteins. However, Ras mutant proteins with the sequence C-G-L-F are capable of undergoing prenylation in vivo (71). Therefore, it remains unclear why this sequence does not direct prenylation of the Gα subunit.

PROTEINS INVOLVED IN PRENYL-DEPENDENT PROCESSING

The maturation of prenylated proteins involves many variations on a single theme, rather than a single series of processing reactions. All known prenylated proteins are initially modified by the attachment of a farnesyl or geranylated group in a thioether linkage to a cysteine residue at or near the carboxyl-terminus. Subsequently, many of these proteins are further modified by proteolytic processing. Finally, the carboxyl-terminal regions of many prenylated proteins are targets for reversible modifications such as palmitoylation, methylation, and phosphorylation. The specific proteins that direct these modifications are critical for mediating the common and divergent biological properties of the processed carboxyl-termini.
Protein Prenyltransferases

BIOCHEMICAL STUDIES Several enzymes, termed protein prenyltransferases, which catalyze the attachment of a specific prenyl moiety to specific protein precursors, have been identified and characterized. At least three distinct protein prenyltransferase activities are present in yeast and mammalian cells. Protein farnesyltransferases (PFT) are soluble enzymes that catalyze the farnesylation of human \( K\text{-}ras \) \( (86, 112) \), a-factor precursor of yeast \( (125) \), and RAS2 of yeast \( (48) \). The substrates for these enzymes are the farnesyl donor farnesyl diphosphate (FPP) and a protein precursor containing an intact CaaX sequence. The farnesyltransferase in rat brain has been purified to homogeneity. The enzyme is a heterodimer composed of nonidentical \( \alpha \) and \( \beta \) subunits; the genes encoding these proteins have recently been sequenced \( (113) \). A second activity, termed protein geranylgeranyltransferase I (PGT I), has also been identified in soluble extracts. This enzyme, which is chromatographically \( (22, 144) \) and genetically \( (40) \) separable from PFT, catalyzes the geranylgeranylation of peptides containing CaaL sequences, using geranylgeranyl diphosphate (GGPP) as a substrate. This enzyme has been only partially purified. However, since antisera directed against the mammalian PFT \( \alpha \) subunit can immunodeplete PGT I activity from crude extracts, it has been hypothesized that mammalian PGT I is also a heterodimer with an \( \alpha \) subunit that is shared with PFT \( (127) \). A third enzyme, PGT II, catalyzes the geranylgeranylation of protein precursors with C-C carboxyl-terminal motifs \( (98) \). This enzyme is chromatographically separable from both PFT and PGT I. Activities have also been identified in mammalian cells that catalyze the geranylgeranylation of C-X-C and C-C-X-X motifs \( (60, 75) \). Although both activities are separable from PFT and PGT I, it is not clear whether either of these activities is distinct from PGT II. In addition, the observation that farnesylation of prelamin A apparently occurs in the nucleus, whereas Ras farnesylation occurs in the cytoplasm, may suggest the existence of a distinct nuclear farnesyltransferase \( (82) \). In this regard, it has been reported that farnesyltransferase activity from bovine extracts fractionates as two peaks on gel filtration chromatography; perhaps one of these peaks corresponds to the putative lamin farnesyltransferase \( (86) \).

The substrate specificities of the protein prenyltransferase enzymes are clearly critical for the attachment of the correct lipid modification to specific prenylated proteins. Current evidence suggests that the specificities of PFT and PGT I are largely determined by the particular amino acids present in the CaaX/CaaL motif itself. Comparison of the sequences of known farnesylated and geranylgeranylated proteins led to the hypothesis that the carboxyl-terminal amino acid of the protein precursor determined whether a particular protein was farnesylated or geranylgeranylated. According to
this hypothesis, proteins containing S, C, M, Q, or A in this position are substrates for PFT, whereas proteins with L or F are substrates for PGT I. This hypothesis has been supported most convincingly for PFT in competition experiments using synthetic CaaX-related peptides (114). The in vitro specificity of PGT I also conforms largely to in vivo predictions (98). Interestingly, both PFT and PGT I can act weakly in vitro on the preferred substrates of the other enzyme (144).

The PGT II enzyme shows striking substrate specificity. Intriguingly, the sequences required for geranylgeranylation by PGT II, unlike PFT and PGT I, are not exclusively located at the carboxyl-terminus (98). Geranylgeranylation by PGT II is not competed by peptides corresponding to the carboxyl-termini of PGT II substrates, nor are these carboxyl-terminal peptides used as substrates by the enzyme. Furthermore, protein precursors containing C-C carboxyl-termini are not weakly prenylated by PFT or PGT I. Thus, the recognition of protein substrates by PGT II may be mechanistically dissimilar to substrate recognition by PFT and PGT I. The internal sequences required to direct geranylgeranylation by PGT II have not been identified.

GENETIC STUDIES In *S. cerevisiae*, studies of the genes encoding protein prenyltransferase subunits have been instrumental in revealing many of the roles of protein prenylation. The yeast genes RAM1 (125) and RAM2 (48) are required for PFT activity. Bacterially expressed RAM1 and RAM2 proteins, upon mixing, can reconstitute active yeast PFT enzyme; thus, these proteins apparently comprise the subunits of the yeast heterodimer (57). The protein sequences of RAM1 and RAM2 are homologous to the mammalian PFT β and α subunits, respectively (77). Mutations in RAM1 have been isolated independently by several groups based on several phenotypes: suppression of RAS2 activation (43, 109), a defect in the production of mature α-factor (138), and interference with the function of the Gy subunit STE18 (94). Thus, the yeast PFT is required for the functions of all the known yeast farnesylated proteins (it is worth noting that lamins have not been identified in yeast cells). Mutations in RAM2 also suppress RAS2 activation and block production of active α-factor (57).

Yeast contains two additional genes that share sequence homology with RAM1. One of these is known as CDC43 or CAL1. Mutations in this gene were identified on the basis of two distinct phenotypes. cal1 mutants arrest specifically at the G2/M transition unless grown in the presence of high levels of calcium (106). These mutants are suppressed by overexpression of yeast (RHO2), which contains the carboxyl-terminal sequence C-I-V-L, suggesting RHO proteins as possible targets of the CDC43 enzyme (Y. Ohya, personal communication). The cdc43 mutants, in contrast, display a temperature-sensitive defect in cytokinesis and the establishment of cell
polarity (1). Mutations in CDC42 and RSRI, which encode proteins that contain the carboxyl-terminal sequence C-T-I-L, also cause this phenotype, suggesting that these proteins are also CDC43 substrates (12). These observations suggest that CDC43/CAL1 encodes the β subunit of a PGT I-type protein prenyltransferase. Experiments using polypeptide substrates containing CaaL sequences demonstrate that CDC43/CAL1 is indeed required for PGT I activity (40).

Two yeast genes may encode PGT I α subunits. The observation that mammalian PFT and PGT I contain a shared α subunit suggested that RAM2 might be the α subunit for yeast PGT I, and the observation that coexpression of CDC43 and RAM2 in bacterial cells results in the production of active PGT I enzyme supports this hypothesis (J. B. Gibbs, personal communication). However, the ram2-1 point mutation caused only a twofold decrease in PGT I activity in vitro (40). This may suggest that multiple yeast genes encode PGTα subunits. Interestingly, a second gene, known as MAD2, has been isolated with sequence similarity to rat PFTα and RAM2. mad2 gain-of-function mutations were first identified on the basis of a defect in the regulation of the G2/M transition (81). Loss-of-function mutations in this gene cause arrest at G2/M, a phenotype similar to that of call mutants (R. Li, personal communication). Furthermore, the MAD2 protein sequence contains calcium-binding motifs. These genetic data suggest that CDC43/CAL1 may form heterodimers with both RAM2 and MAD2. Thus, α subunits, like β subunits, may be shared between protein prenyltransferases. The CDC43-RAM2 heterodimer may prenylate proteins involved in cytokinesis and cell polarity, such as CDC42, whereas CDC43-MAD2 may prenylate proteins such as RHO1 and RHO2 that are involved in the control of passage through G2/M. The cdc43 and call point mutations may specifically affect interaction of the CDC43 protein with RAM2 or MAD2, respectively. Intriguingly, these data also imply that the activity of the CDC43-MAD2 enzyme may be regulated in a cell cycle-specific manner by intracellular calcium signals.

A third gene that encodes a protein with similarity to β subunits, known as BET2, was identified on the basis of mutations that affect the secretory pathway. Since these mutations interact genetically with mutations in YPT1 and SEC4, both of which contain the carboxyl-terminal sequence C-C, BET2 is thought to encode the β subunit of the yeast PGT II (119). bet2 mutations result in a defect in the membrane localization of both YPT1 and SEC4 proteins. In addition, cell extracts of bet2 mutants lack PGT II activity measured using bacterially expressed YPT1 precursor (98). No promising candidates for a PGT II α subunit have been identified. ram2 mutants display no detectable defect in PGT II activity (98), and no genetic interactions have been observed between mad2 and bet2 mutations (R. Li, personal communication). Thus, the α subunit for this enzyme may be encoded by an as yet
unidentified gene. Alternatively, the inability of carboxyl-terminal peptides to compete for PGT II activity in vitro suggests that PGT II may differ in reaction mechanism, and perhaps subunit structure, from PFT and PGT I. Preliminary evidence is consistent with a heterotrimeric structure for the Rab3A PGT II (60). Therefore, the putative other subunit(s) of PGT II might not be homologous to previously characterized α subunits.

Some prenylated proteins may be processed by multiple protein prenyltransferases. For example, yeast RAS2 protein, which is primarily farnesylated by the RAM1-RAM2 farnesyltransferase, also appears to be modified to a lesser degree by a second prenyltransferase activity. Yeast cells carrying a deletion of RAM1 are viable, and a small but detectable amount of RAS2 protein in these strains is in the membrane-associated, mature form (125). In contrast, a deletion of RAM2 is lethal, and no membrane-associated RAS2 is detected in ram2 mutant strains (57). These data suggest that a RAM1-independent, RAM2-dependent activity is capable of weakly prenylating RAS2 in vivo. The β subunit of this enzyme may be CDC43, since overexpression of CDC43 can suppress the growth defect of a ram1 mutant. In addition, ram1 cdc43 double mutants are inviable, but can be rescued by overexpression of RAS2 (C. Trueblood & J. Rine, unpublished data). Since RAM2 and CDC43 appear to comprise a PGT I enzyme, some RAS2 protein may be geranylgeranylated in vivo; determination of the nature of the prenyl moiety of RAS2 in ram1 strains should address this possibility.

Taken together, these genetic data suggest a model in which the β subunits of prenyltransferases confer protein substrate specificity, whereas α subunits provide catalytic function. This model is consistent with biochemical evidence that PFTα, but not PFTβ, binds the peptide substrate (113). Thus, sharing of β subunits may serve to generate divergent prenyltransferase specificities, whereas sharing of α subunits may generate multiple prenyltransferase isozymes with common specificity but divergent regulatory properties.

**Proteases**

The maturation of many prenylated proteins involves specific proteolytic processing. In particular, farnesylated and geranylgeranylated proteins with carboxyl-terminal CaaX sequences are further modified by the proteolytic removal of the three carboxyl-terminal amino acids, a modification necessary for subsequent carboxymethylation. A carboxyl-terminal protease activity active on Ras precursors is present in mammalian cell membranes (8, 52). Thus, whereas farnesylation of Ras precursors occurs in the cytosol, proteolysis most likely occurs at a membrane organelle, perhaps the plasma membrane. The carboxyl-terminal protease is an endopeptidase (8), and requires a farnesylated substrate. The residue one-amino acid from the carboxyl-terminus (i.e. the $a_2$ residue of the $C_7a_2X$ sequence) appears to be
important for the specificity of the mammalian carboxyl-terminal protease, as mutations in this residue can prevent proteolysis, but not farnesylation, of K-ras in vivo (71). Yeast extracts also contain a membrane-associated carboxyl-terminal protease activity (8, 62). Like the mammalian enzyme, this enzyme is an endopeptidase that acts only on prenylated substrates. The membrane-associated protease is not encoded by any known gene required for a-factor or RAS2 processing. Geranylgeranylated peptides are also proteolyzed by a membrane-associated protease; it is presently unclear whether this activity is distinct from the farnesyl-dependent membrane protease (8).

In addition to this membrane protease, a carboxyl-terminal protease active on a-factor is present in the soluble fraction of a yeast extract (8, 62), and has been partially purified (62). This soluble enzyme is a processive exopeptidase that is not farnesyl-dependent. In fact, this enzyme can remove the farnesylated cysteine itself unless it is further modified by carboxymethylation. Therefore, it is not clear that this enzyme is relevant to the processing of prenylated proteins in vivo.

The maturation of lamin A, a-factor, and M-mating pheromone of S. pombe also involves a second type of proteolytic processing. These proteins require endoproteases that cleave the polypeptide precursor at a site amino-terminal to the prenylated cysteine residue, resulting in the release of a prenylated oligopeptide. In vivo, the amino-terminal proteases involved in lamin A and a-factor maturation are active only on farnesylated precursors (11, 124). Presently, little is known about these enzymes. A yeast gene, STE19, has been identified that is required for the production of active a-factor, and is not deficient in the farnesyltransferase, carboxyl-terminal protease, or methyltransferase activities (M. N. Ashby & J. Rine, unpublished). The amino-terminal proteolysis of a synthetic substrate based on a-factor can be catalyzed in vitro by a crude yeast extract (88). Using this assay, it should be possible to determine whether STE19 encodes the amino-terminal protease enzyme.

In addition to the proteases involved in the maturation of prenylated proteins, there are at least two examples of prenyl-dependent degradative proteases. Strains of R. toruloides of the a-mating type produce an endoprotease that cleaves the prenylated mating factor rhodotorurine A (95). This cleavage is necessary for metabolism of and response to rhodotorurine A by a cells. Similarly, S. cerevisiae strains of α-mating type produce a cell surface-associated endoprotease that degrades a-factor (89). This protease inactivates the a-factor pheromone molecule and promotes recovery from growth arrest. Both enzymes specifically degrade farnesylated peptides; peptide analogues that lack the farnesyl group, or are modified with a different alkyl group, are poor substrates for these enzymes. The sxal gene from S. pombe may encode a prenyl-dependent degradative protease; sxal mutants are hypersensitive to the M-factor lipopeptide pheromone (65). The possibility
that prenyl-dependent degradative proteases may play a role in the turnover of prenylated proteins in other organisms remains unexplored.

The primary sequence of the rhodotorucine A precursor suggests the existence of a protease that releases short oligopeptides, which are further processed to produce farnesylated pheromone molecules (2). The details of this process are unclear; it is not known, for example, whether proteolysis occurs before or after farnesylation, or whether the earlier step is a precondition for the later step. At present, rhodotorucine A provides a novel example of prenyl modification of cysteine residues located in an internal region of the primary precursor. If the prenylation of internal cysteine residues is a general biological phenomenon, the details of this type of proteolytic maturation will acquire particular biological significance.

Reversible Modifications

The carboxyl-termini of prenylated proteins are often targets for additional modifications that, unlike proteolysis and prenylation, are reversible. In particular, palmitoylation has been implicated in the functions of several prenylated proteins, including some Ras, Rap, and Rab proteins. Among prenylated proteins, palmitoylation has been studied most extensively for the human Ras proteins. The palmitoyl modification of these proteins has a faster turnover time than the proteins themselves; thus, Ras proteins appear to be palmitoylated and depalmitoylated in a dynamic fashion (50).

A palmitoyltransferase activity has been identified in mammalian cell extracts that can palmitoylate cytosolic N-ras precursors, using palmitoyl-CoA as a substrate (49). This activity is associated with Golgi-rich membrane fractions. Bacterially expressed Ras precursors are poor substrates, implying that the palmitoyltransferase may require a farnesylated substrate. Since the mature N-ras protein is associated with the plasma membrane, and palmitoylation is dynamic, the apparent lack of palmitoyltransferase activity in the plasma membrane is perhaps surprising. Possibly this palmitoyltransferase activity may not be relevant to Ras processing in vivo. It is interesting to note, however, that yeast YPT1 is palmitoylated and localized to the Golgi (97, 128). It is possible, therefore, that this palmitoyltransferase is actually involved in palmitoylation of Rab/YPT proteins. Clearly, mutants in the palmitoyltransferase gene would be invaluable in identifying the relevant targets of this enzyme.

Consideration of the mechanism of the palmitoylation of rhodopsin, an integral membrane protein, may provide insight into the mechanism of Ras palmitoylation. Rhodopsin is posttranslationally modified by a thioester-linked palmitoyl moiety at the rod outer segment, an organelle derived from the plasma membrane (104). Palmitoylation of rhodopsin, like Ras, appears to be dynamic. Surprisingly, the palmitoylation of rhodopsin may occur by a
nonenzymatic mechanism (103). Although rod outer segments can transfer palmitate from palmitoyl-CoA to rhodopsin, this activity is insensitive to boiling, and copurifies with rhodopsin itself. Energetically, it is possible that both the transfer of a palmitoyl moiety from palmitoyl-CoA to cysteine and the subsequent hydrolysis of the cysteine thioester may not require an enzyme for catalysis. However, since palmitoyl is the only fatty acyl moiety that is transferred to rhodopsin, some mechanism must exist to provide this specificity. Precedent exists for specific nonenzymatic modification of proteins; for example, the maturation of rhodopsin involves the nonenzymatic covalent attachment of a specific carotenoid molecule, 11-cis-retinal, to a specific amino acid of the opsin polypeptide (145). The parallels between palmitoylation of rhodopsin and Ras suggest that Ras may become palmitoylated and depalmitoylated at the plasma membrane by a similar nonenzymatic mechanism.

A second modification common to many prenylated proteins is methylation. For example, Ras proteins, B-type lamins, some trimeric G-proteins, and some Rab proteins contain methylated carboxyl-termini. Whereas for Ras proteins the half-life of this modification is identical to that of the protein itself (50), for several other prenylated proteins, such as lamin B, the methyl group is turned over more rapidly (25), suggesting that carboxymethylation can be reversible. Reversible methylation of prenylated carboxyl-termini would presumably require the existence of a methyltransferase and a methylesterase enzyme. In yeast, a membrane-associated methyltransferase has been identified that is encoded by a single gene, \textit{STE14} (61, 63). \textit{STE14} encodes an integral membrane protein required for the methylation of \textit{a}-factor and \textit{RAS2}. Mutants defective in this gene are unable to produce active \textit{a}-factor, but display no other obvious phenotypes. Thus, methylation may have little importance for Ras function, at least in yeast. It is worth noting, however, that proteins such as lamin B and "C-X-C" Rab proteins, for which methylation may be a reversible, regulatory modification, have not been identified in \textit{S. cerevisiae}. Methyltransferase activity has also been detected in crude membrane fractions from mammalian cells (132), and in rod outer segment (ROS) membranes (107). Interestingly, ROS membranes contain an active methylesterase activity (107). Since methylation appears to play an important role in the interaction of transducin with the activated form of rhodopsin, these two enzymes may provide an important function in regulating visual signal transduction by controlling the methylation state of proteins such as transducin, cGMP phosphodiesterase, and rhodopsin kinase.

The phosphorylation of Rap1b protein provides an intriguing example of a reversible modification mediating the effects of an intracellular second messenger on the biological properties of a prenylated protein. Rap1b is phosphorylated on a specific carboxyl-terminal serine residue by cAMP-de-
pendent protein kinase (A-kinase) (56). This modification is important for efficient interaction between the carboxyl-terminus of Rap1b and the GDP exchange factor GDS (131). GDS stimulates both the exchange of GTP for GDP bound to Rap1b and the dissociation of Rap1b from intracellular membranes. Thus, phosphorylation of Rap1b by A-kinase results in an increase in the GTP-bound versus the GDP-bound form of the protein, and transfer of the protein molecule from a membrane location to the cytosol. Interestingly, Ras proteins from both yeast and humans are phosphorylated on specific serine residues; in human N-Ras, the site of phosphorylation has been localized to the carboxyl-terminus (27, 121). In RAS proteins of yeast, only the membrane-bound form is phosphorylated. Perhaps phosphorylation also regulates the interaction of Ras proteins with guanine nucleotide exchange factors.

PRENYL-MEDIATED INTERACTIONS

Although considerable progress has been made recently in identifying the enzymes that direct protein prenylation and their protein targets, the ways in which prenyl modifications function remain largely unknown. In particular, although a primary function of prenylation for many modified proteins is to direct intracellular localization, the mechanisms of these targeting processes, and the cellular factors required for them, have not been determined. Based on recent data (Figure 1), however, it is possible to speculate upon how prenyl-dependent protein targeting might occur. At least three general types of models for prenyl-mediated protein targeting can be proposed.

Prenyl-protein Receptors

Prenylated carboxyl-termini could be directed to intracellular compartments by specifically localized membrane-bound receptors. This type of mechanism is likely to be responsible for the plasma membrane localization of myristoylated Src proteins (115). The putative myristoyl-Src receptor appears to recognize both the lipid moiety and the amino acids in the protein amino-terminus (116). Similarly, hypothetical prenyl-protein receptors would most likely recognize both the prenyl moiety and some specific amino acid sequence or protein structure, since proteins with identical lipid modifications can be localized to different subcellular compartments.

The principle of interaction between prenylated polypeptides and specific receptors has been clearly established by work on fungal mating factors and their receptors. The prenylated mating pheromone α-factor interacts with a specific transmembrane receptor, STE3, to induce growth arrest and morphological differentiation in cells of the α mating type (51). Furthermore, experiments using synthetic analogues of α-factor have demonstrated that the

The receptor is highly specific for both the peptide sequence and the posttranslational lipid modifications. Peptide analogues lacking an alkylated cysteine are essentially inactive. If the farnesyl group is replaced with a different thioether-linked alkyl group, the activity of the resulting peptide is reduced; some analogues, such as S-geranyl a-factor, retain as much as 50% of the activity of authentic a-factor, whereas S-methyl a-factor is only 0.2% as active as authentic a-factor in some assays (5, 87). Methylation is also important for the activity of a-factor; unmethylated a-factor analogues are generally inactive in inducing a-factor arrest (87). a-factor is inactivated by proteolysis (89); therefore, the peptide must also be important for recognition by STE3. The effects of the pheromone rhodotorunic A of R. toruloides and the pheromone tremerogen A-10 of T. mesenterica, whose activities are also likely to be receptor-mediated, also require both the peptide sequence and the lipid modification for biological activity (136).

Interactions between the prenylated carboxyl-terminus of a-factor and a specifically localized transmembrane protein may also be involved in the extracellular secretion of the pheromone. The secretion of farnesylated a-factor is known to require the activity of a transmembrane glycoprotein, STE6. STE6 was identified on the basis of mutations that rendered yeast cells of the a-mating type mating-deficient (118). ste6 mutants are capable of synthesizing mature a-factor; however, this a-factor is not secreted (79). The sequence of STE6 predicts that it encodes a membrane-spanning protein with
significant sequence similarity to bacterial membrane transporters and, in particular, mammalian P-glycoproteins (78, 92). Thus, \textit{STE6} is likely to encode a protein that pumps processed a-factor across the plasma membrane. It is not known whether \textit{STE6} protein specifically recognizes the farnesyl group of a-factor. However, since mutations that block farnesylation of a-factor prevent its secretion by \textit{STE6}, whereas a mutation that blocks methylation does not (90), either farnesylation or one of the proteolytic modifications must be essential for export by \textit{STE6}.

Mammalian cells contain genes that are highly homologous to \textit{STE6}, the MDR (multidrug resistance) or P-glycoprotein genes. Overexpression of the \textit{MDR1} gene in transformed cells results in resistance to a wide range of structurally divergent cytotoxic compounds, including vinblastin, vincristine, actinomycin, colchicine, and adriamycin (3, 28). This phenotype results from an increased efflux of drug from the treated cells, implying that the MDR1 protein is pumping toxins out of the cell (45). Other MDR1 homologues are involved in translocation of MHC peptides into the ER lumen (36). One might also speculate that an MDR-like protein might mediate the apparent prenyl-dependent entry of tremerogen A-10 precursor into the secretory pathway. Although the idea of prenyl-mediated intracellular trafficking mediated by MDR homologues is reasonable, it is important to recognize that no MDR homologue, including \textit{STE6}, has been directly demonstrated to require a prenyl modification for membrane translocation at a peptide substrate.

Another candidate for a prenyl-protein receptor that mediates protein targeting is the lamin B receptor, a 58-kd nuclear membrane protein. This receptor mediates the association of lamin B with the nuclear membrane, a process that also depends on farnesylation (141). The gene for the lamin B receptor, which has been cloned from chicken, is predicted to encode a polytopic integral membrane protein (140). However, it is presently unknown whether recognition by the lamin B receptor specifically requires the farnesyl modification.

\textit{Lipid Interactions}

A second possible mechanism for prenyl-mediated protein targeting is association of prenylated proteins with cellular membranes by interactions between the prenyl group and membrane lipids. Models of this type include the "dumb lipid" model, in which the prenyl group merely provides a nonspecific lipophilic glue that provides affinity for membranes, but does not mediate targeting to a specific membrane organelle, and the "smart lipid" model, in which the lipid modification directs the protein to a specific locale through a nonreceptor-based mechanism. A type of smart lipid model has been proposed to explain the localization of GPI-linked proteins to plasma-membrane sites called caveolae (6). Clustering of GPI-linked proteins, such
as the folate receptor in caveolae, is disrupted by drugs, such as filipin and nystatin, that bind cholesterol (120). Thus, it has been suggested that the specific lipid composition of caveolae attracts GPI-linked proteins through specific lipid interactions.

A lipid interaction model is consistent with much of the information on the localization of prenylated Ras-superfamily proteins. Clearly, farnesylation and the other carboxyl-terminal modifications serve to increase the affinity of Ras proteins for membranes. For K-ras proteins synthesized in vitro, farnesylation, proteolysis, and methylation all have the cumulative effect of increasing the membrane affinity of the Ras protein (52). Similarly, the membrane affinities of mutant H-ras proteins lacking palmitoylation sites and K-ras proteins lacking a polybasic region are markedly less than wild-type forms of these proteins (53). Some experiments with mutant Ras proteins suggest that prenylation is a nonspecific membrane-localization signal. For example, K-ras mutant proteins with a CaaL sequence identical to Rap1a are geranylgeranylated rather than farnesylated, yet are still localized to the plasma membrane rather than the Golgi (53). Similarly, the biological activity of Rap1a, a Golgi-associated protein that suppresses Ras activation, is unaffected by replacing the geranylgeranyl modification with farnesyl (30). In addition, the yeast YPT1 protein, a member of the Rab family that contains the geranylgeranylation sequence C-C at its carboxyl-terminus, retains biological activity when this sequence is altered to produce a CaaX farnesylation sequence (97). These results suggest that targeting of these proteins to specific membrane compartments does not involve the prenyl group, but rather the amino acid sequence of the proteins.

The carboxyl-termini of small GTP-binding proteins contain a hypervariable region that may direct endomembrane targeting. Mutations that affect the polybasic region of the hypervariable domain of geranylgeranylated K-ras do not affect membrane affinity in vitro, nor membrane partitioning in vivo. However, these mutant proteins are targeted neither to the plasma membrane, like K-ras, nor the Golgi, like Rap1a, but to many membranes in an apparently nonspecific manner (53). The hypervariable domain of geranylgeranylated Rab proteins also appears to be critical to the localization of these proteins to specific membrane compartments (24). Thus, in K-ras, Rap1, and at least some Rab proteins, prenylation, proteolysis, and methylation appear to promote nonspecific membrane association, whereas the hypervariable region directs localization to specific membranes.

Although the above results suggest that the farnesyl modification of K-ras is a dumb lipid, experiments with H-ras suggest that the prenyl moiety may provide more than nonspecific lipophilicity. Mutation of the carboxyl-terminal residue of H-ras to leucine (30, 53), or deletion of the CaaX sequence and insertion of an amino-terminal myristoylation sequence (20), results in the
production of mutant H-ras proteins that are either geranylgeranylated or myristoylated, rather than farnesylated. Both mutant proteins are membrane associated; for the geranylgeranylated form, the site of localization is the plasma membrane (53). In addition, activated forms of these proteins were capable of inducing cellular transformation. However, the nonactivated forms of these proteins behaved differently from farnesylated H-ras; geranylgeranylated H-ras exhibits a dominant negative phenotype (30), and myristoylated H-ras has a transforming phenotype (20). Interestingly, H-ras, but not K-ras, is palmitoylated. Perhaps the interactions between the farnesyl group, the palmitoyl group, and the membrane lipids serve to orient the protein in a way that is important for wild-type, but not transforming, function. In this regard, note that palmitoylation has been suggested to be important for orienting rhodopsin in the rod outer segment membrane (103).

**Indirect Prenyl-dependent Targeting**

Prenylation itself need not play a direct role in mediating protein targeting; in some cases, prenylation may instead be a precondition for a secondary modification that actually directs protein localization. The processing of lamin A provides a clear case of an indirect effect of prenylation on protein localization. Farnesylation of prelamin A is clearly required for the assembly of normal lamin A protein into the nuclear lamina, since inhibition of this process results in accumulation of prelamin A in nuclear inclusions (11). However, farnesylation per se is not required for this process. Rather, the essential step is removal of the prelamin A carboxyl-terminus, which, in turn, requires farnesylation, as lamin A precursors lacking the carboxyl-terminal amino acids are assembled into the lamina in the complete absence of prenylation (82). Prenyl-dependent proteolysis may also be essential for the secretion of α-factor of yeast. Although the requirements for STE6-mediated export of α-factor have not been determined, it is possible that the critical processing step required for this process is proteolysis of the amino-terminal pro region of the α-factor precursor.

Reversible prenyl-dependent modifications may be important for the localization and function of prenylated proteins. For example, methylation appears to be required for translocation of transducin βγ from the cytosol to the ROS membrane, where it interacts with transducin α and rhodopsin (105). Activation of rhodopsin with light stimulates the membrane localization of methylated transducin βγ, a stimulation that requires transducin α. Thus, the effect of methylation on the membrane-targeting of transducin βγ appears to result, at least in part, from enhanced protein-protein interactions between transducin α and βγ. The existence of several proteins involved in visual signal transduction, including rhodopsin kinase and cGMP phosphodiesterase, that are similarly farnesylated and carboxymethylated, suggests that reversible
methylation may be critical for the interaction of all these proteins with rhodopsin and with one another. The example of transducin also suggests a similar role for methylation and palmitoylation in the cycling of Rab and Rho proteins between the cytosol and cellular membranes; this possibility remains to be explored.

These models for prenyl-dependent protein targeting are not mutually exclusive. For example, the interaction of geranylgeranylated Rap1b with its GDS requires the geranylgeranyl group, the carboxyl-terminal peptide sequence, and the phosphorylated geranyl serine residue. This interaction is correlated with transit of Rap1b from the Golgi membrane to the cytosol (56). Thus, this process involves a protein-prenyl interaction that is analogous to the prenyl receptor model, yet also requires a secondary modification. The targeting of many prenylated proteins may likewise involve mechanisms that contain elements of several of the models described here.

FUTURE PERSPECTIVES

Although much has been learned about protein prenylation in a relatively short period of time, many critical questions remain unanswered. Clearly, the precise functional role played by the prenyl group in protein localization and function is only beginning to be understood, and understanding this role is therefore a major focus of current research. In addition, the identification and characterization of new protein prenyltransferases and other enzymes involved in prenyl-dependent modifications remains an important area of research, since the existence of new enzymes with novel substrate specificities appears likely. In particular, the combinatorial sharing of protein prenyltransferase subunits could provide both diverse protein and lipid specificities and differential regulation of protein prenylation. The identification of new prenylated proteins may also provide conceptual breakthroughs, particularly if new prenyl modifications or new modification sites are identified.

Understanding protein prenylation may provide insight into two related biological phenomena of medical significance: oncogenesis, and cholesterol metabolism. The Ras proteins are well known for their oncogenic potential; activated Ras genes have been found in a variety of types of human tumors, including colorectal tumors, lung adenocarcinomas, and pancreatic tumors (10). Prenylation has been shown to be important for the functions of Ras proteins, since inhibition of prenylation suppresses the phenotypes of constitutively active Ras proteins in yeast (109). Furthermore, genetic and pharmacological inhibition of Ras processing in animal cells likewise suppresses Ras-induced transformation (31, 64). Thus, a greater understanding of the catalytic mechanism of protein prenyltransferases may aid in the design of PFT inhibitors with potential utility for cancer treatment. Inhibitors of isoprenoid biosynthesis may also be useful for cancer therapy; several studies
have indicated that inhibitors of mevalonate synthesis suppress Ras-induced cell transformation (110, 126, 33).

Protein prenylation may also be important for the regulation of HMG-CoA reductase (HMGR), the rate-limiting step in cholesterol biosynthesis. The HMGR inhibitor mevinolin is commonly used to treat hypercholesterolemia, since mevinolin treatment results in increased synthesis of the LDL receptor (17, 83). However, depletion of nonsterol isoprenoids also causes an increase in the translation of the HMGR message and a decrease in HMGR degradation, resulting in abnormally high levels of HMG-CoA reductase protein (16, 101). Thus, in patients undergoing treatment with mevinolin, the cholesterol biosynthetic potential is actually elevated. Understanding the mechanism of translational and half-life control may provide insights into how this increase in HMGR synthesis could be prevented. Prenylated proteins could mediate both processes, although other models involving mevalonate-binding proteins or prenylated mRNA are also plausible. Further studies should clarify the mechanism of translational regulation of HMGR, and perhaps provide practical insights into more effective treatment of hypercholesteremia.

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