Lipid modifications of proteins – slipping in and out of membranes

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Protein lipid modification, once thought to act as a stable membrane anchor for soluble proteins, is now attracting more widespread attention for its emerging role in diverse signaling pathways and regulatory mechanisms. Most multicellular organisms have recruited specific types of lipids and a suite of unique enzymes to catalyze the modification of a select number of proteins, many of which are evolutionarily conserved in plants, animals and fungi. Each of the three known types of lipid modification – palmitoylation, myristylation and prenylation – allows cells to target proteins to the plasma membrane, as well as to other subcellular compartments. Among the lipid modifications, protein prenylation might also function as a relay between cytoplasmic isoprene biosynthesis and regulatory pathways that control cell cycle and growth. Molecular and genetic studies of an *Arabidopsis* mutant that lacks farnesyl transferase suggest that the enzyme has a role in abscisic acid signaling during seed germination and in the stomata. It is becoming clear that lipid modifications are not just fat for the protein, but part of a highly conserved intricate network that plays a role in coordinating complex cellular functions.

Many extrinsic membrane proteins are anchored to the membrane via mechanisms that are distinct from those employed by *trans*-membrane proteins. Lipid modifications facilitate the attachment of soluble proteins to biological membranes, but they also enable protein–protein interactions and, in some cases, the shuttling of proteins between the plasma membrane and the cytosol or other membrane compartments. These modifications, which are found in all eukaryotic cells, fall into three major classes and are characterized by the type of lipid and the site of modification in the protein. They include *N*-myristylation, palmitoylation and prenylation (Fig. 1). Unlike phosphorylation, lipid modification is limited to a small subset of cellular proteins that often participate in signaling. Certain lipid modifications are reversible, and can therefore have regulatory functions in signal transduction, such as providing a structural basis for the assembly of signaling-protein-complexes on membranes, or terminating a signal cascade by hydrolysis of the lipid and dissociation of the protein from the membrane. In addition, the prenyl lipids used in the modification of signaling proteins are also important branch-point intermediates of isoprene biosynthesis, thereby providing the cell with a potential mechanism for coordinating signaling and a key biosynthetic pathway.

The biochemistry of most lipid modifications is now well established, although not all enzymes that carry out the transfer reactions have been identified. The role of *N*-myristylation and palmitoylation in animal cells[1–3], and protein prenylation in animals, fungi and plants has been reviewed extensively[4–7]. Here we focus primarily on the role of lipid modification in modulating protein functions, particularly the insights gained from the identification of modified plant proteins and the genetic analysis of mutants that affect protein prenylation.

Function of reversible protein palmitoylation

Protein palmitoylation is achieved through esterification of cysteine thiol groups by palmitate (Fig. 1). In contrast with *N*-myristylation and prenylation, a primary sequence motif that would predict sites of palmitoylation in proteins has not been identified. Palmitoylation is often found associated with myristate or prenyl groups, or near hydrophobic sequences of putative *trans*-membrane domains. The palmitoyl group is hydrophobic and therefore increases the affinity of modified proteins to membranes. But unlike other lipid modifications, palmitoylation is a dynamic and readily reversible process. This reversibility has attracted considerable attention because it provides a potential regulatory mechanism for shuttling proteins between the cytosol and the membranes or between the different membrane domains. Agonist-stimulated turnover of palmitate is found in a small number of proteins, including the β-adrenergic receptor and certain G-protein α-subunits[2,3]. However, the instability of palmitoyl modification and the difficulties in achieving efficient metabolic labeling of proteins with palmitic acid have hampered research into the mechanisms of protein palmitoylation. Insights into its precise function are further complicated because depalmitoylation can occur enzymatically and non-enzymatically, and the mechanisms that regulate each of these reactions are unknown.

The most extensively studied examples of palmitoylated proteins are the α-subunits of trimeric GTP-binding proteins (Gα) in animal cells. Following association with an effector, Gα is depalmitoylated and subsequently relocalizes to the cytosol. This might be necessary to facilitate the hydrolysis of GTP to GDP to reset the protein for a new activity cycle[3]. In this case, palmitoylation could regulate the localization and interaction of Gα with receptor and effector proteins. Although Gα-like proteins have been identified in plants[8], their function and palmitoylation status is unknown. To date, the chloroplast D1 protein encoded by *psbA* is the only plant protein for which palmitoylation has been demonstrated[9]. D1 is an integral protein of the photosystem II (PSII) reaction center. The D1 precursor protein first inserts itself into the unstacked stroma lamellae of the thylakoid membrane, where it is processed and subsequently migrates to the stacked grana lamellae where it becomes transiently palmitoylated. The role of this transient lipid modification of D1 is not known, but it is reasonable to suggest that palmitoylation might help to increase the local concentration, as well as the specificity and efficiency of D1 interaction with other PSII components in the lipid environment of the thylakoid membrane.

Palmitoylation is often found associated with myristate or prenyl groups, but the relationship between these different lipid modifications has been investigated in detail for only one member

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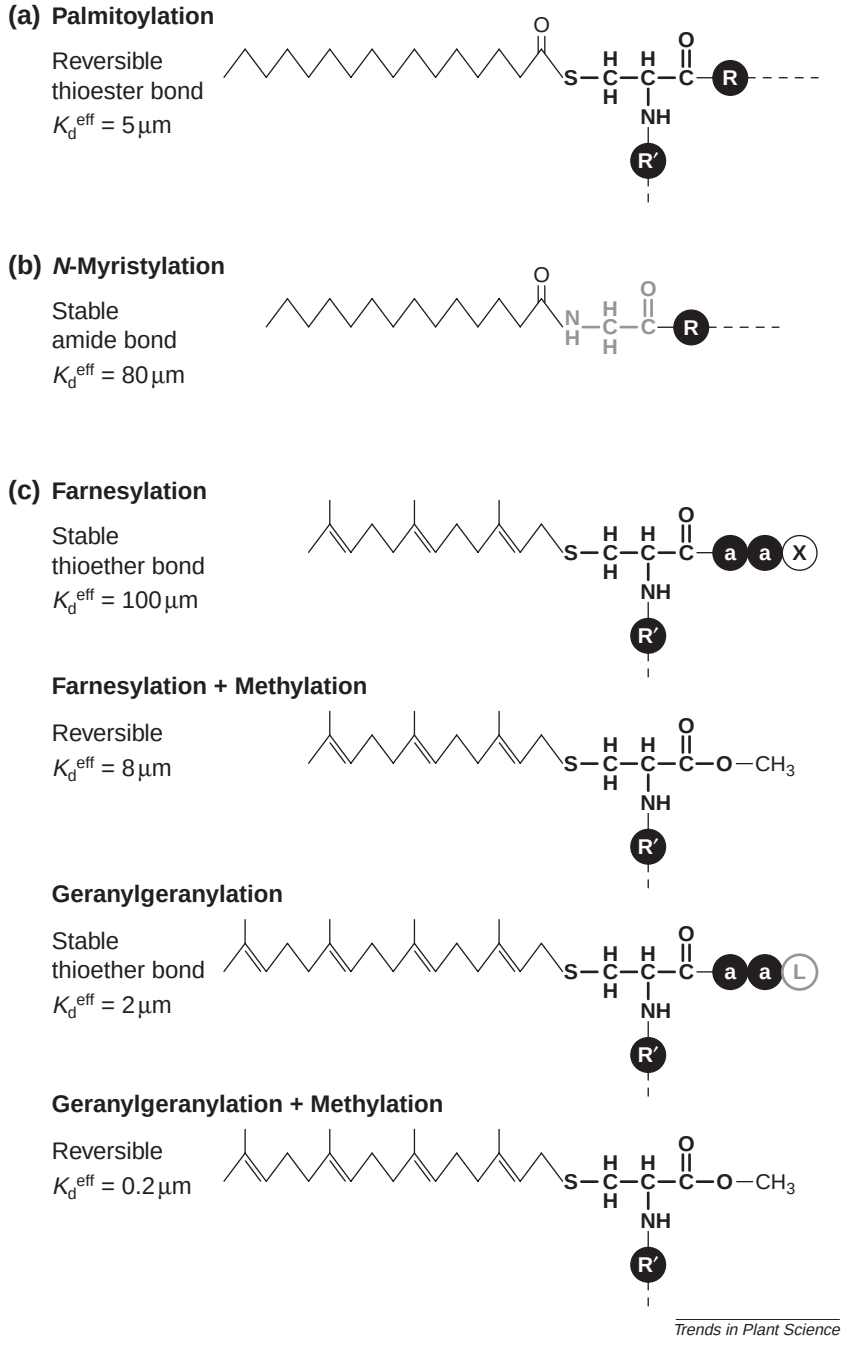

Fig. 1. Three types of protein lipid modifications. (a) In palmitoylated proteins the saturated 16-carbon fatty acid palmitate is attached to cysteines (black bold) via a labile thioester bond[2,3]. (b) *N*-myristylation occurs co-translationally by the attachment of the saturated fatty acid myristate to a conserved acceptor-glycine (gray) next to the initiator-methionine via a stable amide bond. The acceptor glycine is the first amino acid of a consensus sequence M-**G**[SVALQN]xx[STAGCNV]. (c) Protein prenylation involves the attachment of the 15- and 20-carbon isoprenes farnesyl and geranylgeranyl, respectively, to conserved cysteines (black bold) at the C-terminal end of proteins via a non-reversible thioether bond. In proteins modified by FTase or GGTase-I the acceptor cysteines are part of a conserved CaaX-box motif (where 'C' indicates cysteine, 'a' represents an aliphatic amino acid, and 'X' is usually serine, methionine, cysteine, alanine, glutamine or leucine). Following prenylation, the C-terminal -aaX amino acids are proteolyzed followed by methylation of the free cysteine carboxyl group. The methylation is reversible and significantly increases membrane affinity of the prenylated proteins[6]. R and R′ designate C- and N-terminal amino acid positions, respectively, relative to the acceptor amino acid. K_d^{eff} represents the membrane affinities of the respective lipid groups at the exposed lipid concentration at which 50% of a given modified peptide is associated with the membrane[2,49].

of the Gαi protein family. In this case, both palmitoylation and myristylation are required for stable plasma membrane localization, and the rate of palmitate turnover is significantly increased in the non-myristylated protein[10]. Interestingly, myristylated but non-palmitoylated Gαi mutant proteins are distributed between the plasma membrane and internal membranes, and a protein in which the myristylation site was mutated fails to associate with the plasma membrane and is not palmitoylated. However, myristylation *per se* is not required for palmitoylation because when Gαi is tethered to the plasma membrane via association with Gβγ in cells in which these proteins are expressed at high levels, Gαi is readily palmitoylated. These results reinforce the view that palmitoylation occurs only when proteins are already in close proximity to the inner surface of the plasma membrane. Thus, palmitoylation probably strengthens the reversible membrane association of plasma membrane-attached proteins independently of myristylation or prenylation, as demonstrated for Ha-Ras (Ref. 11). Because of the recent progress in the purification and cloning of a mammalian palmitoyl transferase[12] there is now the opportunity to search for palmitoyl transferase homologs in plants, and to gain insights into the biochemistry and function of protein palmitoylation.

Myristylation-mediated membrane attachment requires cooperation with other protein modifications

Several plasma membrane-associated proteins are modified by the 14-carbon-saturated fatty acid, myristate. Covalent linkage of myristate via an amide bond to a glycine in the N-terminal consensus sequence Met-Gly-x-x-x-[Ser/Thr] is catalyzed by *N*-myristyl transferase and occurs co-translationally after the removal of the initiator methionine by M-aminopeptidase. Although the modification is stable, the hydrophobicity of a myristylated peptide alone is not sufficient to anchor the protein in the plasma membrane (Fig. 1). This suggests that additional mechanisms cooperate with myristylation to facilitate stable plasma membrane association of the modified protein.

The best studied examples of myristylated proteins are mammalian members of the Src family of non-receptor tyrosine protein kinases (which participate in intracellular signal transduction) and the MARCKS proteins (myristylated alanine-rich C-kinase substrate; which are substrates of protein kinase C). These proteins have N-terminal polybasic domains with clusters of positively charged amino acids, allowing them to interact with head groups of acidic

phospholilipids that impart a negative charge to the cytoplasmic surface of the plasma membrane. Phosphorylation of serine residues located near to the polybasic domains of the proteins produces negative charges that reduce the net positive charge, thereby weakening the electrostatic interactions and releasing the myristylated protein from the plasma membrane[1,2]. A different mechanism is employed by Recoverin, a myristylated Ca^{2+} sensor in retinal rod cells, which controls the lifetime of photoexcited rhodopsin by inhibiting rhodopsin kinase. In the absence of Ca^{2+}, the myristyl group is masked by a hydrophobic pocket in the protein. Ca^{2+}-binding causes a conformational change in Recoverin that exposes the myristyl group and thereby facilitates the binding of the protein to the membrane[13].

A subset of the known myristylated proteins, including certain members of the Src family and α-subunits of the heterotrimeric G proteins, contain a cysteine next to or adjacent to the myristylated glycine that is usually palmitoylated (Fig. 2). Because palmitoylation is unstable, de-palmitoylation releases the myristylated protein from the plasma membrane[1–3]. Although in this case palmitoylation certainly enhances the efficiency of membrane binding, it is also possible that palmitoylation influences the subcellular localization or protein–protein interaction of the myristylated protein. *N*-myristylation, in conjunction with other functional domains or modifications, enables the reversible association of proteins with the plasma membrane in response to different cellular signals or shuttling of proteins between different membrane compartments.

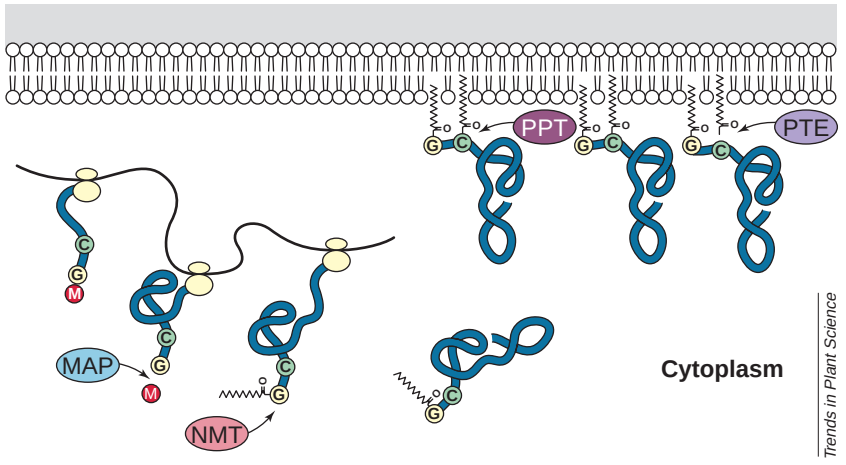

Fig. 2. Mechanisms of protein myristylation and palmitoylation. Co-translational *N*-myristylation at the glycine-acceptor is catalyzed by *N*-myristyl transferase (NMT) after removal of the initiator-methionine by methionine amino peptidase (MAP). Following release from the ribosome the myristylated protein attaches to the membrane. Many myristylated proteins are also palmitoylated at the membrane by protein palmitoyl transferase (PPT) to increase their hydrophobicity for stable membrane attachment. Palmitoylation can occur independently of myristylation if the proteins are initially attached to the plasma membrane (which is appressed to the cell wall) by other mechanisms, such as prenylation or positive surface charges. Palmitoylation is a reversible modification catalyzed by palmitoyl thioesterases (PTE), but the regulation of de-palmitoylation in response to signals is not well understood.

Three groups of plant proteins that contain putative *N*-myristylation sites have been characterized (Table 1), and several more might be identified through ongoing genome sequencing projects. The tomato PTO protein kinase is involved in signaling during pathogen infection, but resistant plants containing the *Pto* locus are also sensitive to the organophosphorous insecticide fenthion. This sensitivity is mediated by FEN, a PTO homolog, whose gene is tightly linked to *Pto* (Ref. 14). Both proteins have putative *N*-myristate-acceptor-glycines, and mutation of the FEN putative *N*-myristate-acceptor-glycine results in loss of fenthion sensitivity[14]. However, a similar mutation in PTO does not affect its function when the protein is ectopically expressed in susceptible transgenic plants[15], suggesting that myristylation is not required for PTO-mediated bacterial resistance. It has not been established if PTO is myristylated, and consequently a mutation of the putative *N*-myristate-acceptor-glycine could interfere with another function of the kinase. Myristylation has been demonstrated for one of the plant Ca^{2+}-dependent protein kinases (CDPKs), whose functions are unknown[16]. In addition, conserved cysteines in positions 4 and/or 5 are present in all the members of this family of kinases, suggesting additional modification by palmitoylation as discussed for the PTO protein kinases. It remains to be seen whether the plasma membrane association of plant CDPKs is regulated in a similar way to Recoverin, in which Ca^{2+}-binding exposes the myristyl group and facilitates membrane localization[13]. It is likely that plants exploit the cooperation between myristylation and palmitoylation to anchor regulatory proteins to the cytoplasmic surface of the plasma membrane.

Protein prenylation is catalyzed by three types of prenyl transferases

Protein prenylation differs from *N*-myristylation and palmitoylation, both in the type of lipid and the protein domains that are used in this modification. The reaction is catalyzed by three types of protein prenyltransferases that attach 15- or 20-carbon isoprenes farnesyl and geranylgeranyl, respectively, to conserved C-terminal

Table 1. Plant proteins with conserved myristylation motifs

Protein	Plant	Sequence	Function
CDPKs[a]	Several	M-**G**[S/x][C/x][C/V][S/x][d]	Ca^{2+}-dependent protein kinases
ATN1[b]	*Arabidopsis*	M-**G**SASGF[d]	Protein kinase, homology with Src kinases
PTO[c]	Tomato	M-**G**SKYSK[d]	Protein kinase involved in disease resistance
FEN[c]	Tomato	M-**G**SKYSK[d]	Protein kinase required for fenthion sensitivity

[a]Genes encoding Ca^{2+}-dependent protein kinases (CDPKs) have been cloned from several different plants, but myristylation has been confirmed for only one protein[16].
[b]Myristylation of ATN1 has not been demonstrated[47].
[c]The acceptor-glycine is required for FEN function, but is disposable for PTO function[14,15].
[d]Acceptor-glycine is shown in bold. Potential palmitoylation cysteine-acceptors and lysines that increase positive protein surface charge are underlined.

Table 2. Three different types of prenyltransferases are conserved in eukaryotes

Enzyme	Subunits	Recognition motifs	Protein substrates (examples)
FTase[a]	α β	CaaX	Ras, Gγ, a-factor, nuclear lamins, IP_3 5-phosphatase, cGMP phosphodiesterase
GGTase-I[a]	α β	CaaL	Rap, Rho, Rac
Rab-GGTase[b]	α, β, REP	CC, CXC, CCX, CCXX, CCXXX	Rab, Ypt

[a]FTase and GGTase-I are heterodimeric enzymes that share a common α-subunit but have distinct β-subunits[6,7].
[b]The subunits of the Rab-GGTase catalytic component are similar (but not identical) to those of FTase and GGTase-I. The catalytic component requires a third protein component, the Rab Escort Protein (REP), for full activity. REP binds non-prenylated Rab proteins, and presents them to the catalytic component[48].
Abbreviations: a, aliphatic amino acid; X, any amino acid.

cysteines of a small subset of cellular proteins (Fig. 1 and Table 2). Protein prenylation was discovered in fungi[17], but has been identified since as a conserved modification in other multicellular eukaryotes (reviewed in Refs 6,7). Interestingly, most of the proteins modified by prenyltransferases have regulatory roles in cellular signaling and vesicle transport. They include almost all members of the Ras superfamily of small GTPases (Ref. 18), several γ-subunits of heterotrimeric G proteins (Ref. 19), nuclear lamins[20], type I inositol 1,4,5-trisphosphate 5-phosphatase (Ref. 21), as well as several other proteins. Since the demonstration that prenylation inhibition could reverse the transformation of mammalian cells by activated Ras mutant proteins[22], much attention has been focused on the structure and function of protein prenyltransferases. However, our understanding of the regulation of these enzymes and their potential function in coordinating isoprenoid synthesis with cellular growth control remains incomplete.

Farnesyltransferase (FTase) and type I geranylgeranyltransferase (GGTase-I) are heterodimeric enzymes that share a common α-subunit but have distinct β-subunits that determine substrate specificity[6]. Both enzymes recognize a conserved C-terminal amino acid sequence motif known as CaaX box, where 'C' indicates cysteine, 'a' represents an aliphatic amino acid, and 'X' is usually serine, methionine, cysteine, alanine, glutamine or leucine (Table 2). If 'X' is leucine, the protein is geranylgeranylated by GGTase-I. The presence of a polybasic domain that is rich in arginine and lysine proximal to the CaaX box greatly increases the substrate affinity of GGTase-I and efficient prenylation[23,24]. Following the attachment of the prenyl group to the cysteine acceptor, the terminal aaX amino acids are cleaved by specific proteases. The exposed carboxyl group of the prenyl-cysteine is then methylated by an *S*-adenosylmethionine-dependent membrane-bound prenylcysteine carboxyl methyltransferase. A similar enzymatic activity has been identified in suspension-cultured tobacco cells using an artificial methyl acceptor[25], suggesting that this post-prenylation modification is conserved in plants as well. Recent evidence obtained for Ras proteins suggests that aaX proteolysis and carboxy methylation occur in the endoplasmic reticulum. The fully modified proteins then traffic to the plasma membrane via the Golgi secretory pathway or perhaps through the cytoplasm[26].

Rab-GGTase is the third prenyltransferase; it appears to modify only Rab GTPases, which regulate secretory vesicle transport. It does not share subunits with FTase and GGTase-I and differs from the other two prenyltransferases by the presence of a third component, Rab Escort Protein (REP; Table 2), which is required for enzyme activity[6,7]. Although Rab-GGTase has been characterized in plants and is thought to be similar to the animal and yeast enzymes[27,28], no molecular studies on the regulation of this prenyltransferase have been reported from plants.

Plant farnesyltransferase and protein substrates yield surprises

In synchronized tobacco tissue culture cells, increase in FTase activity is coincident with the initiation of cell division. Conversely, manumycin, a specific inhibitor of FTase, blocks cell division[29,30]. Thus, early events in the plant cell cycle probably require farnesylation of currently unknown proteins. Therefore, it was surprising when an *Arabidopsis* mutant (*era1*-enhanced response to ABA) was identified during a screen for plants with a hypersensitive response to ABA: the gene for the β-subunit of FTase is deleted in this mutant[31]. Mature *era1-2* has a pleiotropic phenotype, of which certain aspects can be attributed to alterations in ABA perception and/or signaling[32]. Because *era1-2* represents the first FTase null mutant in a multicellular organism that shows only modest effects on cell division and plant growth, it is tempting to conclude that farnesylation is not a critical protein modification for cell cycle regulation in plants. However, such a conclusion might be premature because current information on plant FTase protein substrates is limited, and promiscuous farnesylation of proteins by GGTase-I might account for the viability of *era1*.

The apparent role of plant FTase in the negative regulation of ABA-signaling unmasks only part of the complexity of protein prenylation in cellular functions, and reflects that not all the proteins in the ABA-signal-transduction pathway or other regulatory pathways have been identified. A novel biochemical screen to identify plant prenylation substrates[33], and computer searches with algorithms designed to detect CaaX-box proteins in databases have

Table 3. Plant FTase protein substrates

Protein	CaaX-motif	Plant	Proposed function
ANJ1[a,b]	CaQQ	Several	Molecular chaperone
AP1[b] SQUAMOSA CAULIFLOWER MADS3	CFaa CFaa CYaa CFaT	*Arabidopsis* *Antirrhinum majus* *Arabidopsis* *Betula*	MADS box transcription factors regulating flower development
NAP1[a]	CKQQ	Several	Cell cycle regulation

[a]Homologs have been identified in other organisms.
[b]Prenylation of these proteins has been confirmed *in vivo*: and in the other proteins has been shown using *in vitro* assays. Abbreviations: a, aliphatic amino acid; X, any amino acid.

now yielded several interesting plant candidate proteins (Table 3). Prenylation of most proteins is confirmed, but tobacco ANJ1 is the first plant protein for which farnesyltion was demonstrated *in vivo*[34]. ANJ1 is a plant homolog of the bacterial chaperone DnaJ, a protein found in a complex with the chaperone Heat shock protein70. Although the function of ANJ1 is not known, farnesylation of the protein is required for membrane binding. In yeast, farnesylation of YDJ1 (the yeast homolog of ANJ1) is essential for growth at 37°C. Wild-type ANJ1, but not the farnesyl cysteine-acceptor mutant protein, can complement a *ydj1*^{ts} mutant to restore growth at 37°C (Ref. 34). These results imply that only farnesylated ANJ1 is active at elevated temperatures in yeast, but they do not reveal the function of ANJ1 in plants. Proteins related to ANJ1 have been identified in many divergent plants and in all cases the Cys-Ala-Gln-Gln CaaX-box is conserved, suggesting that farnesylation is also a requirement for protein function in plants.

Table 4. Plant GGTase-I protein substrates

Protein	CaaL-motif	Species	Proposed function
Rac/Rop protein family[a]	CSIL, CaFL, CVFL, CFFL	Several	Polar growth
CaM53[b]	CTIL	*Petunia*	Calmodulin
CaM61	CVIL	Rice	
AIG1	CSIL	*Arabidopsis*	Pathogen response

[a]Homologs have been identified in other organisms.
[b]Prenylation of these proteins has been confirmed *in vivo*: and in the other proteins has been shown using *in vitro* assays. Abbreviation: a, aliphatic amino acid.

Geranylgeranyl transferase-I directs the localization of regulatory proteins

Considering the number of geranylgeranylated plant proteins identified to date (Table 4), the role of GGTase-I in various cellular processes will undoubtedly be complex. Similar to their mammalian and yeast counterparts, most known members of the Rac-related Rop family of small GTPases in plants have conserved C-terminal CaaL-box motifs and a polybasic sequence domain that is proximal to the prenyl acceptor-cysteine. Direct geranylgeranylation has been demonstrated only for one family member[35], but it is likely that most Rops are substrates for GGTase-I. Rac GTPases are implicated in the reorganization of the actin cytoskeleton through the activation of phosphatidylinositol 4-phosphate 5-kinase. It is likely that Rop proteins have a related function in the regulation of polar growth in plant cells, because overexpression of an *Arabidopsis* Rop protein induces isotropic growth in fission yeast, similar to the fission yeast homolog, and the protein is found at the site of growth[36]. In plants, certain Rop proteins localize to the tip of the growing pollen tube, which is consistent with the protein playing a role in polarized cell growth[37].

The effect of geranylgeranylation on membrane localization of GGTase target proteins in plants is now best understood for CaM53, a novel type of calmodulin protein that is not found in yeast or mammalian cells. CaM53 has a typical calmodulin domain but contains a C-terminal extension of 34 amino acids that is rich in lysine and arginine and a typical GGTase-I CaaL motif[23]. Prenylated CaM53 localizes to the plasma membrane, but the cysteine-acceptor mutant protein accumulates in the nucleus. This differential localization, which can be explained by the presence of a polybasic C-terminal domain that also acts as a nuclear localization signal in

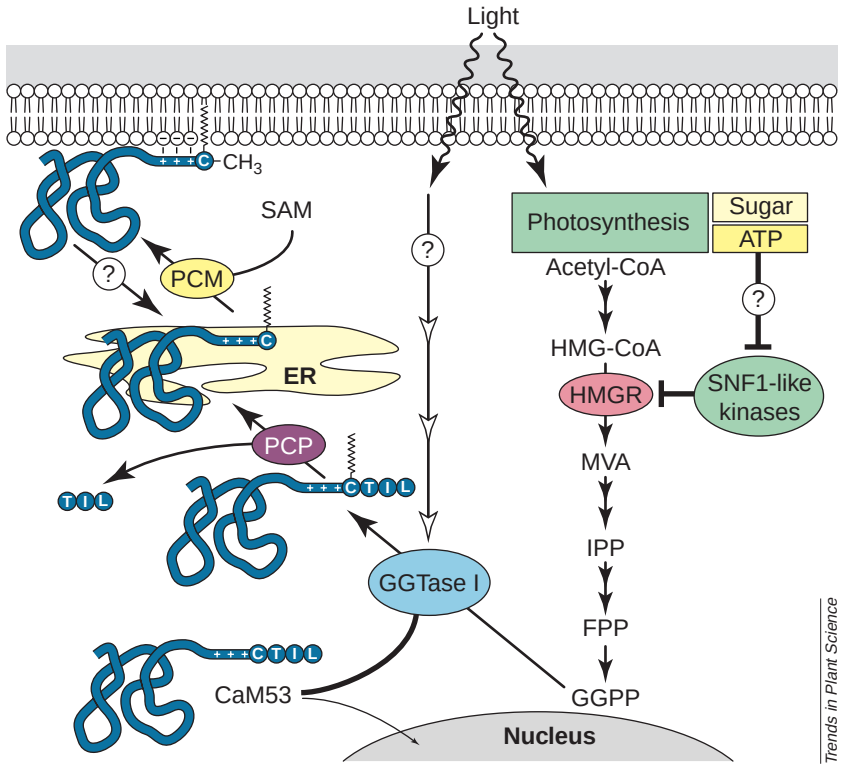

Fig. 3. Regulation of protein prenylation – a physiological relay? CaM53, a novel type of calmodulin protein, provides a model system to investigate the potential role of protein prenylation as a relay between metabolic and signaling pathways. In the light, or in dark-grown plants in the presence of sucrose, CaM53 is prenylated and localizes to the plasma membrane (which is appressed to the cell wall). However, in the absence of sucrose, CaM53 is not prenylated and is found in the nucleus[23]. Because the prenyl transferase substrates FPP and GGPP are intermediates of the mevalonate pathway, changes in the activity of the rate-limiting HMG CoA reductase (HMGR) can influence the prenylation status of proteins. The activity of HMGR is regulated by SNF1 protein kinases, for which homologs have been identified in plants[50]. Members of the SNF1 family of protein kinases are activated by AMP and inhibited by ATP, thus acting as a sensor to the metabolic status of the cells. However, this model does not exclude other regulatory mechanisms, such as activation of prenyl transferases by light, or phosphorylation of the shared FTase/GGTase-I α-subunit that has been detected in mammalian cells[41]. Abbreviations: SAM, *S*-adenosyl methionine; PCP, prenylcysteine protease; PCM, prenylcysteine carboxyl methyltransferase; MVA, mevalonic acid; IPP, isopentenyl pyrophosphate; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; HMGR, hydroxymethylglutaryl CoA reductase; ER, endoplasmic reticulum.

plants, might prove to be an important aspect of CaM53 function, and a convenient marker of physiological changes in the cell. For example, most of the plasma membrane-localized fusion proteins between GFP and the C-terminal domain of CaM53 are targeted to the nucleus in dark-adapted leaf explants. A similar nuclear localization has been detected for the GFP-fusion protein and for the endogenous CaM53 following treatment of seedlings with mevinolin, a potent inhibitor of HMG CoA reductase (HMGR), which catalyzes the rate-limiting step in cytoplasmic isoprenoid biosynthesis. Plasma-membrane-localization of the GFP-fusion protein in dark-shifted leaf explants can be restored by adding sucrose (but not mannitol) to the growth medium[23]. To date, there is no known enzymatic mechanism for cleaving the thioether bond between the prenyl group and the protein, and therefore it is probable that nuclear localization of CaM53 only involves newly synthesized protein, which does not become prenylated. The potential of CaM53 for regulating protein activity in the plasma membrane or the nucleus has interesting implications because prenylation of the protein could be controlled by several physiological mechanisms (Fig. 3).

Regulatory implications of protein prenylation

The evidence to date suggests that protein prenylation can be modulated in response to flux through the cytoplasmic isoprenoid biosynthesis pathway, a finding that is significant because FPP and GGPP are early intermediates and constitute major branch points in plants[38]. However, the situation has become more complex in view of the recently discovered alternative isoprenoid synthesis pathway in plastids[39]. Thus, overall changes in the cellular concentrations of FPP and GGPP might not necessarily reflect local changes in the cytosol that result from changes in:

- Metabolic status of the cells.
- Activity of HMGR.
- Flux between the cytoplasmic and plastid isoprenoid pathways.
- Activities of other enzymes in the cytoplasmic isoprene biosynthesis pathway.

Recent evidence in animals also suggests a regulatory role for prenylation. Activation of isoprene biosynthesis in chicken cardiac cells results in Ras farnesylation, activation of the Ras signaling pathway, and expression of muscarinic receptor and heterotrimeric G-proteins (Ref. 40). Insulin-induced phosphorylation of the FTase α-subunit also enhances Ras farnesylation[41]. Thus, changes in cellular glucose levels or AMP:ATP ratios might regulate protein prenylation via phosphorylation and FTase activation, and probably GGTase-I as well. To date it is not known whether the shared FTase/GGTase-I α-subunit is also phosphorylated in plants.

The above considerations do not preclude other mechanisms that might regulate protein prenylation in plants, such as controlling the expression of FTase and GGTase genes, which has not been extensively investigated[42,43]. The cellular substrate level of a prenylation target protein might also be an important factor because concentrations below the K_m would probably result in inefficient modification. In contrast with palmitoyl transferase and *N*-myristyl transferase, the affinity of prenyl transferases towards different protein substrates varies considerably depending on the amino acid sequence context of the CaaX box[44,45], and in some proteins it also depends on the polybasic domain proximal to the CaaX box[24]. Prenylation of specific signaling proteins might significantly affect the activity of their pathways. Regulation of prenylation might be further complicated by promiscuity between FTase and GGTase-I. Both enzymes inefficiently prenylate substrates that would usually be specific to FTase (CaaX) or GGTase (CaaL), and GTase-I can also utilize FPP. This raises interesting questions because recent experiments with mammalian RhoB suggest a functional relevance for this alternative prenylation. The RhoB GTPase is involved in actin cytoskeleton regulation, and also plays a critical role in Ras-mediated transformation. Metabolic labeling of cells with ^{3}H-mevalonate show that most of RhoB is geranylgeranylated, consistent with the CaaL motif, but a small fraction is farnesylated also. FTase inhibitors (which do not inhibit GGTase-I) suppress cellular transformation by oncogenic $RhoB^{Val14}$ and eliminate the population of farnesylated RhoB from the cells[46]. Together, these results also have potential implications for prenyl-transferase-modified plant proteins, and might explain why *era1-2*, which lacks FTase activity, can survive. With the molecular and biochemical tools now available, it should be possible to clarify these questions and illuminate the cellular role and regulation of lipid modifications in plants.

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