Multiple roles for protein palmitoylation in plants

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Palmitoylation, more correctly known as S-acylation, aids in the regulation of cellular functions including stress response, disease resistance, hormone signalling, cell polarisation, cell expansion and cytoskeletal organization. S-acylation is the reversible addition of fatty acids to proteins, which increases their membrane affinity. Membrane–protein interactions are important for signalling complex formation and signal propagation, protein sequestration and segregation, protein stability, and maintaining polarity within the cell. S-acylation is a dynamic modification that modulates the activity and membrane association of many signalling molecules, including ROP GTPases, heterotrimeric G-proteins and calcium-sensing kinases. Recent advances in methods to study S-acylation are permitting an in-depth examination of its function in plants.

Membrane–protein interactions, protein lipidation and cellular function

In the biochemical soup that comprises the interior of cells, the segregation of functions into different membrane compartments is essential for efficient operation. Signalling at and across membranes requires the coordinated and controlled formation of signalling complexes containing both membrane-associated and integral membrane proteins. Using membranes to hold components that are required for signalling increases the likelihood that interactions will occur and the required complexes will form. Recent evidence indicates that the functions of many proteins, such as ROP (Rho of plants) small GTPases [1], are regulated by a change in membrane affinity. Alternatively, proteins might require release from the membrane so that they can shuttle between different subcellular compartments [2,3] or initiate signalling [4]. Proteins that act within a single membrane compartment can shuttle between domains of varying lipid composition, which might have very different properties [1]. These differing membrane regions are known as membrane microdomains. Many of these proteins' functions within the cellular environment require reversible and controlled changes in membrane affinity.

Soluble membrane-associated proteins can be anchored to membranes by adding lipids, such as 14-carbon myristoyl groups (N-myristoylation) [5], 15-carbon farnesyl or 20-carbon geranylgeranyl groups (prenylation) [6]. Integral membrane, membrane associated and soluble proteins can also have their membrane affinity increased by linking fatty acids to their cysteine residues through thioester

bonds (S-acylation, also known as palmitoylation). The addition of lipids to proteins (lipidation) promotes membrane association by intercalation of the lipid group into the hydrophobic core of the membrane. Lipid modifications are often associated with stretches of basic amino acids within the protein that interact with negatively charged membrane lipid head groups [7], especially phosphatidylinositol 4,5-bisphosphate (PIP2) and phosphatidylinositol 3,4,5-trisphosphate (PIP3) membrane lipids [8]. Combinations of lipid modifications and protein–protein interactions provide proteins with differing strengths of membrane association and affinities for different membrane lipids and microdomains [9]. The S-acylation state of proteins can also change as a result of the activation or deactivation of complexes, leading to enhanced or decreased solubility of the protein in a specific membrane microdomain [1]. S-acylation influences protein–membrane interaction dynamics and function more strongly than the other factors discussed above, and recent advances that are summarized in this review highlight the significance of S-acylation in plants.

S-acylation

S-acylation involves the addition of a fatty acid, frequently palmitate (C16) or stearate (C18), via a thioester bond to the thiol group of a specific cysteine residue in a target protein. Using model membrane systems, it has been shown that an S-acyl group (palmitoyl moiety) on its own provides a membrane association that is about five times stronger than that provided by a single geranylgeranyl group, 100 times stronger than that provided by a single farnesyl group and 10 times stronger than that provided by a single N-myristoyl moiety. Longer S-acyl chains further increase the strength of membrane interaction [10]. Prenylation or N-myristoylation on their own do not promote lengthy membrane residence times; they allow for partitioning into the aqueous phase and for transient interaction with and sampling of membranes, although a geranylgeranyl group provides a significantly stronger membrane association than a farnesyl group [10–12]. S-acylated peptides are much less likely to partition into the aqueous phase but can move between lipid compartments, also allowing sampling of membranes. S-acylation of singly prenylated or N-myristoylated proteins effectively permanently anchors the protein to the membrane [10].

S-acylation is promoted by protein S-acyl transferases (PATs), using acyl-CoA as the acyl donor [13]. S-acylation frequently requires prior membrane association of the

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protein in question. PATs are integral membrane proteins and prior membrane association of the target protein increases the likelihood of interaction with the appropriate PAT [14]. Once S-acylated, substrates become attached to the membrane compartment that the PAT resides on, although this doesn't necessarily prevent its movement away from that compartment through trafficking pathways [15,16]. Different PATs in yeast and mammals associate with distinct membrane compartments [17] although the mechanism by which this preferential association is determined and controlled is unknown. Prior anchoring of substrates by prenylation, myristoylation or protein–protein interactions to specific membrane compartments might help to determine the specificity of a PAT by restricting the potential substrate proteins encountered by each PAT. In addition, only certain forms of a protein might be S-acylated (e.g. active ROP6 is S-acylated but inactive ROP6 is not [1]), and these forms might be restricted to a specific membrane compartment or domain, further reducing the number of PATs that the substrate encounters while in an S-acylatable state. Tissue-specific expression of PATs in all organisms might also limit access to potential substrates.

S-acylation is the only reversible lipid modification of proteins that, when coupled with the high membrane affinity that it provides, offers unique opportunities for rapid and precise regulation. The reversal of S-acylation is catalysed by thioesterases that cleave the thioester bond between the S-acylated protein and the acyl group [18]. Many S-acylated proteins have been observed cycling on and off membranes or in and out of lipid microdomains [1,3]. Thioesterification is important in regulating this cycling, and hence the activity of S-acylated proteins [18].

Enzymology of S-acylation

No consensus amino acid sequence for S-acylation has been reported, but specific cysteines within proteins are S-acylated, indicating that specificity determinants exist. These could involve enzyme–substrate interactions or the chemistry of specific substrate cysteine residues. S-acylation requires free cysteine thiolate groups; basic or aromatic residues that are spatially close to a cysteine reduce the pK_a of that cysteine, making thiolate formation more likely [19]. Binding to an S-acyl transferase might also reduce a cysteine's pK_a by bringing basic or aromatic residues into close proximity. Other post translational modifications might also alter the environment of the cysteine residue. These aspects of S-acylation have been comprehensively reviewed [20].

PATs

S-acylation is catalysed by the DHHC-motif cysteine-rich domain [13,21] found in three related categories of PAT: ankyrin-repeat-containing [13,22,23], heterodimeric [21,24] and monomeric PATs [25]. All PATs identified to date from eukaryotes are integral membrane proteins.

Ankyrin-repeat-containing PATs are generally found once or twice per genome across eukaryotes (P. Hemsley, unpublished). Ankyrin repeats are common protein–protein interaction motifs but no absolute function has been ascribed to the ankyrin repeats of any PAT. Most

Table 1. Plant species contain varying numbers of PATs

Plant species	PATs[a,b]
Lower plants	
Physcomitrella patens (*Physcomitrella*)	9
Gymnosperms	
Pinus taeda (Loblolly pine)	13
Monocots	
Allium cepa (Garden onion)	9
Oryza sativa (Rice)	58
Sorghum bicolour (Sorghum)	13
Triticum aestivum (Wheat)	25
Zea mays (Maize)	50
Dicots	
Arabidopsis thaliana (Thale cress)	23
Medicago truncatula (Barrel medic)	14
Solanum lycopersicum (Tomato)	12
Solanum tuberosum (Potato)	24

[a]PAT, protein S-acyltransferase.
[b]Data obtained from the J. Craig Venter Institute (JCVI; www.tigr.org) and represents independent gene models assembled from ESTs.

PATs contain only the DHHC domain and belong to either the Erf2p-like heterodimeric PATs [21,26] or the GODZ-like monomeric PATs [25]. *Arabidopsis* contains 23 putative PATs (AtPATs; http://openwetware.org/wiki/AtPATs) but only one ankyrin-repeat-containing PAT (TIP1). *tip1* mutants have reduced cell size, reduced pollen tube growth and cell polarity defects, suggesting that S-acyltransferases play important roles within the plant [23,27,28]. It is unclear whether plants contain both monomeric GODZ-like and heterodimeric Erf2p-like PATs. Expressed sequence tags (ESTs) from other plant species indicate that DHHC proteins are found in both lower and higher plants (Table 1). The subcellular localisation of plant PATs is currently unknown.

Functions of known S-acylated proteins in plants

S-acylation is found on a range of proteins that require membrane association, and participates in a range of signalling processes involving both small [1,3,29] and heterotrimeric [15,16] G-proteins, calcium perception [30] [31–34], and responses to pathogens [35]. S-acylation is involved in endocytosis [3] and α–tubulin is also S-acylated [36]. S-acylated proteins that have a known subcellular localisation in plants are illustrated in Figure 1.

S-acylation and GTPase signalling

S-acylation of the active type-I ROP ROP6

Type-I ROPs are small G-proteins that are involved in many aspects of cellular signalling and development. Overexpression of the type-I ROP, ROP6, affects abscisic acid (ABA)-induced stomatal closure [37] and the polar growth of root hairs [38]. Type-I ROPs are predicted to be prenylated, and recent data using transgenic plants over-expressing epitope tagged ROP6 show that ROP6 is S-acylated as a consequence of activation [1] (Figure 1a). Inactive (GDP-bound) ROP6 is not S-acylated. S-acylated ROP6 co-purifies with detergent-resistant membranes (DRMs), whereas the GDP-bound, non-S-acylated form does not. These results are consistent with the idea that the S-acylated ROP associates with specific membrane microdomains. The major acyl lipids identified on ROP6 in these microdomains by GC-MS (gas chromatography-mass spectrometry) were
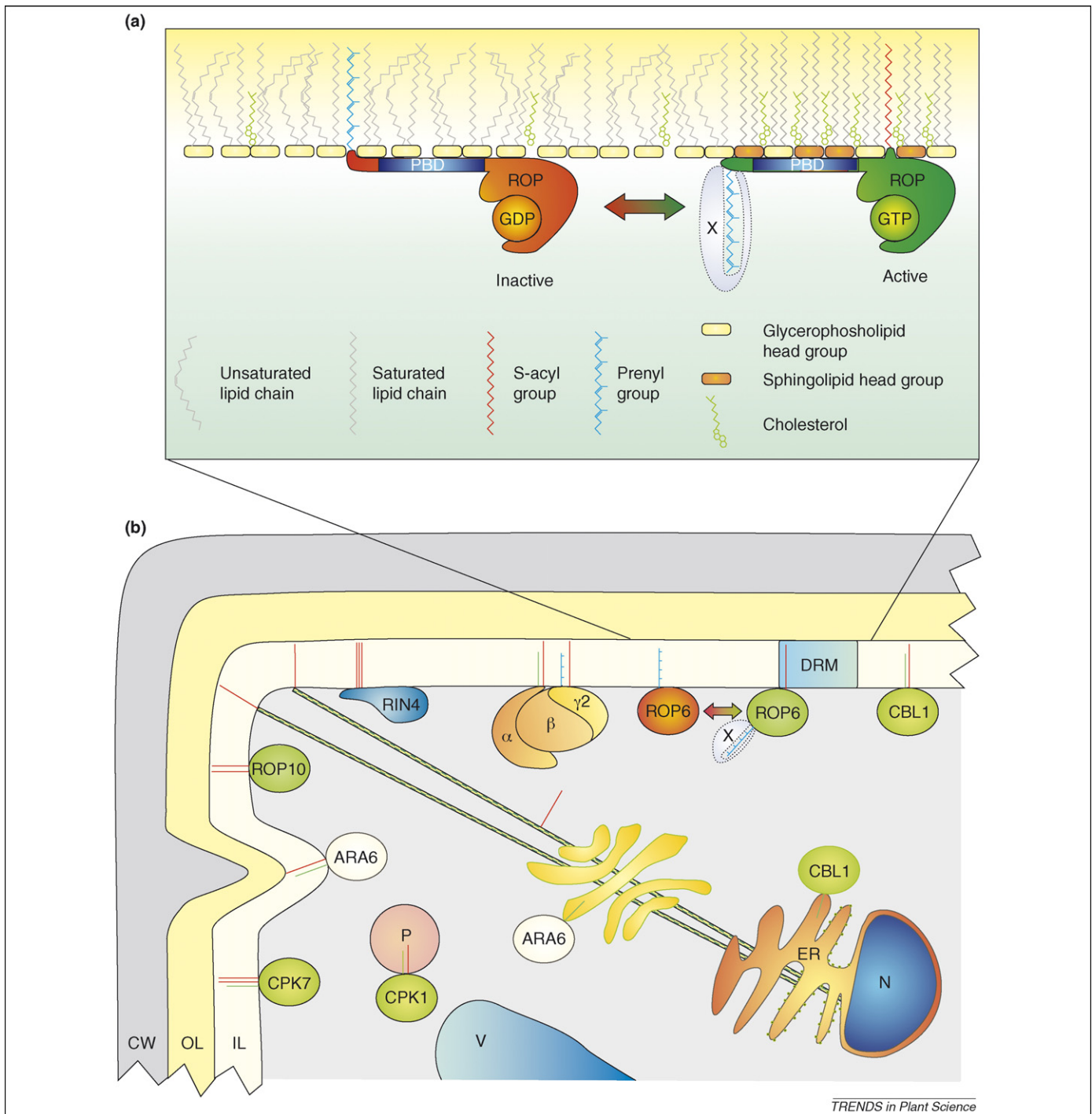
Figure 1. S-acylation promotes and defines the membrane localisation of proteins. **(a)** ROP6 partitions between non-detergent-resistant and detergent-resistant membrane (DRM) fractions. ROP6 was used to demonstrate that GDP-bound (inactive) ROP is prenylated but not S-acylated and is associated with the non-ionic detergent soluble fraction of the membrane. By contrast, the GTP-bound (active) ROP is prenylated and S-acylated. GTP-bound ROP associated with the DRM fraction of membranes, which is frequently interpreted as the saturated long-chain sphingolipid and phospholipid, cholesterol-enriched fraction of the membrane, where signalling complex formation is thought to occur. ROP6 with a cysteine-to-serine point mutation at the presumed acylation site did not partition into DRMs, irrespective of activation state and was not S-acylated. ROP6 was also shown to cycle between the soluble and insoluble fractions of the membrane in response to activation and deactivation cycles [1]. S-acyl groups, but not prenyl groups, are soluble in saturated lipid environments. The partitioning of ROP6 to DRMs following activation might therefore be a direct consequence of S-acylation, with no other factors being involved. Conformation changes that are found in the active state might also change the penetrance and alignment of the attached lipid groups within membranes, leading to altered bilayer interactions [42]. Recent work in mammals also indicates that the prenyl groups of active small GTPases might recruit other proteins, rather than interacting with membranes, contributing to downstream effects [75]. This potential interaction, as yet unproven in plants, is illustrated by protein 'X'. PBD, polybasic domain; GTP, guanosine-5'-triphosphate; GDP, guanosine-5'-diphosphate. **(b)** S-acylated proteins are found on many cellular membranes. All groups of proteins discussed in the main text that have known subcellular localisations are illustrated. To aid clarity only one member of each group of similarly modified proteins is shown; e.g. plasma membrane associated CPKs are represented by CPK7. CBLs and CPKs are involved in calcium signalling. Small GTPases act as molecular switches in a range of signalling processes. Type-I ROP small GTPases, represented by ROP6, are S-acylated and prenylated whereas type-II ROP small GTPases, represented by ROP10, are solely S-acylated. The hypothetical prenyl group binding protein envisaged for type-I ROPs is labelled ''X''. The atypical Rab small GTPase ARA6 is involved in the early stages of endocytosis. RIN4 acts in defence against P. syringae. The heterotrimeric G-protein labelled αβγ2 comprises GPA1 (α-subunit), AGB1 (β-subunit) and AGG2 (γ2-subunit) and is involved in a range of signalling processes. Lipid modifications are shown as lines linking proteins to membrane compartments with red for S-acylation, green for N-myristoylation and blue for Prenylation. Microtubules are represented by green and yellow striped lines. Abbreviations: CW – cell wall, OL- outer plasma membrane leaflet, IL-inner plasma membrane leaflet, DRM- detergent resistant membrane, ER- Endoplasmic Reticulum, G- Golgi, P- Peroxisome, V- Vacuole, N- Nucleus.

stearic acid (C18) and a small amount of palmitic acid (C16) [1]. This might indicate that ROP6 partitions into microdomains composed of longer saturated lipids than those favoured by palmitate-modified proteins. Given that all of the signal detected using ROP6–GFP (green fluorescent protein) fusions is at the plasma membrane (PM) and that ROP6 activation and S-acylation states are tightly linked [1], S-acylation of ROP6 probably takes place at the PM. One of the antibodies used in this study detects recombinant ROP10 and ROP11, both Type-II ROPs, as well as the type-I ROP ROP6 and so might be a general anti-ROP antibody. Results obtained from wild-type plants using this antibody mimic those obtained for ROP6 from transgenic lines, and therefore the conclusions regarding activation state and lipidation might be applicable to all ROPs [1].

Type-II ROPs

Plant type-II ROPs are rare among small GTPases in that they are tethered to the membrane solely by S-acylation and a polybasic domain [7,29], which probably interacts with PIP2 and PIP3 membrane lipids [39]. The polybasic domain of Type-II ROPs is closely followed by two or more S-acylated cysteines within the C-terminus [7,29]. The type-II ROP AtROP10 is involved in regulating responses to ABA [40,41] and is tightly associated with the PM. No AtROP10 signal is detected in cytoplasmic fractions by GFP fusion fluorescence or immunoblot. AtROP10 that lacks either the polybasic domain or S-acylatable cysteines however, is cytoplasmic [7]. This might indicate that the polybasic domain is required for S-acylation to occur. Glycine to alanine mutations adjacent to S-acylatable cysteines within AtROP10 reduced the membrane association of the protein [7] possibly by stearically hindering S-acylation. Valine, leucine, isoleucine, phenylalanine or methionine residues are often found adjacent to the S-acylatable cysteines of type-II ROPs [7]. The hydrophobic nature of these residues might act to push the acyl group through the charged head groups of the membrane further into the bilayer, providing stronger anchorage, protecting against cleavage by S-acyl protein thioesterases (PPTases), and increasing membrane residence time [42].

Heterotrimeric G-proteins

S-acylation contributes to heterotrimeric G-protein signalling in many organisms (e.g. [43–45]). *Arabidopsis* contains one canonical Gα (GPA1 [46]), one Gβ (AGB1 [47]) and two Gγ (AGG1 and AGG2 [48,49]) subunits. GPA1 mutants show defects in cell division [50] and germination [51], whereas AGG2 mutants show defects in gravitropism, germination and basipetal auxin transport [52]. GPA1 contains an N-terminal myristoylation sequence with an adjacent S-acylated cysteine [16]. GPA1 S-acylation is not required for binding to either the Gβγ$_1$ (AGB1–AGG1) or the Gβγ$_2$ (AGB1–AGG2) heterodimers or for trafficking of the heterotrimer to the PM. Instead, S-acylation acts to localize GPA1 to the PM, where it interacts with the Gβγ dimer and helps to stabilize the heterotrimer [16].

Arabidopsis Gγ subunits are prenylated and, unlike other heterotrimeric G-proteins, rely on dimerisation with AGB (AGG1 [16]) or S-acylation (AGG2 [15]) to traffic to the PM (Figure 2). AGG2 is S-acylated *in planta* by an
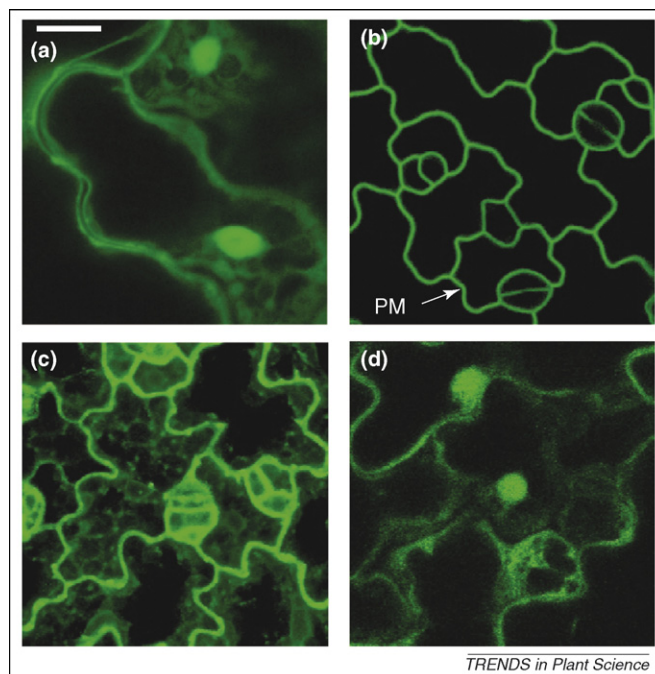

Figure 2. The heterotrimeric G-protein gamma subunit AGG2 is prenylated and S-acylated and localises to the plasma membrane (PM) in *Arabidopsis* leaf pavement cells. **(a)** Free yellow fluorescent protein (YFP). **(b)** Prenylated and S-acylated YFP–AGG2 fusions localize to the PM, whereas **(c)** YFP–AGG2 Cys^{95}Ser fusions lacking the S-acylatable cysteine are observed on the PM and many internal membraneous structures that are positive for Golgi markers. **(d)** YFP–AGG2 Cys^{97}Ser fusions are not prenylated and show a distribution similar to free YFP. This indicates that prenylation anchors AGG2 to membranes and is required for S-acylation of AGG2, but S-acylation is required for full targeting and anchorage of AGG2 solely to the PM [15]. Scale bar represents 10 μm. All images are of stably transformed plants and were obtained using confocal microscopy. Data provided by Qin Zeng and Mark Running.

unknown S-acyl transferase [36] at the Golgi before trafficking to the PM [15,16]. S-acylation might act to target and restrict AGG2 signalling to the PM or to cycle AGG2 in and out of PM sub-domains in response to stimuli. There is currently no evidence for S-acylation of AGG1, and AGG1 is found at the PM and Golgi [15]. S-acylation might therefore be involved in determining the downstream effects of AGG2 by restricting its cellular mobility and localisation. Lipid modifications contribute to the unique nature of heterotrimeric G-protein signalling in plants, and it will be interesting to determine the roles of lipidation in known heterotrimer signalling processes [52].

S-acylation of Ara6

Ara6 is a plant-specific Rab5 GTPase homologue that is myristoylated and S-acylated rather than geranylgeranylated like other Rabs [3]. Ara6 co-localises with the sterol-reactive dye filipin on a subset of early endosomes. S-acylation is essential for this localisation, with solely myristoylated Ara6 remaining largely on the endoplasmic reticulum (ER) [2]. This indicates that N-myristoylation and S-acylation of Ara6 enable association with, and potentially endocytosis of, sterol-enriched PM.

S-acylation and calcium signalling

CBLs

Calcineurin B-like proteins (CBLs) are calcium-binding proteins that bind to CBL-interacting protein kinases

(CIPKs) in a calcium-dependent manner [53]. CBL1 is involved in integrating plant responses to abiotic stresses [8,54]. It is myristoylated on Gly^2, S-acylated on Cys^3 and associates with the PM as a dimer with CIPK1. Lipid modification is not, however, required for CBL1–CIPK1 dimer formation. Solely myristoylated CBL1 is found primarily on the ER but also on other cellular membranes, whereas CBL1 that lacks both lipid modifications is cytoplasmic. This indicates that S-acylation specifies a PM localisation for CBL1, whereas myristoylation promotes sampling of membranes or ER retention until it encounters the CBL1 S-acyltransferase. As myristoylated CBL1 is mainly associated with the ER, it is likely that the CBL1 S-acyl transferase also resides in the ER [30].

CPKs

Calcium-dependent protein kinases (CPKs) are calcium-sensing kinases that have been linked to abiotic stress signalling. Many CPKs are N-myristoylated and contain cysteine residues near to this modification that might be S-acylated (reviewed [55]). The rice CPK OsCPK2 is the only CPK shown biochemically to be modified by myristate and palmitate, and its S-acylation depends upon prior myristoylation [34]. The tomato CPK LeCRK is observed at the PM when fused to GFP, LeCRK1–GFP Gly^{2}Ala myristoylation-site mutants are cytoplasmic, whereas LeCRK1–GFP Cys^{4,5}Ala putative S-acylation-site mutants are observed on all cellular membranes. This indicates a role for S-acylation in specifying LeCRK localisation at the PM and that myristoylation is required for the S-acylation of Cys^{4,5} [32].

Arabidopsis contains 34 CPKs, of which 27 have an N-myristoylation site followed by one to two cysteines that are potential candidates for S-acylation. Subcellular localisation studies of nine AtCPKs indicated that those with one (AtCPK9, AtCPK16, AtCPK21 and AtCPK28) or two (AtCPK7 and AtCPK8) cysteine residues adjacent to a myristoylated glycine bind to the PM [31]. AtCPK1 and AtCPK2 also have one cysteine following the myristoylation site but localize to peroxisomes [31] and the ER [33], respectively. The non-myristoylated CPK AtCPK4 is cytoplasmic, whereas the solely myristoylated CPK AtCPK3 transiently associates with membranes [31]. These data indicate that although myristoylation and S-acylation aid anchoring of CPKs to membranes, there are other factors that determine target-membrane specificity. Specific membrane localisation might therefore be achieved by association with other proteins, by protein sequence or by other post-translational modifications.

Other S-acylated proteins
RIN4

RIN4 acts as part of a system that monitors foreign proteins in *Arabidopsis* defences against *Pseudomonas syringae*. RIN4 is C-terminally S-acylated at three cysteines and is anchored to the PM [35]. Membrane localisation of RIN4 is required for its interaction with the membrane-bound *P. syringae* type-III effectors AvrRpm1 and AvrB, which leads to RIN4 phosphorylation and increased resistance to *P. syringae* [56].

The cytosolic *P. syringae* type-III effector protease AvrRpt2 cleaves RIN4, leaving the S-acylated portion of RIN4 on the membrane while the remaining cytosolic portion is degraded by the proteasome [35]. This, in turn, leads to increased RPS2 activation and defence against *P. syringae* [4,57,58]. Thus, S-acylation acts to prevent constitutive degradation of RIN4 and enables interaction with AvrRpm1 and AvrB.

Tubulin

The yeast TUB1 α–tubulin subunit is S-acylated and the major site for S-acylation is Cys^{377} [59]. Plant α–tubulin subunits contain a Cys residue that is analogous to that in yeast, and *Arabidopsis* α–tubulin subunits were recently shown to be S-acylated [36]. The exact role of S-acylation in microtubule function is unknown but it appears to aid in spindle positioning and astral microtubule turnover in budding yeast during cell division [59]. S-acylation also reduces the polymerisation competence of α–tubulin monomers over repeated polymerisation-depolymerisation cycles *in vitro* [60], although the possibility that this is an artefact of the reduced solubility of S-acylated tubulin in aqueous buffer has not been addressed. Although as yet untested, S-acylation of tubulin could act to bind cortical microtubules to membranes and aid in maintaining microtubule alignment, which could, in turn, affect cell wall deposition. S-acylated microtubules could also link to, and maintain the positions of, membrane compartments within the cell that might be important for directional growth and cellular organization.

More S-acylated proteins in plants?

In yeast, the SNARE Tlg1p is ubiquitinated and rapidly degraded. S-acylation at a site immediately adjacent to the N-terminus of the transmembrane domain of Tlg1p blocks its ubiquitination [61]. This reveals another distinct role for S-acylation. *Arabidopsis* contains numerous SNAREs that have Cys residues preceding their transmembrane domain in a similar manner to Tlg1p. This makes plant SNAREs likely candidates for S-acylation.

S-acylation of G-protein coupled receptors (GPCRs) frequently regulates the activity of these receptors in animals and fungi. Although plants have few identified GPCRs, they are potential S-acylation targets. Many more S-acylated plant proteins are likely to be identified in the future and potential approaches are outlined below.

Tools for studying S-acylation

Despite the difficulties associated with predicting protein S-acylation, two complementary programs, NBA-Palm and CSS-Palm, which use two different approaches, have been published as being able to predict S-acylation sites [62,63]. As yet there are no reports of previously unknown S-acylated proteins being identified by these methods.

Mutation of candidate cysteine residues for S-acylation to serine or alanine often provides the first evidence of protein S-acylation: the localisation of fluorescent fusion proteins, or protein membrane association after cell fractionation, can be examined. The palmitate analogue 2-bromopalmitate (2-bromohexadecanoic acid) is a potent inhibitor of S-acylation [64]. 2-bromopalmitate has been

used in plants to examine both phenotypes [23] and changes in the subcellular localisation of fluorescent fusion proteins [15,29] that are associated with S-acylation. Cerulenin [65] and tunicamycin [14,66] also inhibit S-acylation but have secondary effects; neither of these has been used to date in plant S-acylation studies.

In vivo labelling with [^{3}H]- [23] or [^{125}I]- [67] palmitic acid has been the main method for assaying S-acylation state. This is a simple, if costly and time-consuming, procedure for culture grown cells but is limited in its application to whole plants or tissues and does not identify which acyl group would ordinarily be attached. The biotin switch method for assaying S-acylation of plant proteins [36] is both fast and sensitive and can be performed in any laboratory familiar with western blotting. This method does not require special equipment, *in vivo* labelling or the use of radioactive sources, but it only identifies the presence of acylation not the type of acyl group attached [68]. An antibody that is reactive against the protein of interest or an epitope-tagged version of the protein of interest is also required. An extension of this assay has been used on a proteomic scale to identify *Saccharomyces cerevisiae* S-acylated proteins and to assign them to PATs [69]. GC-MS analysis allows direct quantitative identification of lipids that are attached to proteins, including the differentiation of acyl groups, but requires large quantities (50 g) of tissue [1] and is not readily available in all laboratories. This method also requires purification of the protein of interest before analysis by GC-MS necessitating the use of chromatographic equipment and/or epitope-tagged versions of the protein of interest. All of these analyses require point mutants to identify the cysteine residue that is S-acylated. Epitope tagging of proteins is convenient and cheaper than raising antibodies that are specific to the protein of interest but care must be taken not to disrupt potential S-acylation sites with the epitope tag. Epitope tagging also requires the use of transgenic plants and frequently uses promoters, such as 35S, that drive expression at non-native levels. All of the normal precautions taken when using transgenic plants need to be taken into account in any analysis of S-acylation using epitope-tagged proteins. Future, as yet undeveloped, approaches using tandem mass spectroscopy (MS-MS) would hopefully eliminate the need for point mutagenesis and biochemical assay by allowing direct identification of the size and site of acyl groups within a protein.

Microscopical analysis of the membrane association of fluorescent fusion proteins has proven useful for analysis of S-acylated proteins [1,3,7,15,16,29,32,41]. FRAP (fluorescence recovery after photobleaching) [70–72], FLIM-FRET (fluorescence lifetime imaging microscopy-fluorescence resonance energy transfer) and immunogold electron microscopy [73] have also been used in mammalian cells to examine S-acylation but have yet to be used in plants.

The future of S-acylation research in plants

S-acylation affects many aspects of protein function, acting as a specific membrane targeting and retention signal [3,15,16,31,34,41,74] and preventing protein degradation [35]. The reversibility of S-acylation also makes it important for rapid microdomain partitioning [1] and cycling of proteins between compartments [3]. The large number of S-acyl transferases in plants indicates that there are likely to be many more S-acylated proteins than have currently been identified. It is likely that new roles for S-acylation will be uncovered as the number of known S-acylated plant proteins increases. Assigning S-acylated proteins to their respective S-acyltransferases might aid in understanding the phenotypes observed in S-acyltransferase mutants [23]. The phenotypes of proteins with mutated S-acylation sites might help answer many of the outstanding questions about the role of S-acylation in protein function. It should be noted, however, that the kinetics of S-acylation has not yet been addressed in any organism.

We have highlighted the new approaches that might be taken by researchers to examine potentially S-acylated proteins and the function of their S-acylation. With these tools, it should now be possible to fill many of the gaps in our knowledge of S-acylation in plants.

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