Current perspective on protein S-acylation in plants: more than just a fatty anchor?

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Abstract

Membranes are an important signalling platform in plants. The plasma membrane is the point where information about the external environment must be converted into intracellular signals, while endomembranes are important sites of protein trafficking, organization, compartmentalization, and intracellular signalling. This requires co-ordinating the spatial distribution of proteins, their activation state, and their interacting partners. This regulation frequently occurs through post-translational modification of proteins. Proteins that associate with the cell membrane do so through transmembrane domains, protein–protein interactions, lipid binding motifs/domains or use the post-translational addition of lipid groups as prosthetic membrane anchors. S-acylation is one such lipid modification capable of anchoring proteins to the membrane. Our current knowledge of S-acylation function in plants is fairly limited compared with other post-translational modifications and S-acylation in other organisms. However, it is becoming increasingly clear that S-acylation can act as more than just a simple membrane anchor: it can also act as a regulatory mechanism in signalling pathways in plants. S-acylation is, therefore, an ideal mechanism for regulating protein function at membranes. This review discusses our current knowledge of S-acylated proteins in plants, the interaction of different lipid modifications, and the general effects of S-acylation on cellular function.

Key words: Acylation, lipid raft, lipidation, lipids, membrane, microdomain, palmitoylation, plant, post-translational modification, S-acylation.

Introduction: the cellular context of protein lipidation

Membranes, and in particular the plasma membrane, are the primary site of signal transduction in the cell and provide a stable platform for protein organization and a site to integrate and transduce extracellular signals. Environmental factors such as water and nutrient availability or pathogen presence, as well as plant-derived factors such as hormones and signalling factors, are perceived by an array of receptors and associated proteins resident in or at the plasma membrane. Many of these proteins undergo rapid post-translational modification upon signal perception to activate responses. The importance of post-translational modifications such as phosphorylation, ubiquitination, and SUMOylation in modulating cell signalling has been firmly established, but lipid-based post-translation modifications are now emerging as major factors regulating the function of membrane associated proteins and complexes. Four major classes of protein lipidation have been identified in plants; N-myristoylation, prenylation, S-acylation, and GPI/GIPC anchors. Much effort is now being focused on how these lipid-based post-translational modifications affect protein function. GPI and GIPC anchors are complex glycolipids and are restricted to proteins found in the outer leaflet of the plasma membrane. All other known lipid modifications of proteins are found on proteins associated with, or integral to, the cytoplasmic leaflet of membranes.
N-myristoylation was first described in 1982 (Aitken et al., 1982; Carr et al., 1982) and involves the addition of myristic acid to N-terminal glycine residues through an amide bond by N-myristoyl transferase. Despite N-myristoylation providing a weak membrane–protein association it plays an important role in protein–membrane interactions and signal transduction events. Prenylation was discovered in the late 1980s and involves the addition of isoprenyl groups, either farnesyl (15 carbon) or geranylgeranyl (20 carbon), to C-terminal cysteine residues in a CaaX motif by a thioether linkage. It is catalysed by farnesyl transferase and geranylgeranyl transferase enzymes, respectively (Casey and Seabra, 1996). Both N-myristoylation and prenylation in plants have recently been reviewed (Hemsley, 2015) and will not be discussed further other than in the context of the main focus of this review—protein S-acylation.

S-acylation: a unique lipid modification of proteins

S-acylation is historically referred to as palmitoylation but, as other acyl groups such as stearic acid can be added instead of palmitic acid, the use of S-acylation is becoming more common (Sorek et al., 2007; Batistic et al., 2008). Protein S-acylation emerged as a new type of post-translational protein modification during the 1970s when palmitate was shown to be added to vesicular stomatitis virus glycoprotein (Schmidt and Schlesinger, 1979). Despite this comparatively early start, S-acylation is still the least-understood post-translational lipid modification of proteins (Hemsley and Grierson, 2008; Sorek et al., 2009). It is known that S-acylation allows proteins directly to associate with membranes and provides a membrane association strength equivalent to that of a transmembrane domain (Shahinian and Silvius, 1995). S-acylation is also unique amongst intracellular lipid modifications of proteins for two reasons: (i) it is reversible, and (ii) different lipid groups can be added. Reversibility offers the potential to regulate protein function in response to a stimulus in a similar manner to phosphorylation or ubiquitination, rather than just acting as a membrane anchor as proposed for prenylation and N-myristoylation. This may explain why many integral membrane proteins are S-acylated (Hemsley et al., 2013) as S-acylation is obviously not required to provide membrane attachment. The importance of adding different length and saturations of lipid groups to proteins is essentially unknown, although some data are now emerging that certain S-acylated cysteine residues in proteins are preferentially and consistently modified by specific lipids (Brett et al., 2014). Some of the concepts and ideas surrounding the function of S-acylation are shown in Fig. 1 and will be discussed further below.

Enzymatic regulation of S-acylation in plants

S-acyl groups are added to cysteine residues through a thioester bond. S-acylation also frequently appears to require prior membrane association, either via a transmembrane domain, protein–protein interaction or other lipid modification such as myristoylation or prenylation. While there is no known consensus sequence for S-acylation to occur, it is widely accepted that S-acylation frequently occurs on cytosolic cysteine residues adjacent to transmembrane domains, prenylated cysteines or N-myristoylated glycines (Roth et al., 2006).

Protein S-acyl transferases

Spontaneous, enzyme independent, S-acylation of proteins in vitro by acyl-CoA has been reported in rat and yeast extracts (Bizzozero et al., 1987; Duncan and Gilman, 1996). However, the levels of acyl-CoA used were orders of magnitude greater than physiological concentrations making the in vivo relevance questionable. Subsequently, with a few notable exceptions such as the transport particle Bet3, S-acylation has been shown to be dependent on a family of proteins known as Protein S-acyl Transferases (PATs) in vivo. PATs were originally identified in Saccharomyces cerevisiae (Lobo et al., 2002; Roth et al., 2002) and are integral membrane proteins with an Asp-His-His-Cys (DHHC) motif conserved across eukaryotes. The DHHC motif is thought to be the active site and is required for the PAT to modify target proteins (Mitchell et al., 2010). In Arabidopsis, PATs are a family of 24 proteins (Hemsley et al., 2005; Hemsley and Grierson, 2008; Batistic, 2012). Research with mammalian systems has shown that not all DHHC-type PATs exhibit S-acyl transferase activity, but this possibility has not yet been investigated in plants (Batistic et al., 2008; Ohno et al., 2012). In yeast Ykt6, a unique PAT activity that does not have the DHHC motif, has been described (Dietrich et al., 2004). Ykt6 is a SNARE protein that is required for vesicle fusion and S-acylation of vac8 at the vacuolar membrane (Dietrich et al., 2004). Plant Ykt6-like proteins do not appear to possess the motif required for S-acyl transferase activity, but it does invite the idea that there may be other undiscovered PAT activities that could regulate protein S-acylation.

**Fig. 1.** Proposed functions for S-acylation in plants. (A) S-acylation can act as a means of recruiting an otherwise soluble protein to membranes. (B) S-acylation can change the hydrophobic character of proteins such that it can move a protein from one microdomain to another. As S-acylation is reversible, the movement of proteins from one domain to another can be regulated which, in turn, may determine protein–protein interactions or alter protein activity or function. (C) S-acylation can promote the formation of specific complexes either through altering membrane microdomains to include and exclude specific proteins or by direct interaction of the S-acyl group with other S-acyl groups or hydrophobic regions of proteins. (D) S-acylation can also alter the conformation of proteins in membranes. In particular, the tilting of transmembrane helices by S-acylation to prevent hydrophobic mismatch appears to be important to stop proteins being recognized as mis-folded and degraded. Proteins are shown in blue, S-acyl groups are shown in red, and alternative microdomain environments are shown as yellow or green balls.
Arabidopsis PATs are localized in the ER, Golgi, various vesicles, and the tonoplast but appear to be mainly localized to the plasma membrane (nine out of 24) (Batistic et al., 2012). This is different from mammalian and yeast PATs which are mainly localized to the Golgi (Rocks et al., 2010). This suggests that regulation of proteins by PATs at the plasma membrane may be more important or involve more substrates in plants than in animals. In metazoan and fungal systems, the majority of PATs appear to be involved in regulating protein transport from the ER/Golgi (Rocks et al., 2010). Only two plant PATs, PAT24/TIP1 and PAT10 that localize either to the Golgi or to the tonoplast, respectively, have been described in any detail in the literature. The remaining 22 PATs have yet to be characterized and remain an unexplored yet potentially fruitful area for understanding the importance of S-acylation in plants.

PAT24/TIP1 localizes to the Golgi in Arabidopsis (Batistic et al., 2012). tip1 mutants show defects in pollen tube and root hair growth in addition to impaired cell expansion, increased shoot branching, and early senescence (Schiefelbein et al., 1993; Ryan et al., 1998; Hemsley et al., 2005). PAT10 localizes to the tonoplast but not the Golgi or post-Golgi vesicles (Batistic et al., 2012). Loss of PAT10 causes a semi-dwarf phenotype and defective growth in the reproductive organs.

S-acylated proteins in plants

More than 500 proteins have been proposed to be S-acylated in plants through a proteomics study (Hemsley et al., 2013), with even more having been identified subsequently (PA Hemsley, unpublished data). While this work undoubtedly highlights the potential importance and wide-ranging roles of S-acylation in plants, the remainder of this review will focus primarily on proteins with a described role for S-acylation in their function. A large number of S-acylated proteins are also involved in plant–pathogen interactions but these are covered in depth by another review in this issue (Boyle and Martin, 2015) and will not be discussed further here.

Solely S-acylated proteins

As the study of S-acylation is in its infancy, and the S-acylation of most proteins was discovered fortuitously while investigating other phenomena such as N-myristoylation or prenylation, proteins that are lipid modified solely by S-acylation are in the minority. A number of S-acylated Receptor-Like Kinase (RLK) proteins were recently identified and while S-acylation appears to be important for their function the exact mechanism has yet to be described (Hemsley et al., 2013).

LIP1 and LIP2 are receptor-like cytoplasmic kinasins (RLCK, part of the RLK superfamily) and are involved in guiding pollen tube growth towards ovules. LIP1 and LIP2 proteins are solely S-acylated and transformation of lip1 lirp2 mutants with LIP1 or LIP2 lacking the S-acylation sites fails to complement the lip1 lip2 mutant phenotype (Liu et al., 2013). This indicates that S-acylation is crucial for the functioning of these proteins. Other proteins identified as being S-acylated in plants also include those found in other systems such as ABC transporters, tetraspanins, SNAREs, ion channels, and various ATPases but a role for S-acylation in their function has yet to be determined in any depth (Hemsley et al., 2013). Plant-specific S-acylated protein families include the NDR1/NHL stress response proteins, most members of the cellulose synthase superfamily, remorins, and many proteins involved in pathogen responses (Hemsley et al., 2013). CBL proteins interact with and recruit CIPKs, a family of ser/thr protein kinases during calcium signalling. CBL2 is an S-acylated tonoplast-localized Ca2+ sensor. Vacuolar targeting of CBL2 is dependent upon multiple S-acylation modifications to its N-terminus, potentially by PAT10 (Batistic et al., 2012; Zhou et al., 2013). Loss of the three S-acylation sites causes CBL2 to localize to the cytoplasm and nucleus rather than the tonoplast. Transformation with S-acylation-defective and, consequently, mis-localized CBL2 fails to complement the ABA hypersensitivity of cbl2 mutants during seed germination indicating an absolute requirement for S-acylation in CBL2 function (Batistic et al., 2012).

Acyl protein thioesterases/palmitoyl protein thioesterases

De-S-acylation is also enzymatically regulated by two families of serine hydrolases; acyl protein thioesterases (APT) and palmitoyl protein thioesterases (PPT). In animal systems cytoplasmic APTs regulate de-S-acylation and are involved in altering a protein’s function or fate (Yeh et al., 1999). PPTs act at the lysosome during protein catabolism to recycle acyl groups (Verkruyse and Hofmann, 1996). Nothing is known about numbers of APTs or PPTs in plants, although there are potential homologues of mammalian APTs and PPTs in plant genomes.
It has long been known that the yeast α-tubulin protein TUB1 is S-acylated (Caron et al., 2001). More recent research in plants (Hemsley and Grierson, 2008) showed that plant tubulin is also S-acylated. S-acylation of yeast TUB1 is important during cell division for correct spindle positioning and astral microtubule turnover; however, plants do not possess astral microtubules. It is possible that tubulin S-acylation in plants is involved in anchoring the microtubule cytoskeleton to cellular membranes. It is also possible that S-acylation could promote interactions between proteins that decorate the cytoskeleton and the cytoskeletal network. However, no phenotype has yet been established for S-acylation-deficient tubulin mutants in plants, but could prove an interesting area of future research.

N-myristoylated and S-acylated proteins

Peripheral membrane proteins are often modified by other lipids before S-acylation occurs. It has been suggested that N-myristoylated or prenylated proteins with the potential to become S-acylated interact weakly or transiently with membranes through these other lipid groups (Shahinian and Silvius, 1995). This enables interaction with their cognate PAT and, subsequently, they become S-acylated and fixed at the membrane (Rocks et al., 2010). N-myristoylation and S-acylation are frequently observed together and appear to make up the majority of proteins described as S-acylated in the literature despite comprising only a small part of the overall S-acyl proteome (Roth et al., 2006; Hemsley et al., 2013). This is presumably due to the ease of predicting that a cysteine residue adjacent to an N-myristoylation site is likely to be S-acylated.

Thioredoxins play a critical role in stress responses by balancing the cells redox state through thiol-disulphide exchange reactions. Some H-type thioredoxins are N-myristoylated and become S-acylated in the ER before export to their final destination of the plasma membrane, while others are solely N-myristoylated (Hemsley et al., 2013; Traverso et al., 2013). S-acylation of cysteine residues adjacent to the N-myristoylation site is crucial for H-type thioredoxin localization to microdomains in the plasma membrane.

The plant Ca<sup>2+</sup>-dependent nucleases, CAN1 and CAN2, are N-myristoylated and S-acylated proteins involved in DNA and RNA degradation during programmed cell death (Lesniewicz et al., 2012; Hemsley et al., 2013). The localization of these nucleases is unique for their class of protein, but the in vivo relevance of this has yet to be elucidated. CBL1 is a plasma membrane-localized protein that complexes with CIPK23 to activate potassium channels in the membrane. S-acylation is also required for plasma membrane targeting of CBL1 to the plasma membrane as mutation of the S-acylation site causes retention of CBL1 in the ER (Batistic et al., 2008). Calcium-dependant protein kinases (CDPK) are the most abundant ser/thr kinases in plants. OSCPK2, a rice CDPK, must be both myristoylated and S-acylated for it to be correctly targeted to the plasma membrane. Non-S-acylated OSCPK2 is localized to the cytoplasm, highlighting the necessity of S-acylation in OSCPK2 membrane-association.

It was speculated that, as S-acylation is reversible, changes in the state of OSCPK2 S-acylation in response to various stimuli may change its sub-cellular distribution and affect downstream pathways (Martin and Busconi, 2000), but this has yet to be shown in vivo.

ARA6/AtRabF1 is a Rab GTPase involved in regulating vesicle transport and fusion with target membranes. ARA6 function is dependent on both N-myristoylation and S-acylation; wild-type ARA6 is mainly localized to early endosomes whereas solely N-myristoylated ARA6 localizes to the ER (Ueda et al., 2001). The N-terminal 34 amino acids that contain the S-acylation site are able to confer plasma membrane localization, but an additional eight amino acids from the C-terminus are necessary for ARA6 endosomal targeting. This indicates that there are other factors at play besides lipid modification in determining sub-cellular localization. ARA6 is currently the only plant vesicle trafficking protein with a confirmed role for S-acylation in protein function.

POLTERGEIST and PLL1 are protein phosphatases that transduce signals from membrane receptors to cis-regulatory elements in the WOX genes that they target. POL and PLL1 are essential for root and shoot meristem maintenance. They are both S-acylated and N-myristoylated and it was suggested that S-acylation stabilized the N-myristoylation-mediated plasma membrane association. S-acylation- and N-myristoylation-defective PLL exerts a dominant negative effect on plants, indicating a requirement for these lipid modifications in maintaining correct function of PLL by regulating its cellular distribution (Gagne and Clark, 2010). Interestingly, this phenotype was remarkably similar to other plant lines that are defective for NMT1 and NMT2 (N-myristoyltransferase) (Pierre et al., 2007). This suggests that N-myristoylation is required prior to S-acylation for correct POL and PLL1 function.

G-protein S-acylation

GTP binding-proteins (G-proteins) are major signalling regulators in eukaryotes and plants are no exception. This family of proteins contains some of the best-characterized S-acylated proteins in plants.

Heterotrimeric G-proteins

Heterotrimeric G-proteins are composed of three subunits, α, β, and γ. GPA1 encodes the Arabidopsis Gα subunit and glycine 2 is N-myristoylated, with the proximal cysteine residue probably being S-acylated. Both of these lipid modifications are required for the plasma membrane localization of Gda (Adjobo-Hermans et al., 2006). Transient expression of the Gβ subunit AGB1 or Gγ subunit AGG1 alone was not sufficient for their localization to the plasma membrane, but co-expression allowed plasma membrane localization to occur. By contrast, AGG2 expressed in the absence of other subunits was able to localize to the plasma membrane. The Arabidopsis Gγ subunits AGG1 and AGG2 are prenylated.

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(Zeng et al., 2007; Sorek et al., 2013). AGG2 has also been proposed to be S-acylated at a cysteine adjacent to the prenylation site and S-acylation is essential for correct localization to the plasma membrane. Mutation of the equivalent cysteine in AGG1 did not alter its localization within the cell (Adjobo-Hermans et al., 2006; Zeng et al., 2007) leading to the suggestion that AGG1 is not S-acylated. Subsequent analysis by GC-MS failed to identify S-acyl groups on AGG1 (Sorek et al., 2011) but S-acylation assays of AGG1 appear to indicate that AGG1 may be S-acylated, albeit at lower levels than AGG2 (Hemsley et al., 2013). This apparent contradiction has yet to be resolved.

ROP small GTPases

ROP proteins (Rho Of Plants) are a family of membrane-associated small GTPases involved in many signal transduction events (Fu et al., 2002) and are split into two families. Type-I ROPs can be both membrane-bound and cytosolic while Type-II ROPs are exclusively membrane-associated. Type-I ROPs are prenylated and, depending on their activation state, can also be S-acylated (Lavy and Yalovsky, 2006; Sorek et al., 2007). In their inactive GDP-bound state, Type-I ROPs are found in both membrane-bound and cytosolic pools and are not S-acylated. The cytosolic pool is created and maintained by the action of guanine nucleotide dissociation inhibitors (GDI) thereby preventing ROP activation by guanine nucleotide exchange factors (GEF) at the membrane (Bischoff et al., 2000). GEF activity promotes the exchange of GDP for GTP in response to a stimulus (Michaelson et al., 2001) and Type-I ROPs in this GTP-bound state can be S-acylated (Sorek et al., 2007). ROP6 is the best-characterized member of the ROP family in terms of S-acylation. Expression of constitutively active ROP6 mutant constructs that are S-acylation-deficient can still hydrolyse GTP but are impaired in their ability to affect growth polarity, reactive oxygen species distribution, and endocytic pathways when compared with constitutively active S-acylated ROP6. However, S-acylation-deficient ROP6 can still interact with downstream effectors (Sorek et al., 2007, 2010). It is possible that S-acylation promotes the localization of ROP6 to discrete domains within the plasma membrane. Once localized to these domains, ROP6 could then correctly orchestrate cell polarity mechanisms.

Type II ROPs are similar to Type I ROPs, but they are entirely membrane-associated and do not appear to have a cytosolic pool. Type II ROPs undergo S-acylation on 2C or 3C-terminal cysteine residues, although if and how Type II ROPs undergo S-acylation state changes in response to stimuli is not known. However, it is clear that S-acylation is essential for the function of type-II ROPs (Lavy et al., 2002; Lavy and Yalovsky, 2006).

Non-enzymatic S-acylation in plants

BET3 is an S-acylated protein involved in tethering vesicles to the Golgi membrane. Mutation of the S-acylation site does not noticeably impair its membrane-localization or protein function, indicating that S-acylation does not act as a membrane anchoring system for BET3 (Turnbull et al., 2005). Rather, BET3 is permanently S-acylated inside a hydrophobic pocket and this is required for correct folding and stability of BET3 (Kummel et al., 2006).

The chloroplast D1 protein is involved in photo-oxidative stress repair of PSII (Nanba and Satoh, 1987). D1 is encoded by the PsbA gene and is known to become S-acylated in a light-dependent manner. D1 remains the only chloroplast-localized protein reported to be S-acylated (Mattoo and Edelman, 1987). Interestingly, no DHHC PATs are localized to the chloroplast (Batistic et al., 2012) although it is possible that prokaryotic non-DHHC PAT proteins exist. However, as auto-S-acylation has previously been reported (Bizzozero et al., 1987; Duncan and Gilman, 1996), it is possible that D1 could auto-S-acylate in the chloroplast as it is the main site of fatty acid biosynthesis in plant cells and, therefore, acyl-CoA levels are much higher than in the rest of the cell (Ohlrogge et al., 1978).

General effects of S-acylation on cellular function

Membrane microdomains

In the work covered by this review, S-acylation has largely been shown to promote membrane localization or differential localization within the cell. Research also shows that it has a potential, more subtle, role in protein localization to distinct membrane microdomains. Biochemical methods isolating ‘detergent-resistant’ (DRM) or ‘detergent-insoluble’ (DIM) membranes that are sterol-rich have been proposed to represent microdomains and formed the basis of the lipid raft hypothesis. However, recent work indicates that the lipid raft hypothesis and associated biochemical methods, while useful tools, are an oversimplification of the in vivo state of affairs. By contrast, many varied, diverse and mutually exclusive microdomains appear to exist in the plasma membrane (Jarsch et al., 2014). Emerging data suggest that protein clustering into microdomains is based on proteins with similar physico-chemical properties grouping together in the membrane to form many different types of microdomain. The addition of lipid groups to proteins could alter the surrounding lipid environment to attract or drive out other proteins from the microdomain. As S-acylation is the only major hydrophobic characteristic of proteins so far described as being able to be altered, it is the ideal mechanism to regulate microdomain formation and content. Changing the S-acylation state of proteins would therefore allow protein relocalization into, or formation of, microdomains that, for example, contain binding partners required for signal transduction to occur (Fig. 1).

Until recently, the available microscopy tools had been unable to resolve these hypothesized nano-scale microdomains. However, recent advances in fluorescent microscopy sensitivity and resolution have revealed discrete, reproducible, and distinct...
patterns of protein localization at the plant plasma membrane (Jarsch et al., 2014). The Arabidopsis plasma membrane is highly organized into a discrete patchwork of different microdomains around 100–500 nm across. These domains are not static entities, but do appear relatively stable in size and localization in the plasma membrane over a 20 min sampling period (Jarsch et al., 2014). The main group of proteins used to demonstrate these phenomena are the membrane-associated remorin family (Jarsch et al., 2014). Remorins are a family of 16 proteins in Arabidopsis that do not contain an apparent transmembrane domain (Raffaele et al., 2009), but many contain S-acylation sites (Konrad et al., 2014). Interestingly, while S-acylation has varying effects on the membrane association and sub-cellular distribution of different remorins, it does not appear to be a strict prerequisite for microdomain formation. However, S-acylation-defective SYMREM1 remorin does show an altered microdomain pattern compared with the wild type (Konrad et al., 2014), but whether the protein composition, lifetime or stability of microdomains formed by mutant non-S-acylated remorins differs from their wild-type counterparts has yet to be investigated.

TMD tilting and hydrophobic mismatch

Another theory put forward on the role of S-acylation is that it induces tilting of transmembrane helices relative to the membrane plane (Joseph and Nagaraj, 1995). It has also been suggested that S-acylation can realign transmembrane domains, causing them to reorient in the lipid bilayer, potentially exposing protein-interaction sites (Fig. 1). In mammalian systems, this theory has been used to explain the effect that S-acylation has on several proteins (Abrami et al., 2008). The plasma membrane is thicker than the ER or Golgi membranes and plasma membrane-associated proteins generally have longer transmembrane domains. This can lead to a hydrophobic mismatch between the protein and the ER or Golgi membrane environment during synthesis and trafficking. The ability of S-acylation to tilt transmembrane helices in the membrane reduces the effective transmembrane domain length and reduces the hydrophobic mismatch. This, in turn, prevents the protein from being recognized by the ER quality control machinery and degraded (Abrami et al., 2008). Transmembrane helix tilting may also act to prevent inappropriate protein–protein interactions until the protein is in the correct membrane environment.

Protein stability

While no data exist in plants on how S-acylation may affect protein degradation, turnover or stability there is evidence from yeast and animal systems that S-acylation does play a role in these processes. LR6P S-acylation is required for trafficking from the ER to the PM and non-S-acylated LR6P is recognized by the ER quality control machinery, ubiquitinated, and rerouted for degradation (Abrami et al., 2008). The yeast SNARE TLG1 is also targeted for degradation by ubiquitin ligases in the absence of S-acylation (Valdez-Taubas and Pelham, 2005). While both of these proteins show an obvious requirements for S-acylation in maintaining their stability, it is unknown whether S-acylation and de-S-acylation are actively used to regulate their abundance and turnover in the cell or if their ubiquitination and degradation is simply the result of non-S-acylated protein being recognized as aberrant.

Potential protein-independent effects of S-acylation

Another possible function of protein S-acylation is causing direct membrane deformation by altering membraneleaflet tension. This interesting phenomenon has not yet been demonstrated in plants, but has been shown in mammalian systems. In animal cells, filopodia formation can be induced using only the regions of GAP43 or paralemmin that contain the S-acylation site fused to GFP. This, therefore, could be a physical effect of inserting the S-acyl group into the plasma membrane, independent of the described and presumed function of full-length GAP43 or paralemmin (Gauthier-Campbell et al., 2004). The cause of this phenomenon remains to be explained but it could be due to the acyl group physically altering the tension of the membrane. While this has not been reported in plants, pollen tubes and root hairs grow in a similar manner to filopodia, making this mechanism a possibility in plants.

Conclusion and future perspective

Until recently, little was known concerning the role or extent of S-acylation in plants. Proteomic work has now expanded the range of S-acylated proteins in plants considerably (Hemsley et al., 2013). Research in plants to date has largely focused on S-acylation acting as a membrane anchor. However, evidence is emerging in the literature of a more interesting role for S-acylation in regulating plant protein function (Sorek et al., 2007). The reversible nature of S-acylation suggests that it could play a similar role to phosphorylation in regulating the activation status of proteins or controlling signalling network activation. With the exception of ROPs, little in planta work has been performed to substantiate this theory.

S-acylation is now emerging as a major player in the regulation of cellular processes across eukaryotes and the diverse repertoire of S-acylated proteins in plants indicates that plants are no exception to this. Future efforts need to be centred on determining how changes in protein S-acylation state are regulated and understanding how this impacts upon protein function and downstream events, if the field of plant protein S-acylation is to keep pace with developments in other systems and in other fields of post-translational modifications.

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