Posttranslational Modifications of Chloroplast Proteins: An Emerging Field[1]

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Posttranslational modifications of proteins are key effectors of enzyme activity, protein interactions, targeting, and turnover rate, but despite their importance, they are still poorly understood in plants. Although numerous reports have revealed the regulatory role of protein phosphorylation in photosynthesis, various other protein modifications have been identified in chloroplasts only recently. It is known that posttranslational N^{α}-acetylation occurs in both nuclear- and plastid-encoded chloroplast proteins, but the physiological significance of this acetylation is not yet understood. Lysine acetylation affects the localization and activity of key metabolic enzymes, and it may work antagonistically or cooperatively with lysine methylation, which also occurs in chloroplasts. In addition, tyrosine nitration may help regulate the repair cycle of photosystem II, while *N*-glycosylation determines enzyme activity of chloroplastic carbonic anhydrase. This review summarizes the progress in the research field of posttranslational modifications of chloroplast proteins and points out the importance of these modifications in the regulation of chloroplast metabolism.

The constantly changing environment challenges plants both in the short and long term. Environmental fluctuations affect gene expression by relaying signals that adjust the accumulation of proteins in various cell compartments according to ambient requirements. In addition to these relatively slow changes, rapid shifts in enzyme activities are sometimes required to meet the needs of plant metabolism at a given moment. Enzyme activity, interactions with other molecules, targeting, and turnover rate of a protein may be determined by a number of different posttranslational modifications (PTMs), which refer to the covalent processing of a protein after it has been fully translated. Although PTMs have long been known to provide enormous functional diversity and regulatory complexity in mammalian systems, an emerging interest in plant PTMs has developed only recently. Studies focusing on PTMs in plants are required not only because of fundamental interest in the regulation of physiological responses, but also because of the possibility of producing correctly modified biological compounds in plants for medical purposes. Despite the importance of chloroplast metabolism in the growth and development of the plant, PTMs of chloroplast proteins, apart from phosphorylation, have been poorly studied. It appears from recent progress that the numerous recently identified PTMs of chloroplast proteins provide additional regulation and signaling in chloroplasts. This review will introduce and summarize current developments in this emerging research field and will emphasize the importance of PTMs in chloroplast metabolism (Fig. 1).

PTMs OF CHLOROPLAST PROTEINS

Phosphorylation

Reversible phosphorylation is the best characterized PTM in eukaryotes, and the concerted action of kinases and phosphatases is of utmost importance in the regulation of virtually all cellular processes. In plants, there are approximately 1,000 genes encoding potential kinases, which phosphorylate Ser, Thr, and Tyr residues (Sugiyama et al., 2008). However, of all chloroplast phosphoproteins, 72% were identified as Ser phosphorylated and 27% as Thr phosphorylated, while no Tyr phosphorylation has been reliably detected to date (Reiland et al., 2009). Phosphorylation of chloroplast proteins has been a target of intense research for more than 3 decades, and the high light-induced phosphorylation and consequent dephosphorylation of the PSII core protein D1 have been shown to play a crucial role in the PSII repair cycle (Aro et al., 1993; Komenda et al., 2012).

In addition to the PSII core proteins, the proteins of the light-harvesting complex (LHC) undergo reversible, light-dependent phosphorylation (Allen et al., 1981; Rintamäki et al., 2000), which regulates the balance of excitation energy between PSII and PSI (Bellafiore et al., 2005). Two distinct Ser/Thr kinases are responsible for these phosphorylation events: STATE TRANSITION8 (STN8), which phosphorylates the PSII core proteins (Bonardi et al., 2005; Vainonen et al., 2005) and STN7, which phosphorylates the LHC proteins (Bellafiore et al., 2005). Also, the LHC phosphatase has been identified and characterized (Pribil et al., 2010; Shapiguzov et al., 2010). Among the 174 chloroplast phosphoproteins that have been identified (Reiland et al., 2009) are PROTON

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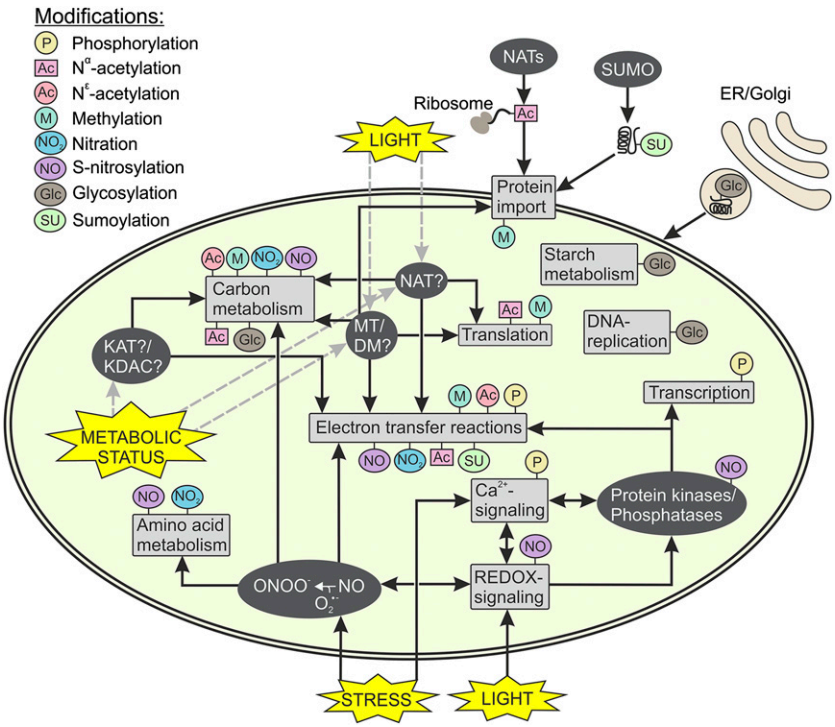

Figure 1. Overview of the possible PTMs of chloroplast proteins. Changes in internal and external conditions (yellow stars) require rapid regulation of many processes inside the chloroplast. The main processes regulated via PTMs are shown in gray boxes. Different types of modifications affecting these processes are marked with colored pins, and the enzymes/molecules executing the modifications are marked with dark-gray ellipses. Enzymes as yet uncharacterized are indicated with question marks. Black arrows mark known interactions, and hypothetical relations are marked with dashed gray arrows. KAT, Lys-acetyltransferase; KDAC, Lys-deacetylase; NAT, N^{α}-acetyltransferase; MT, methyltransferase; DM, demethylase; SUMO, sumoylation machinery.

GRADIENT REGULATION5-LIKE PROTEIN1, a possible modulator of cyclic electron transfer (Reiland et al., 2011) and CALCIUM SENSING RECEPTOR, which is involved in calcium signaling (Vainonen et al., 2008; Stael et al., 2012) and various components of the chloroplast transcription machinery (Ogrzewalla et al., 2002), emphasizing the importance and functional diversity of phosphorylation in the fine-tuning of chloroplast metabolism (Fig. 1).

Acetylation

Acetyl CoA, a universal metabolite required for numerous biochemical pathways, is also a substrate for protein acetylation. Three types of protein acetylation have been identified this far: *O*-acetylation, N^{α}-acetylation, and N^{ε}-acetylation (Lys acetylation). *O*-acetylation occurs at the hydroxyl group of Ser or Thr residues, N^{α}-acetylation is at the α-amino group of the N-terminal amino acid residue of proteins, and N^{ε}-acetylation is at the amino group of the side chain of an internal Lys residue. N^{α}-acetylation and N^{ε}-acetylation are known to take place in plants, whereas protein *O*-acetylation has so far been described only in the bacterium *Yersinia pestis* (Mukherjee et al., 2006).

N^{α}-Acetylation

Irreversible N^{α}-acetylation of the N-terminal amino acid residue of a protein is one of the most prevalent protein modifications of eukaryotes, including plants (Baerenfaller et al., 2008; Bienvenut et al., 2012). It neutralizes the positive charge on the N terminus, thus shifting the pI of the protein to be more acidic. N^{α}-acetylation in the cytoplasm is catalyzed by a family of N^{α}-ACETYLTRANSFERASE complexes (NATA/NATB/NATC/NATD/NATE), which are often associated with ribosomes (Polevoda et al., 2009). N^{α}-acetylation may be involved in a number of processes in different organisms, e.g. protein targeting (Behnia et al., 2004), determination of protein half-life (Hwang et al., 2010; Bienvenut et al., 2011), and mediation of protein-protein interactions (Scott et al., 2011). Although scant knowledge is available concerning the effects of N^{α}-acetylation in plants, a recent study of the mutated cytoplasmic NATB complex revealed that defects in N^{α}-acetylation result in abnormalities in flowering time, gametogenesis, fertilization, and leaf development in Arabidopsis (*Arabidopsis thaliana*; Ferrández-Ayela et al., 2013) as well as in circadian rhythm in *Chlamydomonas reinhardtii* (Matsuo et al., 2012). Nevertheless, the exact mechanism leading to this kind of pleiotropic developmental phenotype has not yet been determined.

Chloroplast proteins show three different types of N^{α}-acetylation (Fig. 1; Table I). First, transit peptides of several nuclear-encoded proteins are cotranslationally N^{α}-acetylated in the cytoplasm (Van Damme et al., 2011). N^{α}-acetylation of preproteins is required for organelle import, as plants lacking the homolog of NATC show disturbed structure and function of photosynthetic machinery (Pesaresi et al., 2003). Moreover, in plants lacking TOC159 (subunit of TRANSLOCON OF OUTER ENVELOPE MEMBRANE OF CHLOROPLAST), the N^{α}-acetylated chloroplast preproteins accumulate in the cytosol (Bischof et al., 2011). The second type of

Table I. *Summary of the PTMs identified in chloroplast proteins*

For each type of modification, the origin of the target proteins (nuclear or chloroplast encoded), the target of modification (preprotein or mature protein), and the location of modification are indicated.

Modification Type	Nuclear-Encoded, Chloroplast-Localized Targets?	Chloroplast-Encoded Targets?	Location of Modification: Cytoplasm (c) or Chloroplast (p)
Ser/Thr phosphorylation	Yes (mature protein)	Yes (mature protein)	p
N^{α}-Acetylation	Yes (preprotein)	Yes (mature protein)	c/p
Lys acetylation	Yes (mature protein)	Yes (mature protein)	p
Lys/Arg methylation	Yes (mature protein)	Yes (mature protein)	p
Tyr nitration	Yes (mature protein)	Yes (mature protein)	p
S-Nitrosylation	Yes (mature protein)	Yes (mature protein)	p
Glycosylation	Yes (preprotein)	No	c
Sumoylation	Yes (preprotein)	No	c

N^{α}-acetylation occurs when nuclear-encoded proteins are posttranslationally acetylated inside the chloroplast after transit peptide excision. For instance, the mature form of both FERREDOXIN-NADP^{+} OXIDOREDUCTASE (FNR), which functions in the last step of linear electron transfer of photosynthesis (Lehtimäki et al., 2014), and PYRUVATE ORTHOPHOSPHATE DIKINASE, which functions in C_4 photosynthesis (Chen et al., 2014), have been shown to be N^{α}-acetylated. Third, many proteins encoded by the chloroplast genome, such as the PSII core polypeptides D1, D2, the PSII CP43 reaction center protein, Rubisco LARGE SUBUNIT (RBCL), and the ATP SYNTHASE ε SUBUNIT (ATPE; Michel et al., 1988; Mulligan et al., 1988; Zybailov et al., 2008; Hoshiyasu et al., 2013), are targets of either co- or posttranslational N^{α}-acetylation in the chloroplast (Table I). However, the enzyme(s) catalyzing N^{α}-acetylation in chloroplasts has not been identified, and no NAT homologs are predicted to be chloroplast located (Bienvenut et al., 2012). Although there is little information available about the role of N^{α}-acetylation in chloroplasts, it has been recently shown that, upon drought stress, the N^{α}-acetylated form of ATPE is more stable against degradation by metalloaminopeptidases than is the nonacetylated ATPE (Hoshiyasu et al., 2013). In addition, because the accumulation of N^{α}-acetylated chloroplast FNR (Lehtimäki et al., 2014) and chloroplast ribonucleoproteins CP29A and CP29B (Wang et al., 2006) has been shown to be dynamic and light responsive, it seems likely that N^{α}-acetylation of chloroplast proteins provides another level of regulation for adaptation to changing environmental cues.

Lys Acetylation

N^{ε}-acetylation of Lys residues in histones was described in the 1960s and is well known to regulate chromatin remodeling, which, in turn, controls transcriptional activity (Jenuwein and Allis, 2001). By contrast, Lys acetylation of nonhistone proteins has been a focus of intense research only more recently. More than 100 Lys-acetylated proteins have been identified in plants, and more than 20 chloroplast proteins, including two chloroplast-encoded proteins (RBCL and ATP SYNTHASE β SUBUNIT), have been found to be Lys acetylated (Finkemeier et al., 2011; Wu et al., 2011; Nallamilli et al., 2014). However, no Lys acetyltransferase or deacetylating enzymes have been identified in the chloroplast (Rao et al., 2014), and it has been suggested that organellar Lys acetylation might occur in part via nonenzymatic autoacetylation (Table I; König et al., 2014). Although most of the Lys-acetylated chloroplast proteins appear to have only one acetylation site, RBCL contains nine and PHOSPHOGLYCERATE KINASE three of these sites. Also, a recent study showed that chloroplast FNR isoforms possess multiple and differential Lys acetylation sites and that one of the acetylated Lys residues is positioned in close proximity to the catalytic center (Lehtimäki et al., 2014). In RBCL, the acetylated Lys residues are located in the catalytic center of the enzyme (Cleland et al., 1998; Finkemeier et al., 2011) at the dimer-dimer interface between the two RBCL subunits (Knight et al., 1990; Finkemeier et al., 2011) as well as in the region that is important for the formation of tertiary structure of Rubisco (Knight et al., 1990).

Because the acetylation of Lys residue neutralizes the positive charge of the side chain and disturbs hydrogen and ionic bonding, it is likely to affect enzyme activity and interactions with other biomolecules. It was shown that partial in vitro deacetylation of Arabidopsis RBCL increases the maximum catalytic activity of Rubisco by 40% (Finkemeier et al., 2011). Intriguingly, also Rubisco SMALL SUBUNIT and Rubisco ACTIVASE are targets of Lys acetylation (Finkemeier et al., 2011; Wu et al., 2011). In addition to the reported changes in enzyme activity, Lys acetylation has been suggested to regulate association of LHCB1 and LHCB2 with PSII. The pool of LHC tightly bound to PSII showed a lower level of Lys acetylation than did the peripheral, loosely bound LHCII pool, but the level of acetylation did not depend on short-term light/dark changes (Wu et al., 2011). Taken together, these results imply that the efficiency of carbon assimilation is regulated in part by Lys acetylation, although the effect might be relayed through multiple mechanisms (Fig. 1). In bacteria, central metabolic pathways are controlled via Lys acetylation, which reflects the types of available carbon sources and the metabolic status of the cells (Wang et al., 2010; Weinert et al., 2013; Mo et al., 2015). It is tempting to speculate that, as an ancient and conserved modification, Lys

acetylation provides a similar regulatory mechanism also in chloroplasts.

Lys and Arg Methylation

Protein Lys methyltransferases catalyze the transfer of one to three methyl groups from *S*-adenosyl-Met to the ε-amine of a Lys residue (Schubert et al., 2003). By contrast, protein Arg methyltransferases transfer one or two methyl groups to the distal nitrogen atoms of the guanidine group of an Arg residue (Bedford and Clarke, 2009). Identification of Lys demethylases has indicated the reversible nature of this modification (Lanouette et al., 2014), but no Arg demethylases have been reliably characterized to date. Addition of methyl group(s) increases the basicity and hydrophobicity of Lys and Arg residues without altering their charge, thus possibly changing the stability, localization, activity, or protein-protein interactions of the modified protein (Rice and Allis, 2001).

Lys methylation of an increasing number of nonhistone proteins has been identified both in prokaryotes and eukaryotes (Huang and Berger, 2008), and a member of the Lys methyltransferase enzyme family (Rubisco LARGE SUBUNIT METHYLTRANSFERASE [RLSMT]) has been identified in chloroplasts (Table I; Klein and Houtz, 1995; Wang et al., 1995; Zybailov et al., 2008). RLSMT was shown to exist in complex with Rubisco (Raunser et al., 2009) in which the Lys-14 is trimethylated in several species belonging to the Fabaceae, Solanaceae, and Cucurbitaceae families but not in wheat (*Triticum aestivum*), spinach (*Spinacia oleracea*), or Arabidopsis (Houtz et al., 1992; Mininno et al., 2012). Despite numerous studies, the effects of Lys-14 trimethylation of RBCL have not been defined, and RLSMT knockdown plants do not show marked defects in growth and CO_2 assimilation (Mininno et al., 2012). Moreover, three isoforms of chloroplast FRU-1,6-BISPHOSPHATE ALDOLASE have been found to contain trimethylated Lys residues in close proximity to their C termini. No differences, however, could be detected in the catalytic activity, stability, or oligomerization between the trimethylated and unmodified enzymes (Mininno et al., 2012).

A recent study has identified 23 chloroplast methylproteins (Alban et al., 2014), including the previously described ALDOLASE isoforms (Mininno et al., 2012) and PLASTID RIBOSOMAL PROTEIN L11 (Yamaguchi and Subramanian, 2000). Four out of these 23 proteins contained one or more methylated Arg residues, while 17 proteins had Lys methylation. RBCL and ATP SYNTHASE β-subunit were methylated at both Lys and Arg residues (Alban et al., 2014). In addition to Rubisco and FRU-1,6-BISPHOSPHATE ALDOLASE, five additional proteins functioning in CO_2 assimilation were methylated (Alban et al., 2014). Interestingly, chloroplast protein methylation is, at least partly, induced by light (Niemi et al., 1990). Even if no functional differences between the unmethylated and trimethylated forms of the proteins studied have been described yet, it is plausible that methylation plays a regulatory or signaling role in carbon metabolism (Fig. 1). Moreover, it is notable that a specific Lys residue may be targeted for either acetylation or methylation, and thus these modifications may have either antagonistic or cooperative effects in vivo (Rice and Allis, 2001), not to mention various other types of possible modifications. Hence, the recently identified acetylation of Lys-14 in Arabidopsis RBCL (Finkemeier et al., 2011) may prevent the well-documented interaction of RLSMT with Rubisco in a catalytically productive way (Mininno et al., 2012).

Tyr Nitration and *S*-Nitrosylation

Protein nitration is an enzymatic or nonenzymatic process in which a nitro group (-NO_2), originating from nitric oxide (NO), is added to Tyr, Trp, Cys, or Met residues of a target protein (Corpas et al., 2009). Protein *S*-nitrosylation, in turn, refers to the covalent binding of an NO group to a Cys residue, often located in a hydrophobic pocket and surrounded by acidic and basic amino acids (Seth and Stamler, 2011). Because Tyr nitration and *S*-nitrosylation are associated with signaling processes, these PTMs are presented in more detail here.

In Tyr nitration, a nitro group is covalently attached to an *ortho*-carbon of the aromatic ring (Gow et al., 2004), which alters this residue into a hydrophilic moiety with negative charge (Corpas et al., 2009). Tyr nitration is mediated by peroxynitrite ($ONOO^-$), which has strong nitrating activity, resulting from a rapid reaction between superoxide radicals ($O_2^{\cdot-}$) and NO (Corpas et al., 2008a). It is proposed that nitrated Tyr residues are located in loops that contain a large solvent-accessible region in close proximity to a basic amino acid residue and a negative charge (Lozano-Juste et al., 2011). Although there is some evidence concerning the existence of Tyr denitrase, which could specifically remove the nitro group, the reversible nature of this PTM is still controversial (Gow et al., 2004). In animal cells, protein Tyr nitration is shown to affect protein conformation, activity, and turnover, and in general, Tyr nitration is a sign of pathogen attack and nitrosative stress (Corpas et al., 2008b). A recent study focusing on the Arabidopsis nitro-Tyr proteome identified 127 proteins containing nitrated Tyr residues. Most of the Tyr-nitrated proteins were predicted to be located in organelles where $ONOO^-$ is produced, e.g. in chloroplasts and mitochondria (Table I; Lozano-Juste et al., 2011). In accordance with this finding, several photosynthesis-related proteins involved in the Calvin-Benson cycle were targeted by Tyr nitration in *Citrus aurantium* (Tanou et al., 2012; Fig. 1).

Another study identified 126 Tyr-nitrated and 12 Trp-nitrated proteins in the Arabidopsis thylakoid proteome (Galetskiy et al., 2011a). Tyr nitration inhibits the activity of both FNR (Chaki et al., 2011) and chloroplast SUPEROXIDE DISMUTASE3, an important scavenger of superoxide anion ($O_2^{\cdot-}$; Holzmeister et al., 2014).

Moreover, the activities of GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH; Lozano-Juste et al., 2011) and β-CARBONIC ANHYDRASE (CA), which are involved in photosynthetic carbon metabolism, were decreased upon Tyr nitration (Chaki et al., 2009). It has been hypothesized that Tyr nitration may disturb the electron transfer chain, because (1) Tyr nitration of the Tyr-262 in the D1 protein may lead to the release of the secondary electron-accepting plastoquinone of PSII from the binding pocket and (2) the double Tyr nitration of PSI subunit D may disturb the flow of electrons from PSI to FERREDOXIN (Galetskiy et al., 2011b). It has also been shown that numerous proteins in PSII monomers, dimers, and PSII-LHCII supercomplexes possess low levels of phosphorylation and high levels of nitration under standard growth conditions, but under high-light conditions, the situation is reversed (Galetskiy et al., 2011b). Thus, the complex and conversely regulated nitration and phosphorylation events might control the stability, turnover, and reassembly of the PSII core complexes and the LHC antenna upon light stress (Galetskiy et al., 2011b).

S-Nitrosylation has been shown to affect protein activities, localization, and protein-protein interactions in mammalian systems (Hess et al., 2005). *S*-Nitrosylation is reversible, and it may occur either enzymatically or nonenzymatically (Benhar et al., 2009). A number of plant proteins are targets for *S*-nitrosylation, and several *S*-nitrosylated proteins have been identified in chloroplasts. Many of the *S*-nitrosylated chloroplast proteins function in photosynthesis, both in the light-harvesting reactions and in carbon fixation, in fatty acid metabolism, in amino acid biosynthesis, and in the maintenance of redox homeostasis (Lindermayr et al., 2005; Fig. 1). Although *S*-nitrosylation may increase the catalytic activity of an enzyme (Astier et al., 2012), the chloroplast proteins studied instead showed a loss of function upon *S*-nitrosylation. For example, *S*-nitrosylation markedly decreased the activity of Rubisco, GAPDH, and DEHYDROASCORBATE REDUCTASE (Lindermayr et al., 2005). It was recently suggested that *S*-nitrosylation is an important determinant in signaling crosstalk, as *S*-nitrosylation of various kinases and phosphatases results in changes in protein phosphorylation status (Hess and Stamler, 2012).

Glycosylation

Protein glycosylation is the addition of carbohydrates to proteins. In *N*-glycosylation, carbohydrates are attached to the amide group of Asn, and in *O*-glycosylation, they are linked to the hydroxyl group of Ser, Thr, Hyl, or Hyp. Both glycosylation types typically occur in the endoplasmic reticulum (ER) and Golgi apparatus (GA). For *N*-glycosylation, a consensus sequence Asn-X-Ser/Thr (X being any amino acid except Pro) has been defined (Bause, 1983), whereas no such clear consensus sequence has been determined for *O*-glycosylation. In Arabidopsis, there are 3,597 proteins predicted to contain a signal peptide for secretion at the N terminus and one or more consensus glycosylation sites (Song et al., 2011). Additionally, 2,186 *N*-glycosylation sites have been experimentally identified (Zielinska et al., 2012). Although it has been shown that glycosylation of a given enzyme affects its catalytic activity, thermostability, folding (Lige et al., 2001), and subcellular localization (Frigerio et al., 1998), as well as plant immunity (Häweker et al., 2010), it was also shown that defects in glycosylation machinery result in only mild effects on plant development and growth (von Schaewen et al., 1993; Farid et al., 2011).

It was long thought that there are no glycoproteins in the chloroplast proteome, because the transfer of the bulky proteins across the double-membrane envelope via the TRANSLOCON OF THE INNER ENVELOPE MEMBRANE OF CHLOROPLAST was considered improbable. However, during the past few years, some chloroplast glycoproteins have been identified. First of all, CA containing an ER-targeting signal peptide was unexpectedly found to be localized in the chloroplast stroma (Villarejo et al., 2005). The CA protein possesses five predicted glycosylation sites (Villarejo et al., 2005), which are occupied by high Man-type and complex-type glycans (Burén et al., 2011). *N*-Glycosylation was shown to take place in the ER and Golgi apparatus, followed by a transport to the chloroplast via a vesicular Golgi-to-plastid transport pathway (Fig. 1; Table I; Villarejo et al., 2005; Kitajima et al., 2009). *N*-Glycosylation of CA is required for proper folding and catalytic activity (Burén et al., 2011). Moreover, rice (*Oryza sativa*) α-AMYLASEs (AMYLs) are a group of plastid-located proteins that contain an ER-targeting signal peptide in their N termini (Chen et al., 1994, 2004). AMYL1 has a single *N*-glycosylation site, and the protein was susceptible to ENDOGLYCOSIDASE H digestion, indicating that the protein has an *N*-linked carbohydrate side chain (Asatsuma et al., 2005). Also, a NUCLEOTIDE PYROPHOSPHATASE/PHOSPHODIESTERASE of rice and barley (*Hordeum vulgare*) catalyzing the cleavage of ADP-Glc has been shown to be chloroplast localized and *N*-glycosylated (Nanjo et al., 2006). The functional role of *N*-glycosylation for these proteins has not been elucidated, but considering their function, it seems likely that the glycosylation status of these proteins serves as a signal of the overall energy status of the cell and thus contributes to the regulation of carbon metabolism.

A number of *O*-glycosylated proteins are involved in cell wall formation and plant immunity (Velasquez et al., 2012). However, to our knowledge, only the P43 DNA-binding protein from *Pisum sativum* chloroplasts has been shown to contain a series of *O*-glycosylated Hyp residues in its N terminus, with Ara being the major sugar added (Gaikwad et al., 1999). Glycosylation of P43 was shown to stimulate DNA polymerase activity, even though the deglycosylated P43 could bind DNA as efficiently as the glycosylated form (Gaikwad et al., 1999).

Sumoylation

Covalent binding of the small ubiquitin-like modifier (SUMO) protein, which contains approximately 100 amino acids, to other proteins affects diverse cellular processes such as transcriptional regulation and stress responses in plants (Miura et al., 2007). Attachment of SUMO often occurs via formation of an isopeptide bond between its C-terminal Gly and the ε-amino group of Lys in the ψKXE/D consensus target sequence (ψ is a large hydrophobic and X is any amino acid) found in most target proteins of SUMO (Rodriguez et al., 2001). Sumoylation requires the activity of three enzymes: SUMO-activating enzyme, SUMO-conjugating enzyme, and SUMO ligase, whereas removal of SUMO is catalyzed by SUMO proteases (Vierstra, 2012). Although sumoylation machinery has not been identified in chloroplasts, many chloroplast proteins, including FERREDOXIN, PSII subunit O, and subunits of PSI, PSII, and LHC, have been shown to be sumoylated (Elrouby and Coupland, 2010; López-Torrejón et al., 2013). This suggests that SUMO attachment occurs in the cytoplasm and that the modified proteins are then imported through the chloroplast envelope to the plastid (Fig. 1; Table I). In general, sumoylation has been shown to influence localization, interactions, and activity of the modified protein (Vierstra, 2012), but currently, the effects of sumoylation on the function of chloroplast proteins are not clear and thus require further studies.

CONCLUSION AND FUTURE PERSPECTIVES

Recent methodological progress in PTM identification, introduced in several excellent reviews (Choudhary and Mann, 2010; Van Damme et al., 2011; Ruiz-May et al., 2012; Huang et al., 2014; Lanouette et al., 2014), has paved the way for elucidation of PTM-mediated regulatory mechanisms not only in mammals, but also in plants and cyanobacteria. Several studies have provided evidence about the evolutionary conservation of many PTMs, and Ser/Thr/Tyr phosphorylation (Yang et al., 2013) and Lys acetylation (Mo et al., 2015) have recently been shown to regulate various cellular processes, including photosynthesis, also in cyanobacteria. The identification of further PTMs, the characterization of cross-signaling pathways (Deribe et al., 2010), and the detailed determination of the physiological effects of PTMs both in cyanobacteria and in chloroplasts are likely to be the focus of future research. Progress in this field will likely reveal more detailed evolutionary relationships and regulatory potential of these PTMs as they adjust chloroplast metabolism in response to continually changing environmental conditions.

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