Enzymes in Agricultural Sciences

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Chapter: Enzymes in Plant Growth

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Enzymes in Plant Growth

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Abstract

Living organisms, including plants, rely on metabolic processes for growth and development; enzymes play a pivotal role in growth, as they are biological catalysts of metabolic reactions. As autotroph organisms, plants absorb mainly inorganic elements from the external environment. Furthermore, converting light energy into chemical bonds, they can energize fixation of carbon dioxide into carbohydrates; these will be, in turn, used for the synthesis of all the organic molecules needed, including proteins.

In this context, mechanisms of ions uptake, carbon fixation and nitrogen assimilation, are crucial for ensuring plants with essential nutrients and building blocks for biomolecules. To accomplish these tasks plants have evolved specific enzymes with distinctive structural and operational (including regulatory) features. Among these some master enzymes can be indicated, whose function is crucial for the overall process.

In this chapter the role of plasma membrane proton pumps (PM H^+-ATPase), ATP synthase, photosynthetic carbon fixing enzymes (Rubisco) and nitrogen assimilation enzymes (Glutamine synthase) will be described, considering aspects of their function, structure and regulation.

Introduction

Plants are sessile organisms that became adapted to different environments by developing appropriate mechanisms for the use of natural resources in order to sustain growth and reproduction. Cultivated crops have been developed considering an unlimited supply of nutrients and water; nowadays limitation in nutrient availability, environmental concerns and climate change ask for the adoption of sustainable agricultural managements of cultivated land.

As autotroph organisms, plants acquire water and mineral nutrients (generally in ionic forms) from the soil, while oxygen and carbon dioxide come from the atmosphere. Generally essential elements need to be accumulated in plant tissues at concentrations higher than those found in the external media (e.g. due to soil constraints) or the concentration in the environment may be not suitable for optimal use efficiency by internal mechanisms (CO_2 vs. O_2 concentration in the air). These factors can limit plant growth and crop yield; furthermore, this situation might become even worse due to intensive agriculture and global warming. This implies a profound knowledge of mechanisms that plants evolved to acquire water and nutrients and to build up their structural and functional biomolecules; understanding how these mechanisms are regulated by internal and exogenous (environmental) factors is also crucial for the development of new efficient crops genotypes.

Nutrients' uptake from the soil solution, translocation and transport of solutes to different organs and cells within the plant are processes that need specific protein structures located at the cell plasma membranes allowing the passage of uncharged solutes and ions and, especially, capable to generate an electric potential favorable for trans membrane transport. The plasma membrane (PM) H^+-pumping ATPase has a unique role in solute transport in plant cells [1]. On the other hand, ATP used in metabolic processes needs to be previously synthesized by photophosphorylation in the chloroplast or oxidative phosphorylation in the mitochondrion via the ATP synthase proteins.

Fixation of CO_2 is the key step in the photosynthetic biomass accumulation in higher plants. This task is accomplished by Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase), a chloroplast-located enzyme that maintained highly conserved function during evolution; although partly inefficient due to the double catalytic function (carboxylase/oxygenase); this enzyme play a central role in carbon fixation even in plants that developed variants to this mechanism [2]. Ammonia assimilation into glutamine allows the formation of glutamate and further synthesis of plant amino acids. This reaction is catalyzed by glutamine synthase, an enzyme with different isoforms localized in plastids/chloroplasts and in the cytosol [3]. Structural and functional features of these enzymes will be described and discussed in relation to their role in plant growth and response to environmental conditions.

Plasma Membrane H^+-ATPase: A Master Enzyme for Plant Nutrition

Movement of uncharged solutes and ions across biological membrane is essential to ensure metabolic reactions. This movement can be a passive as well an active process and involves the operation of channels and carriers. Both passive and active transport of ions across biological membranes need that a transmembrane electric potential is generated and maintained. In plants, this task is accomplished by the plasma membrane (PM) H^+-ATPase that is an electrogenic enzyme coupling the hydrolysis of ATP to the transport of H^+ from the cytosol into the apoplast. This activity creates a transmembrane gradient of electric potential (negative inside) and pH (more acidic outside), which can be exploited in a variety of physiological processes such as the transport of nutrients and metabolites, preservation of intra- and extra-cellular p^H, cell turgor and related processes [1]. In roots, that are exposed to changing external conditions, such as p^H, concentration of nutrients and toxic elements and rhizosphere signals, the PM H^+-ATPase plays a pivotal role for mineral nutrition and the response to abiotic stress.

The plasma membrane H^+-ATPase belongs to the family of P-type ATPases, which use ATP and form a phosphorylated aspartyl intermediate during the reaction cycle. It is a single subunit protein with molecular mass of about 100 KDa containing ten trans membrane helices and four cytoplasmic domains (Figure 1): the nucleotide binding domain (N-domain), the phosphorylation domain (P-domain), the phosphatase domain (A-domain) and the regulatory domain (R-domain, consisting of the C-terminal of the protein). The N-terminal end is directly involved in controlling the pump activity state interacting with the C-terminal end [4]. The C-terminal region exerts a self-inhibiting function on the enzyme activity by binding to the large cytoplasmic domain. This inhibition is removed by binding of 14-3-3 proteins, that is dependent on the phosphorylation of the penultimate Thr residue [5-7]. The fungal phytotoxin, fusicoccin stimulates H^+ pumping by blocking the complex of 14-3-3 proteins and the PM H^+-ATPase.

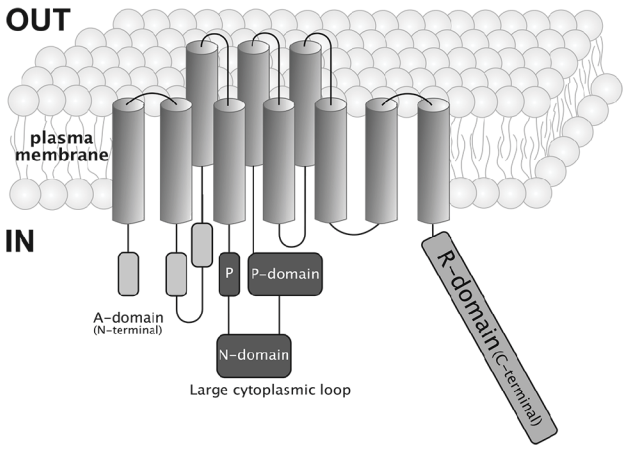

Figure 1: Structure of PM H^+-ATPase.

In several plant species, it has been found that the PM H^+-ATPase is encoded by a multigene family (about 10 genes) belonging to 5 sub-families [8]. It has been suggested that the heterogeneity of isoforms can be linked to the multicellular nature of plants and the need for a fine regulation of the enzymatic activity [9]. Analysis of expression patterns based on available micro array data shows that most of the H^+-ATPase isoforms are expressed at a relative constant level and expression level does not change when a related isoform is deleted or reduced [10]. Regulatory events involving modifications of activity, amount and gene expression have been reported in response to change in the concentration of ionic nutrients in the root external solution.

A close relationship between the activity of the PM proton pump of the root cells and transmembrane transport of anionic nutrients has been demonstrated in the case of NO_3-. Nitrate uptake is an energy-dependent proton-coupled process. Exposure of maize roots to nitrate can cause the induction of the high-affinity transport system; this behavior is paralleled by a concomitant increase in activity and amount of the PM H^+-ATPase and by the preferential expression of the genes MHA3 and MH4, belonging to sub-family II of the PM H^+-ATPase in maize [11]. Spatial and temporal expression patterns of the two genes were also observed along primary maize roots that paralleled changes in enzyme activity and anion transport rates [12]. Other isoforms of the proton pump (MHA1, belonging to sub-family I) have been suggested to be involved in nitrate transport in maize [13].

Another example of regulation of the PM H^+-ATPase has been described in response to limited amounts of phosphate. When grown at low available phosphate white lupin (*Lupinus albus*, L.) release substantial amounts of carboxylates from specialized root structure, called cluster roots, and concomitantly acidify the rhizosphere. The burst of citrate exudation is accompanied by a strong acidification of the external medium and alkalization of the cytosol. The increase in proton secretion is due to both an increased transcription level of one of the two PM H^+-ATPase genes found in white lupin (LHA1 and LHA2) and a post-translational modification of H^+-ATPase protein involving binding of activating 14-3-3 protein [14]. More recently, an RNA-Seq study showed that six H^+-ATPase transcripts exhibited Pi -dependent expression changes in white lupin roots [15].

Rhizosphere acidification mediated by the activity of PM H^+-ATPase has been reported to be also part of the response to limited iron availability in roots of dicots and non-graminaceous plants. It has been shown that under conditions of Fe deficiency, the activity and the quantity of the PM H^+-ATPase increase [16], with the enzyme, which appears to be, concentrated particularly in the rhizodermal and root hair cells of the sub-apical root area [17]. It has also been found that in cucumber plants the expression of the PM H^+-ATPase CsHA2 gene, found both in roots and leaves, was not influenced by the Fe nutritional status of the plant, while the CsHA1 gene, expressed exclusively in the roots, was up-regulated by Fe deprivation [18].

Increases in PM H^+-ATPase enzyme activity have also been observed in the roots of plants adapted to acidic soils [19] and, particularly in response to Al toxicity, a condition which often occurs in that kind of soils. It has been shown that resistant plants release a high amount of organic acid anions from the sub-apical regions of the roots and that the PM H^+-ATPase could be involved in this process [20]. The observed activation of the enzyme due to the presence of Al, has been ascribed to an increased phosphorylation of a threonine residue localized in the auto inhibitory (C-terminal) domain of the PM H^+-ATPase, suggesting an involvement of 14-3-3 proteins. However, it is worth to note that citrate exudation in P-deficient white lupin plants exposed to Al was suggested to be uncoupled from PM H^+-ATPase activity and linked to K^+ rather than to H^+ extrusion; furthermore, a poor relationship between Al-induced oxalate exudation and PM H^+-ATPase activity was reported in tomato roots [21].

There is a number of reports indicating that PM H^+-ATPase could be involved in cell elongation; this function would imply a direct action of auxin on activity and, possibly, synthesis of the proton pump or an indirect one through induced changes in cytoplasmic pH or altered ion fluxes across plasma membrane [22]. Whatever the mechanism, other signal compounds, like those present in the soil solution (e.g. humic molecules or hormone-like compounds released by rhizosphere micro-organisms) have been shown to affect enzyme activity with different mechanisms and, in some cases, acting on the amount of the protein and of gene transcripts [13,23,24]; these effects have been related to root growth [24], apoplast acidification [25] and nutrient uptake [23,26,27].

ATP Synthase: An Extraordinary Nano-Engine

To directly sustain the energy necessary for growth and metabolism, living organisms use mainly that trapped in the phosphoranhydride bound contained in ATP molecules. The majority of ATP is regenerated from ADP and inorganic phosphate (Pi) by the ATP synthase complex. In plants, ATP is mainly produced via two mechanisms: the photophosphorylation in chloroplasts and oxidative phosphorylation in mitochondria. In chloroplasts, plants use the photosynthetic reaction centers to transport protons from the stroma into the lumen and create an electrochemical potential through the membrane of the thylakoids. In mitochondria, a series of complexes present in the respiration chain pumps protons and charge the inner mitochondrial membrane. Nevertheless in both organelles, the final step is catalyzed by the same type of enzyme, the ATP synthase, which transforms the energy of the electrochemical gradient across the membrane of the thylakoid or across the internal membrane of the mitochondrion into the chemical bound contained in the ATP molecule.

The structure of the ATP synthase is a complex of two main subunits, F_0 and F_1. The complex (F_0, F_1) forms a rotary engine that is able to convert the transport of protons, or sodium ions in bacteria, into chemical energy and this complex can also work in reverse mode as an H^+(or Na^+)-ATPase. F0 is embedded in the membrane and consists of at least 1 a, 1 b_2 and 10-15 c subunits; the c subunits form a ring in the membrane which spins with the passage of protons. The F_1 part is a water-soluble complex of proteins, which binds ADP and Pi, catalyze the synthesis of ATP and by conformational changes induced by the rotation of the F_0 counterpart release ATP [28,29]. Many animations illustrating the structure and the rotational mechanism of the ATP synthase can be seen on this website (Mitochondrial Biology Unit).

Due to the dual activity of the ATP synthase a complex regulation mechanism controls its activity. In mitochondria, when the availability of oxygen drops, the electrochemical gradient of protons across the inner mitochondrial membrane decreases and the ATP synthase starts to consume ATP to reestablish the gradient. Under these conditions, the ATP production is sustain by the glycolysis and the factor regulating the ATP synthase, natural inhibitor protein IF1, binds to the ATP synthase due to the drop of pH in the mitochondria matrix [30].

In chloroplast, where no IF1 protein has been identified, the ATPase activity of the ATP synthase is repressed in the dark to prevent wasteful consumption of ATP. Under these conditions, accumulation of Mg^{2+}-ADP and different modifications on the F_1 subunits, including a disulfide bound, inhibit the ATPasic activity [31-33]. Reversely when plants are exposed back to light, photosynthesis centers acidify the stroma, which induce the removal of the inhibiting ADP and the disulfide bound is reduced. Moreover the C-terminal domain of the Epsilon subunit, within the F_1 part, seems to act similarly to the auto inhibitory (C-terminal) domain of the PM H^+-ATPase (see above), inhibiting the ATP hydrolyzing activity the chloroplastic ATP synthase [33]. To sum up, the ATP synthase regulation is just starting to be revealed, but still needs further research.

Another interesting aspect about the functionality of the ATP synthase is the relative importance of the pH gradient and of the electrical potential across the membrane. It is a common believe that it is the pH gradient, which is the main driven force for the synthesis of ATP; however, it depends on the organelles considered. For example, in chloroplast, the electrical potential across the membrane is small but the pH gradient is high. In these conditions, chloroplastic ATP synthase has a large c ring (14 units) [34], as each c unit transport one proton, this enzyme needs many protons for each ATP synthetize but less electrical potential per protons. On the other hand, mitochondrial one has a smaller c ring (10) [35], thus a better proton per ATP ratio, due to the high electrical potential maintained by the respiratory chain for a similar, in comparison to chloroplast, pH gradient across the membrane. Therefore in mitochondria, the electrical potential is the main driving force and in chloroplast is the pH gradient [36].

Rubisco: An Old Enzyme for Future Challenges

Biomass accumulation and crop yield is strictly related to photosynthetic rate and efficiency. Plants have been grouped depending on the first compound that is generated upon incorporation of CO_2 in a pre-existing carbon skeleton.

C3 plants fix carbon of CO_2 into ribulose 1,5-bisphosphate (RuBP) generating primarily two molecules of 3-phosphoglycerate (PGA, a 3-carbon compound). Other variants of photosynthetic carbon assimilation are represented by C4 (the first compounds has 4 C atoms) and *Crassulacean* acid metabolism (CAM) plants, which rely on CO_2-concentrating mechanisms. Notwithstanding these differences Rubisco (ribulose-1,5 bisphosphate carboxylase/oxygenase) plays a central role in CO_2 fixation.

Rubisco of higher plants belongs to the form I of the enzyme, found also in algae and in most photosynthetic bacteria. It is a complex protein, with eight large subunits (four large subunit mass of about 50-52 kDa) and eight small subunits (mass of about 14-15 kDa) arranged in a L_8S_8 structure (four large subunit dimers along with eight non-catalytic small subunits capping the large ones).

The large subunits have the catalytic sites. Each subunit comprise an N-terminal domain and a larger C-terminal domain that forms a α /β -barrel; L2 dimers, formed by head-tail arrangement, have two active sites located at the L-L interface. The small subunits consist of four stranded antiparallel β-sheets with two α-helices; they are not essential for catalysis but provide structural stability to the Rubisco complex [37]. L-subunits are synthesized from the single rbcL gene of the plastid genome; nucleus-encoded factors [38], chaperones [39] and post-translational modification of N-terminal domain [40] would help avoiding misfolding and protect the newly forming protein from proteolytic degradation.

Multiple copies of the rbcS gene, coding for the S-subunit, are located in the nucleus. An N-terminal transit peptide allows transfer of the S-subunits synthesized in the cytosol through the chloroplast envelope translocon complexes into the plastid [41]. Within the stroma, the S-subunits undergo further posttranslational modification (transit peptide cleavage, Met-1 aN- methylation) prior to assembly into L_8S_8 complexes [42].

The activity of Rubisco is highly regulated. The enzyme is inactive in the dark and is converted to an active form upon illumination. Activation is mediated by several environmental and systemic factors, including temperature, pH, light, heavy metal concentrations, natural inhibitors, and by the activity of an ancillary protein: Rubisco activase [43].

Prior to catalysis, Rubisco needs to be preactivated; activation is the result of the binding of CO_2 to the 201-lysine residue near the catalytic site (position may slightly change depending on the species). The carbamate that is formed is then stabilized by Mg^{2+} binding [37]. Carbamylation changes the conformation of the large subunit activating the enzyme that can bind RuBP and catalyzes a complex

five-step reaction involving a CO_2 and a water molecule before the release of two 3-phosphoglycerate (3PGA) molecules. Carbamylation is essential for Rubisco activation, as the non-carbamylated Rubisco binds RuBP too tightly to allow catalysis. The first, rate-limiting, step in carboxylation is the enolization of RuBP via the carbamate side chain; pH values lower than 8.0 may lead to the generation of Xylulose-1,5-bisphosphate that inhibit the enzyme activity (see below).

Another protein, Rubisco activase, is also involved in mediating the light activation of Rubisco. This nucleus-encoded protein uses the energy of ATP to remove active-site bound sugar-phosphate inhibitors, such as 2 carboxyarabinitol 1-phosphate (CA1P) or xyulose-1,5-bisphosphate (XuBP), d-glycero-2,3-pentodiulose-1,5-bisphosphate (PDBP) and, under some conditions, RuBP itself [44]. While XuBP and PDBP can be by-products of reaction intermediates, CA1P occurs naturally in the leaves of several plants and is a strong inhibitor of Rubisco. The affinity of Rubisco for CA1P is much stronger than that for RuBP, the substrate. As a result, CA1P, which accumulates in leaves during the night, inactivates Rubisco by blocking the binding sites. During the day (or on illumination), the bound CA1P is released from Rubisco by the concerted action of Rubisco activase and CA1P phosphatase.

The action of Rubisco activase may be crucial for maintaining Rubisco activity under low CO_2 supply and the sensitivity of Rubisco activase to high temperature might explain the decrease in Rubisco efficiency under these environmental conditions [45,46] (Figure 2).

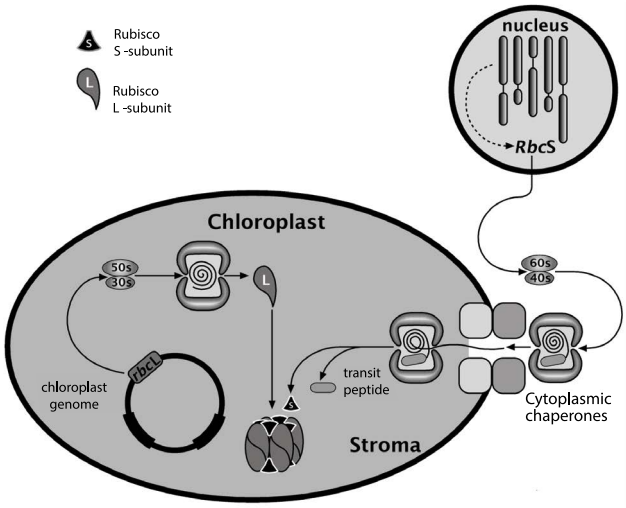

Figure 2: Assembly of large (L) and small (S) subunits of Rubisco.

Besides the carboxylation reaction, Rubisco reacts with oxygen to form one molecule of 2-phosphoglycolate and one of PGA; this reaction is the first step of the photorespiration pathway that leads to the release of previously fixed CO_2, NH_3 and energy. Due to photorespiration, C-fixing reaction has a reduced efficiency and a large amount of protein is needed to support adequate photosynthetic rates (Rubisco accounts for an average of 50% of leaf protein). Photorespiration if favoured by prolonged drought stress conditions and high temperature.

C4 plants have evolved biochemical mechanisms to elevate levels of CO_2 that rely on spatial separation of the initial fixation of atmospheric CO_2 from the Calvin cycle. Phosphoenolpyruvate carboxylase (PEPC) catalyzes CO_2 fixation to PEP in mesophyll cells producing oxaloacetate. Four-C acids (malate and aspartate) are then transported to bundle sheath cells, where they provide CO_2 to Rubisco, after undergoing decarboxylation. A pyruvate, phosphate dikinase (PPDK) catalyses the regeneration of PEP from pyruvate in mesophyll cells [47,48].

As compared to C3 plants, in C4 plants Rubisco shows lower affinity for CO_2 but higher carboxylation rates with minimal photorespiration; this would in turn lead to a higher biomass accumulation for a given amount of energy derived from sunlight. C4 plants can sustain high photosynthetic rates with a lower level of Rubisco; this implies that a lower amount of nitrogen is needed (higher nitrogen use efficiency). C4 plants can operate efficiently under low CO_2 levels, alleviating the need for wide stomata apertures, thereby reducing water loss [49].

Increasing CO_2 concentration associated with global warming might render C3 plants more efficient with respect to their Rubisco activity, although this might require increasing nitrogen supplies. Due to the low efficiency of Rubisco in C3 plants and the huge demand of Nitrogen to sustain the enzyme, strategies to improve Rubisco activity have received much attention in the last years.

Indeed it has been demonstrated that a high variability exists among vascular plant with respect to the catalytic properties of Rubisco; for example plants adapted to dryer environments showed a higher selectivity between CO_2 and O2 [50]. On the other hand, it has also been noted that an increased specificity for the substrate may be accompanied by a decrease in the catalytic rate. Indeed, Rubisco of C4 plants show lower affinity than in C3 plants, as they are adapted to a relative high CO_2 concentration.

The possibility to introduce new Rubiscos in crops by conventional breeding has been exploited [2]; furthermore biotechnological approaches have been tempted to modify Rubisco content and performance [51]. Plastome transformation has evidenced that changes in L-subunit determine changes in photosynthetic rate; although not directly involved in catalysis, transformation of the nucleus-encoded S-subunits are also attractive. Another possible way to increase Rubisco efficiency is through introduction of CO_2-concentrating mechanisms (or C4-like features) in C3 plants. Bioengineering plants for improved thermal tolerance of Rubisco activase [52], and a limited abundance of naturally occurring Rubisco inhibitors [53] may represent indirect targets for improving Rubisco performance.

Glutamine Synthetase: The Eye of the Needle in Nitrogen Assimilation

Plant nitrogenous compounds, including proteins, are all virtually built up starting from glutamine that is the product of the ATP-dependent ammonia addition to a glutamate molecule. This reaction is catalyzed by glutamine synthetase (GS), the first enzyme of nitrogen assimilation (and re-assimilation), which plays a key role in plant growth and productivity as well as in nitrogen use efficiency (NUE) [54,55].

In higher plants, with the exception of conifers, the enzyme is present as plastidial (GS1) and cytolsolic (GS2) isoforms encoded by a multigene family. The presence of isoenzymes in different plant organs has been referred to specific functions [56]. Due to its abundance, this protein can be also used as nitrogen storage in plants [57].

Ammonia for GS activity derives from a wide variety of primary and secondary metabolic processes, including nitrate and ammonium uptake, N2 fixation, photorespiration, protein and amino acid catabolism and phenylpropanoid biosynthetic pathway [58]. This implies that different isoenzymes are involved in production of organic nitrogen in source tissues and the subsequent N assimilation in sink tissues.

Plant GS has been categorized as the type II commonly occurring in prokaryotes. Using X-ray crystallography it has demonstrated that the enzyme has a decameric structure composed of two face-to-face pentameric rings and possesses 10 active sites, each localized at the interface between the N-terminal and C-terminal domains of two neighbouring subunits [59]. The subunit of cytosolic GS1 has a molecular mass of 38–40 kDa, while the plastid GS2 form range from 42 to 45 kDa. Glutamine synthesis is a two-step reaction involving the production of the activated intermediate γ-glutamyl phosphate from ATP and glutamate and the deprotonation of a bound ammonium ion to form ammonia, which attacks the carbonyl C to form glutamine (Figure 3).

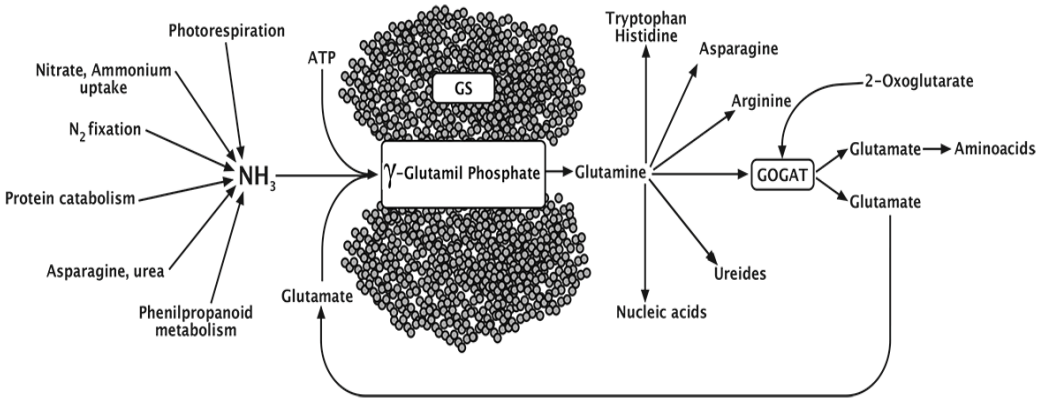

Figure 3: Reaction of Glutamine Synthetase (GS) and fate of glutamine.

Due to its role as a cornerstone in nitrogen metabolism, it is not surprising that GS is a highly regulated enzyme, with regulation occurring at transcriptional and post-translational level.

Using different plant species, a number of 3 to 5 genes encoding for the cytosolic GS1 has been generally identified; on the other hand, a single gene encodes the chloroplastic GS2. Each of the GS genes appears to participate in different metabolic processes, based on where and how they are expressed [60]. The expression of GS1 genes and the presence of the enzyme differ considerably at the tissue and cellular levels [54]. The GS2 gene is of nuclear origin and is targeted to the plastid; it is highly expressed in the mesophyll of leaves and other photosynthetic tissues [61]. Expression is influenced by developmental and environmental cues, such as soil N availability, plant N status, external and internal C status, as well as changes in plant hormones [62,63].

A cytosolic gene has been shown to be highly expressed in infected cells of leguminous root nodules where ammonium produced by N_2 fixation has to be assimilated [64]. The localization of a specific cytosolic GS isoenzyme in the vascular tissues has been reported for several species and related to N transport function [65]. The plastidic GS2, besides its major role in ammonia assimilation for amino acid synthesis, is involved in re-assimilation of ammonium released by photorespiration in photosynthetic tissues [66].

GS1 has been implicated in assimilation of nitrogen in sink tissues. Increasing importance of GS1 relative to GS2 in senescing leaves suggests a role for the former in the mobilization of N to be delivered to different sinks (e.g. developing seeds) [67]. QTL analyses have shown that cytosolic GS is necessary for grain filling [68]. It has also been shown that cytosolic GS protein can accumulate in mesophyll cells of plants in response to stress, such as pathogen attack [69], suggesting a role for this isoform in the re-assimilation of the nitrogen released during the disassembly of the photosynthetic apparatus.

Conifers do not possess GS2 gene, but rather they show two GS1 genes (GS1a and GS1b); based on light stimulation and parallelism between GS1a expression and chloroplast development it has been proposed that this gene might act similarly to GS2 in angiosperms [70]. Interestingly the overexpression of cytosolic GS1a resulted in improved chemical characteristics of field grown hybrid poplars [71].

Understanding the role for the cytosolic GS1 and plastidic GS2 isoforms are being elucidated, mainly using mutant plants. This task is particularly difficult for GS1, due to the variable number of genes found in different plant species. Experiments using model and cultivated plants point to a non-redundancy of the different proteins, rather to a specific role for each of them [58]. These aspects still deserve further research efforts.

Different kinds of post-translational modifications have been reported, involving Ca^{2+}-dependent kinases and phosphorylation [72] or binding of 14-3-3 proteins [73]; furthermore selective phosphorylation of Ser^{97} residue and subsequent binding to 14-3-3 proteins, which causes proteolytic breakdown to an inactive product has been reported for plastidic GS2 [74]. Recently, ubiquitination of GS polypeptides has also been proposed as a reversible post-translational regulatory mechanism [75].

The possibility to alter the expression of either GS1 or GS2 and the enzyme activity has attracted attention of researcher due to the possible effects on N metabolism and NUE [76]. Several transgenic approaches have been used, mainly based on overexpression or knockdown mutants [55].

Several studies have reported increased biomass and yield when GS genes are over-expressed in greenhouse and hydroponics experiments (e.g. [77]). Although the outcomes of these studies have been variable, they have clearly indicated that cytosolic GS1 can be important for efficient nitrogen assimilation, plant growth and biomass accumulation [78]. As compared to GS2, GS1 might be a key component of plant NUE [78].

However it has also been evidenced that post-translational modification of GS might significantly affects the over-expression of this enzyme, thereby influencing its ability to increase NUE in the field. Further research is needed on these aspects; moreover, it has been proposed that gene stacking experiments utilizing GS along with other genes of interest for N metabolism might be a suitable strategy to get a clear effect on NUE. The variable effects of environmental conditions, especially N supply, observed in gene expression experiments [60] should be taken also into account when designing for transgenic approaches to evaluating NUE.

Concluding Remarks

Adaptation of plants to different environments and an efficient use of natural resources to sustain adequate crop productivity are strictly related to the coordinated action of metabolic processes within the plant. Several enzymes play crucial roles in this framework guaranteeing the basis for autotrophic behavior of higher plants. The enzymes analyzed in this chapter amply demonstrate this feature, as they catalyze key reactions in nutrient acquisition, energy production, carbon and nitrogen assimilation. Advances in protein structure definition, genomic approaches and identification of regulatory routes have made it possible to better understand the function of these enzymes, paving the way for designing plants adapted to changing environment and able to respond to new challenges of modern agriculture. Further improvement of our knowledge could conceivably derive from exploiting natural variation and by using transgenic approaches.

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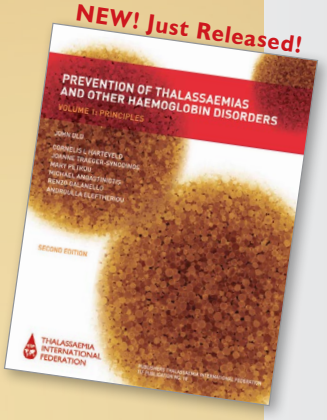

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