

completed by a transamination step catalyzed by the same BCATs that are involved in the formation of valine and isoleucine (see Section 7.5.2).

## 7.6 Glutamate-derived amino acids

**Proline**, **arginine**, and the (nonproteinogenic) amino acid **ornithine** are biosynthetically derived from **glutamate**. Proline has received major attention in plant biology because of its accumulation in responses to salt, drought, and metal stress. Although biochemical studies have rather neglected the synthesis of arginine in plants in the past, genomic annotations have provided missing information.

### 7.6.1 Proline metabolism is a target for metabolic engineering of stress tolerance

Understanding and manipulating proline biosynthesis in plants is largely motivated by observations that this cyclic secondary amino acid is one of several organic compounds that can function as **compatible solutes**. Compatible solutes are molecules that can accumulate to high concentrations in the cytoplasm without disrupting cellular activity and allow plants to lower their water potential and maintain turgor during dry or saline conditions (see Chapter 22). Proline also scavenges **reactive oxygen species** (ROS), which are formed under stressful conditions (see Chapters 21 and 22), and its function in stress situations is thus multifaceted. Water stresses cause considerable loss of agricultural productivity, making it desirable to develop **drought-tolerant plants** by conventional or transgenic strategies. Studies of genetically engineered plants that accumulate high levels of proline have provided clear evidence for the ability of proline to confer increased osmotic tolerance.

### 7.6.2 In plants, proline may be produced by two distinct pathways

Proline can be synthesized by two different pathways in plants. One originates from glutamate, the other from ornithine, although the details of the ornithine pathway are less clear (Fig. 7.44). The committing enzyme of the glutamate pathway, **pyrroline-5-carboxylate synthetase** (P5CS), is a bifunctional enzyme in plants. Its first activity,  **$\gamma$ -glutamyl kinase**, catalyzes the ATP-dependent phosphorylation of L-glutamate, and the  $\gamma$ -glutamyl phosphate produced is then reduced to **glutamic  $\gamma$ -semialdehyde** (GSA) by the NADPH-dependent GSA reductase. This intermediate spontaneously cyclizes to form  $\Delta^1$ -pyrroline-5-carboxylate (P5C, a Schiff base). The *Arabidopsis* genome harbors two closely related P5CS genes with nonredundant functions (see Section 7.6.3). P5C is then converted to proline by the NADPH-dependent enzyme P5C reductase.

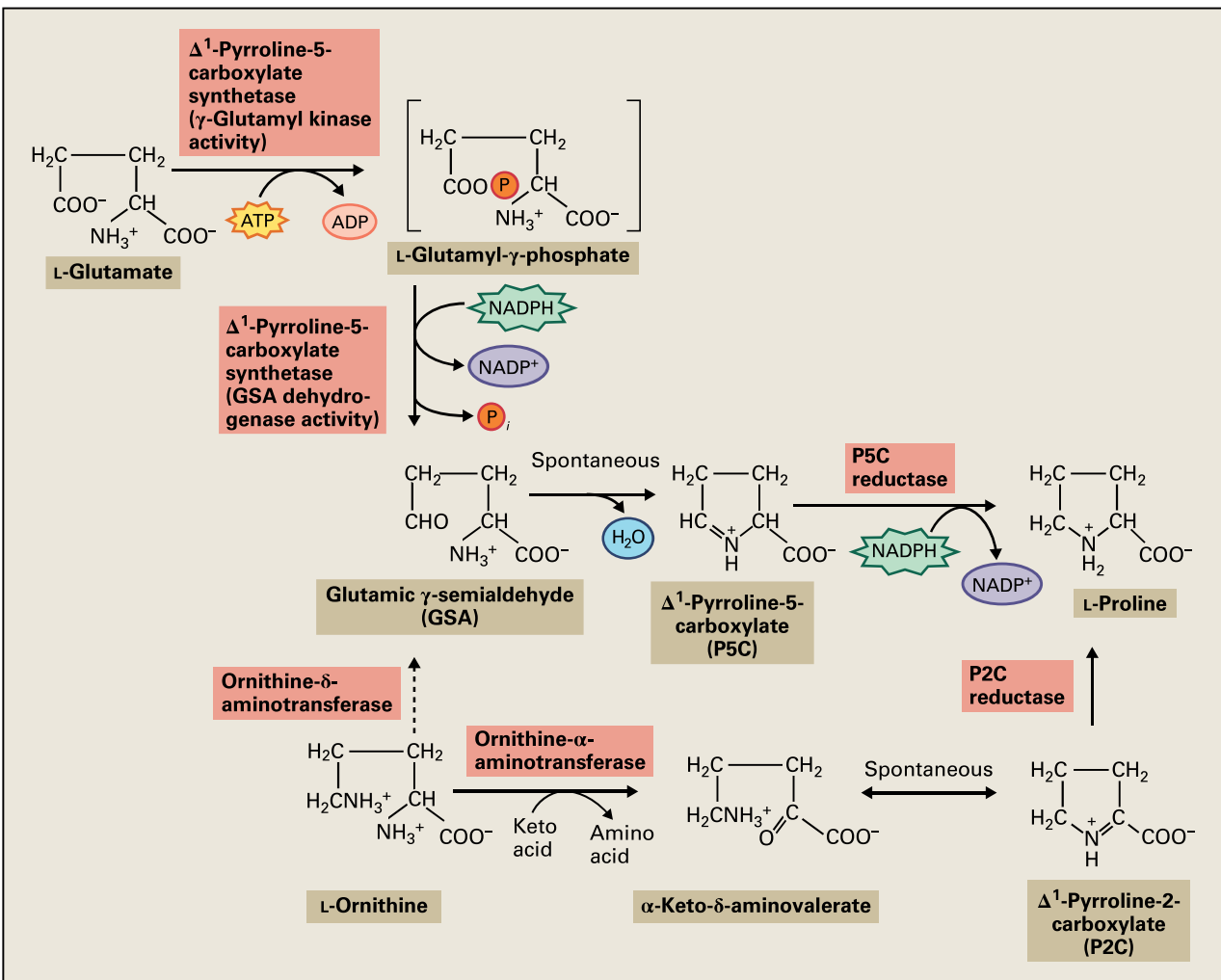
Two routes proposed for the synthesis of proline from ornithine (Fig. 7.44) differ as to whether  $\Delta^1$ -pyrroline-5-carboxylate or  $\Delta^1$ -pyrroline-2-carboxylate (an imino acid) is the final intermediate in the pathway. In the first instance, transamination of ornithine occurs at the  $\delta$ -C, while in the second instance it occurs at the  $\alpha$ -C. Ornithine  $\delta$ -aminotransferase ( $\delta$ OAT) has been characterized at both the biochemical and molecular levels in plants.

### 7.6.3 Proline synthesis and breakdown are environmentally regulated in plants

Regulation of proline accumulation in plants occurs both at the enzyme level and through changes in gene expression. The committing enzyme P5CS appears to be rate limiting in plants, and its transcript as well as free proline increase rapidly in response to desiccation, salt stress, and treatment with abscisic acid, and they return to normal level when dehydrated plants are watered. In several plant species, PC5S overexpression (but not that of P5C reductase) leads to plants with increased soluble proline content and reduced sensitivity to osmotic stress. As the  $\gamma$ -glutamyl kinase activity of PC5S is feedback-inhibited by proline, this feedback loop paradoxically appears to counteract the effect of increased expression of the enzyme. Loss of feedback inhibition by a conformational change of the P5CS protein under stress conditions has been proposed. Expression of a wild-type feedback-sensitive, and a mutagenized feedback-insensitive, *Vigna aconitifolia* PC5S in tobacco (*Nicotiana tabacum*) revealed a higher accumulation of proline in control conditions. Proline accumulated to even higher levels under salt stress in those plants expressing the feedback-insensitive PC5S, indicating that feedback inhibition is not (fully) abolished under normal conditions.

Of the two PC5S genes of *Arabidopsis*, PC5S1 responds to osmotic stress, and the loss-of-function mutant displays increased ROS levels and hypersensitivity to stress. Mutant *pc5s2* plants, on the other hand, have developmental defects, in particular embryo abortion during late seed development (desiccation stage). Tissue-specific expression patterns of the two genes differ considerably, and the two genes, therefore, do not complement each other.

The contribution of the ornithine pathway to proline synthesis was investigated both in  $\delta$ OAT loss-of-function mutants and in transgenic plants overproducing  $\delta$ OAT. In *Arabidopsis* loss-of-function mutants, proline accumulates normally under stress conditions, whereas arginine and ornithine can no longer be catabolized. This indicates a purely catabolic function of  $\delta$ OAT in arginine degradation, which occurs in mitochondria where  $\delta$ OAT is localized. On the other hand, transgenic *Nicotiana plumbaginifolia* and rice (*Oryza sativa*) expressing *Arabidopsis*  $\delta$ OAT accumulate more proline and are less sensitive to osmotic stress than wild-type plants. This discrepancy has not yet been resolved, but expression studies have shown that  $\delta$ OAT transcript levels actually decreased slightly under salt stress, whereas those of P5CS



**FIGURE 7.44** Multiple pathways are proposed for proline biosynthesis in plants. A glutamate-derived pathway is well established (black arrows), and increasing evidence suggests the importance of ornithine as a precursor (red arrows). The relative importance of the pathways through ornithine- $\delta$ -aminotransferase (dotted red arrow) or ornithine- $\alpha$ -aminotransferase (pathway with all red arrows) remains to be determined. In plants, the  $\gamma$ -glutamyl kinase (*proB* in *E. coli*) and GSA dehydrogenase (*proA* in *E. coli*) are synthesized in a protein fusion. This activity is referred to as  $\Delta^1$ -pyrroline-5-carboxylate synthetase.

increased. This would again point to the predominance of the glutamate-dependent pathway of proline biosynthesis.

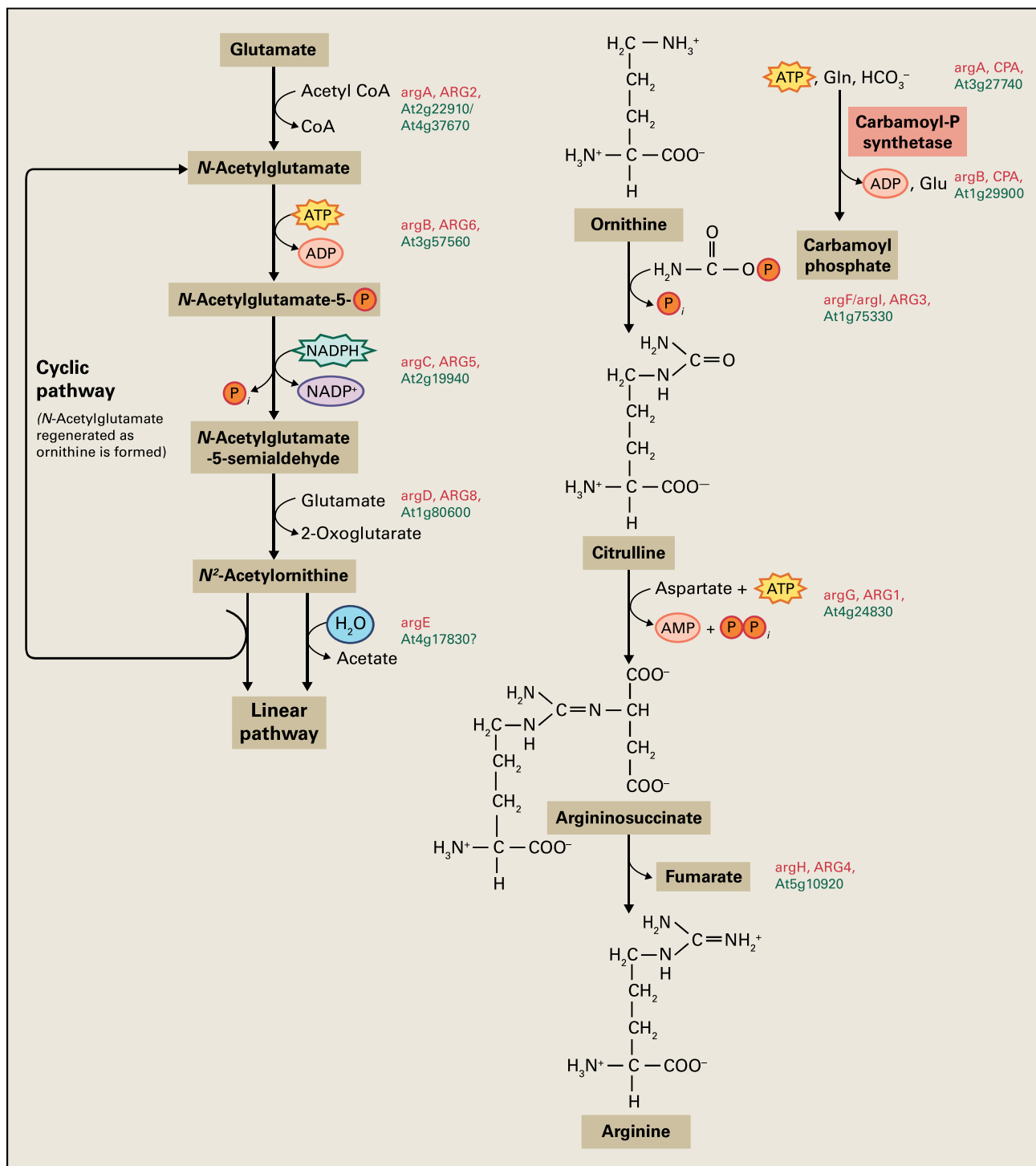
As discussed in Section 7.4.4, catabolism can play an important role in determining the concentration of a free amino acid. We can expect this particularly for proline, because its levels fluctuate considerably with environmental conditions. **Proline dehydrogenase** catalyzes conversion of proline back to  $\Delta^1$ -pyrroline-5-carboxylate (P5C). The enzyme is a flavoprotein and is localized to the inner mitochondrial membrane. As P5C is in equilibrium with glutamic  $\gamma$ -semialdehyde (GSA), an  $NAD^+$ -dependent P5C dehydrogenase will form glutamate, which can move into the cytosol. The resulting **proline-glutamate exchange** between the cytosol and the mitochondria feeds electrons into the mitochondrial electron transport chain. Proline dehydrogenase mRNA is regulated in a manner reciprocal to that of the biosynthetic enzyme P5CS during both dehydration/rehydration of a plant and salt stress and recovery. This suggests that proline degradation is suppressed during periods of rapid de-

novo synthesis to prevent a futile cycle, and is then activated to bring proline concentrations back to prestress conditions.

When expression of an *E. coli* proline-insensitive  $\gamma$ -glutamyl kinase and a  $\gamma$ -glutamylphosphate reductase in tobacco or *Arabidopsis* is combined with simultaneous suppression of proline catabolism by expression of a *ProDH* antisense construct, the level of free proline increases up to 50-fold when the anabolic enzymes are targeted to plastids, and less when they are targeted to the cytosol. Apparently, proline can be synthesized both in the cytosol and in plastids.

#### 7.6.4 Arginine is an amino acid rich in nitrogen

**Arginine** has a high N:C ratio (4:6) and serves as **nitrogen storage compound** in seeds, either in free form (e.g., in pea seeds) or in protein-bound form. In addition, it serves as a



**FIGURE 7.45** Biosynthesis of arginine. Structures are only shown for the pathway from ornithine to arginine. Structures in the ornithine pathway correspond to those also seen in Figure 7.42, with the notable difference that they are N-acetylated.

precursor in the biosynthesis of **polyamines**, certain alkaloids, and the signal molecule **nitric oxide (NO)**. In terrestrial (*ureotelic*) vertebrates, it is the immediate precursor of **urea**, the nitrogen excretion product in urine. The urea cycle and arginine biosynthesis pathway, therefore, figure prominently in textbooks of general biochemistry. A limited number of biochemical and in silico genome mining studies have

allowed elucidation of the pathway of arginine biosynthesis in plants, and these reactions are more or less identical to those in other organisms (Fig. 7.45). The pathway can be divided into two parts: from glutamate to ornithine, and from ornithine to arginine.

The ornithine pathway begins with the N-acetylation of glutamate by **N-acetylglutamate synthase (NAGS)**. The

following  $\gamma$ -phosphorylation and subsequent reduction to the semialdehyde are analogous to the reactions of free glutamate in proline biosynthesis (see Fig. 7.44). Because acetylation of the amino group prevents spontaneous cyclization to the pyrroline carboxylate, *N*-acetylglutamic semialdehyde can be transaminated to give rise to ***N*-acetylornithine**, from which ornithine is released either by hydrolysis or by transfer of the acetyl moiety to glutamate (transacetylation) within the cyclic pathway shown in Fig. 7.45.

Arginine synthesis is initiated by the carbamoylation of the  $\delta$ -amino group of ornithine with **carbamoyl phosphate** as the activated intermediate in a reaction catalyzed by **ornithine carbamoyl transferase** (OCT) that produces **citrulline** (Fig. 7.45). Carbamoyl phosphate is also required in pyrimidine synthesis, and the enzyme catalyzing its synthesis, carbamoyl phosphate synthetase (CPS), is feedback-inhibited by UMP and activated by IMP. Ornithine thus serves the needs of both pathways. The N atom required to convert citrulline to arginine is transferred from aspartate via **argininosuccinate**, which is cleaved to yield arginine and fumarate. Direct measurement of enzyme activities, chloroplast proteome data, and prediction of putative target sequences that direct the respective proteins to plastids, have demonstrated that the pathway is likely localized entirely in chloroplasts.

Only a limited number of reports detail either the overexpression or downregulation/knockout of any enzyme of the ornithine and arginine pathway, respectively. Overexpression of tomato (*Solanum lycopersicon*) NAGS in *Arabidopsis* increases ornithine content and enhances salt tolerance. A metabolomic analysis of the *ven3* and *ven6* mutants of *Arabidopsis* (which have a defect in mesophyll development and, therefore, exhibit a reticulate leaf phenotype) revealed that the mutants have an increased ornithine and a decreased citrulline content and can be rescued by citrulline complementation. The two *VEN* genes encode subunits of CPS, and the functionality of the respective polypeptides in CPS has been confirmed. How the arginine biosynthetic pathway functions in leaf development remains to be shown.

As pointed out above, arginine serves as a nitrogen storage pool. The branch point enzyme in the formation of ornithine and arginine, NAGS, is subject to control by a protein named **PII**, which was first discovered in *E. coli* as a coordinator of C:N interactions and then found to be highly conserved in bacteria, archaea, and plants. The plant homolog is targeted to the plastid and binds  $\alpha$ -ketoglutarate and ATP. High N conditions reduce  $\alpha$ -ketoglutarate concentrations because of its function in N assimilation (see Section 7.2), and this favors the interaction of PII and NAGS, which leads to activation of arginine synthesis and eventually to increased N storage.

## 7.7 Histidine

The elucidation of the **histidine** biosynthetic pathway in *Salmonella typhimurium* more than 50 years ago was a milestone in the biological sciences, not only because of the

novel and complex reactions it involved, but because of the insight provided into the regulation of the pathway, which was instrumental in the development of the operon concept. Comparatively little work had been done on the plant pathway until the 1990s, when histidine biosynthesis was considered a potential target for the development of novel herbicides.

The plant research was instigated by the findings that a number of herbicidal compounds interfere with the biosynthesis of aromatic and branched-chain amino acids (see Sections 7.3.1 and 7.5.2). To date, plant genes encoding all of the eight enzymes required for histidine synthesis shown in Figure 7.46 have been identified. Although several textbooks list histidine as a member of the glutamate family, this is incorrect, because glutamate and glutamine contribute only N atoms to histidine synthesis rather than the C skeleton, as we saw for glutamate in the case of proline and arginine.

Histidine synthesis begins with the attachment of **5-phosphoribose** from 5-phosphoribosyl-1-pyrophosphate to ATP in an N-glycosidic linkage. Note the similarity of this reaction with the phosphoribosylation of anthranilate in tryptophan biosynthesis (see Fig. 7.25). Release of pyrophosphate by hydrolysis produces 5'-phosphoribosyl AMP. The six-membered ring of AMP opens by hydrolysis, and then isomerization to the ketone occurs, again as in the corresponding step in tryptophan biosynthesis (see Fig. 7.27). Ammonia provided by hydrolysis of glutamine in a **glutamine amidotransferase** reaction and being guided through a tunnel (see Fig. 7.24) affects the production of **imidazole glycerol phosphate** (IGP) and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR). AICAR is used in a salvage pathway to regenerate ATP, while IGP is converted by consecutive dehydration, transamination, hydrolysis of the phosphate group, and two dehydrogenation steps to histidine (Fig. 7.46). This is a significant difference to tryptophan biosynthesis, where tryptophan synthase replaces the glycerol phosphate side chain of **indole glycerol phosphate** with serine to produce tryptophan directly (see Fig. 7.29).

All proteins involved in the histidine pathway carry an N-terminal extension either proven or assumed to direct the proteins to the plastids. It is generally accepted that the complete pathway operates in the plastid.

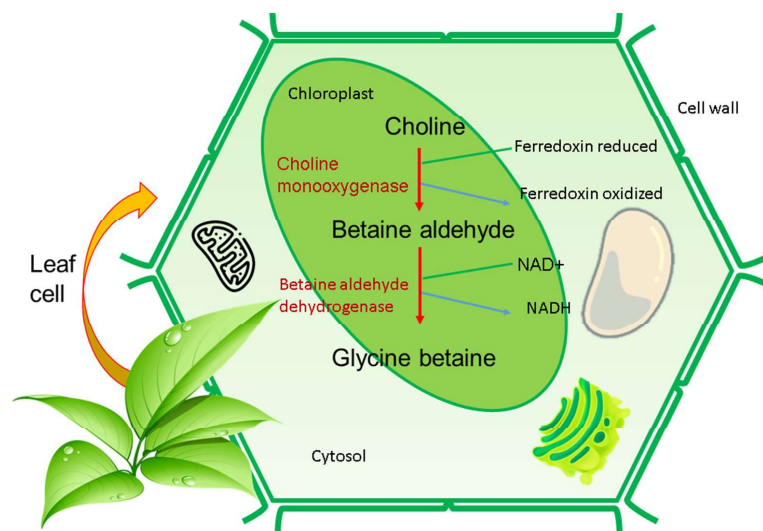
Of all the genes of the histidine biosynthetic pathway, only overexpression of either of the two genes encoding **ATP-phosphoribosyltransferase** (ATP-PRT), the first committed enzyme of the pathway, significantly increased the levels of free histidine in transgenic *Arabidopsis* (up to 40-fold). This indicates that ATP-PRT controls substrate flux through the pathway, which conforms with its feedback inhibition by histidine. ATP-PRT overproducing plants had a specifically increased Ni tolerance (the imidazole ring of histidine is an efficient Ni chelator; this is why His-tagged proteins bind to Ni columns and can be specifically eluted with imidazole), and in wild-type plants, Ni tolerance correlates with ATP-PRT transcript levels. Plants overexpressing ATP-PRT are less

the oxygen evolving complex, photosystem II, RuBisCo, and energy-(ATP) producing processes [11,12]. Furthermore, heat stress-induced disturbance of the electron transport chain leads to excessive reactive oxygen species (ROS) production in different cell organelles, such as mitochondria and chloroplasts, causing severe damage to DNA and cell membranes by inducing lipid peroxidation and ultimately causing cell death [13]. Increasing the capacity of heat stress-induced excessive ROS scavenging is considered an efficient defense strategy for ameliorating heat stress in plants [13]. A plant's thermotolerance ability is attributed to enhanced plasma membrane thermostability and reduced toxic ROS levels [14]. Plants have naturally adapted various defense mechanisms to counteract harsh environmental conditions such as heat stress. These defense mechanisms include an antioxidant machinery, osmolyte accumulation, maintenance of membrane integrity, and increased biosynthesis of heat-shock proteins (HSPs) by upregulating their associated genes' expression [15,16]. These defense mechanisms are involved in cellular defense against heat stress. Osmolyte accumulation has a significant role in mediating stress tolerance in plants (reviewed in Zulfiqar et al. [17]). Various studies have reported enhanced GB accumulation in plants under heat stress, revealing the positive role of this osmolyte in heat stress tolerance [18–22]. Sorwong and Sakhonwasee [23] stated that exogenous GB application alleviated the heat stress-induced reduction in leaf gas exchange traits.

There are no critical reviews on the role of GB in heat stress tolerance. Here, we summarize the fundamental impact of GB in inducing heat stress tolerance in economically important crops.

## 2. GB Structure and Biosynthesis in Plants

Glycinebetaine is an *N,N,N*-trimethylglycine quaternary ammonium compound that is naturally produced in numerous living organisms, including plants [24]. At physiological pH, GB is electrically a neutral molecule, but dipolar in nature. There are two pathways related to GB biosynthesis in plants [25]. The initiating metabolites for these pathways are choline and glycine [26]. GB biosynthesis in plants occurs via *N* methylation of glycine or the oxidation of choline [27]. GB biosynthesis, particularly in higher plants, is a two-step pathway beginning with choline, which is catalyzed by a ferredoxin-dependent Rieske-type protein, namely, choline monooxygenase (CMO), and by a soluble  $\text{NAD}^+$ -dependent enzyme [28,29]. Betaine aldehyde is oxidized by  $\text{NAD}^+$ -dependent betaine aldehyde dehydrogenase (*BADH*) to produce GB. Both *BADH* and *CMO* generally exist in chloroplast stroma [24,30] (Figure 1).



**Figure 1.** Biosynthesis of GB in plant cells (Figure adapted from Sakamoto and Murata [24]; Ashraf and Foolad [31]).

Free polyamines covalently combine with a small molecular substance, such as a phenolic compound and a derivative thereof in the amide bond to form a binding PA, which is also known as a PSCC-PA. The phenolic compound may be hydroxy cinnamic acid, coumaric acid, caffeic acid, or ferulic acid (Luo et al., 2009; Martin-Tanguy, 2010). This kind of PA forms the largest pools of PAs in plants (Kusano et al., 2008; Bassard et al., 2010). Many studies have confirmed that PSCC-PAs act as secondary metabolites, and participate not only in the local allergic reaction of plants against external infestation (Kumar et al., 1997), but also in plant morphogenesis (de Oliveira et al., 2016; De Oliveira et al., 2018; Mustafavi et al., 2018).

Free polyamines covalently bind to biomacromolecules, such as proteins, nucleic acids, uronic acids, or lignin by ionic and hydrogen bonds to form bound PAs, also known as PISCC-PAs. In the physiological pH range, F-PAs are fully protonated and positively charged, and can electrostatically combine with negatively charged biomacromolecules, such as acidic proteins, membrane phospholipids, and nucleic acids in the organism to become NCC-PAs (Igarashi and Kashiwagi, 2015). The NCC-PAs are associated with the regulation of enzyme activity, DNA replication, gene transcription, cell division and membrane stability, and have a wide range of biological functions in plant growth and development. Generally, the more the amino groups, the stronger the physiological activity.

Recent studies using exogenous PAs, PA synthesis inhibitors, and transgenic methods have intensively investigated the role of PAs in plant development and their mechanism of action. Such studies have shown that PAs are closely associated with plant growth, the stability of nucleic acids and membrane structure, stress resistance, and even plant survival (Agudelo-Romero et al., 2013; Pál et al., 2015; Sequeramutiozabal et al., 2016).

In this review, we provide a comprehensive and critical assessment of the published literature on the relationship between plant PAs and plant growth and development. We summarize recent research on the effects of PAs on the

development of plants from flowering to embryonic development to senescence, and explore their roles in the responses to several stresses. The aim of this paper is to reveal the roles that PAs play in plant growth and development and provide a basis for future research on the mechanism of action of PAs in plant growth and development. We also discuss the ways in which exogenous PAs can be used to regulate and promote plant growth and development in production.

## DISTRIBUTION AND METABOLISM OF PAs IN PLANTS

### PAs Distribution

Polyamines are ubiquitous in eukaryotic and prokaryotic cells (Liu et al., 2016, 2017), and are found even in plant RNA viruses and plant tumors. They have potent biological activities. There are numerous forms of PAs. In higher plants, PAs are predominantly present in their free form. The most common PAs in higher plants are Put, Spd, Spm, thermospermine (Tspm) (Kim et al., 2014; Sobieszczuk-Nowicka, 2017; Takahashi et al., 2017b), and cadaverine (Cad) (Regla-Márquez et al., 2015; Nahar et al., 2016) (Table 1). Other PAs are found only in certain plants or under special conditions.

Polyamines show tissue- and organ-specific distribution patterns in plants. For example, the most abundant PA in leaves was found to be Put, and its levels were three times higher than those of Spd and Spm, whereas Spd was found to be the most abundant PA in other organs (Takahashi et al., 2017b). Different types of PAs also show different localization patterns within cells. In carrot cells, Put was found to accumulate in the cytoplasm, and Spm in the cell wall (Cai et al., 2006). The distribution patterns of PAs may be related to their unique functions. In general, more vigorous plant growth and metabolism is associated with greater PA biosynthesis and higher PA contents (Zhao et al., 2004; Cai et al., 2006).

### Polyamine Biosynthesis

Putrescine is the central product of the common PA biosynthetic pathway. It contains two amino groups and is a synthetic precursor of Spd and Spm (Xu et al., 2009). There are three different routes of Put biosynthesis in plants (Figure 1). In the first route, the No. 8 carbon atom is removed from arginine (Arg) by arginine decarboxylase (ADC) to form agmatine (Agm) and CO<sub>2</sub>; the No. 2 nitrogen atom is removed from Agm to form N-carbamoyl Put (NCPA) and NH<sub>3</sub>; and then NCPA is hydrolyzed by N-carbamoylputrescine amidohydrolase (NCPAH) and its carbamoyl group is removed to form Put, CO<sub>2</sub>, and NH<sub>3</sub>. This is the main Put synthesis pathway in plants (Docimo et al., 2012; Pegg, 2016). In the second route, ornithine (Orn) is produced from Arg by arginase; and then ornithine decarboxylase (ODC) removes the carboxyl group of the no.1 carbon atom of Orn to form Put and CO<sub>2</sub> (Docimo et al., 2012; Pegg, 2016). The ODC gene has been lost from *Arabidopsis thaliana* and many members of the *Brassicaceae* (Hanfrey et al., 2010), indicating that the ornithine pathway is not essential for normal growth. In the third route, Arg is first converted into citrulline (Cit), which is then decarboxylated by citrulline decarboxylase (CDC)

**Abbreviations:** PAs, polyamines; F-PAs, free polyamines; CC-PAs, covalently conjugated polyamines; NCC-PAs, non-covalently conjugated polyamines; PSCC-PAs, perchloric acid-soluble covalently conjugated polyamines; PISCC-PAs, perchloric acid-insoluble covalently conjugated polyamines; Put, putrescine; Spd, spermidine; Spm, spermine; Tspm, thermospermine; Cad, cadaverine; Arg, arginine; ADC, arginine decarboxylase; Agm, agmatine; NCPA, N-carbamoylputrescine; NCPAH, N-carbamoylputrescine amidohydrolase; Orn, ornithine; ODC, ornithine decarboxylase; Cit, citrulline; CDC, citrulline decarboxylase; DFMA, difluoromethylarginine; DFMO, difluoromethylornithine; AIH, agmatine iminohydrolase; SPDS, spermidine synthase; CHA, clohexylamine, the inhibitor of SPDS; SPMS, spermine synthase; ACL5, thermospermine synthase; SAM, s-adenosylmethionine; SAMDC, S-adenosylmethionine decarboxylase; SAMS, S-adenosylmethionine synthase; ACC, 1-amino-1-carboxycyclopropane; ACS, 1-aminocyclopropane-1-carboxylate synthase; ACCO, 1-aminocyclopropane-1-carboxylate oxidase; MGBG, methylglyoxal bis (guanylyl-drazone), the inhibitor of SAMDC; DeSAM, decarboxylated SAM; MTA, 5'-methyl-thioadenosine; DAO, diamine oxidase; PAO, polyamine oxidase; PYRR, pyrroline; GABA,  $\gamma$ -aminobutyric acid; PYRR-DH, pyrroline dehydrogenase; FAD, flavin adenine dinucleotide; Dap, 1,3-diaminopropane; PBCP, polyamines back-conversion pathway; ABA, abscisic acid; IAA, auxin; ETH, ethylene; GA, gibberellic acid; Cyt, cytokinins; ESM, embryogenic suspensor mass; ROS, reactive oxygen species; PEG, polyethylene glycol; CAT, catalase; SOD, superoxide dismutase; GR, glutathione reductase.



### 13.2.2 Hexose phosphate pools exist in both the cytosol and the plastid stroma

The plant cytosol and plastids both contain hexose phosphate pools; depending on the plastid type, however, the capacity for the transport of hexose phosphates across the envelopes differs.

Most leaf chloroplasts have a low capacity for hexose phosphate transport, and their hexose phosphate pools are used in metabolic pathways distinct from those in the cytosol. Instead, carbohydrate exchange between these compartments is primarily by transport of the three-carbon intermediates **dihydroxyacetone phosphate**, **3-phosphoglycerate**, and **phosphoenolpyruvate**, and the five carbon intermediate **xylulose 5-phosphate**. Phosphorylated intermediates are exchanged for **inorganic phosphate** via **antiporters** (see Chapter 3) that belong to the same family of integral membrane proteins. The counter-exchange mechanism is strictly coupled, and there is little transport of phosphate in the absence of an exchangeable substrate. Indeed, the major protein in the chloroplast inner membrane is the triose phosphate translocator (TPT; see Fig. 13.4), which is responsible for the export of the products of the Calvin–Benson cycle (see Chapter 12). The nonphosphorylated sugars derived from starch breakdown (see Section 13.7), glucose and maltose, can also cross the chloroplast envelope, but without exchange for phosphate (see Fig. 13.4).

Other plastid types, including colored **chromoplasts** and colorless, starch-storing **amyloplasts**, possess another antiporter, the **glucose-6-phosphate/phosphate translocator** (GPT). This protein can transport glucose 6-phosphate across the plastid envelope, as well as triose phosphates and xylulose 5-phosphate, in exchange for inorganic phosphate ( $P_i$ ). The presence of the GPT implies a direct interaction between the cytosolic and plastid hexose phosphate pools. Chloroplasts in some cells or tissues (e.g., in guard cells, in the cells adjacent to the leaf vasculature, or in developing fruit) also possess the GPT. These chloroplasts likely perform specialized functions, and their metabolism differs from that of chloroplasts performing photosynthetic carbon assimilation. Under exceptional circumstances, such as drought or artificial feeding of leaves with high concentrations of sucrose, expression of a chloroplast hexose phosphate translocator can also be induced, and this is associated with major changes in metabolic fluxes within the leaf mesophyll cells.

## 13.3 Sucrose biosynthesis

**Sucrose** is a major product of photosynthesis in green leaves and accounts for much of the  $CO_2$  fixed during photosynthesis. It also serves as the principal long-distance transport compound in most plants and as a storage compound in some [including sugar beet (*Beta vulgaris*), sugar cane (*Saccharum* sp.), and carrot (*Daucus carota*)]. Sucrose is suited to its transport role: By joining the carbonyl carbons of

glucose and fructose in a stable glycosidic bond, sucrose formation prevents these groups from becoming oxidized through nonenzymatic reactions with other cellular components. For this reason, sucrose is described as a nonreducing sugar (Fig. 13.10).

Sucrose synthesis occurs exclusively in the cytosolic compartment of plant cells using carbon from the hexose phosphate pool. During the day, carbon enters this pool via the gluconeogenic flux from triose phosphates, which are exported from the chloroplast. This flux is regulated at the step of **fructose-1,6-bisphosphatase** (FBPase; see Section 13.6). During the night, hexose phosphates for sucrose synthesis are derived from other sources, such as by catabolism of transitory starch stored in the chloroplast. In this case, carbon exported from the chloroplast as free glucose or maltose is metabolized to hexose phosphates in the cytosol (see Section 13.7).

### 13.3.1 Glucose 1-phosphate can be reversibly converted to UDP-glucose

**Uridine diphosphate-glucose** (UDP-glucose; Fig. 13.11) is one of the two substrates required for sucrose synthesis (the other is fructose-6-phosphate; see Section 13.3.2). UDP-glucose is also a substrate for other anabolic reactions, including cell wall synthesis (see Chapter 2). The enzyme **UDP-glucose pyrophosphorylase** generates UDP-glucose and **pyrophosphate** ( $PP_i$ ) from glucose 1-phosphate and UTP (Reaction 13.3).

#### Reaction 13.3: UDP-glucose pyrophosphorylase

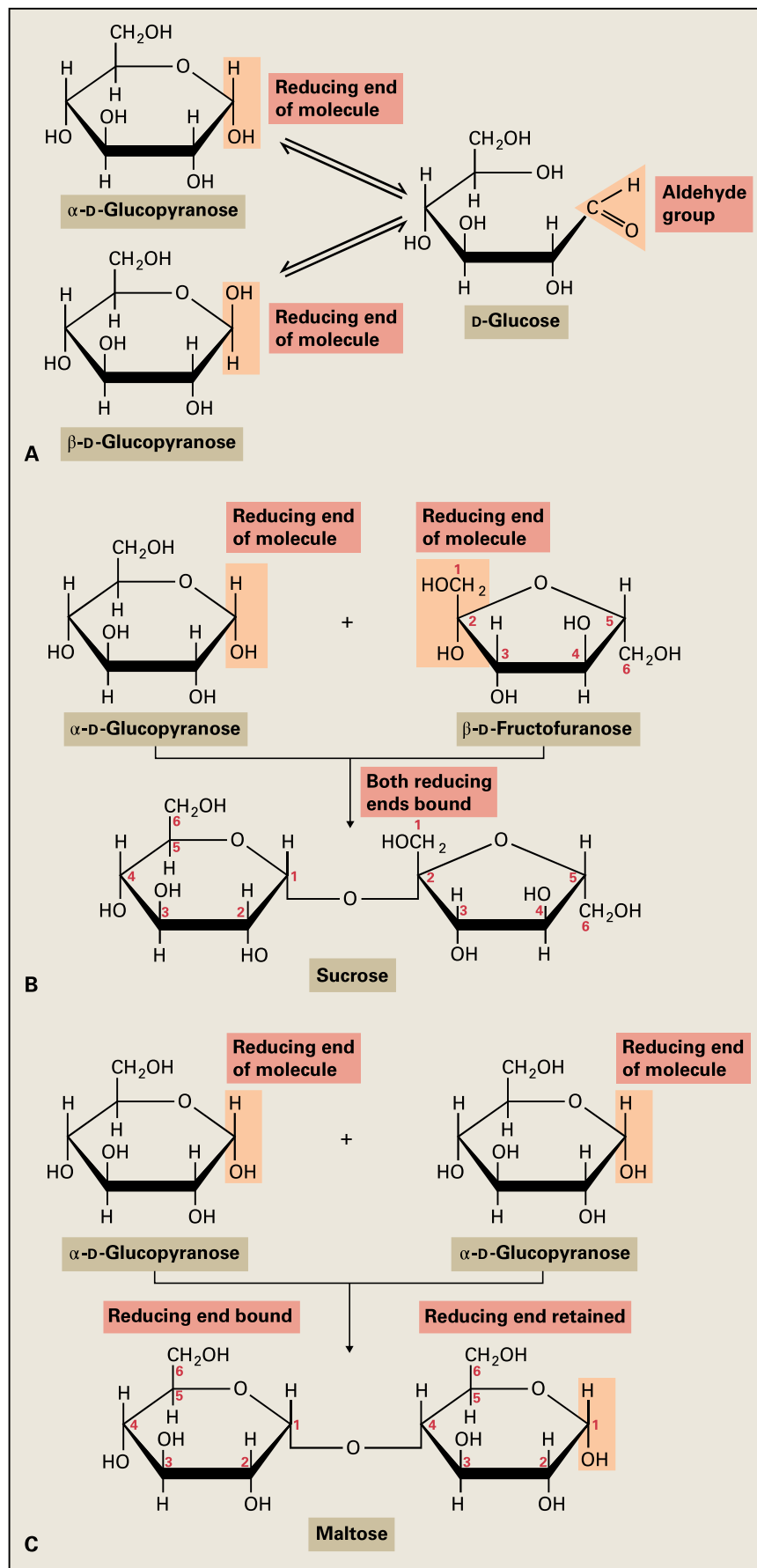


The reaction catalyzed by UDP-glucose pyrophosphorylase is readily reversible, and whether carbon enters or leaves the hexose phosphate pool depends on the concentrations of the respective metabolites. During sucrose synthesis, the flux through this step is driven by the consumption of UDP-glucose. Interestingly, cytosolic concentrations of pyrophosphate remain relatively constant in plants. In this way, plant metabolism differs from that of animals. The cytosol of animal cells contains a **pyrophosphatase** that rapidly hydrolyzes any  $PP_i$  produced during biosynthetic reactions; the metabolism of  $PP_i$  in the plant cell cytosol, however, is coupled with other metabolic processes (see Box 13.1).

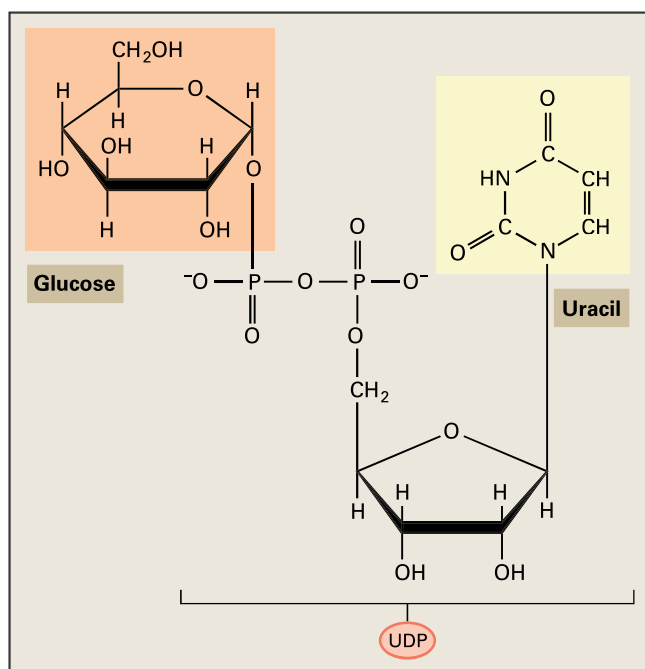
### 13.3.2 Sucrose is synthesized from UDP-glucose and fructose 6-phosphate

The principal route of sucrose synthesis involves the reactions catalyzed by two enzymes, **sucrose-phosphate synthase** and **sucrose-phosphate phosphatase** (Fig. 13.12).



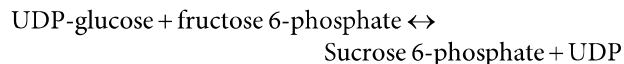


**FIGURE 13.10** Some sugars can act as reductants. (A) Glucose undergoes a conversion at the anomeric carbon (C-1) that yields an aldehyde (D-glucose) and two hemiacetal isomers ( $\alpha$ -D-glucopyranose and  $\beta$ -D-glucopyranose). The aldehyde group can react with (reduce) organic and inorganic substrates and is, therefore, described as the reducing end of the molecule. (B) During sucrose synthesis, the anomeric carbon of fructose (C-2) is joined to the anomeric carbon of glucose (C-1) by a glycosidic bond. This bond protects the reducing ends of both monomers, and defines sucrose as a nonreducing sugar. (C) In the disaccharide maltose, one of the anomeric carbons of glucose remains unbound and reactive. For this reason, maltose is a reducing sugar. In trehalose, also a disaccharide of glucose, the two anomeric carbons are bound, rendering it a nonreducing sugar (not shown).

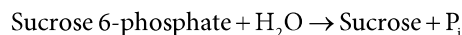


**FIGURE 13.11** Structure of uridine diphosphate-glucose (UDP-glucose).

#### Reaction 13.4: Sucrose-phosphate synthase



#### Reaction 13.5: Sucrose-phosphate phosphatase



Sucrose-phosphate synthase transfers the glucosyl moiety of UDP-glucose to fructose 6-phosphate, yielding sucrose 6-phosphate, and the subsequent hydrolysis of sucrose 6-phosphate by sucrose-phosphate phosphatase yields sucrose and phosphate. Overall, the formation of sucrose by this route has a large negative free energy change ( $\Delta G^{\circ} = -25 \text{ kJ mol}^{-1}$ ), which renders this sequence of reactions essentially irreversible in vivo. This is primarily due to the large negative free energy change ( $\Delta G^{\circ} = -16.5 \text{ kJ mol}^{-1}$ ) associated with the dephosphorylation of sucrose; the preceding steps (the formation of UDP-glucose and of sucrose 6-phosphate) have relatively small negative free energy changes ( $\Delta G^{\circ}$  of  $-2.88 \text{ kJ mol}^{-1}$  and  $-5.7 \text{ kJ mol}^{-1}$ , respectively).

### BOX 13.1

#### Pyrophosphate is a mysterious player in plant metabolism

Pyrophosphate (PP<sub>i</sub>) is generated in many biosynthetic reactions (e.g., the synthesis of nucleotide sugars, such as UDP-glucose and ADP-glucose, the activation of amino acids for protein synthesis, and the polymerization of nucleic acids). In animals and many microorganisms, hydrolysis by pyrophosphatase removes the pyrophosphate produced in these reactions, and the large change in free energy ( $\Delta G^{\circ}$  of  $-33.4 \text{ kJ mol}^{-1}$ ) drives otherwise energetically unfavorable reactions in the direction of synthesis.

The stroma of plastids, the site of many biosynthetic reactions in plants, also contains a pyrophosphatase, but the plant cytosol, where sucrose and cell wall biosynthesis take place, can accumulate a substantial concentration of PP<sub>i</sub> (up to 0.3 mM). This cytosolic PP<sub>i</sub> pool is important; overexpression of a cytosol-localized bacterial pyrophosphatase in transgenic tobacco (*Nicotiana tabacum*) significantly inhibits growth. However, the way in which PP<sub>i</sub> metabolism is linked to other cellular processes is not fully understood.

The discovery of this cytosolic PP<sub>i</sub> pool followed the discovery of one of the enzymes that can metabolize it: the PP<sub>i</sub>-dependent phosphofructokinase (PFK; see Section 13.6). PFK can remove or produce PP<sub>i</sub> while interconverting fructose 1,6-bisphosphate and fructose 6-phosphate in a reversible reaction. Its presence is an enigma because it acts alongside two other cytosolic enzymes catalyzing similar, yet irreversible reactions. The conversion of fructose 6-phosphate to fructose 1,6-

bisphosphate is a principal regulatory step in glycolysis in other organisms. However, regulation differs in plants due to their autotrophic nature, and it has been proposed that PFP serves other functions, including:

- maintenance of a stable pyrophosphate concentration in the cell
- generation of pyrophosphate required for the breakdown of sucrose by way of the sucrose synthase pathway (see Section 13.4)
- bypass of PFK during times of phosphate starvation, when the amounts of ATP may be insufficient for the PFK reaction (see Box 13.5)
- stimulation of carbon flow in glycolysis during anaerobic conditions
- equilibration of the hexose and pentose phosphate/triose phosphate pools during increased biosynthetic activity.

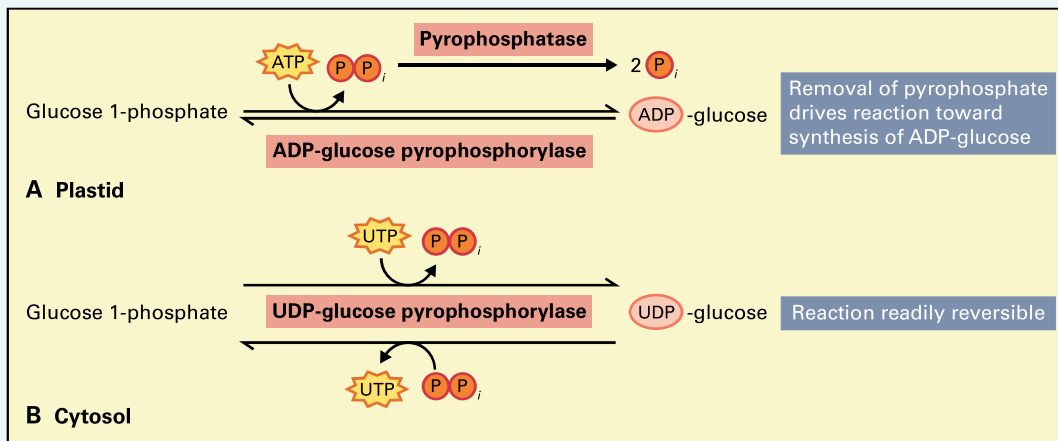
PFP has been repressed or mutated in a range of species. In general, such plants appear normal, but examination of the metabolite levels and fluxes reveals differences in each case, including minor reductions in starch content of potato (*Solanum tuberosum*) tubers, increases in the sucrose content of sugar cane (*Saccharum* sp.) stems, slight inhibition of glycolysis, and changes in the balance of hexose phosphates to triose phosphates. These studies suggest PFP influences fluxes between the hexose phosphate and triose

phosphate pools, but it is not an essential enzyme. Thus, its function remains unresolved. It may be that, under normal laboratory or greenhouse conditions, the enzyme enhances growth and fitness by a margin that is too small to measure, but large enough to ensure conservation of the enzyme during evolution. Alternatively, the enzyme may be important only under certain conditions not tested in the laboratory.

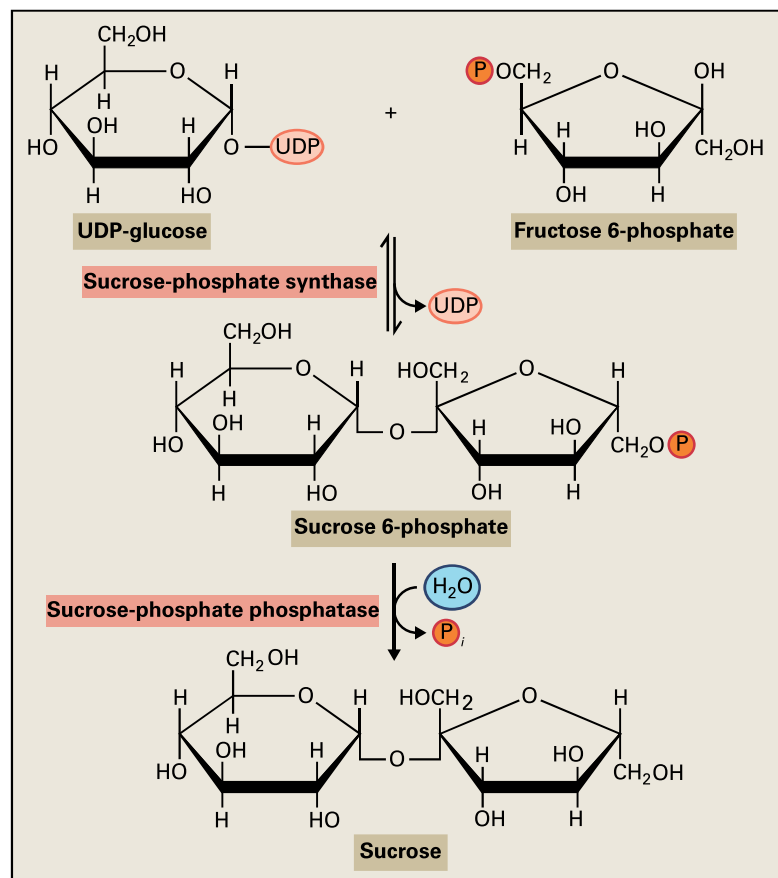
Another player in  $PP_i$  metabolism is the proton-pumping pyrophosphatase located in the tonoplast (see Chapter 3). Energy released by pyrophosphate hydrolysis drives the pump, acidifying the vacuole and generating an

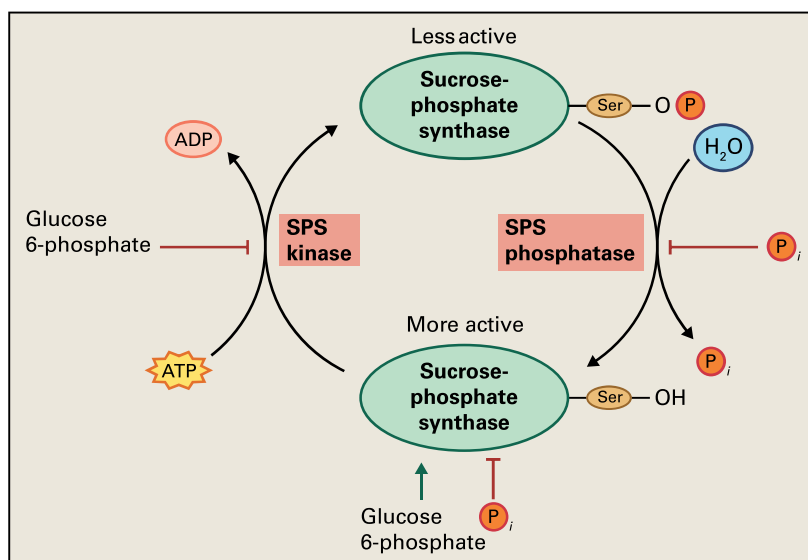
electrochemical gradient used to power transmembrane transport. This  $H^+$ -pyrophosphatase may participate in removing cytosolic pyrophosphate produced during biosynthesis. Analysis of *Arabidopsis* mutants deficient in this pump supports this idea: *Arabidopsis* seedlings convert storage lipids in their cotyledons into sucrose via gluconeogenesis, and pump-deficient mutants have reduced sucrose content and stunted growth due to accumulation of inhibitory levels of  $PP_i$ .

Despite these discoveries, we still do not fully understand how or why plant cells maintain a stable pool of cytosolic  $PP_i$ .



**FIGURE 13.12** Sucrose synthesis by sucrose-phosphate synthase (SPS) and sucrose-phosphate phosphatase.





**FIGURE 13.13** Regulation of sucrose-phosphate synthase (SPS). SPS is regulated by both allosteric effectors and phosphorylation/dephosphorylation of a serine residue in the enzyme. The active enzyme is dephosphorylated and can be activated further, allosterically, by glucose 6-phosphate. SPS is inhibited by inorganic phosphate and possibly by sucrose. In addition, glucose 6-phosphate inhibits the SPS-kinase, and inorganic phosphate inhibits the SPS-phosphatase. As a result, a high ratio of glucose 6-phosphate to inorganic phosphate maintains the enzyme in its active form, whereas a low ratio inhibits the enzyme and promotes its conversion to the inactive form.

There is evidence, mainly from work on spinach (*Spinacia oleracea*) in the 1990s, that sucrose synthesis is regulated by mechanisms that coordinate the activity of sucrose-phosphate synthase with the status of the hexose phosphate pool (Fig. 13.13). First, sucrose-phosphate synthase is subject to **allosteric modulation** by glucose 6-phosphate, which activates the enzyme, and by P<sub>i</sub>, which inhibits it. When hexose phosphates are abundant, the enzyme is activated. Furthermore, a decrease in free P<sub>i</sub>, which generally accompanies high concentrations of hexose phosphates, lifts inhibition. Second, sucrose-phosphate synthase is regulated by multisite, reversible protein phosphorylation. One kinase that phosphorylates Ser<sup>158</sup> and downregulates sucrose-phosphate synthase activity is itself inhibited by glucose 6-phosphate. The phosphatase that upregulates the activity of sucrose-phosphate synthase is inhibited by high concentrations of P<sub>i</sub>. Sucrose-phosphate synthase is also phosphorylated at other sites. Phosphorylation at Ser<sup>229</sup> allows interaction with 14-3-3 proteins, which also inhibits its activity. By contrast, phosphorylation at Ser<sup>424</sup>, which can occur during osmotic stress, can activate the enzyme. The effects of allosteric and post-translational modifications combine to provide sensitive control of sucrose synthesis. There is also evidence that sucrose levels can feed back on sucrose-phosphate synthase activity, preventing overproduction; the mechanism of this feedback, however, is not understood.

While such biochemical findings have laid the foundation for understanding the control of sucrose synthesis, the situation is likely more complex. Multiple conserved genes encode various isoforms of both sucrose-phosphate synthase and sucrose-phosphate phosphatase, and the properties of each of these isoforms have not been rigorously investigated (see Box 13.2). This may complicate the interpretation of some biochemical

studies in which a uniformity of enzyme activity or activation state is assumed. For instance, one class of sucrose-phosphate synthase isoforms found in wheat (*Triticum aestivum*) and other monocotyledonous Poaceae lack the phosphorylation sites associated with 14-3-3 protein binding and osmotic stress activation, and their regulatory features are unknown.

## 13.4 Sucrose metabolism

After its production in source tissues, sucrose is transported within the plant via the phloem to sink tissues, where it can enter destination cells by two routes. In most cells, it moves by **symplasmic transport**, diffusing through the **plasmodesmata**. In this case, the sucrose is broken down in the cytosol or sequestered in the vacuole to maintain passive import. In some tissues, however, sucrose is unloaded from the phloem into the **apoplast** and must cross the plasma membrane to enter the destination cell, often against a concentration gradient. This **apoplastic pathway** is particularly important to developing embryos, which have no direct cytoplasmic connections to the tissues of the maternal plant. The sucrose can be either transported into these cells directly, or is first hydrolyzed to glucose and fructose in the apoplast. The potential significance of the various means of sucrose transport and degradation is receiving more attention, given that carbohydrates, in addition to being intermediates in metabolism, play a role in regulating the expression of many genes. Thus, the form of carbohydrate taken up may influence the activity of the cell (see Chapters 8 and 16).

Sucrose can be degraded by either **sucrose synthase** (Fig. 13.14) or **invertase** (Fig. 13.15):