

Plant Chloroplasts and Other Plastids

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Plastids are a group of organelles that are characteristic of plant cells. They are able to perform many specialized functions that are essential for plant growth and development, such as photosynthesis, nitrogen assimilation, the synthesis of amino acids and of fatty acids, storage of carbohydrates and lipids or the formation of colours in some fruits and flowers.

Introduction

It is generally accepted that plastids originally derived from a free-living photosynthetic prokaryote. Chloroplasts still exhibit many prokaryotic features. For example, plastids contain their own genetic system consisting of a circular DNA; they reproduce by division as bacteria do; the protein synthesizing machinery of plastids is similar to that of prokaryotes; and the import pathways of nuclear-encoded proteins across the internal membranes of chloroplasts, the thylakoids, are in part of prokaryotic origin as are parts of the lipid-synthesizing machinery. During evolution, most of the genes originally present in the prokaryote have been transferred to the nuclear genome. Since plastids possess a wide range of metabolic and biosynthetic capabilities that are indispensable to plant growth and development, the functioning of the plant cell relies strongly on a complex metabolic network that links reactions in the plastid and in the extraplastidial compartments.

Plastids – A Family of Semiautonomous Organelles

All plastids derive originally from proplastids, small (0.5–1.0 μm diameter) undifferentiated and colourless plastids that are present in meristematic and rapidly dividing cells of shoots and roots. Proplastids represent the precursors of the other more highly differentiated plastids. When exposed to light, proplastids develop into chloroplasts, which are green because of the presence of the photosynthetic pigment chlorophyll. The prominent inner membrane system found in chloroplasts, the thylakoid membrane, is almost absent in proplastids. Chloroplasts, 4–6 μm in diameter, represent the most important class of plastids and are the site of photosynthesis and the fixation of atmospheric carbon dioxide. In addition, they are involved in several other biosynthetic processes such as the incorporation of nitrogen into organic compounds

Introductory article

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(ammonia assimilation), the biosynthesis of fatty acids and of amino acids, and the temporary storage of starch. An individual plant cell may contain as many as 50 to 100 chloroplasts, each containing multiple copies of circular DNA that encodes only about 100 proteins. The vast majority of the plastidial proteins are encoded in the nucleus.

Like other plastids, chloroplasts are bounded by a double membrane, the outer and the inner envelope membrane (Figure 1). In addition, they contain the chlorophyll-containing thylakoid membrane system, which is embedded within the stroma. The thylakoids are the site of photosynthesis leading to the light-driven formation of energy (in the form of ATP) and reducing

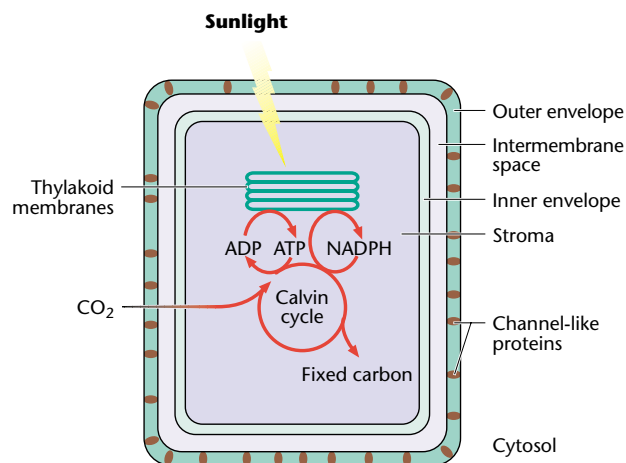


Figure 1 Chloroplasts are the site of photosynthesis and fixation of atmospheric CO_2 . Like other plastids, chloroplasts are double membrane-bound organelles. In addition to the outer and the inner envelope membranes, chloroplasts contain chlorophyll-containing thylakoid membranes, the site of photosynthesis. The products of the light reaction, ATP and NADPH, are used to convert atmospheric CO_2 into organic compounds by enzymes of the stroma-localized Calvin-Benson cycle. ADP, adenosine diphosphate; ATP, adenosine triphosphate; NADPH, nicotinamide adenine dinucleotide phosphate (reduced form).

power (in the form of NADPH). They represent an interconnected membrane system consisting of tightly stacked membranes (grana thylakoids resembling a stack of coins), which are connected by stroma thylakoids traversing the stroma. In the absence of light, proplastids first develop into etioplasts, which contain semicrystalline prolamellar bodies composed of tubular membranes. Upon illumination, etioplasts develop into chloroplasts, whereby the prolamellar bodies are transformed into thylakoids.

In nongreen heterotrophic tissues, proplastids develop into leucoplasts, unpigmented, colourless plastids. These plastids are capable of synthesizing and storing a variety of substances, for example, proteins, oils or starch. Amyloplasts, found in potato tubers or maize kernels, for example, are the most prominent leucoplasts and are carbohydrate-importing organelles in which carbon is stored in the form of starch. Upon exposure to light, amyloplasts can be converted into chloroplasts.

In some flowers and fruits, chloroplasts develop into highly coloured chromoplasts. In these plastids, chlorophyll and the thylakoid membrane system break down, while large amounts of carotenoids are synthesized that are responsible for the yellow, orange or red colours as observed, for example, in red pepper fruits, apples, tomatoes or carrot roots. Chloroplasts also contain carotenoids, which are essential parts of the photosynthetic machinery and can act as antioxidants protecting the photosynthetic apparatus against reactive oxygen species. The carotenoids are synthesized inside the plastids via a novel route, the deoxyxylulose 5-phosphate (DOXP) pathway, which is only present in photosynthetic organisms and some eubacteria but not in mammals. It uses triose phosphates and pyruvate to form DOXP, which is then the starter molecule for a variety of isoprenoids such as carotenoids, phytol (the hydrophobic membrane anchor of the chlorophyll molecule) or the prenyl chain of plastoquinone (a component of the photosynthetic electron transport chain in chloroplasts).

The carotenoids are normally masked by the green colour of chlorophyll and become visible only in the late stages of leaf development when the chlorophyll molecules are degraded during leaf senescence. This process is accompanied by a transition of chloroplasts to gerontoplasts representing the senescence-specific form of plastids. In contrast to other plastids, the metabolism of gerontoplasts is solely catabolic. Stromal components and the thylakoid membrane are gradually degraded while the envelope membrane remains intact. The mobilized nutrients are exported from the senescent leaf and can then be reused in other parts of the plant, for example in younger leaves or in growing seeds.

The common progenitors of all plastids are the proplastids that can develop into any type of plastid. Mature plastids are highly interconvertible (**Figure 2**). For example, any type of plastid can be transformed into

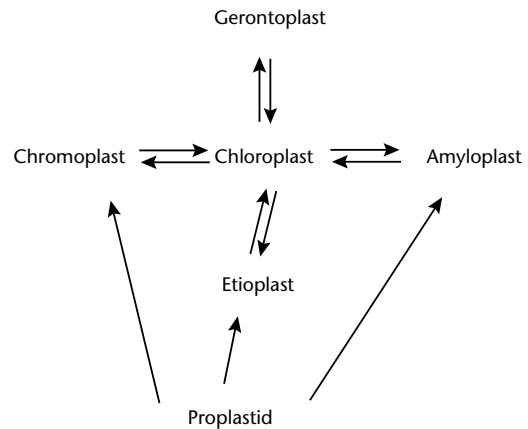


Figure 2 Interconversion of various types of plastids. All mature plastids derive from proplastids that are converted into etioplasts in leaves if plants are grown in the dark. Upon light, etioplasts develop into chloroplasts that can reversibly differentiate into chromoplasts in flowers and fruits. Leucoplasts (e.g. amyloplasts) of nongreen tissues can re-green into chloroplasts. During leaf senescence, chloroplasts are transformed into gerontoplasts, which can, in some cases, re-develop into chloroplasts.

chromoplasts, which are, in turn, able to re-green into chloroplasts. A good example of the relative ease with which plastids can change from one type to another is the observation that even fully developed gerontoplasts can be transformed back into chloroplasts.

Plastid Division

Plastids replicate by dividing into equal halves, which is characteristic of bacteria. Since all plastids are derived from proplastids of meristematic cells, the proplastids must divide to ensure that, following cell division, both daughter cells contain proplastids. In mature leaves, the greater portion of the final plastid population will, however, derive from the division of mature plastids. Division of both proplastids and chloroplasts, the two best-studied examples, seems to take place primarily by a process termed binary fission that involves constriction of the envelope membranes. During division, an electron-dense two-ring torus ('plastid dividing ring') around the constriction site can be observed. This structure is composed of an internal ring on the stromal side of the inner envelope membrane and an external ring on the cytosolic side of the outer envelope membrane. It has been shown that the *Arabidopsis* genome encodes two eukaryotic homologues of the bacterial cell division protein FtsZ, an essential component of the cell division machinery of prokaryotes. FtsZ is structurally homologous to eukaryotic tubulins, which can undergo dynamic GTP-dependent assembly into long polymers *in vitro*. Such microtubules are involved, for example, in the intracellular transport of organelles or the separation of chromosomes

during mitosis. One of the two *Arabidopsis* FtsZ-like proteins is localized in the plastid stroma (FtsZ1) and the other in the cytosol (FtsZ2). It is speculated that both proteins are components of the plastid dividing rings that function together on opposite sides of the chloroplast envelope to effect constriction of the organelle. The complete process of plastid division, however, is only poorly understood.

Specialized Functions of Envelope Membranes: Import of Proteins and Exchange of Metabolites

Import of proteins

Since the initial endosymbiotic event, the major part of the plastid genes has been transferred to the nucleus. In consequence, most of the plastid proteins are coded for by nuclear genes and are synthesized in the cytosol. These proteins have to be targeted to the plastids and sorted to their appropriate plastid subcompartments. These subcompartments are, in the case of chloroplasts, the outer and the inner envelope membrane, the intermembrane space, the stroma, the thylakoid membrane and the thylakoid lumen (**Figure 1**). Most of the precursor proteins carry an N-terminal extension (the transit peptide, up to about 100 amino acid residues in length) that is responsible for the correct targeting to the plastid and that is removed by a proteolytic cleavage upon entry into the plastids. With only few exceptions, outer envelope membrane proteins are synthesized without a cleavable transit peptide, suggesting that the targeting information of these proteins is contained within the mature protein. On the other hand, proteins destined for the thylakoid lumen contain a bipartite transit peptide containing the information both for targeting to the stroma and for crossing the thylakoid membrane. Such domains are consecutively removed by processing proteases localized in the stroma and the thylakoid lumen, respectively.

The general import pathway into the plastids starts with the interaction of the transit peptide with components of translocation machineries of both the outer and the inner envelope membranes. The energy-independent binding of the precursor protein to the plastid surface is followed by an energy-dependent partial translocation across the outer envelope membrane (docking) and a complete and ATP-dependent translocation across both membranes. Binding and docking of the precursor proteins requires components of the Toc complex (translocon at the outer envelope of chloroplasts), whereas components of the Tic complex (translocon at the inner envelope membrane of chloroplasts) mediate the complete translocation. The Toc complex consists of Toc159 (an ATP- and GTP-binding protein, functioning as a receptor that is responsible for the

initial binding of the precursor proteins), Toc75 (constituting the major unit of the translocation channel of the outer envelope membrane), and Toc33 and Toc34 (GTP-binding proteins, components of the receptor complex whose precise function is unknown). It has been shown that chloroplast biogenesis is strongly impaired in plants that are defective in receptor components such as Toc159 or Toc33. The Tic complex contains Tic110, which is presumably a receptor for the precursor proteins in the intermembrane space or/and can interact with stromal chaperons and additional components (Tic55, Tic40, Tic22, Tic20) with yet unknown functions (the numbers correspond to the molecular masses in kilodaltons of the different components).

The precursor proteins are transported across the envelope membrane in an unfolded state. Various molecular chaperons located in the cytosol, the intermembrane space and the stroma, are therefore required for unfolding and refolding of the translocated proteins and, presumably for the ATP-dependent pulling of the precursor proteins into the plastid through repeated cycles of binding and release.

Luminal proteins, once imported into the stroma, have to be translocated further across the thylakoid membrane. Four different pathways have been described. (1) Spontaneous insertion into the membrane without the requirement of energy and a translocation machinery as is the case for the CF_oII subunit of the thylakoid ATP synthase. (2) An ATP-dependent SecA/SecY pathway, which is reminiscent of the bacterial Sec system consisting of a SecA translocation ATPase and a heterotrimeric SecY/E/G translocation channel. The 33 kDa subunit of the oxygen-evolving complex and plastocyanin are transported by this pathway. (3) A GTP-dependent signal recognition particle (SRP)-like pathway that shows similarities to the SRP-system of the endoplasmic reticulum and of bacteria. This pathway is used, for example, by the light-harvesting complex protein. (4) A ΔpH-dependent pathway that uses the thylakoid transmembrane pH-gradient to drive precursor translocation. All precursor proteins that are transported via this pathway (e.g. the 23 kDa and the 17 kDa subunits of the oxygen-evolving complex) possess a twin arginine motif in their transit peptides. This pathway is also of prokaryotic origin and it is unique in that the proteins can be transported in a tightly folded state.

Exchange of metabolites

Transport of photoassimilates

Metabolites that are exchanged between the plastids and the surrounding cytosol also have to be transported across the two envelope membranes. The outer envelope membrane contains different channel-like proteins that allow the passage of small molecules, whereas the inner envelope membrane is the actual permeability barrier for solutes and

the site of different metabolite translocators that coordinate the metabolism in both compartments. As already pointed out, one of the main function of chloroplasts is the light-dependent transformation of atmospheric carbon dioxide into organic compounds (triose phosphate (trioseP), a three-carbon carbohydrate). These products of photosynthesis are exported from the chloroplast into the cytosol by the triose phosphate/phosphate translocator (TPT; **Figure 3**). This pathway represents the 'day path' of carbon. In the mature leaves of most plants, the photo-assimilates exported by the TPT are used in the formation of sucrose and amino acids, which are the main products allocated to heterotrophic plant organs. Sucrose and amino acids are actively loaded into the sieve element/companion cell complex of the vascular tissue by specific H^+ symporters.

The TPT was the first plant membrane transport system for which the primary sequence was determined. Analyses of TPT sequences from various plants showed that all TPTs are nuclear-encoded and possess N-terminal transit peptides that direct the adjacent protein to the chloroplasts.

The TPT is present almost exclusively in photosynthetically active tissues. studies of transgenic plants with a reduced activity of the TPT showed that most of the daily fixed carbon is no longer available for sucrose biosynthesis but is maintained within the plastids and directed into the accumulation of starch. These plants, however, are able to compensate the reduced flux of carbon to sink tissues by rapidly mobilizing and exporting the major part of the daily accumulated carbon (i.e. transitory starch) during the following night period and thus balancing the reduced carbon supply of sink tissues during the day. These transformants use, at much higher rates than wild-type plants, the 'night path' of carbon, that is they degrade the accumulated starch into hexoses, which are subsequently exported by a hexose translocator and are then used for sucrose formation (**Figure 3**). Such a hexose translocator, located at the inner envelope membrane of chloroplasts, has been identified and characterized at the molecular level.

Transport of phosphoenolpyruvate

Chloroplasts and nongreen plastids from most plants, depending on the developmental stage of the tissue, are unable to convert hexose phosphates and/or trioseP into phosphoenolpyruvate (PEP). However, PEP is used for a variety of metabolic pathways confined to plastids, for example the biosynthesis of PEP- and pyruvate-derived amino acids, for fatty acids, or as precursor for the shikimate pathway. The aromatic compounds synthesized from PEP via the shikimate pathway are not only constituents of proteins (aromatic amino acids that are essential nutrients in animal diets) but are also utilized as precursors for the biosynthesis of a large number of

secondary metabolites (e.g. alkaloids, flavonoids and lignin) that are important in plant defence mechanisms and stress responses. Plastids therefore rely on the supply of PEP. A transporter that enables the transport of PEP in plastids has been identified. It transports PEP preferentially in exchange with inorganic phosphate, which is released during the biosynthetic processes (**Figure 3**). This translocator, named PEP/phosphate translocator (PPT),

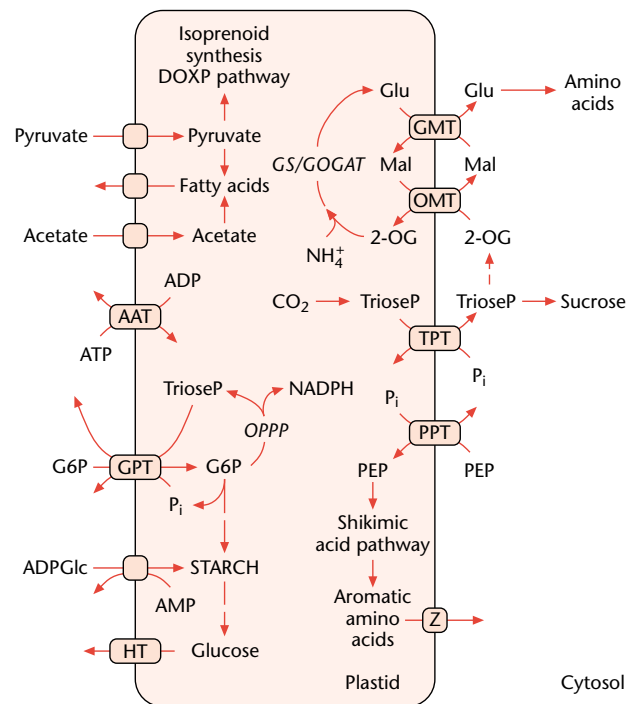


Figure 3 Exchange of substrates between plastids and the cytosol to connect metabolic processes in both compartments. The net product of photosynthetic CO_2 fixation, triose phosphates (trioseP), are exported via the triose phosphate/phosphate translocator (TPT). Nitrogen assimilation requires the import of carbon (2-oxoglutarate (2-OG) by the oxoglutarate/malate translocator, OMT) and the export of amino acids (glutamate (Glu) by the glutamate/malate translocator). Phosphoenolpyruvate (PEP), imported via the PEP/phosphate translocator (PPT), is the starting molecule for the shikimic acid pathway leading to the formation of aromatic amino acids that are exported via as yet unknown transporters. The biosynthesis of isoprenoids, for example carotenoids, requires the uptake of pyruvate that can also be used for the synthesis of fatty acids that can subsequently be exported from the plastids for use in other compartments. Fatty acid synthesis is also driven by acetate. The ATP/ADP translocator (AAT) provides nongreen plastids with ATP for biosynthetic processes, for example starch biosynthesis. In amyloplasts, starch synthesis starts with glucose 6-phosphate (G6P) that is imported via the glucose 6-phosphate/phosphate translocator (GPT). G6P can also be exchanged for trioseP, the product of the oxidative pentose phosphate pathway (OPPP) that leads to the generation of reducing power in form of NADPH. In some grasses, starch can alternatively be synthesized from ADPGlucose (ADPGlc) that is imported by an ADPGlc translocator. The products of starch degradation, for example glucose, are exported by a hexose translocator (HT). GS/GOGAT, glutamine synthetase/glutamate synthase; Mal, malate; DOXP, deoxyxylulose 5-phosphate; P_i , inorganic phosphate; ADP, adenosine diphosphate.

transports the substrates of the TPT only poorly and shares only about 35% amino acid identity with members of the TPT family. An *Arabidopsis* knockout mutant that is defective in the PPT shows a severe phenotype and is unable to synthesize sufficient amounts of secondary metabolites but can be rescued by feeding the seedlings with aromatic amino acids.

Transport of hexose phosphate

Nongreen plastids of heterotrophic tissues that are unable to photosynthesize (e.g. amyloplasts), rely on the import of carbon that is converted into starch. In most plants, hexose phosphates (glucose 6-phosphate; G6P) that are formed from sucrose delivered from source tissues is the preferred hexose phosphate taken up. A G6P/phosphate translocator (GPT) has been identified that shares only limited similarities to members of both the TPT and PPT families. It transports inorganic phosphate, trioseP and G6P, about equally well, whereas PEP is only poorly transported. The imported G6P serves two different functions. It is the precursor for the biosynthesis of starch and of fatty acids, during which processes inorganic phosphate is released and exported via the GPT (**Figure 3**). In addition, G6P is the substrate of the oxidative pentose phosphate pathway yielding trioseP that is exported by the GPT in exchange with G6P. The reducing power generated by the oxidation of G6P can be used, for example, for the biosynthesis of fatty acids and amino acids or for the reduction of nitrite.

Transport of dicarboxylates

Chloroplasts are not only the site of CO₂ fixation but also the site of assimilation of ammonia derived from nitrate reduction or from glycine decarboxylation during photorespiration. In ambient air, the rate of photorespiration accounts for about one-quarter of the rate of CO₂ fixation and is therefore several times higher than that of nitrate reduction. Carbon skeletons (2-oxoglutarate) formed from C₃ compounds exported from the chloroplasts have to be imported into the plastids in order to be converted into amino acids (**Figure 3**). For this, carbon skeletons (2-oxoglutarate) formed from C₃ compounds exported from the chloroplasts have to be imported into the plastids. The stroma-located glutamine synthetase/glutamate synthase (GS/GOGAT) reaction yields glutamate that is exported to the cytosol. Two different dicarboxylate antiport systems are involved in this process: the 2-oxoglutarate/malate translocator (DiT1, OMT) transporting 2-oxoglutarate (but not amino acids) and a glutamate/malate translocator (DiT2, GMT, a general dicarboxylate translocator) exporting glutamate. Glutamate as the key compound of nitrogen metabolism in plants and other amino acids can then be further loaded into the sieve tubes via specific amino acid transporters. DiT1/OMT was identified as a 45 kDa component of the inner envelope membrane and a corresponding cDNA clone was isolated.

DiT1 has a transmembrane topology with a 12-helix motif resembling that of other plasma membrane transporters from prokaryotes and eukaryotes that presumably all function as monomers. Database searches showed that DiT1 possesses no similarities to other proteins except for not yet characterized transporters from bacteria, for example from *Helicobacter pylori* or *Chlamydia*, both pathogens of humans.

Transport of adenylates

Nonphotosynthesizing plastids that are unable to produce ATP possess an ADP/ATP antiport system (AAT) for the supply of the plastids with ATP as the driving force for biosynthetic processes, such as the biosynthesis of starch and fatty acids (**Figure 3**). Such a transporter has been identified in plants. It exhibits similarities to the ADP/ATP translocase from the bacterium *Rickettsia prowazekii* but has no homology to the known mitochondrial adenylate translocators. The AAT contains 12 potential transmembrane helices and thus belongs to the group of transporters with a 12-helix motif as is the case for DiT1/OMT. Antisense potato plants with a reduced activity of the ATP/ADP translocator are impaired in starch biosynthesis, demonstrating that starch biosynthesis in nongreen plastids can be limited by the availability of plastidial ATP. In contrast, plants overexpressing this transporter accumulated increased amounts of starch in tubers.

Starch Metabolism

Starch is the most abundant polysaccharide in plant cells. It is used for industrial applications as well as for animal feed. Starch is an $\alpha(1,4)$ homopolymer of glucose units that occurs as unbranched amylose (making up $\sim 30\%$ of starch) and as an $\alpha(1,6)$ branched glucan, amylopectin (making up $\sim 70\%$ of starch). Amylopectin is thus similar to glycogen, a highly branched but soluble polysaccharide used for energy storage in animal cells. The structure of the insoluble starch granule is primarily determined by amylopectin, which is responsible for its semi-crystalline nature. There are two different types of starch: transitory starch is temporarily synthesized as a carbon reserve in chloroplasts during ongoing photosynthesis and is mobilized as a carbon source during the following night period. Starch can serve as a permanent carbon store that is deposited in the amyloplasts of sink tissues (e.g. potato tubers).

Transitory starch is synthesized from trioseP, the product of photosynthesis, which is either exported into the cytosol for sucrose biosynthesis (during which process phosphate is released) or maintained within the chloroplasts for starch synthesis (**Figure 4**). As outlined above, in transgenic plants with a reduced ability to export trioseP, the fixed carbon is mainly used for starch biosynthesis. The

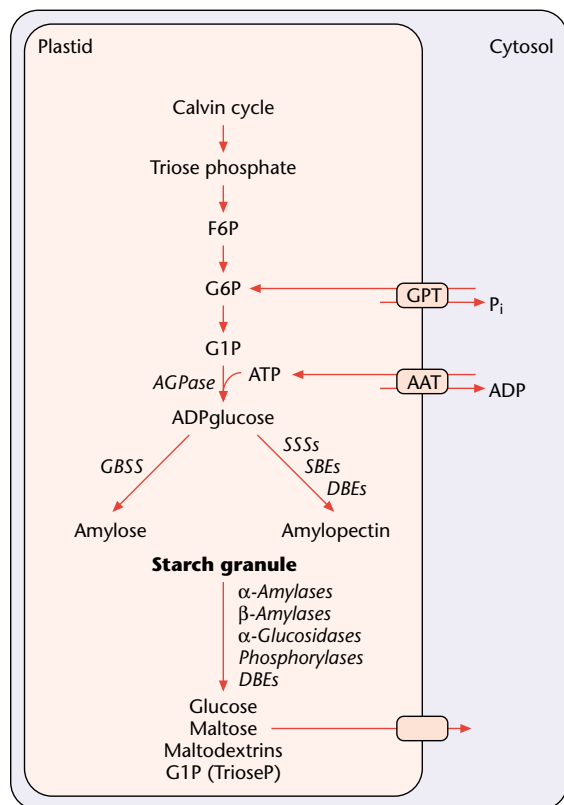


Figure 4 Starch metabolism in plastids. In chloroplasts, starch biosynthesis starts with triose phosphates provided by the Calvin cycle. In plastids of nongreen tissues, glucose 6-phosphate (G6P), imported by the glucose 6-phosphate/phosphate translocator (GPT) is the precursor for starch biosynthesis. ATP, required for the reaction of the ADP-glucose pyrophosphorylase (AGPase), is imported by the adenylate translocator (AAT). The biosynthesis of amylose and amylopectin from ADP-glucose involves the combined action of granule-bound starch synthase (GBSS), soluble starch synthases (SSSs), starch branching enzymes (SBEs) and debranching enzymes (DBEs). Starch breakdown by α -amylases, β -amylases, α -glucosidases, starch phosphorylases and debranching enzymes results in the production of various breakdown products that can be exported from the plastid. F6P, fructose 6-phosphate; G1P, glucose 1-phosphate; TrioseP, triose phosphate; P_i , inorganic phosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate.

key enzyme of starch synthesis is ADPglucose pyrophosphorylase (AGPase), which converts glucose 1-phosphate into ADPglucose, a highly activated form of glucose, from which the glucose moiety is transferred to the growing starch molecule. This enzyme is activated by a low stromal phosphate/3-phosphoglycerate ratio, that is under conditions of reduced sucrose biosynthesis. Plants with a defect in the AGPase activity are unable to accumulate starch and can survive only if sucrose is synthesized and exported during the day at higher rates than in wild-type plants. Mobilization of the transitory starch at night ensures that heterotrophic tissues of the plant can be supplied with carbon even in the absence of photosynthesis.

The biosynthesis of amylose and amylopectin requires the action of several enzymes (Figure 4). A granule-bound starch synthase (GBSS) leads to the synthesis of amylose; accordingly, the repression of GBSS results in amylose-free starch. The biosynthesis of amylopectin is much more complicated (and poorly understood) and involves the action of different granule-bound and soluble starch synthases, of branching enzymes introducing $\alpha(1,6)$ branches, and of debranching enzymes that reduce $\alpha(1,6)$ branches in the amylopectin molecule. Mutants defective in the debranching enzymes possess only soluble and highly branched amylopectin molecules (reminiscent of the highly branched animal glycogen and therefore named, 'phytglycogen') but never insoluble starch granules.

According to textbooks, the degradation of starch can proceed either hydrolytically by α -amylases (endoamylases that cleave 1,4- α -glycosidic bonds), β -amylases (exoamylases that catalyse the successive removal of the disaccharide maltose from the polysaccharide chain), α -glucosidases (maltases, hydrolysing maltose and short maltosaccharides) and debranching enzymes. The combined action of these enzymes should finally yield glucose that can be exported from the plastids via a glucose translocator. Alternatively, starch can be degraded by starch phosphorylases resulting in the formation of glucose 1-phosphate that can be transformed to glucose 6-phosphate, which, in turn, can be converted to triose phosphates via the oxidative pentose phosphate pathway. Triose phosphates can then be exported by the TPT and used for sucrose synthesis in the cytosol. There is increasing evidence that the hydrolytic pathway is the major path of starch mobilization (the 'night path' of carbon, see above). Although significant progress has been made in understanding starch metabolism, the detailed picture of starch synthesis and breakdown is far more complicated than previously thought (and outlined here) and is a topic of ongoing research.

Synthesis of Lipids

Lipids are essential constituents of any cells. In plants, the major site of fatty acid biosynthesis is within the plastid; this is in contrast to animals, which produce fatty acids in the cytosol. Reducing power required for fatty acid biosynthesis is provided by NADPH formed during photosynthesis in chloroplasts or via the oxidative pentose phosphate pathway in plastids of nongreen tissues.

Acetyl-coenzyme A (acetyl-CoA) is the general precursor of fatty acid biosynthesis. It can be formed from glucose 6-phosphate in nongreen plastids, which are able to import this compound. Glucose 6-phosphate can be converted into pyruvate and further into acetyl-CoA via the glycolytic pathway and the subsequent action of the plastidial pyruvate dehydrogenase. However, many

plastids, except plastids of lipid-storing tissues, do not possess the whole set of glycolytic enzymes and are therefore unable to convert C_6 or C_3 compounds into pyruvate. In these plastids, uptake of pyruvate (or acetate, which is converted to acetyl-CoA inside the plastids) is thus required (Figure 3).

Acetyl-CoA is first transformed to malonyl-CoA by the ATP-dependent acetyl-CoA carboxylase, which shares similarities with the corresponding bacterial enzyme. The malonyl moiety is then transferred from CoA to the acyl carrier protein (ACP) and ACP carries all of the intermediates of the subsequent formation of saturated 16- or 18-carbon fatty acids (palmitic acid, stearic acid). These reactions are catalysed by seven individual enzymes that form a functional fatty acid synthase complex, similar to that in bacteria. This is in contrast to the animal system, in which virtually the same reactions are catalysed by a large homodimeric multifunctional polypeptide located in the cytosol. Stearic acid can be desaturated to oleic acid (18:1) while still being bound to ACP. Stearic acid and palmitic acid can be released from ACP, yielding the free acids that are now available for export from the plastid (Figure 3). Alternatively, the fatty acids can be transferred to glycerol 3-phosphate to form phosphatidic acid, which is then dephosphorylated to diacylglycerol. Diacylglycerol can also be synthesized outside the plastids, mainly at the endoplasmic reticulum. In this case, the two free hydroxyl groups of glycerol 3-phosphate are preferentially acylated with 18-carbon fatty acids instead of both 16- and 18-carbon fatty acids. Diacylglycerol synthesized in the plastids as well as in the endoplasmic reticulum is a key intermediate in the synthesis of glycerolipids representing the major class of plant membrane lipids, for example phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine and diphosphatidylglycerol (cardiolipin), found preferentially in extraplastidial membranes, and of monogalactosyldiacylglycerol and digalactosyldiacylglycerol, which are the major lipids in chloroplast membranes (envelope membranes and the thylakoid membrane). Further desaturation of membrane lipids is catalysed by desaturases and results in the formation of linoleic acid (18:2) and linolenic acid (18:3). Both are essential fatty acids that cannot be synthesized by mammals.

Diacylglycerol is also used for the synthesis of triacylglycerols that contain three fatty acids bound to glycerol and that primarily serve as a storage for carbon in the form of oil, for example in seeds.

Plants grown in colder temperatures have a higher content of highly unsaturated fatty acids that are able to keep the membranes in a fluid state. In turn, high temperatures lead to a reduction in unsaturated fatty acids. It has been shown recently that transgenic tobacco plants with a decreased content of plastidial linolenic acid due to a defect in the corresponding desaturase showed

resistance to high temperatures. Conversely, *Arabidopsis* mutants that are deficient in the activity of the 18:1 desaturase of the endoplasmic reticulum showed a severe chill-sensitive phenotype when grown at lower temperatures. Most probably, the fatty acid desaturases represent a valuable tool for the genetic engineering of temperature tolerance in plants.

Outlook

Plastids play a key role in plant cell metabolism and development. The molecular basis of most of the processes is far from being completely understood. Various international sequencing programmes will provide sequences of complete higher plant genomes and future work will concentrate on unravelling the functions of all these genes. This will lead to a deeper understanding of the molecular basis of plant cell metabolism and how these processes are controlled. It will also provide clues as to how to specifically engineer metabolic pathways in transgenic plants to produce, for example, plastid-derived commercially important products such as modified starch or oils. It is also conceivable to create plants with an increased content of natural metabolites (synthesized primarily from products of the shikimic acid pathway) that are beneficial to health because they contain elevated contents of specific natural antioxidants or that strengthen plant resistance against viral and fungal pathogens.

Further Reading

- Flügge UI (1998) Metabolite translocators in plastids. *Current Opinion in Plant Biology* **1**: 201–206.
- Keegstra K and Cline K (1999) Protein import and routing systems of chloroplasts. *Plant Cell* **11**: 557–570.
- Murakami Y, Tsuyama M, Kobayashi Y, Kodama H and Iba K (2000) Trienoic fatty acids and plant tolerance of high temperature. *Science* **287**: 476–479.
- Ohlrogge J and Browse J (1995) Lipid biosynthesis. *Plant Cell* **7**: 957–970.
- Pyke KA (1999) Plastid division and development. *Plant Cell* **11**: 549–556.
- Smith AM (1999) Making starch. *Current Opinion in Plant Biology* **2**: 223–229.
- Streetfield SJ, Weber A, Kinsman EA *et al.* (1999) The phosphoenolpyruvate/phosphate translocator is required for phenolic metabolism, palisade cell development, and plastid-dependent nuclear gene expression. *Plant Cell* **11**: 1609–1622.
- Tjaden J, Möhlmann T, Kampfenkel K and Neuhaus HE (1998) Altered plastidic ATP/ADP-transporter activity influences potato (*Solanum tuberosum* L.) tuber morphology, yield and composition of tuber starch. *Plant Journal* **16**: 531–540.
- Weber A, Servaites JC, Geiger DR *et al.* (2000) Identification, purification and molecular cloning of a putative plastidic glucose translocator. *Plant Cell* **12**: 787–801.