Starch Degradation

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Abstract

Recent research reveals that starch degradation in *Arabidopsis* leaves at night is significantly different from the "textbook" version of this process. Although parts of the pathway are now understood, other parts remain to be discovered. Glucans derived from starch granules are hydrolyzed via β-amylase to maltose, which is exported from the chloroplast. In the cytosol maltose is the substrate for a transglucosylation reaction, producing glucose and a glucosylated acceptor molecule. The enzyme that attacks the starch granule to release glucans is not known, nor is the nature of the cytosolic acceptor molecule. An *Arabidopsis*-type pathway may operate in leaves of other species, and in nonphotosynthetic organs that accumulate starch transiently. However, in starch-storing organs such as cereal endosperms and legume seeds, the process differs from that in *Arabidopsis* and may more closely resemble the textbook pathway. We discuss the differences in relation to the biology of each system.

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INTRODUCTION

Our aim in this article is to discuss new information about the pathway of starch degradation in *Arabidopsis* leaves at night, and to use it to reassess our understanding of starch degradation in other plant organs. Although starch degradation has been extensively studied in germinating cereal endosperm (8, 29, 76), the nature and regulation of the process in this and other plant organs is poorly understood. There is good a priori reason to think that the process in endosperms differs from that in other organs because the mature endosperm is not a living tissue whereas starch degradation in all other plant organs occurs within living cells. Biochemical analyses show that many plant organs possess a wealth of isoforms of several different types of enzymes capable of degrading starch and related glucans. However, discovering the roles and importance of each of these forms in catalyzing starch degradation in vivo has been hampered by a lack of tools for this purpose. In the last five years, the genetic and genomic resources available in *Arabidopsis* have facilitated new approaches to the pathway in leaves. We present below the picture that has emerged for *Arabidopsis* leaves, then discuss to what extent this is applicable to leaves of other species, and to other plant organs.

THE PATHWAY OF STARCH DEGRADATION IN *ARABIDOPSIS* LEAVES

During the day, starch and sucrose are synthesized together as the products of photosynthetic carbon assimilation in *Arabidopsis* leaves. Sucrose is exported to nonphotosynthetic parts of the plant, and starch accumulates in the chloroplasts. The ratio of starch-to-sucrose synthesis varies with environmental conditions, but in our "standard" growth conditions (12 h light, 20°C, and about 180-μmol quanta of photosynthetically active radiation m^{-2} s^{-1}) about half of the newly assimilated carbon is partitioned into starch, and the content at the end of the day is 10–15-mg g^{-1} fresh weight (14, 117). During the subsequent night, the starch is degraded to provide substrates for sucrose synthesis to allow continued export to nonphotosynthetic parts of the plant, and to provide carbon skeletons, energy, and reductant within the leaf cell. The supply of carbohydrate provided by nighttime starch degradation is essential for the normal

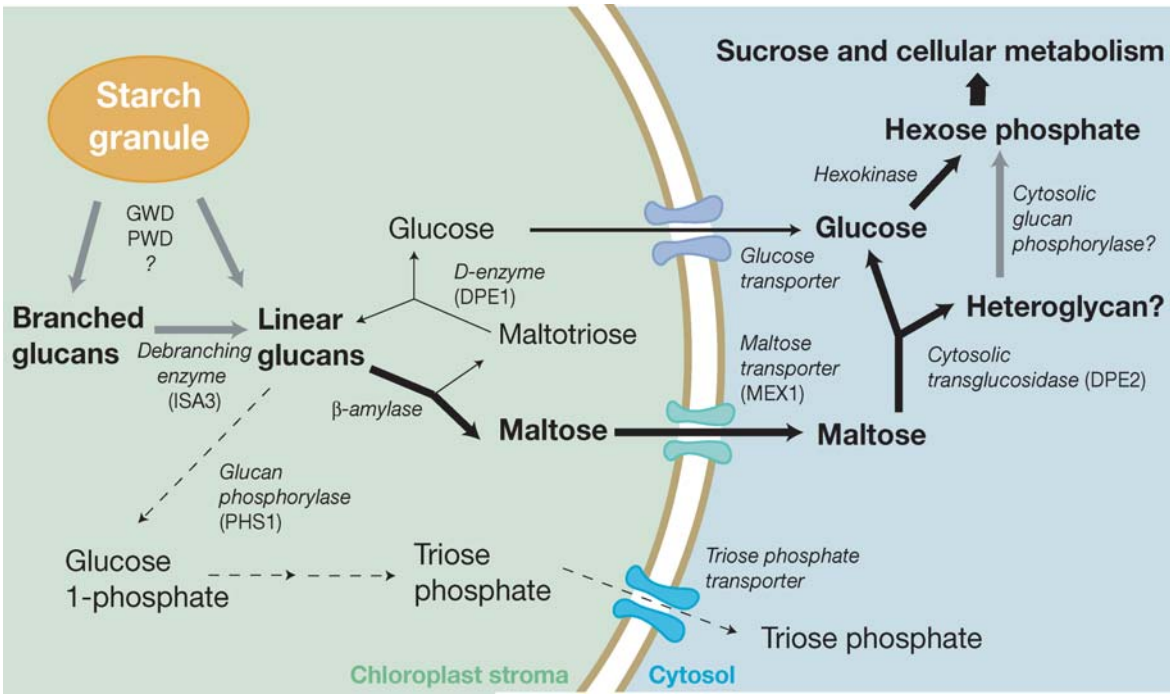


Figure 1

Proposed pathway of starch degradation in *Arabidopsis* leaves at night. Steps about which uncertainty remains are represented as stippled or dashed arrows, and with question marks. GWD is glucan, water dikinase and PWD is phosphoglucan, water dikinase. Further details of the enzymes and reactions involved are in **Table 1**.

growth of the plant. Mutants that synthesize less starch during the day, or have a reduced capacity to degrade it at night, have reduced growth rates under many environmental conditions (13, 85). In the following sections we discuss the steps involved in converting granular starch in the chloroplast into hexose phosphate in the cytosol. The scheme we describe is presented in **Figure 1**, and further details on the proteins involved are in **Table 1**.

The Attack on the Granule Surface

Starch in chloroplasts—like starch in other parts of plants—is in the form of granules composed of branched polymers of glucose. Most of these polymers are amylopectin—an α-1,4, α-1,6-linked polymer with a distinctive branching pattern that enables its organization into semicrystalline arrays (119, 120). The first step in the pathway of starch degradation must therefore be catalyzed by an enzyme capable of metabolizing polymers at the surface of a semicrystalline granule, rather than in a soluble form. Although several different types of enzymes are capable of releasing soluble glucans from purified starch granules in in vitro experiments (82, 95, 101), the only enzyme generally believed to do this in planta is the endoamylase α-amylase. In germinating cereal endosperm, α-amylases hydrolyze α-1,4 linkages within polymers exposed on the surface or in channels within granules, releasing soluble glucans that are the substrate for further degradation (see below).

Three proteins predicted to be α-amylases are encoded in the *Arabidopsis* genome, and one of these—AMY3 (**Table 1**)—is located in the chloroplast. It would seem reasonable to suppose that AMY3 catalyzes the attack on the granule surface in the chloroplast at night. However, surprisingly, none of these isoforms

TABLE 1 Starch-degrading enzymes in *Arabidopsis* leaves*

Enzyme	Locus	Reaction
Chloroplastic α-amylase; AMY3 EC 3.2.1.1	At1g69830	The enzyme is an endoamylase that hydrolyzes internal α-1,4 linkages of linear or branched glucans. It produces a mixture of linear and branched malto-oligosaccharides from amylopectin.
Glucan, water dikinase; GWD or GWD1 EC 2.7.9.4	At1g10760	The enzyme adds the β-phosphate group of ATP to either the 3- or the 6-carbon of a glucosyl residue of amylopectin (78). Glucan + ATP + water = Glucan-P + AMP + inorganic phosphate
Phosphoglucan, water dikinase; PWD or GWD3 EC 2.7.9.4	At4g24450	The *Arabidopsis* enzyme catalyzes the same reaction as GWD, but amylopectin must already be phosphorylated and the phosphate group is added to the 3- rather than the 6-position of glucosyl residues of amylopectin.[a]
Limit dextrinase EC 3.2.1.142	At5g04360	The enzyme hydrolyzes α-1,6 linkages of amylopectin and β-limit dextrin. The yeast α-1,4, α-1,6-linked glucan pullulan is a good substrate; glycogen is not.
Isoamylase; ISA1 and ISA2 EC 3.2.1.68	At2g39930 At1g03310	Recombinant ISA1 from potato is active; recombinant ISA2 from potato is not (12). Most of the isoamylase activity in potato (45) and *Arabidopsis*[b] is a function of a heterotetramer consisting of ISA1 and ISA2 proteins. The enzyme hydrolyzes α-1,6 linkages of amylopectin, β-limit dextrin, and glycogen, but not the yeast glucan pullulan.
Isoamylase; ISA3 EC3.2.1.68	At4g09020	Recombinant ISA3 from potato strongly prefers β-limit dextrin over amylopectin as a substrate (12).
Chloroplastic β-amylase EC 3.2.1.2	At3g23920 At4g00490 At4g17090 At5g55700	The enzyme is an exoamylase that hydrolyzes alternate α-1,4 linkages sequentially from the nonreducing end of a glucan chain, producing maltose. It cannot pass α-1,6 linkages, so the product of β-amylolysis of amylopectin is a dextrin with outer chains of two or three glucosyl units (a β-limit dextrin).
Chloroplastic glucan phosphorylase EC2.4.1.1	At3g29320	The enzyme catalyzes the conversion of the terminal glucosyl unit at the nonreducing end of glucan chains to glucose 1-phosphate, using inorganic phosphate. It cannot pass α-1,6 linkages. Chloroplastic isoforms prefer linear malto-oligosaccharides rather than large branched glucans as substrates (25, 64). Glucan(n) + inorganic phosphate = glucan(n-1) + glucose 1-phosphate.
Disproportionating enzyme; DPE1 2.4.1.25	At5g64860	The enzyme catalyzes the transfer of part of one α-1,4-linked glucan molecule (the donor) to another (the acceptor). The smallest donor molecule is maltotriose and the smallest acceptor is glucose, but the enzyme can use very large glucans as donors and acceptors. The preferred substrate is maltotriose, which is converted to glucose and maltopentaose (15).
Maltose transporter; MEX1	At5g17520	The *Arabidopsis* protein is located in the inner membrane of the chloroplast envelope and facilitates the transfer of maltose across the membrane (71). The mechanism is not known.
Cytosolic transglucosidase; DPE2	At2g40840	The *Arabidopsis* and potato enzymes catalyze the release of one of the glucosyl moieties of maltose and the transfer of the other to a glucan acceptor (14, 60). The nature of the endogenous acceptor is not known.
Cytosolic glucan phosphorylase; PHS2 EC 2.4.1.1	At3g46970	The enzyme catalyzes the same reaction as the chloroplastic isoform, but strongly prefers branched glucans rather than malto-oligosaccharides as substrates (25, 64).

*The table displays the EC numbers and chromosomal locations of *Arabidopsis* enzymes discussed in the text and shown in **Figure 1**, together with information about the reactions they catalyze. For amylases, phosphorylases, limit dextrinase, and disproportionating enzyme, catalytic properties have been characterized for several plant sources. In most of these cases the catalytic properties of the *Arabidopsis* enzymes have not been examined, but there is no reason to think that they will be substantially different from those of the enzymes from other plant species. For glucan, water dikinase, phosphoglucan, water dikinase, isoamylases, the maltose transporter, and the cytosolic transglucosidase, catalytic properties have been examined for the proteins from only one or two sources. The extent to which these properties will be shared by the proteins from other plant sources is not yet known.

[a]G. Ritte, personal communication.

[b]T. Delatte & S. Zeeman, unpublished data.

is necessary for starch degradation. T-DNA insertion mutants (referred to as knockout mutants) lacking either AMY1 (At4g25000), AMY2 (At1g76130), or AMY3 have normal rates of starch degradation in leaves at night. A triple mutant lacking all three α-amylases also has normal rates of starch degradation (128). It appears either that the initial attack on the granule surface does not require an endoamylase, or that *Arabidopsis* contains a novel endoamylase not recognizable from its primary amino acid sequence. These findings also leave open the role of AMY3. The AMY3 protein has α-amylase activity (128). It seems likely that it is involved in starch degradation, but—at least under the growth conditions we use—its absence can be compensated for by another enzyme.

The Importance of Starch Phosphorylating Enzymes

Whatever the mechanism of release of soluble glucans from the starch granule, it is clear that this process requires a newly discovered enzyme called glucan, water dikinase (GWD) (**Table 1**). Studies of the enzyme from potato show that GWD transfers the β-phosphate of ATP to either the 6- or the 3-position of glucosyl residues within amylopectin (67, 78). This phosphorylation probably occurs during both starch synthesis and starch degradation in vivo (70, 80, 112). Although phosphate groups occur at a low frequency in *Arabidopsis* leaf starch (about 1 in 2000 glucosyl residues is phosphorylated) (116), the presence of an active GWD appears to be essential for normal starch degradation. Mutations that eliminate the GWD protein or affect the dikinase domain of the enzyme dramatically reduce both the amount of phosphate in the amylopectin and the rate of starch degradation. Mature leaves of these mutants (*starch excess 1*, or *sex1*) accumulate amounts of starch up to seven times greater than those in wild-type leaves (13, 116, 117).

The simplest explanations for the involvement of GWD in starch degradation are that either the phosphate groups or the protein itself are necessary for the actions of the unknown enzyme(s) that attack the granule surface. The phosphate groups may influence the packing of the glucose polymers within the granule and hence the susceptibility of the granule surface to attack by enzymes (5, 6, 116). The GWD protein consists of both a dikinase domain and a large (approximately 120-kD) N-terminal domain of unknown function. This domain could be involved in promoting the activity of a granule-degrading enzyme, through an interaction with either the starch surface (see 79) or the enzyme itself.

Further complexity was recently added to this picture with the discovery that a second GWD-like enzyme [GWD3, or phosphoglucan, water dikinase (PWD)] (**Table 1**) is also required for normal starch metabolism (126, 127). Knockout mutants lacking this enzyme have increased amounts of leaf starch. However, unlike *sex1* mutants, the amount of phosphate in the starch of mutants lacking PWD is not dramatically affected. PWD is predicted to be chloroplastic. Although its C-terminal domain, like that of GWD, is closely related to those of other dikinases (67), its N-terminal domain is different from that of GWD. Recombinant PWD will phosphorylate amylopectin that already contains phosphate groups but, unlike GWD, it will not act on unphosphorylated glucans. This suggests that PWD action in vivo requires the presence of active GWD. It is possible to speculate that GWD and PWD actions together create a pattern of phosphorylation of amylopectin that makes it accessible to degradative enzymes at the granule surface, but much more information on these proteins and their products is necessary before their precise roles can be defined. There is also no information on the fate during starch degradation of the phosphate groups introduced by these enzymes.

Debranching

The *Arabidopsis* genome encodes four proteins predicted to catalyze the hydrolysis of α-1,6 linkages in glucan polymers (**Table 1**). One of

these belongs to the limit dextrinase class and the remaining three to the isoamylase class of debranching enzymes (68). Limit dextrinase is present in high activity in germinating cereal endosperm, and is thought to be involved in the hydrolysis of the α-1,6 linkages during starch degradation in this organ (see below). However, a knockout mutant of *Arabidopsis* lacking limit dextrinase has normal rates of starch degradation in the leaf at night (T. Delatte & S. Zeeman, unpublished data). This indicates that one or more isoamylases is involved.

Understanding which debranching enzymes are involved in starch degradation is complicated by the fact that isoamylase also plays a role in starch synthesis. In *Arabidopsis* leaves, cereal endosperms, and potato tubers, reducing or eliminating measurable isoamylase activity causes abnormal starch synthesis. Starch granule numbers are increased, and at least some of the starch is replaced with a soluble α-1,4, α-1,6-linked glucan known as phytoglycogen (10, 12, 48, 122). The way in which isoamylase suppresses the initiation of starch granules and phytoglycogen particles in normal circumstances is not known. In *Arabidopsis*, mutations in either the *ISA1* or the *ISA2* (*DBE1*) gene cause the loss of detectable isoamylase activity and the disruption of normal starch structure (122; T. Delatte & S. Zeeman, unpublished data). In potato, the products of the *StISA1* and *StISA2* genes together form a heterotetramer responsible for the measurable isoamylase activity in the tuber (12, 45). It seems likely that the same is true for the enzyme in *Arabidopsis*.

Although ISA1 and ISA2 are indispensable for normal starch synthesis in *Arabidopsis* leaves, they are not necessary for hydrolysis of α-1,6 linkages during starch degradation at night. In *isa1* and *isa2* (*dbe1*) mutants, starch and phytoglycogen are both completely degraded at night (122; T. Delatte & S. Zeeman, unpublished data). In fact, given that phytoglycogen has a higher ratio of α-1,6 to α-1,4 linkages than amylopectin, the rate of degradation of α-1,6 linkages is probably faster than in wild-type leaves.

The lack of requirement for limit dextrinase, ISA1, or ISA2 in starch degradation suggests that ISA3 may be important in this process. Preliminary analyses of knockout mutants lacking ISA3 show that starch contents are higher than in wild-type leaves, consistent with a role for ISA3 in the hydrolysis of α-1,6 linkages at night (T. Delatte & S. Zeeman, unpublished data). The possibility that a further, unidentified activity is also involved cannot be ruled out at this stage.

Metabolism of Soluble, Linear Glucans

The initial stages of starch degradation proposed above will produce linear glucans that are soluble in the chloroplast stroma (**Figure 1**). Enzymes known to be present in the chloroplast can potentially catalyze two alternative pathways of further degradation. First, chloroplastic glucan phosphorylase (58, 118) (**Table 1**) can release glucose 1-phosphate, which can be converted to triose phosphate and exported from the chloroplast in exchange for inorganic phosphate via the triose-phosphate transporter (38). Second, β-amylase can catalyze the production of maltose from linear glucans (**Table 1**). Four of the nine β-amylases encoded in the *Arabidopsis* genome are predicted to be chloroplastic, and one of these proteins has been shown to be in the chloroplast (54).

Our recent results show that degradation of linear glucans in the *Arabidopsis* chloroplast usually proceeds via β-amylases rather than glucan phosphorylase. First, knockout mutants lacking chloroplastic glucan phosphorylase have normal rates of starch degradation under our growth conditions (121). Second, knockout mutants lacking one of the chloroplastic β-amylases have reduced rates of starch degradation (D. Fulton, H. Dunstan & S. Smith, unpublished data). It is not yet clear whether more than one β-amylase is required for normal rates of starch degradation. Third, plants lacking proteins required for maltose metabolism accumulate massive quantities of maltose in a pattern consistent with its production from

starch, and have reduced rates of starch degradation (see below).

Fate of Maltose and Maltotriose

There are several ways in which maltose produced by β-amylolysis might be metabolized inside a chloroplast, but none of these appears to operate in *Arabidopsis*. For example, a maltose phosphorylase (EC 2.4.1.8) reported from pea leaf chloroplasts converted maltose to glucose 1-phosphate and glucose (52). However, this enzyme has not been reported from *Arabidopsis* and the genome does not encode a protein related to the maltose phosphorylase of bacteria (7). The genome encodes five enzymes classified as α-glucosidases (EC 3.2.1.20), at least some of which may be capable of hydrolyzing maltose to produce glucose, but none of these is predicted with confidence to be plastidial.

There is now strong evidence that maltose produced during starch degradation is exported to the cytosol via a specific transporter rather than metabolized inside *Arabidopsis* chloroplasts (**Figure 1**). Mutations at the *MEX1* locus cause accumulation of both starch and maltose in *Arabidopsis* leaves. Maltose levels are at least 40 times those of wild-type leaves. They rise at night and fall during the day, consistent with a block in the metabolism of maltose produced by β-amylolytic degradation of starch at night (71). *MEX1* encodes a protein located in the inner membrane of the chloroplast envelope (**Table 1**). When expressed in a mutant of *E. coli* lacking a component of the endogenous maltose transporter, MEX1 can restore the ability of this strain to grow on maltose (71). These data indicate that MEX1 is a maltose transporter responsible for the export to the cytosol of maltose produced during starch degradation in the chloroplast at night.

In addition to maltose, β-amylolytic degradation is also expected to produce a smaller amount of maltotriose because it is unable to act on chains of less than four glucosyl residues (e.g., 59). The only known maltotriose-metabolizing enzyme encoded by the genome and predicted to be chloroplastic is disproportionating enzyme, or α-1,4 glucanotransferase (DPE1) (**Table 1**), which can potentially convert two maltotriose molecules to one maltopentaose and one glucose. Evidence that this enzyme is responsible for maltotriose metabolism during starch degradation comes from a knockout mutant, which has a reduced rate of starch degradation and accumulates maltotriose at night to a far greater extent than wild-type leaves (15). We suggest that in the wild-type leaf the maltopentaose produced by disproportionating enzyme is acted on by β-amylase, and the glucose is exported from the chloroplast via the glucose transporter of the inner envelope (109) (**Figure 1**).

The Metabolism of Maltose in the Cytosol

The most obvious fate for maltose in the cytosol would be hydrolysis via an α-glucosidase, followed by conversion of the resulting glucose to glucose 6-phosphate via hexokinase. This is not, however, the pathway that appears to operate. Instead, maltose is metabolized via a transglucosylation reaction. Evidence for this fate for maltose comes from examining knockout mutants lacking a predicted transglucosidase (DPE2) (**Table 1**), structurally related to amylomaltases (7), which are involved in maltose metabolism in bacteria. Extracts of leaves of the mutants lack a transglucosidase activity, present in wild-type leaves, that can transfer one of the glucosyl moieties of maltose to branched polyglucans such as glycogen and release the other as glucose (14). The mutants have a phenotype similar to that of *mex1* mutants that lack the maltose transporter. Maltose levels are many times higher than those of normal plants, and starch degradation is inhibited (14, 62), suggesting that metabolism via DPE2 is the major or sole fate of maltose exported to the cytosol during starch degradation.

It is reasonable to assume that the free glucose released from maltose via DPE2 is converted to hexose phosphate via hexokinase. The fate of the second glucosyl moiety of maltose remains to be discovered. Presumably it is

transferred by DPE2 to a cytosolic carbohydrate molecule, which is subsequently acted on by another enzyme that releases the glucosyl moiety either as glucose or as glucose phosphate (**Figure 1**, and see below).

Further Characterization of the Pathway

As described above, there are still major gaps in our understanding of the conversion of starch to hexose phosphate in *Arabidopsis* leaves. Here we suggest possible solutions to two of these outstanding problems and provide further support for novel parts of the pathway proposed above. The first outstanding problem is the nature of the enzyme responsible for attacking the starch granule. It remains entirely possible that the attack is catalyzed by an enzyme not recognizable as a α-1,4 endoglucanase from its predicted amino acid sequence. Continuing screens for mutant plants unable to degrade starch may lead to the identification of such an enzyme. An alternative possibility is that the attack on the granule is via β-amylase. β-amylase is an exoamylase that removes maltosyl units from the nonreducing ends of chains. It can act only on the outer chains of a branched glucan and cannot hydrolyze linkages beyond branch points. However, it could act in concert with a debranching enzyme, perhaps ISA3, to degrade starch granules progressively to maltose and maltotriose. The extent to which β-amylase can attack chains at the granule surface might be determined by the actions of GWD and PWD because the distribution of phosphate groups added to amylopectin by these enzymes affects the degree of crystalline packing of chains within the granule matrix (6). If this view of granule degradation is correct, the soluble linear and branched glucans postulated as the first products of starch degradation in **Figure 1** would not exist. Although unconventional, this scheme is appealing for its simplicity. Earlier studies concluded that β-amylase is not capable of degrading native starch granules (4, 24), but a chloroplastic β-amylase from potato leaves was recently shown to release maltooligosaccharides from potato tuber starch granules (82).

The second major problem in understanding the pathway is the fate of the glucosyl moiety transferred by DPE2 from maltose to a carbohydrate acceptor. One possible candidate for the endogenous acceptor is a specific type of soluble arabinogalactan present in leaves of several species of plant (28, 114). This molecule is a mixed-linkage glycan consisting mainly of arabinose and galactose residues, with glucose, rhamnose, xylose, fucose, and mannose as minor components. Leaf and protoplast fractionation experiments indicate that it may be cytosolic: It is within the cell rather than associated with the wall, and is outside the chloroplast. Unlike cell-wall derived arabinogalactans, it is a good substrate in vitro for the ubiquitous cytosolic isoform of glucan phosphorylase (PHS2) (**Table 1**), which can transfer glucosyl moieties to the heteroglycan from glucose 1-phosphate. Thus, it is possible that glucosyl moieties from maltose are transferred to chains within this heteroglycan by DPE2 and released again as glucose 1-phosphate by PHS2 (**Figure 1**). This scheme is appealing because the PHS2 is likely readily reversible in vivo. PHS2 could potentially allow glucosyl moieties from the heteroglycan acceptor to be converted to hexose phosphate at a rate dependent on the rate of hexose phosphate utilization in the cytosol. The heteroglycan could thus act as a buffer between the rate of appearance in the cytosol of maltose produced in starch degradation and the rate of consumption of hexose phosphate in sucrose metabolism and cellular metabolism. We emphasize that this is speculation. Objective approaches are required to identify the acceptor and its further metabolism in vivo.

Although the first and latter stages of the pathway have yet to be discovered, there is good supporting evidence for the central portion in which chloroplastic maltose and maltotriose are converted to cytosolic hexose. In addition to the evidence discussed above, the creation of double mutants supports the view that the pathway presented in **Figure 1** accounts for most or

all of the flux of carbon out of starch. Mutants lacking both MEX1 (so unable to metabolize maltose) and DPE1 (so unable to metabolize maltotriose) would be expected to be severely compromised, as no carbon from starch would be available for sucrose synthesis or cellular metabolism at night. The same should also be true of mutants that lack DPE1 and DPE2. Consistent with these expectations, *mex1/dpe1* (71) and *dpe2/dpe1* (T. Chia & A. Smith, unpublished data) mutants are extremely slow growing, much more so than their parental lines. In contrast, mutants lacking both MEX1 and DPE2 are very similar in appearance to their parental lines (T. Chia & A. Smith, unpublished data), consistent with the idea that MEX1 and DPE2 are consecutive steps on the same branch of the pathway.

CONTROL OF FLUX THROUGH THE PATHWAY IN *ARABIDOPSIS* LEAVES

Understanding the control of the pathway of starch degradation in *Arabidopsis* leaves presents two major challenges: switching on the pathway in response to darkness and other environmental signals, and controlling flux through the pathway at night. In standard growth conditions (see above) little or no starch degradation occurs while starch is being synthesized during the day. Pulse-chase experiments—in which $^{14}CO_2$ is supplied for a short period and then replaced with unlabeled carbon dioxide—indicate that carbon incorporated into granular starch is not released again until the light level falls below that required for net carbon dioxide assimilation (119). These experiments suffer from the technical difficulty that ^{14}C may be very rapidly buried by newly formed ^{12}C starch during the chase period, so that turnover of glucan chains at the surface is underestimated. However, release of ^{14}C was not observed even when light levels were lowered during the chase to a point only just sufficient for net assimilation, of which incorporation of ^{12}C into starch was minimal. Thus, under these growth conditions starch degradation is switched on by transition to conditions in which there is insufficient light for net carbon assimilation.

After the onset of the night, the rate of starch degradation increases over the first two hours and then remains relatively constant until the end of the night (117; authors' labs, unpublished data). At this point almost all of the starch in the leaf has been consumed. The rate of starch degradation is thus controlled such that all of the starch accumulated during the day is used to provide the cell with hexose phosphate at an almost constant rate throughout the night. This remarkable phenomenon occurs under a wide range of conditions. Mutations and environmental changes that decrease the accumulation of starch during the day result in a decrease in the rate of its consumption at night so that the supply of starch again allows a constant rate of hexose phosphate synthesis through the night (57). Except for photoperiods of more than 16 hours, alterations in the length of the photoperiod result in alterations of the rates of accumulation and degradation of starch so that the supply of starch again provides for a constant rate of hexose phosphate synthesis and is exhausted at the end of the night (34). These observations suggest that the control of the rate of degradation during the night is entrained to the amount of starch available for consumption and the expected length of the night. The importance of this control in the carbon economy of the plant is illustrated by experiments in which plants are subjected to a further period of darkness at the end of a normal 12-hour night. Within a few hours, massive changes in the transcriptome of the leaf indicate that the plant is entering carbon starvation. For example, expression of genes encoding enzymes involved in amino acid, lipid, and cell wall degradation increases (34, 106).

Regulation at the Level of Gene Expression

How are the switching-on and subsequent control of the rate of degradation achieved? The mechanisms are likely complex and are not yet understood, but some possibilities have

emerged. The discovery that transcripts for several putative enzymes of starch degradation show strong diurnal rhythms and are under circadian control led to the suggestion that the diurnal control of starch degradation is primarily transcriptional (36). However, our examination of the diurnal pattern of transcript change for the enzymes in **Figure 1** casts strong doubt on the idea that transcription is of primary importance in switching on or controlling flux through the pathway (90).

First, diurnal patterns of transcript change are not the same as the pattern of change in the flux through the pathway of starch degradation. For several of the enzymes essential for starch degradation (DPE1, DPE2, ISA3, GWD, PWD), plus other glucan-metabolizing enzymes that may be involved (PHS1, PHS2, AMY3), there is a very similar pattern of transcript change in which levels are highest toward the end of the light period and lowest at the start of the light period (90). Although not mirroring changes in flux, this pattern provides high transcript levels at the time of day when starch degradation commences, and low levels at times when degradation does not usually occur. However, transcript levels for the four chloroplastic β-amylases and the maltose transporter MEX1 each show different diurnal patterns, none of which is obviously related to the pattern of starch degradation.

Second, there is no evidence so far that amounts of any protein necessary for starch degradation vary diurnally in *Arabidopsis* leaves in a manner consistent with a role in control of degradation. For several key enzymes with strong diurnal transcript changes, there is very little diurnal change in the amount of enzyme protein, and assayable activities remain high throughout the light period (90; authors' labs, unpublished data). A diurnally fluctuating endoamylolytic activity was reported from *Arabidopsis* leaves (50). However, neither this enzyme nor its subcellular location has been identified, and endoamylases appear to be unnecessary for normal rates of starch degradation in *Arabidopsis* leaves (see above). Levels of StDPE2 protein fluctuate diurnally in potato leaves grown in natural light, peaking in the first half of the night, but—in spite of strong diurnal changes in transcript levels—no equivalent fluctuation is seen in levels of DPE2 protein in *Arabidopsis* leaves grown in a controlled environment (60, 90).

Third, it is clear that the capacity for starch degradation remains high throughout the light as well as the dark period. Under appropriate conditions, high rates of starch degradation can be achieved in the middle of the light period. For example, if plants are transferred to low carbon dioxide and high oxygen concentrations (conditions that prevent net carbon assimilation and promote photorespiration) during the normal light period, starch degradation commences at once at a rate comparable with that in the dark period (S. Weise & T. Sharkey, personal communication).

Regulation of Enzyme Activity

pH, redox potential, and malto-oligosaccharide levels may all play a role in controlling starch degradation, via their effects on enzyme activities. All three of these factors undergo changes within the chloroplast during a light-dark transition. The pH of the stroma drops from about 8 to about 7 during a light-dark transition. This may promote activity of starch-degrading enzymes, but it is unlikely that it could switch on starch degradation or cause a major change in flux through the pathway (discussed in 98).

Redox potential presents interesting possibilities for flux control. Several chloroplast enzymes undergo very large changes in activity on light-dark transitions, mediated via oxidation and reduction of the sulfhydryl groups of cysteine residues. Reduction of the sulfhydryl groups is brought about by thioredoxin, which is reduced during photosynthesis by electrons from PSI, transferred via ferredoxin. Many enzymes interacting with thioredoxin were recently discovered, including a β-amylase from spinach (2). Recent work on ADPglucose pyrophosphorylase, the enzyme exerting most of the control over starch synthesis in *Arabidopsis* leaves, shows that it is activated by

reduction (40). The level of activation is strongly influenced in a complex manner by sugars. It is tempting to suggest that starch degradation might be controlled in an analogous manner by oxidative activation of one or more key enzymes. Thus, degradation would be prevented during the day by reduction of the enzyme(s). Oxidation of this enzyme(s) at the onset of darkness would switch on the degradative process, and flux would be controlled by modulation of the redox state of the enzyme by sugars, linking the rate of degradation to the level of sucrose in the cytosol. The possibility that redox potential is involved in the control of starch degradation was dismissed in the past because phosphorolytic starch degradation in isolated chloroplasts is unaffected by dithiothreitol and because activities of chloroplastic α-amylase and phosphorylase are not modulated by thioredoxin or dithiothreitol (98). The discovery that neither α-amylase nor phosphorylase is necessary for starch degradation in vivo in *Arabidopsis* leaves reopens the possibility that redox potential may be involved in the control of the process.

Malto-oligosaccharide levels might act to control the rate of starch degradation by inhibiting a step in the pathway at or close to the attack on the granule, thus linking the rate of degradation of the granule to the rate of consumption of the products of degradation. We assume that this is what happens in the *dpe2*, *mex1*, and *dpe1* mutants, which accumulate abnormally high levels of maltose or maltotriose. The DPE1, MEX1, and DPE2 proteins do not attack the starch granule. However, losing any one of these enzymes causes a reduction in the rate of starch granule degradation (14, 15, 62, 71). It is likely that the malto-oligosaccharides inhibit an enzyme involved in the attack on the granule, perhaps by competing with granular starch for a starch-binding domain necessary for attack on the granule (113). Maltose also inhibits some β-amylases at high concentration (59).

Strong evidence for the involvement of protein phosphorylation in control of starch degradation was recently obtained from study of a starch-excess mutant *sex4*. We discovered that mutations at *sex4* lie in a gene encoding a protein phosphatase (authors' labs & J. Chen, unpublished data). This mutant accumulates up to three times more starch than the wild type under standard growth conditions because of a very low rate of degradation at night (117, 118). Amounts of the chloroplastic α-amylase AMY3 are reduced in this mutant (118). However, AMY3 is not encoded at the *sex4* locus and knockout mutants for AMY3 have normal rates of starch degradation (see above), so it is unlikely that the decrease in starch degradation in *sex4* is caused by its effect on AMY3 levels. The targets of this enzyme are not yet known, but it seems highly likely that either an enzyme of starch degradation or a protein controlling flux indirectly is a target for inactivation by phosphorylation. Whether this mechanism is of major importance in controlling flux in vivo, and whether it interacts with other possible regulatory mechanisms such as redox potential, remains to be established. However, discovering a protein phosphatase necessary for normal starch degradation presents the first direct evidence for a specific regulatory mechanism for this process and should allow further rapid process in this area.

The timing and pattern of starch degradation in plants growing in natural conditions is likely different from that in controlled environments. In some species there is good evidence that degradation of leaf starch can occur in the light when light levels are relatively low at the start and end of the day (e.g., 31). Starch degradation in *Arabidopsis* leaves is also switched on in the light by conditions that promote high rates of photorespiration (T. Sharkey, personal communication). Thus, the mechanisms that control the rate of degradation probably respond to complex and varying environmental and metabolic inputs in natural conditions. Data obtained from controlled environments will not necessarily reveal the full range of factors that can affect the onset and rate of degradation.

THE PATHWAY IN LEAVES OF OTHER SPECIES

Little direct evidence is available for any species other than *Arabidopsis* about which enzymes are important in leaf starch degradation. Activities of starch-degrading enzymes have been reported in leaves of numerous species, but most reports do not identify the isoforms that contribute to activity or their subcellular locations. Indirect evidence suggests that there may be variation in the pathway of starch degradation between species and with changing developmental and environmental conditions. Interspecific variations might be expected because there are enormous differences between species in the extent of accumulation of starch during photosynthesis and its importance in supplying carbon to the plant at night. Some species accumulate little or no starch in their leaves during the day and rely primarily on vacuolar sucrose and, in grasses, fructans for a continued carbohydrate supply at night. In other species the diurnal pattern of starch accumulation and degradation changes dramatically through leaf development. In tobacco, for example, there is a strong diurnal change in starch content at night in younger leaves whereas in older leaves progressively less of the starch is utilized at night and levels increase. During senescence all of this starch is degraded and the products are exported from the leaf (65, 66). Below we discuss likely similarities and differences between species in the pathway of degradation of leaf starch.

The Production of Soluble, Linear Glucans

There is no robust information about which enzyme attacks the starch granule in leaves of any plant species. Chloroplastic α-amylases similar to AtAMY3 are probably widespread. The three amylases of *Arabidopsis* are representatives of three distinct families of these enzymes found in higher plants. Evidence of expression of family 3 amylases—the family to which AMY3 belongs—has been reported for a wide range of dicots and for the gymnosperm *Pinus taeda*, and this family may also be represented in cereals (94). Endoamylase activity is present in chloroplasts of pea, spinach, sugar beet, and maize (bundle sheath), although the reported level of chloroplastic activity varies considerably between species and between different studies of single species (26, 56, 73, 74, 123). However, there is no direct evidence that endoamylases are necessary for starch degradation in leaves.

It seems likely that GWD is involved in starch degradation in leaves of a wide range of species. GWD protein and transcript have been reported for leaves of several species (77–79), and the amylopectin of leaf starches thus far examined contains phosphate groups (5, 116). For potato leaves, there is good evidence that GWD is necessary for normal rates of starch degradation, as in *Arabidopsis* leaves. Leaves of transgenic plants with reduced levels of GWD (formerly known as R1 protein) have a higher starch content than those of untransformed plants and fail to degrade all of their starch even after prolonged periods in the dark (61). The widespread occurrence of GWD may indicate that the nature and control of the attack on the starch granule is conserved between leaves of different species.

Proteins closely related to all four of the debranching enzymes present in *Arabidopsis* leaves are found in a wide range of plant species. Although the roles of isoamylases similar to AtISA1 and AtISA2 have not been examined in leaves other than those of *Arabidopsis*, it is likely that they are involved in starch synthesis rather than degradation in plant organs generally. StISA1 and StISA2 are necessary for normal starch synthesis in tubers of potato (12, 45), and ISA1-type isoamylases are involved in starch synthesis in cereal endosperms (11, 48, 53). Unlike *Arabidopsis*, limit dextrinase (pullulanase) is required in maize leaves for normal starch metabolism. The rates of both degradation and synthesis of starch are reduced in knockout maize plants lacking this enzyme (22). Nothing is yet known about the function of ISA3-type isoamylase in maize or in any species

other than *Arabidopsis*. It may be that both ISA3 and limit dextrinase are capable of hydrolyzing α-1,6 linkages during starch degradation in leaves, and that their relative importance differs between species.

Hydrolytic versus Phosphorolytic Degradation

The debate about the relative importance in leaves of hydrolytic degradation via β-amylase versus phosphorolytic degradation via glucan phosphorylase (see **Figure 1**) is long standing. It is clear that chloroplasts from many species possess the capacity for both pathways. Work with transgenic and mutant plants has provided both direct and indirect evidence that degradation via β-amylase makes an important contribution to chloroplast starch degradation in a range of species. There is good direct evidence that β-amylase is necessary for normal starch degradation in leaves of potato. Transgenic plants with reduced activities of a chloroplastic isoform have reduced rates of starch degradation (82).

Good indirect evidence for the operation of a hydrolytic pathway of starch degradation comes from mutant and transgenic plants in which conversion of chloroplastic triose phosphate to cytosolic hexose phosphate is blocked, for example by lack of the triose phosphate transporter or cytosolic FBPase. Such plants have drastically reduced rates of export of carbon from the chloroplast during the day, and consequently accumulate much larger amounts of starch than wild-type plants. At night, all of this starch is degraded and the products are used for sucrose synthesis (38, 39, 87, 125). This implies that products of starch degradation are not exported from the chloroplast as triose phosphate at night but rather as hexose units and/or maltose. Nuclear magnetic resonance experiments also show that carbon for sucrose synthesis is exported from chloroplasts as hexose units or maltose, rather than triose phosphate, in intact bean and tomato leaves in the dark (83). There are strong indications that the chloroplast envelope has little or no capacity to transport hexose phosphate (30, 110). However, isolated chloroplasts from several sources produce maltose and glucose during starch degradation and can transport both of these compounds across the envelope (41, 75, 81, 86, 99, 111). Thus, these indirect lines of evidence are consistent with a predominantly hydrolytic route of starch degradation.

The involvement of β-amylase does not preclude the operation of a phosphorolytic pathway of degradation via a chloroplastic glucan phosphorylase. The activity of this enzyme in the *Arabidopsis* chloroplast is very low (58, 118) and probably insufficient to catalyze the observed rate of starch degradation, but in some other species phosphorylase activity in the chloroplast is much higher (for example, pea: 35, 73, 97). Isolated chloroplasts of spinach and pea are capable of phosphorolytic starch degradation (96, 98, 99). However, there is no evidence for a major role for glucan phosphorylase in starch degradation in leaves of any species. Carbohydrate metabolism was apparently normal in leaves of transgenic potato plants with no detectable chloroplastic glucan phosphorylase (91). It has been suggested that phosphorolytic starch degradation provides carbon for specific functions within the photosynthetic cell—for example for respiration at night (100), for the chloroplastic oxidative pentose phosphate pathway at night (121), or to maintain levels of Calvin cycle intermediates under photorespiratory conditions during the day (S. Weise & T. Sharkey, personal communication). The relative rates of phosphorolytic and hydrolytic degradation may thus vary with developmental stage and environmental conditions.

Maltose Metabolism

Consistent with the widespread involvement of β-amylase in leaf starch degradation, the proteins that act on its immediate products in *Arabidopsis* leaves—the maltose transporter MEX1, the chloroplastic disproportionating enzyme DPE1, and the cytosolic transglucosidase DPE2—are also of widespread occurrence. Genomic sequences or ESTs for proteins

closely related to those in *Arabidopsis* have been reported from a wide range of species including both dicots and monocots (14, 62, 71, 104). The importance of MEX1 in starch degradation in leaves other than those of *Arabidopsis* has not yet been examined. Potato plants with drastically reduced levels of DPE1 grow more slowly than normal plants, suggesting a role for this enzyme in leaf starch metabolism (104). Leaves of potato plants with reduced levels of DPE2 have reduced rates of starch degradation, implying that this is a major, if not the sole, route of maltose metabolism (60). However, the DPE2 of potato is reported to be chloroplastic (60) whereas that in *Arabidopsis* is reported to be cytosolic (14, 62). A chloroplastic location for this transglucosidase implies a different route for starch degradation from that presented in **Figure 1**. This potentially major difference between potato and *Arabidopsis* is under further investigation.

THE PATHWAY IN OTHER PLANT ORGANS

A huge amount of information is available about the occurrence of starch-degrading enzymes in nonphotosynthetic organs of plants. For the most part, this information cannot be used to deduce the pathway of starch degradation because individual isoforms and their subcellular locations are not known, and the precise role and importance of enzymes cannot be assessed. Whereas in leaves starch degradation can be studied over a defined, short, and controllable period, the process in many other organs is much less amenable to study. Starch degradation may occur over long periods, during which there are profound changes in the developmental state of the organ concerned. Frequently, the extent and rate of degradation cannot be accurately measured because it may occur in parallel with starch synthesis and at different rates in different parts of the organ. Nonetheless, there are general reasons to believe that starch degradation in some nonphotosynthetic organs proceeds by a pathway different from that in *Arabidopsis* leaves. First, in some organs starch degradation does not occur inside a plastid. Second, the nature of the starch granule and its appearance during degradation differ substantially between plant organs. It is also clear that the control of degradation—if not the pathway—differs between organs. A host of developmental, metabolic, and environmental factors influence starch degradation in various organs. In a few instances control of expression of genes encoding specific enzymes of degradation has been studied in detail (e.g., 7, 51), but for the most part—reflecting the lack of knowledge of the pathway itself—the control of flux through the pathway as a whole is not understood. Rather than attempt to review the whole body of literature on the pathway of starch degradation and its control in nonphotosynthetic organs, we consider some examples for which there is sufficient information to make meaningful comparisons with the pathway presented in **Figure 1**. We deal first with starch-storing organs such as seeds and tubers in which large amounts of starch are stored over long periods (see **Figure 2**), then consider the pathway in cells in which starch forms a relatively transient store of carbon over a particular developmental period.

The Attack on the Granule in Storage Organs

For some starch-storing storage organs, there is good reason to think that the attack on the granule is catalyzed by α-amylase. The onset of starch degradation during germination of cereal seeds is accompanied by massive de novo synthesis of α-amylase. The control of this synthesis in the aleurone and scutellum, and the secretion of the enzyme into the starch-storing endosperm, is understood in detail at a molecular level (29, 76). The pattern of attack on the starch granule and the nature of the limit dextrins that appear in the endosperm as starch is degraded are both consistent with the idea that α-amylase participates in granule degradation (24). Experiments in vitro show that α-glucosidase from cereal endosperm can also attack cereal starch granules, and that this enzyme

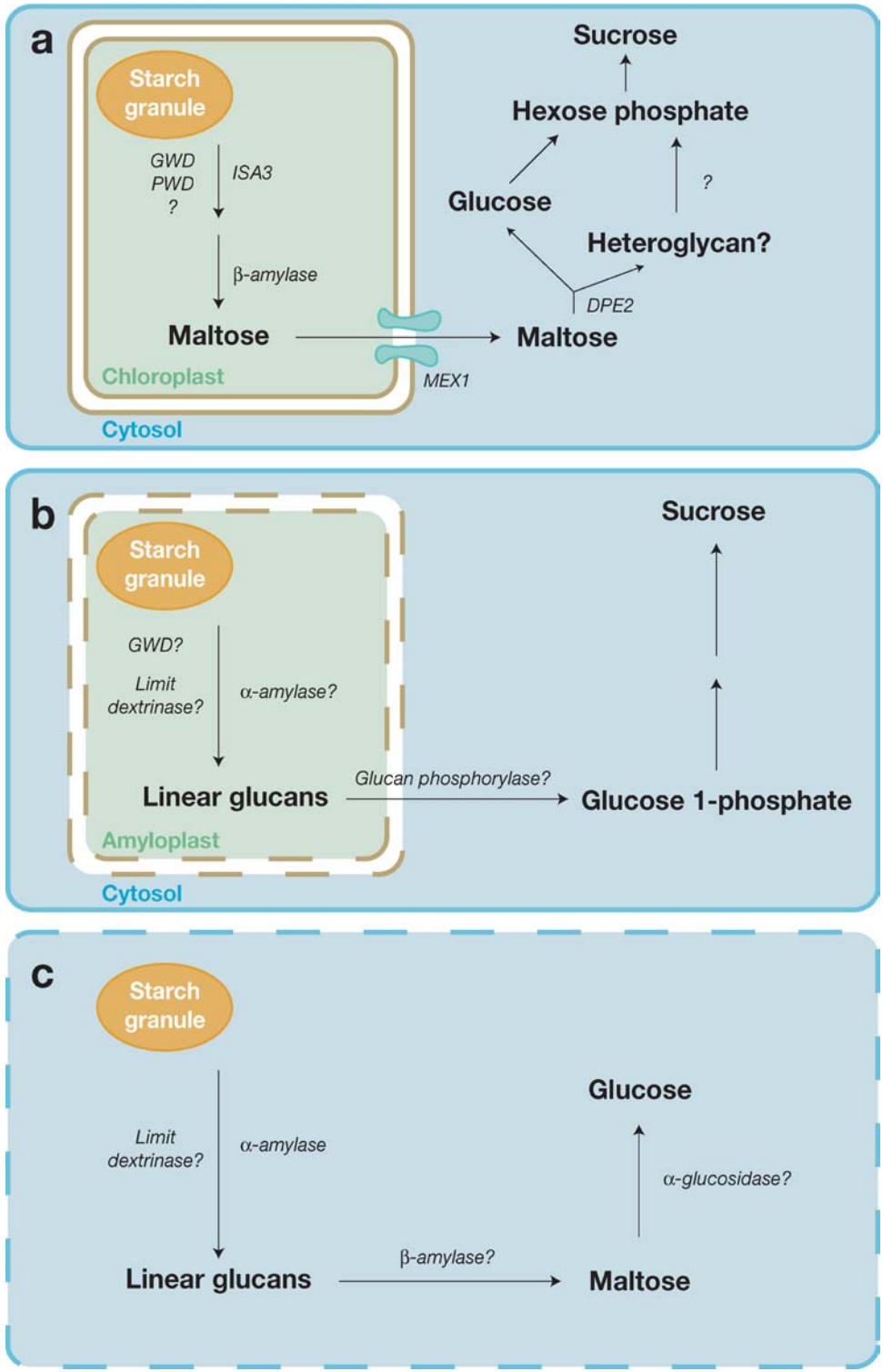
and α-amylase interact synergistically to promote granule degradation (88, 102). The extent of involvement of α-glucosidase in the attack on starch granules in vivo is not known.

The very substantial increases in α-amylase activity during the first few days of germination of starch-storing legume seeds are consistent with the idea that here too it is responsible for the attack on the starch granule (16, 105, 115). Changes in the properties of starch during degradation in germinating pea seeds also point to an endoamylolytic attack (49). An endoamylase is also implicated in the attack on the granule in one of the most spectacular examples of starch degradation in plants: the thermogenic spadix of *Arum maculatum*. In this organ—and those of other thermogenic members of the Araceae—enormous rates of degradation occur over just a few hours, fueling uncoupled respiration that heats the spadix to temperatures 10 or more degrees above ambient. Rates of starch degradation are typically more than 60 times greater on a fresh weight basis than those in *Arabidopsis* leaves under standard growth conditions at night (1). During spadix development prior to thermogenesis, the activity of an endoamylase increases dramatically, and the appearance of oligosaccharides in the spadix during starch degradation is consistent with the idea that this amylase is responsible for granule degradation (9).

For other types of starch-storing organs the nature of the attack on the starch granule is much less clear. In sprouting potato tubers, starch degradation is not initiated in a uniform manner across the tuber, and there are no consistent reports of accompanying substantial increases in either amylolytic or phosphorolytic starch-degrading enzymes (18–20). Starch degradation—accompanied by increases in sugars—also occurs when tubers are stored at low temperatures (cold-induced sweetening). A specific isoform of β-amylase increases in activity during this process, but its importance in degradation is not known (21, 69). The balance of information indicates that degradation during sprouting and cold-induced sweetening occurs within plastids. Although amyloplast membranes were reported to disappear during cold-induced sweetening (72), other reports, and the rapidly reversible nature of this process (21, 46, 47, 92), argue against loss of amyloplast integrity during starch degradation prior to tuber senescence.

An indication that starch degradation proceeds differently in cereal endosperms and potato tubers comes from the appearance of the granules during degradation. Granules from cereal endosperm have abundant channels leading from pores on the surface to the interior (3, 27, 42, 43). During degradation—both in vitro and in the germinating endosperm—they become deeply pitted, with loss of internal material surrounding the channels before much of the surface has been attacked. In contrast, granules from potato tubers have few if any pores or channels running inward from the surface. They are highly resistant to enzymic attack, and damage appears on the surface as degradation proceeds (23, 32, 33, 55). Because of these very different properties, it is tempting to suggest that the type of enzyme responsible for the attack on the granule in potato tubers may be different from that in cereal endosperm. Further information about the process in potato tubers is required to resolve this issue.

Starch in storage organs other than cereal endosperm is extensively phosphorylated, implying a possible role for GWD in controlling degradation. In mung bean seeds the level of phosphate in the starch is comparable with that in leaves, in potato tubers the level is up to 10 times that in *Arabidopsis* leaves, and in rhizomes of *Curcurma zedoaria* (ginger family) it is 3 times higher than that in potato tubers (5, 6). GWD homologues occur in a very wide range of species, and the enzyme may be ubiquitous in higher plants (78). For potato tuber, there is good indirect evidence that GWD is required for normal rates of starch degradation. Transgenic potatoes with greatly reduced levels of GWD had very low levels of phosphate in tuber starch and were less prone to starch loss and sugar accumulation when stored at low temperatures (cold-induced sweetening, see above), implying that mobilization of starch was



impaired in these conditions (61). In contrast, the starch of most cereal endosperms contains almost undetectably low levels of phosphate. It is doubtful whether GWD plays a role in degradation in these organs (5, 6).

Degradation of Soluble Glucans in Storage Organs

The pathway of degradation of soluble glucans released from starch granules likely varies between organs because of the very different physical circumstances in which degradation occurs. In cereal endosperm, starch degradation takes place in a nonliving tissue—effectively in an acidic, apoplastic environment in which no intracellular or intercellular compartmentation remains. In starch-storing legume seeds, starch degradation takes place within living cells of the cotyledons, but probably not within the plastid in which the starch was synthesized. The plastid envelope is believed to disintegrate prior to germination so that degradation occurs within the cytosol (37, 108) (**Figure 2**). In potato tubers, the balance of evidence indicates that degradation takes place within intact plastids (see above).

In cereal endosperm, the degradation of glucans released from starch granules probably proceeds via limit dextrinase, α- and β-amylase, and α-glucosidase to maltose and glucose, which can enter the embryo (**Figure 2**). These enzymes are either synthesized within surrounding cell layers or mobilized within the endosperm as degradation proceeds. Study of a knockout mutant of maize shows that limit dextrinase is necessary for normal rates of starch degradation in the endosperm during the early stages of germination (up to seven days) but not thereafter (22). Questions about the importance of limit dextrinase in starch degradation in barley endosperm have been raised by the demonstration that newly synthesized enzyme in aleurone cells is targeted to the plastids and not to the secretory pathway. The extent to which the enzyme enters the endosperm and participates in starch degradation during germination is unclear (11, 84). The possibility remains open that another type of debranching enzyme also participates in starch degradation in cereal endosperm. A protein that binds to and strongly inhibits limit dextrinase in vitro (limit dextrinase inhibitor) is synthesized during endosperm development and present in the germinating seeds of some cereals (63, 93), but its importance in determining the course of starch degradation during germination is unclear. The extent to which β-amylase and α-glucosidase are necessary for starch degradation in cereal endosperm has been the subject of considerable debate (e.g., 17, 24, 103), and both this and the way in which the products of degradation in the endosperm are exported to the embryo (e.g., 44) remain unresolved.

The degradation of glucans produced from starch in germinating pea embryos is proposed to proceed via limit dextrinase and glucan phosphorylase in the cytosol (**Figure 2**), although

Figure 2

Comparison of the pathway of starch degradation in leaves with those proposed for germinating legume cotyledons and germinating cereal endosperm. (*a*) Major pathway by which starch is converted to sucrose in leaves (see **Figure 1** for more detail). Starch degradation occurs within the chloroplast, and maltose is exported to the cytosol for further metabolism to sucrose. (*b*) Proposed pathway by which starch is converted to sucrose in germinating legume cotyledons. The amyloplast membrane disintegrates so that starch degradation is catalyzed by cytosolic enzymes. Glucan phosphorylase is important in the degradation of soluble glucans. Sucrose synthesized from starch is exported from the starch-storing cells to the growing root and shoot of the seedling. (*c*) Proposed pathway by which starch is converted to maltose and glucose in germinating cereal endosperm. Both the amyloplast envelope and the plasma membrane disintegrate so that starch degradation takes place in a nonliving tissue. The granule is attacked by α-amylase. The precise roles and importance of α-amylase and other starch-degrading enzymes in metabolizing soluble glucans are not clear. Glucose and maltose produced in the endosperm are exported from the endosperm into the scutellum, then converted to sucrose for the growth of the embryo.

direct information about the importance of these enzymes is lacking. Limit dextrinase activity is present both inside and outside the plastid during embryo development. The activity of the extraplastidial form increases as the seed matures and remains high in the germinating embryo (124). Activity of a cytosolic isoform of glucan phosphorylase is low during seed development and then increases dramatically during the first five days of germination (64, 108).

The pathway of degradation of soluble glucans derived from starch in potato tubers remains unclear. Tubers of transgenic plants with reduced activity of cytosolic glucan phosphorylase have normal starch contents and actually sprout better than normal tubers, suggesting that this enzyme is not necessary for starch degradation (25). Tubers with reduced activities of disproportionating enzyme produce sprouts more slowly than normal tubers (104), and reductions in DPE2 have no effect on sprouting (60). Unfortunately these observations provide little information about the roles of the enzymes in tuber starch degradation: Activities of the enzymes have been reduced rather than eliminated in tubers and effects on starch degradation have not been directly assessed.

The Pathway in Vegetative Tissues

In many plant organs and cell types, starch accumulates and then disappears on a timescale of a few days (89). In *Arabidopsis*, for example, starch appears transiently during development of the embryo and testa (mature seeds contain little or no starch) and in the root cap prior to sloughing off. Although the pathway of degradation of these temporary starch reserves is not known, preliminary indications in *Arabidopsis* are that it may be the same as that presented for leaves in **Figure 1**. First, mutations that affect starch degradation in the leaf also affect the process in several other parts of the plant. In a study of the *sex1* mutant (lacking GWD) Caspar et al. (13) reported that starch accumulated to levels greater than those in the wild type in seeds, roots, petals, and anthers as well as leaves. Starch is present in mature seeds of the starch-excess mutants lacking disproportionating enzyme (*dpe1*), the chloroplastic maltose transporter (*mex1*), and the cytosolic transglucosidase (*dpe2*). Again consistent with observations in leaves, starch content is unaffected in developing seeds and other plant parts of mutants lacking plastidial glucan phosphorylase or the plastidial α-amylase AMY3 (authors' labs, unpublished data). Second, genes encoding enzymes important in starch degradation in leaves are widely expressed in other parts of the plant. For example, MEX1 was originally identified as a gene expressed in root caps (*RCP1* or root cap1) (107). We emphasize that these are preliminary observations rather than systematic analyses, but at present there is no good reason to believe that starch degradation proceeds via distinctly different pathways in different organs of the *Arabidopsis* plant.

CONCLUSION

Although we can present an experimentally based model of the pathway of starch degradation in *Arabidopsis* leaves, this is not possible at present for any other plant organ. However, the balance of evidence suggests that the pathway may be the same or similar in other leaves, and perhaps in nonphotosynthetic tissues in which starch is stored transiently. In some starch-storing organs in which starch is a major, long-term reserve compound, the pathway of degradation is different. These differences are probably related to the different subcellular locations in which the process is believed to occur (the apoplast in cereal endosperms, the cytosol in legume seeds, and the plastid in potato tubers) and the different ways in which the products of degradation are utilized in these organs.

We suggest that rapid progress in understanding this vital and commercially important pathway should now be possible. Information from *Arabidopsis* leaves, together with a wealth of existing descriptive and correlative observations for other species, allows hypotheses about the nature of the pathway to be formulated for other plant organs. The increasing availability of transformation, TILLING,

and knockout technologies for crop and other model species should allow these hypotheses to be tested definitively in the near future. However, we emphasize that our understanding of the process in *Arabidopsis* is far from complete. Much further work is required to define this pathway fully, to test whether the present picture holds under natural environmental conditions, and in particular to understand how flux through the pathway is controlled.

SUMMARY POINTS

1. Forward and reverse genetic tools combined with biochemical approaches have produced a radically new picture of the pathway of starch degradation in *Arabidopsis* leaves at night.
2. The first steps in the pathway, in which glucans are released from the starch granule, remain unknown. Surprisingly, α-amylases are not required. A glucan water dikinase and a phosphoglucan water dikinase, which add phosphate groups to amylopectin, are necessary, but their influence on granule degradation is not understood.
3. Metabolism of glucans released from the starch granule is via β-amylase rather than glucan phosphorylase. The maltose product is exported from the chloroplast to the cytosol via a recently discovered maltose transporter.
4. Maltose in the cytosol is metabolized via a transglucosylation reaction. Details of this and downstream reactions in the conversion of maltose to hexose phosphate remain to be discovered.
5. Flux through this pathway is clearly subject to complex control, but the control mechanisms are poorly understood. Although levels of transcripts for several of the enzymes vary considerably on a diurnal basis, there are good reasons to think that control of flux is primarily posttranslational rather than transcriptional.
6. Information is sparse about starch degradation in leaves of other species of plant, and in nonphotosynthetic plant parts in which starch accumulates transiently. At present it seems likely that pathways similar to those in the *Arabidopsis* leaf may operate in these organs.
7. Knowledge of starch degradation in starch-storing organs such as cereal endosperm and legume seeds is likewise incomplete, but the process probably differs considerably between different types of storage organs and between storage organs and *Arabidopsis* leaves.

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