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Starch: A Flexible, Adaptable Carbon Store Coupled to Plant Growth

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Abstract

Research in the past decade has uncovered new and surprising information about the pathways of starch synthesis and degradation. This includes the discovery of previously unsuspected protein families required both for processes and for the long-sought mechanism of initiation of starch granules. There is also growing recognition of the central role of leaf starch turnover in making carbon available for growth across the day-night cycle. Sophisticated systems-level control mechanisms involving the circadian clock set rates of nighttime starch mobilization that maintain a steady supply of carbon until dawn and modulate partitioning of photosynthate into starch in the light, optimizing the fraction of assimilated carbon that can be used for growth. These discoveries also uncover complexities: Results from experiments with *Arabidopsis* leaves in conventional controlled environments are not necessarily applicable to other organs or species or to growth in natural, fluctuating environments.

Contents

1. INTRODUCTION

Our understanding of starch metabolism in plants has progressed hugely in the past decade. Although a textbook description of starch synthesis and degradation has been in place for years, recent discoveries of previously unknown proteins essential for normal starch turnover reveal the process to be far more complex than originally envisaged. The textbook description also largely failed to recognize the importance of control of leaf starch turnover for plant growth. New discoveries have revealed a sophisticated control mechanism that adjusts the rate of starch degradation according to the length of the night, optimizing carbon supply for growth in the hours of darkness. In this review we describe the present state of knowledge of starch metabolism, highlighting new concepts and areas that remain to be explored.

Much of the research on starch metabolism in the past decade has been conducted on *Arabidopsis* leaves. In addition to its general value as a reference organism for plant biology, *Arabidopsis* has specific advantages for the study of starch metabolism. In many species, leaf starch metabolism changes profoundly through development and with plant age and maturity, making it difficult to establish a robust experimental system. By contrast, starch metabolism in *Arabidopsis* leaves varies rather little with leaf age. Measurements made on whole rosettes are thus reasonably representative of most of the individual leaves.

Another important source of new information about starch metabolism is research on cereal endosperms, fueled by the need for new genetic markers and targets for yield and quality improvement and the parallel expansion of genetic and genomic information for major cereals. Although many studies of endosperm starch metabolism are descriptive, they have the potential to broaden our understanding of starch metabolism and offer informative comparisons with leaf starch metabolism.

In the first part of the review we discuss starch granules themselves—their nature, their growth, and how new granules are initiated. In the second part we discuss the function of starch in the plant—the control of starch synthesis and degradation and its importance for plant growth.

2. STRUCTURE OF STARCH GRANULES

Although consisting entirely of α-1,4-linked and α-1,6-linked glucose polymers, starch granules have a complex hierarchy of internal organization that stems largely from the architecture of

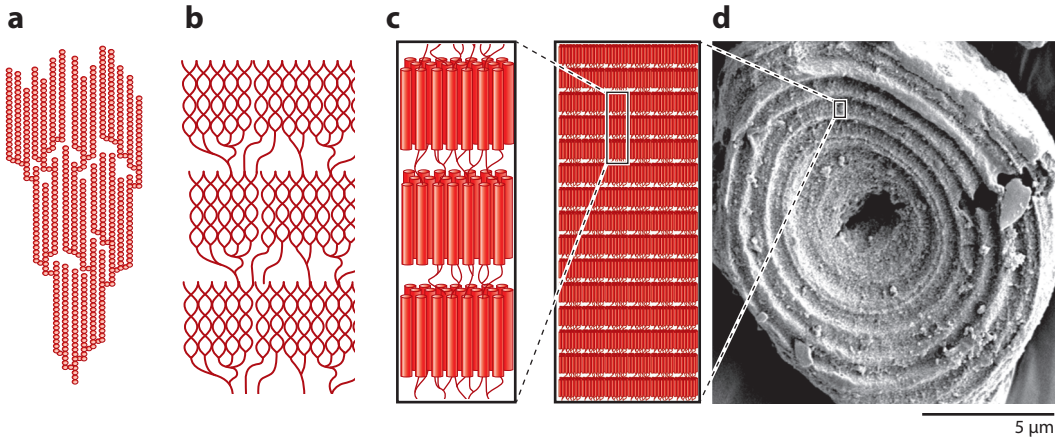

Figure 1

The structure of a starch granule. (*a*) Part of an amylopectin molecule, consisting of clusters of linear chains of α-1,4-linked glucose residues connected by α-1,6 linkages. (*b*) Double-helix formation between adjacent linear chains within clusters. (*c*) Representation of the ordered (crystalline) arrays formed by the packing of double helices, interspersed with amorphous lamellae in which the α-1,6 linkages are located. The repeat distance is 9–10 nm. (*d*) An inner face of a maize endosperm starch granule, etched to show the growth rings. Each ring spans tens of the semicrystalline repeats shown in panel *c*.

the major glucose polymer, amylopectin. This massive, branched molecule consists of clusters of chains of α-1,4-linked glucose units (typically 12–20 units) that arise from regions rich in branch points (α-1,6 linkages). Longer chains span two, three, or more clusters, giving rise to a polymodal distribution of branch lengths. The pattern of alternating linear and branched regions is repeated many times along the axis of the molecule (**Figure 1*a***). Adjacent linear chains within the clusters form double helices, which pack together in ordered arrays (**Figure 1*b***). The result is a semicrystalline structure of alternating crystalline (ordered) and amorphous (containing the branch points) lamellae, with a periodicity of 9–10 nm. Although the relative widths of crystalline and amorphous lamellae and the pattern of packing of helices in the crystalline lamellae vary among starches from different sources, this repeat distance is remarkably conserved. The formation of the semicrystalline structures in starch may arise because amylopectin has properties akin to side-chain liquid crystalline polymers, which are self-organizing structures (87, 88). According to this view, the chains in the amorphous lamellae serve as flexible spacer arms that permit the double helices to align in an ordered manner (**Figure 1*c***).

In addition to organization at the nanometer scale, many starch granules also have periodic growth ring organization at a scale of hundreds of nanometers. Growth rings comprise a zone of semicrystalline repeats, described above, and a more amorphous zone in which the glucan structure is not well defined (**Figure 1*d***). The genesis of the rings is not understood. It is tempting to imagine that they result from different rates of granule growth during the day and the night, as carbohydrate supply from photosynthetic organs fluctuates. Studies of plants grown in constant light or dark conditions have not resolved this question. Growth of potato plants in constant light and microtubers in constant darkness on sucrose-containing media did not abolish growth rings (132). Some studies of cereal grains grown in constant light reported loss of growth rings from endosperm starch, but other such studies reported that growth rings were retained (16, 69, 132, 187). Alternative explanations that remain to be explored are that branch density increases with granule growth, leading to periodic breakdown of lamellar organization in the matrix, or that the circadian clock governs granule deposition and organization processes over a 24-h period. Leaf

starch granules usually lack growth rings because of their small size and extensive diel turnover (203).

In addition to the 9–10-nm and growth-ring scales of organization, evidence from atomic force and scanning electron microscopy of inner surfaces of storage starch granules indicates that the alternating crystalline and amorphous lamellae within a growth ring may be organized into structures of approximately 25–100 nm, known as blocklets (9, 64).

The second glucose polymer in starch granules is amylose, which consists of long α-1,4-linked glucose chains with few branch points. Leaf starches generally contain less than 10% amylose, whereas starch in storage organs contains 20–30%. It should be noted that different measurement methods can give substantially different amylose contents for the same sample. Iodine-binding, differential precipitation, and lectin-binding methods identify amylose as material containing long unbranched chains, while other methods distinguish amylose from amylopectin by its smaller molecular size. Amylose is believed to occur largely as single unstructured helices in the amorphous regions of the matrix (88, 92) and to play no role in the organization of the matrix: Amylose-free mutants (see Section 3.1) have normal starch contents, granule sizes, and shapes.

3. GROWTH OF STARCH GRANULES

3.1. Pathway of Starch Synthesis

The dedicated pathway of starch synthesis starts with the generation of the glucose donor adenosine diphosphate (ADP)-glucose by the enzyme ADP-glucose pyrophosphorylase (AGPase). In all plant organs except grass and cereal endosperm, this enzyme is located exclusively in the plastid. In chloroplasts, its glucose 1-phosphate substrate is derived from the Calvin-Benson cycle, while in nonphotosynthetic plastids it is derived from hexose phosphates imported from the cytosol (**Figure 2**). Most AGPase activity (typically 80% or more) in grass and cereal endosperm resides in the cytosol, and the remainder is plastidial (84, 180). ADP-glucose produced by the cytosolic enzyme enters the amyloplast via a dedicated transporter, BRITTLE1 (Bt1), in the inner membrane of the amyloplast envelope (158). Plant AGPases consist of two similar subunits [the large subunit (LSU) and the small subunit (SSU)] that form an active tetramer. LSUs are usually encoded by small multigene families, whereas SSUs are encoded by one or two genes. The properties of the enzyme are conferred by synergistic interactions between the SSU and LSU, the latter being important for modulation of activity. LSUs have different spatial patterns of expression, potentially giving rise to AGPases with distinct properties in different parts of the plant (28, 185). The plastidial enzyme is under strong allosteric control by metabolites including 3-phosphoglycerate, sugar phosphates (activators), and inorganic phosphate (an inhibitor). Its activity is also modulated posttranslationally by redox activation (7, 78, 184).

Its position as the first enzyme in the starch synthesis pathway and its regulatory properties led to the idea that AGPase catalyzes a rate-limiting step. This idea is not fully supported by quantitative data. AGPase exerts significant control over flux into starch in saturating light in *Arabidopsis* leaves but less control in limiting light (125). In potato tubers, AGPase exerts significant control over flux into starch in nonstressed conditions (65, 66, 173) but less control under osmotic stress (65). In practice, control of flux into starch is likely partitioned between enzymes on the committed pathway and is likely dependent on genetic, developmental, and environmental factors. While AGPase can play an important role, the general use of the term rate-limiting step is misleading.

Three enzymes are responsible for the conversion of ADP-glucose to starch polymers: starch synthases (SSs), starch-branching enzymes (SBEs), and debranching enzymes. ADP-glucose is the substrate for SSs, which add its glucosyl moiety to glucose chains at the granule surface. The glucose chains become branched through the action of SBEs that transfer the terminal part of a

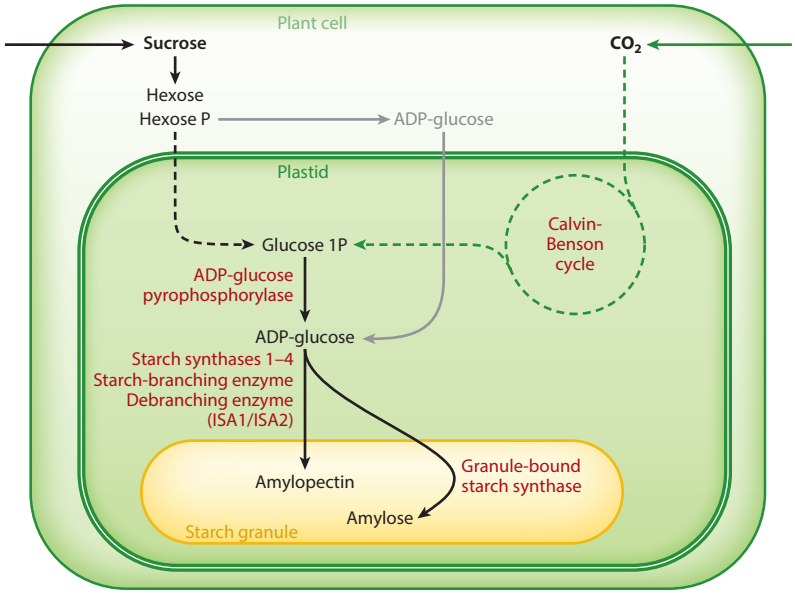

Figure 2

Pathways of starch synthesis. The starch granule is contained within the double membrane of the plastid envelope. In a photosynthetic cell (*dashed green lines; right*), CO_2 is assimilated in the Calvin-Benson cycle and the Calvin-Benson cycle intermediate fructose 6P is used for starch synthesis. Fructose 6P is converted to glucose 1P, the substrate for synthesis of ADP-glucose. The glucosyl moiety of ADP-glucose is transferred to starch polymers by starch synthases. Elaboration of the chains to form amylopectin is carried out by starch-branching enzymes and the debranching enzymes ISA1 and ISA2. In nonphotosynthetic cells (*dashed black lines; left*), hexose phosphate derived from imported sucrose is transported into the amyloplast. Conversion to starch is as in the photosynthetic cell. An exception is the endosperm of cereals (*gray lines*); here, glucose 1P is converted into ADP-glucose in the cytosol and then imported into the amyloplast. Dashed lines indicate multiple steps that are not shown. Abbreviations: ADP, adenosine diphosphate; ISA, isoamylase-type starch-debranching enzyme; P, phosphate.

chain onto the side of an adjacent chain, creating an α-1,6-linkage. Removal of some branches by debranching enzymes then gives rise to a structure that can become organized as part of the granule matrix. These enzymes have been reviewed in detail recently (71, 123, 131, 179, 180). The diverse properties of their multiple isoforms are believed to be important for the periodic branching pattern of amylopectin and hence the structure of the granule matrix. In organisms other than plants, α-1,4-linked and α-1,6-linked glucans are generally synthesized by single forms of synthase and branching enzyme. The resultant glycogen polymers have unimodal rather than polymodal distributions of branch lengths (139) and exist as small amorphous particles, limited in size when the packing density of peripheral branches is too great to permit further synthesis.

Five classes of SS isoforms are conserved across starch-synthesizing organisms. They share a C-terminal catalytic domain resembling that of bacterial glucan synthases but differ from one another in the length and amino acid sequence of their N termini (35, 131, 174). These regions variously contain carbohydrate-binding modules (CBMs) for substrate binding and coiled-coil domains that may be involved in protein-protein interactions. Three classes are primarily responsible for elongation of chains at the granule surface: SSI, SSII, and SSIII. Mutants lacking individual members of this group can synthesize semicrystalline starch but typically have isoform-specific alterations in amylopectin chain-length distributions. The properties of the isoforms have been

defined by examining their products when purified from heterologous expression systems and by comparing amylopectin structure in mutants lacking single isoforms (129, 174, 207). They differ with respect to—for example—their affinities for chains of different lengths, their propensity to bind to starch granules, and their capacity to form complexes with other starch-synthesizing enzymes (discussed in Section 3.2). The precise role of each isoform during starch synthesis in vivo remains difficult to define. Loss of one isoform in a mutant plant changes the distribution of chain lengths available as substrates for the remaining isoforms, alters the ratio of SS to SBE activity, and perturbs the formation of complexes involving other enzymes (see Section 3.2). Loss of single isoforms can also have pleiotropic effects on the abundance and location of other enzymes (163, 191).

Despite these caveats, there is a consensus from in vivo and in vitro research that SS1 preferentially elongates short chains of amylopectin (6 or 7 glucoses) and that SS2 further elongates them to 13–20 glucoses—sufficient to form double helices with neighboring chains within amylopectin clusters. SS3 is also proposed to contribute to the elongation of shorter chains and to generate longer, cluster-spanning chains (24, 35, 40, 43, 48, 59, 62, 114, 118, 174, 205–207).

The remaining two SS isoforms—SS4 and granule-bound starch synthase (GBSS)—have roles in the initiation of starch granules (discussed in Section 3.3) and in amylose synthesis, respectively. Unlike other SS isoforms, GBSS is located exclusively within the starch granule. It uses ADP-glucose to elongate short maltooligosaccharides, thereby generating amylose inside the amylopectin matrix (35, 41, 42, 177). It was long thought that GBSS was the sole protein required for amylose synthesis: Amylose-free mutants of a wide range of starch-storing crops and of *Arabidopsis* were shown to lack GBSS (20, 83, 162). However, the reason why GBSS is exclusively within the starch granule remained obscure: It has no obvious CBM or other means of binding to starch. This mystery was partly solved by the discovery in *Arabidopsis* chloroplasts of a protein that physically delivers GBSS to starch granules; PROTEIN TARGETING TO STARCH1 (PTST1) (101, 156). PTST1 interacts with GBSS via C-terminal coiled coils and with starch via an N-terminal CBM. Without PTST1, GBSS cannot bind efficiently to starch and is degraded in the stroma. Hence, *Arabidopsis ptst1* mutants, like *gbss* mutants, are amylose free. This unexpected discovery may have broad significance. PTST1 is widely conserved in plants, and recent mutation of the cassava *PTST1* gene using clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9) resulted in a low GBSS content and a halving of amylose content in the starchy storage roots (14).

Most plants have two distinct classes of SBE isoforms, SBE1 (or SBEI) and SBE2 (or SBEII). In vitro, the enzymes typically act on linear chains of greater than 12 or 13 glucoses, transferring terminal sections of six or more glucoses to the same or an adjacent chain. SBE1 and SBE2 from cereals differ considerably in their substrate preferences and products. For example, SBE1 has high activity on long linear chains and can cleave α-1,4 linkages behind existing branch points, whereas SBE2 isoforms preferentially act on outer chains of branched structures (73, 103, 131, 146). Species examined thus far typically have a single isoform of SBE1 and one or more isoforms of SBE2. The SBE2a and SBE2b isoforms of cereals have different expression patterns; for example, in maize and rice SBE2a is essential for normal starch synthesis in leaves but not in endosperm, and SBE2b is unimportant in leaves but essential in endosperm (the *sbe2b* or *amylose-extender* mutant of maize is the basis of the commercial high-amylose cultivars of this crop) (10, 11, 116, 199). *Arabidopsis* is notable because there is no SBE1 encoded in its genome (the gene annotated as *BE1* encodes a protein of unknown function) (46, 130, 188, 193). Loss of both SBE2 proteins from *Arabidopsis* (annotated as BE2 and BE3) prevents starch formation and leads to accumulation of maltose, probably because the linear chains produced in the absence of SBE activity are hydrolyzed to maltose by a β-amylase (46).

The possession of distinct forms of SBE has been assumed to be important in creating the polymodal chain-length distribution of amylopectin, although SBE1 is generally agreed to be less important than SBE2. The loss of SBE1 from potato, maize, and rice has relatively little impact, whereas the loss of SBE2 markedly alters amylopectin structure (10, 145, 148). Nonetheless, in almost all cases the amylopectin of *sbe2* mutants retains sufficient branching to allow formation of starch granules. The complexities of interactions between enzymes and starch structures in vivo render it impossible to define precisely the roles of SBE1 and SBE2 from the study of starch structure in the respective mutants or from SBE properties measured in vitro on soluble substrates (which may not reflect conditions in which the enzyme acts at the starch granule surface in vivo). The *Arabidopsis be2 be3* double mutant—lacking SBE but containing the other starch biosynthetic enzymes—has been used to study the properties of different SBE isoforms. The results largely confirmed the chain-length specificities deduced from in vivo studies and defined the relationship between SBE activity and amylopectin structure (103). Interestingly, expression of a glycogen-branching enzyme from *Escherichia coli* in *be2 be3* also resulted in small amounts of insoluble, semicrystalline granules. These had abnormal polymer branching patterns and low crystallinity, but the result is nonetheless informative given that *E. coli* makes soluble glycogen rather than semicrystalline material (13, 103). It indicates that the formation of glucose polymers capable of crystallization does not strictly require multiple, distinct forms of SBE.

Chain elongation and branching could conceivably be sufficient for the production of starch, but in fact some debranching (hydrolysis of α-1,6 linkages) also occurs during normal amylopectin biosynthesis. Most plant species have multiple genes encoding distinct isoforms of debranching enzymes, some implicated in amylopectin synthesis and others in starch degradation. Plant debranching enzymes are classified as isoamylase type (encompassing classes ISA1, ISA2, and ISA3) and limit dextrinase type (LDA; also called pullulanase). All are specific for the α-1,6 linkage but differ in their substrate preferences (91). ISA1, ISA3, and LDAs are active hydrolases, but ISA2 appears to be inactive due to nonconservative substitutions in amino acids essential for catalysis (131). While ISA3 and LDA are monomeric enzymes (176), ISA1 forms heteromultimers with ISA2 (e.g., in *Arabidopsis*, bean, and potato) (38, 39, 82, 176) and is inactive and/or unstable in its absence. In cereal endosperms, however, ISA1 forms both active homomultimers and heteromultimers with ISA2 (91, 95, 186).

The requirement for debranching in normal amylopectin synthesis was established through the analysis of mutants in cereals, *Chlamydomonas*, *Arabidopsis*, and potato, all of which were deficient in isoforms of the ISA1 type (15, 38, 85, 119, 124, 194). ISA1 deficiency results in glucans with an increased frequency of branches, a fraction of which fails to crystallize into a granular form and remains soluble (so-called phytoglycogen). These observations led to the suggestion that SSs and SBEs create a branched glucan that is selectively debranched (trimmed) by isoamylase to form crystallization-competent amylopectin. Without trimming, misplaced or excessive branches may impede the formation and packing of double helices and hence the formation of the semicrystalline matrix (6, 122, 195).

The extent of phytoglycogen accumulation in isoamylase-deficient plants depends on the species and tissue type. In *Chlamydomonas*, starch synthesis is abolished and phytoglycogen is synthesized in its place (119), but more commonly, *isa* mutants synthesize both phytoglycogen and some starch (with altered amylopectin architecture) (38, 97). The ratio of phytoglycogen to starch varies widely (38, 60, 97, 124) and may depend on the complement and/or relative amounts of other starch metabolic enzymes. First, LDA and ISA3, although primarily involved in degradation, may also contribute to debranching during synthesis (44, 61, 95, 170, 195). Second, the requirement for debranching to facilitate glucan crystallization will depend on the structure produced by SSs and SBE. This idea is supported by work in maize, rice, and *Arabidopsis*, where additional

null mutations (e.g., in SS isoforms) introduced into ISA1-deficient backgrounds can increase or decrease the severity of the phytoglycogen-accumulating phenotype (27, 59, 129). Third, the actions on glucans of starch-catabolizing enzymes such as α-amylases and β-amylases can modify or delay the formation of higher-order structures (38, 170).

Several aspects of debranching enzyme function in starch biosynthesis remain unclear. These include the occurrence of an exclusively multimeric enzyme (ISA1/ISA2) rather than a single isoform in some organs but not others (49, 171), the precise selectivity and mode of action of the enzyme during amylopectin synthesis in vivo, and the evolutionary origins of a debranching requirement for granule synthesis—particularly given that some combinations of other starch-metabolizing enzymes can create insoluble polymers without debranching (170).

The most successful attempt thus far to simulate aspects of starch biosynthesis in vitro or in heterologous systems is the creation of semicrystalline starch-like glucans in the baker's yeast *Saccharomyces cerevisiae* (130). This was achieved by (*a*) eliminating *S. cerevisiae* genes encoding enzymes of glycogen metabolism, thus avoiding interference with an introduced pathway, (*b*) introducing a bacterial AGPase to supply ADP-glucose for the plant SSs [yeast glycogen synthases use uridine diphosphate (UDP)-glucose], and (*c*) expressing genes encoding *Arabidopsis* starch biosynthetic enzymes in different combinations. When all core enzymes (SS1–SS4; BE2 and BE3; ISA1 and ISA2) were expressed, the cells synthesized insoluble, semicrystalline glucans with lamellar repeat structures, albeit with less order and a larger repeat (13–14 nm rather than 9–10 nm) than in *Arabidopsis* starch. Comparisons of the amounts and structures of glucans in strains expressing different enzyme combinations enabled the roles of specific proteins to be deduced. This work reinforced the importance of debranching enzymes for the formation of insoluble glucans and revealed the requirement for particular SS and SBE combinations to create a suitably branched precursor. For example, the presence of SS1 increased the numbers of short chains in the synthesized glucans and reduced their tendency to crystallize. SS1 and SBEs together produced a soluble glucan that was not converted to a crystallization-competent form by ISA1/ISA2 but instead appeared to be degraded by this enzyme, highlighting the need for additional SS isoforms (130).

The combination of many genetic, biochemical, and synthetic biology approaches such as those cited above has defined AGPase, SSs, SBEs, and ISAs as the core starch biosynthetic components. Nevertheless, other proteins—both long known and newly discovered—have also been implicated in starch granule synthesis. Some are widely conserved and likely have a direct influence on starch synthesis. Others may have species-specific and/or indirect effects but may still influence the rate of starch synthesis and/or starch structure in particular organs, species, and conditions. Examples include the highly conserved plastidial starch phosphorylase PHS1, which catalyzes the phosphorolysis of glucose residues from the nonreducing ends of chains to yield glucose 1-phosphate. A possible role in starch degradation is discussed in Section 4.4.1. In some circumstances it may also elongate glucan chains during starch synthesis (36, 145). The recently characterized PTST and early starvation (ESV) protein families are other examples. PTSTs bring starch synthesis enzymes into proximity with their glucan substrates within the plastid (e.g., PTST1, described above), while ESVs are starch-binding proteins that appear to influence the structure of the granule matrix (50, 156).

The study of cereal mutants with abnormal endosperm appearance (e.g., *opaque* or *floury endosperm* mutant) has led to identification of further genes that influence starch accumulation in grains. These genes function in processes including the synthesis of plastidial membranes, plastidial glycolysis, protein-protein interactions, and transcriptional control (17, 58, 102, 121, 135, 159, 192, 204). Despite the inclusion of transcription factors in this list, it is notable that no master regulator of the starch biosynthesis pathway has been found. It seems likely that there are

multiple controls at transcriptional and posttranslational levels, depending on the organ and species concerned.

3.2. Cooperative Actions of Enzymes of Starch Synthesis

It is generally agreed that starch granules grow through the cooperative actions of SS, SBE, and ISA: Chains at the granule surface arising from SBE action are elongated by SS isoforms until they are long enough to form double helices and to serve as substrates for further branching. Removal of extraneous branches by ISA renders amylopectin competent for crystallization. Since the substrate for each enzyme is produced by the concerted action of other enzymes, loss of any one enzyme can have far-reaching consequences for structure. There is also good evidence that starch biosynthetic enzymes can associate into specific complexes. Such complexes could modulate the activities of participating enzymes, channel glucan substrates and products toward the formation of specific structures, and colocate synergistic enzyme activities at the granule surface (32, 99).

Numerous examples have been reported from cereal endosperm of complexes that contain SS and SBE isoforms, and in some cases plastidial starch phosphorylase (e.g., 30, 79, 178). Complex formation requires phosphorylation of some of the constituent proteins (e.g., 31, 181, 182). Good evidence that complex formation is important for normal amylopectin synthesis comes from the study of two near-isogenic lines of maize containing different mutant alleles of SBE2b. One mutant has no SBE2b protein [a null mutant: *amylose extender 1.1* (*ae1.1*)], while SBE2b is catalytically inactive in the other (98). The two *ae* mutants differ from the wild type and from each other in multiple respects, including the composition of complexes containing starch-synthesizing enzymes, the profile of granule-bound proteins, and the composition and properties of starch. These data show that the composition of complexes of starch-synthesizing enzymes can profoundly influence starch synthesis. More work is required to understand the relationship between complex constituents and the structures of nascent starch polymers.

3.3. Initiation of Starch Granules

Until recently, the question of how starch granules are first initiated remained unanswered. Although there are well-supported proposals about the elaboration of the periodic lamellar structure of amylopectin, these assume the existence of a suitable glucan substrate. Early ideas about granule initiation came from the study of phase-separation behavior of glucan polymers in solution (208) or from extrapolating knowledge of glycogen initiation in fungi and animals (based on self-glucosylating glycogenin proteins) and have gained little traction in plants (155). Nonetheless, it is clear that there is control over numbers and sizes of granules per plastid. These traits can be so characteristic of the organ in which starch is synthesized that archaeological researchers use starch granule morphologies as reliable indicators of species used as crops in prehistory (26). The cereals exemplify this point: Maize endosperm contains a uniform population of simple starch granules, each thought to result from a single initiation event; rice endosperm contains compound granules resulting from multiple initiations in a single plastid followed by partial fusion; and wheat and barley endosperm contains both large lenticular A granules and small spherical B granules, thought to arise from two successive rounds of granule initiation (113, 155). Overall, there is remarkable diversity of starch granule morphology and size across the plant kingdom (e.g., 86, 113, 155).

Transitory starch granules found in chloroplasts are generally small, flattened, and discoid, occupying stromal spaces between the thylakoid membranes. *Arabidopsis* chloroplasts reportedly

contain 6–10 granules, and the numbers are positively correlated with chloroplast volume (33). There has been significant progress in identifying the genetic determinants of granule initiation in *Arabidopsis*, starting with the discovery that SS4 plays a key role (140). Chloroplasts of *ss4* mutants contain very few or no starch granules and accumulate high levels of the SS substrate ADP-glucose (34, 136, 175). The few granules that initiate are almost spherical rather than lenticular. The dual roles of SS4 in initiation and determination of granule shape seem to be attributable to different domains of the protein. The C-terminal glucosyl transferase domain promotes initiation, while the N-terminal coiled-coil-containing domain is critical for the subchloroplastic localization of SS4 and for guiding granule growth (155). The remaining SS isoforms can initiate some granules in the absence of SS4, albeit very inefficiently (175).

MFP1: MAR BINDING FILAMENT-LIKE PROTEIN1

MRC: MYOSIN-RESEMBLING CHLOROPLAST PROTEIN

SS4 is able to multimerize and to interact with other recently discovered proteins that also contribute to starch granule initiation. First, SS4 can directly bind PTST2, which, like its homolog PTST1, has a coiled-coil-containing domain and a CBM. Although SS4 has coiled coils in its N terminus, it seems that PTST2 interacts directly with the C-terminal glucosyl transferase domain (153). PTST2 is proposed to bind long maltooligosaccharides (degree of polymerization $\geq$10) and deliver them as primers for SS4, allowing their elaboration into a granule precursor. The loss of PTST2 severely reduces granule number, a phenotype exacerbated by loss of its close homolog PTST3 (153). Searches for further protein interactors of SS4 and PTST2 identified the chloroplastic proteins MAR BINDING FILAMENT-LIKE PROTEIN1 (MFP1) and MYOSIN-RESEMBLING CHLOROPLAST PROTEIN (MRC; also called PII) (154, 189). Both lack recognizable protein domains but are rich in predicted coiled-coil motifs that are probably important for their interactions with PTST2 and SS4.

As for SS4, loss of any one of these new interacting proteins (PTST2 and PTST3; MFP1 and MRC) severely reduces starch granule numbers. Expression in *Arabidopsis* of fluorescently tagged versions of these proteins reveals that MFP1 is associated with the thylakoid membranes and MRC occupies discrete domains within the chloroplast stroma (154). These observations have led to the proposal that granule-initiation complexes formed of PTSTs, MRC, and SS4, clustered adjacent to thylakoid membranes through their association with MFP1, produce a locally high concentration of elongated glucan chains that can be acted on by other starch-synthesizing enzymes to form a granule precursor (155). The exact way in which such initiation domains result in a specific number of granule precursors per chloroplast is currently unclear.

The emergence of new players in starch granule initiation raises many questions. What are the dynamics of the protein-interaction network? Are other enzymes of amylopectin synthesis involved, and are there other crucial unidentified components? What events trigger crystallization of newly formed glucans? There are early indications that granule initiation in other organs involves at least some of the proteins identified in *Arabidopsis*. Rice and barley mutants lacking orthologs of PTST2 [*floury endosperm6* (128) and Franubet (142), respectively] have abnormal patterns of starch granule initiation.

4. FUNCTION OF STARCH IN THE PLANT

With few exceptions, starch in plant cells acts as a reserve that can be mobilized to provide sugars for growth and maintenance when photosynthesis is not possible. Most plants spend more than half of their lives in this situation. Photosynthesis is not possible in leaves at night, during seed germination and emergence, during bud outgrowth following dormancy in deciduous plants, and following defoliation by herbivores. The basic pathway of starch synthesis is well conserved, but there is great variation among species, organs, and circumstances in the way the starch is subsequently mobilized to produce sugar. The control mechanisms determining the rate and

magnitude of starch accumulation and the timing of onset and rate of starch mobilization are likely to be complex and diverse but are presently poorly characterized.

We discuss first the pathway of starch mobilization and the control of starch turnover in leaves. This is best understood in *Arabidopsis*, where the diel control of starch turnover is of paramount importance for growth. Numerous *Arabidopsis* mutants defective in starch synthesis during the day or starch mobilization at night grow more slowly than wild-type plants in short days. We then consider how starch may be accumulated and remobilized in other plant organs and different circumstances. Starch turnover is a huge topic that cannot be explored in depth here; we attempt below to provide an overview and to identify general principles.

4.1. Pathway of Mobilization in *Arabidopsis* Leaves

Leaves synthesize both sucrose and starch as primary products of photosynthesis during the day and convert starch to sucrose for growth and maintenance during the night. Starch is synthesized from the Calvin-Benson cycle intermediate fructose 6-phosphate. At night, the rate of starch degradation is such that almost all starch is consumed by dawn. Knowledge of the degradation pathway is based largely on analysis of mutants selected on the basis of high starch contents at dawn and the offspring of crosses between these mutants.

4.1.1. The attack on the granule surface. The attack on the granule involves two types of hydrolytic enzyme, the exoamylase β-amylase, which cleaves α-1,4 linkages, and the debranching enzyme isoamylase, which cleaves α-1,6 linkages. At least four, and possibly up to six, isoforms of β-amylase are chloroplastic in *Arabidopsis*. Two, β-AMYLASE3 (BAM3) and BAM4, are essential for normal rates of starch degradation. Knockout mutants lacking either isoform continue to turn starch over during the day-night cycle but have much higher levels of starch than wild type. BAM1 can also contribute to starch degradation. Although the *bam1* mutant does not accumulate excessive starch, mutants lacking both BAM3 and BAM1 have higher starch contents than mutants lacking BAM3 alone (63). Remarkably, BAM4 is not an active β-amylase (63, 96). The recombinant protein has no detectable activity and lacks amino acid residues believed to be essential for catalysis. Its role in starch degradation is not known but is independent of those of BAM3 and BAM1: Loss of BAM4 increases starch accumulation in *bam3* and *bam3 bam1* mutants, implying that it does not simply modulate BAM3/BAM1 activity (63) (**Figure 3**).

The debranching enzymes required for starch granule degradation, ISA3 and LDA, are distinct from ISA1 and ISA2, which are involved in starch synthesis. LDA is redundant with respect to ISA3 in a wild-type background, but mutant analysis shows that it hydrolyzes α-1,6 linkages in an *isa3* mutant background. The *isa3 lda* double mutant has a much higher starch content than *isa3* and contains large amounts of small, soluble branched glucans (39). These are released from the granule by endoamylolytic cleavage of α-1,4 linkages but cannot be metabolized further in the absence of debranching activity (170). Further loss of the chloroplastic endoamylase α-AMYLASE3 (AMY3; in the *isa3 lda amy3* mutant) prevents the accumulation of soluble branched glucans, confirming that they arise from amylolysis in the *isa3 lda* mutant.

The major product of the actions of β-amylase and ISA3 on the starch granule is the disaccharide maltose. Some maltotriose is also produced from hydrolysis of chains with an odd number of glucose residues; β-amylase cannot further hydrolyze this short maltooligosaccharide. Maltotriose is metabolized by a disproportionating enzyme, DPE1 or D-enzyme, which transfers glucan moieties between nonreducing ends of chains and thus converts two maltotrioses to maltopentaose (five glucoses; a substrate for β-amylase) and free glucose. Mutants lacking DPE1 accumulate maltooligosaccharides at night and have slightly elevated starch contents (29). The fate of

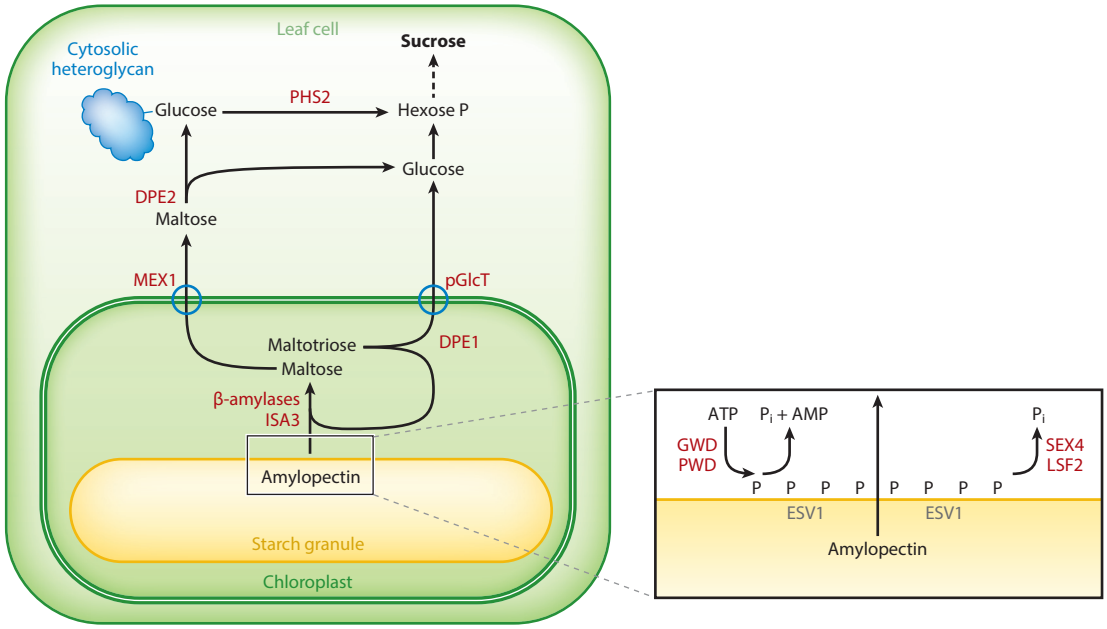

Figure 3

Pathway of starch degradation in leaves. (*Left*) Conversion of starch to sucrose. The starch granule (*yellow*) is attacked by β-amylases and the debranching enzyme ISA3, yielding maltose and maltotriose. Maltotriose is further metabolized by DPE1 to yield glucose and products susceptible to further amylolytic attack. Maltose and glucose are exported from the chloroplast by specific transporters (MEX1, pGlcT: *blue circles*) in the envelope. In the cytosol, maltose is acted on by the disproportionating enzyme DPE2, releasing one glucose and transferring the other to a cytosolic heteroglycan. This glucosyl moiety is converted to glucose 1P by the glucan phosphorylase PHS2. Glucose and glucose 1P are converted to sucrose. Dashed lines indicate multiple steps that are not shown. (*Right*) The attack by β-amylases and ISA3 requires phosphorylation of the granule surface by GWD1 and PWD and then release of the phosphate by the glucan phosphatases SEX4 and LSF2. The granule-bound protein ESV1 modulates the impact of these enzymes on the extent of granule surface hydrolysis. Abbreviations: AMP, adenosine monophosphate; ATP, adenosine triphosphate; DPE1, disproportionating enzyme 1; ESV1, early starvation 1; GWD1, glucan, water dikinase 1; ISA3, isoamylase-type starch-debranching enzyme 3; LSF2, like sex four 2; MEX1, maltose excess 1; P, phosphate; P_i, inorganic phosphate; pGlcT, plastidic glucose translocator; PHS2, cytosolic glucan phosphorylase; PWD, phosphoglucan, water dikinase; SEX4, starch excess 4.

maltose and glucose produced by the combined actions of β-amylase, ISA3, and DPE1 is discussed in Section 4.1.3.

In addition to the amylases, *Arabidopsis* chloroplasts also contain PHS1, which can generate glucose 1-phosphate from starch. Collectively, these enzymes can catalyze a network of reactions, with both pathway and isoform redundancies. Indeed, many of them appear essentially redundant in a wild-type background (including BAM1, AMY3, LDA, and PHS1). However, as already noted for BAM1, LDA, and AMY3, their activities are apparent in mutant backgrounds where the reaction network is already compromised (39, 63, 201, 202). Loss of PHS1 does not affect starch turnover in standard growth conditions (202), but its loss from *ss4*, *mex1*, and *dpe2* (Section 4.1.3) mutants reduces granule number and alters granule morphology (108–110).

Evidence for subfunctionalization within the reaction network comes from the specific activation of some components by abiotic stresses including heat, cold, and drought (90, 183). For example, acute osmotic stress activates BAM1 and AMY3 in the leaf mesophyll, resulting in conversion of starch to sugars as part of an adaptive response (183). Some enzymes that are redundant for starch degradation in the leaf mesophyll are important elsewhere in the plant; starch degradation in guard cells is discussed below.

4.1.2. Granule phosphorylation and dephosphorylation. The rate at which the granule is degraded by hydrolytic enzymes depends on the activities of proteins that influence the organization of amylopectin molecules at the granule surface. Two sets of enzymes respectively phosphorylate and dephosphorylate glucose residues within amylopectin molecules. Glucan, water dikinase [GWD, also called STARCH EXCESS1 (SEX1)] and phosphoglucan, water dikinase (PWD) add the β-phosphate of adenosine triphosphate (ATP) to glucose residues, releasing AMP and inorganic phosphate (8, 93, 138, 200). Analyses of starch in *gwd* and *pwd* mutants and studies with recombinant enzymes have established that GWD preferentially phosphorylates at the 6-position of glucose residues and PWD at the 3-position (137) (**Figure 3**).

Both GWD and PWD are essential for normal starch degradation. In *gwd* mutants, starch accumulates to very high levels and there is little net synthesis or degradation (200). The loss of 6-phosphorylation in the *gwd* mutant prevents the action of PWD, so that starch is almost unphosphorylated in these mutants (77, 137). The *pwd* phenotype is less severe than that of *gwd*—the level of starch in the leaf is elevated, but diel turnover continues. In vitro studies of the effects of phosphorylation by GWD and PWD on the physical properties of starch-like substrates have provided an explanation of their role in starch degradation. Phosphorylation decreases the degree of crystallinity of starch-like substrates, probably by interfering with the packing of double helices. The resulting hydration and opening of the granule surface renders it more susceptible to hydrolytic enzymes (12, 47, 76, 77).

Dephosphorylation of the glucose residues phosphorylated by GWD and PWD is also necessary for normal starch degradation. Loss of the major glucan phosphatase SEX4 causes elevated starch levels and accumulation of linear phosphorylated glucans in the chloroplast stroma (94, 126). It appears that although phosphorylation renders the granule surface susceptible to β-amylolysis, the phosphate groups must then be removed to allow the progressive degradation of linear chains by β-amylase. Loss of SEX4 in *sex4* mutants blocks β-amylolysis. In this circumstance, the hydrolases ISA3 and AMY3 release soluble phosphorylated glucans that cannot be completely converted to maltose.

A second glucan phosphatase, LIKE SEX FOUR 2 (LSF2), is also involved in amylopectin dephosphorylation during starch degradation (143, 198). Unlike SEX4, which can remove both 6- and 3-phosphates from glucose residues, LSF2 removes only 3-phosphates. Its loss increases the amount of 3-phosphorylation in starch but has little effect on starch levels except in the absence of SEX4: levels of starch and starch-bound phosphate are higher in *lsf2 sex4* than in *sex4*. A third protein, LSF1—closely related to SEX4 and LSF2—is essential for normal starch degradation but via a different mechanism. Its loss elevates starch levels, and a double mutant lacking both LSF1 and SEX4 has higher starch levels than either parent (25). However, *lsf1* does not accumulate soluble phosphorylated glucans, and recombinant LSF1 has no detectable phosphatase activity. Recent research suggests that LSF1 is a starch-binding protein that forms granule-surface-located complexes with either BAM1 or BAM3 (147). It may thus facilitate the β-amylolytic attack on the granule surface during starch degradation.

In addition to the cycle of phosphorylation and dephosphorylation of the granule surface, normal patterns of starch degradation also require the widely conserved granule-bound protein ESV1. Loss of ESV1 accelerates starch degradation rather than causing starch accumulation. Starch degradation in *esv1* mutants is very rapid early in the night, and starch reserves are exhausted before dawn (50). The ESV1 protein has no known catalytic activity but possesses a large domain of tryptophan-rich near repeats. Genetic analysis shows that it functions upstream of other enzymes of starch degradation, including the starch-phosphorylating and -dephosphorylating enzymes. It is proposed that ESV1 may modulate the structure of the starch granule, perhaps preventing glucan degradation in the absence of phosphorylation. In the *esv1* mutant the granule may be intrinsically

more susceptible to attack, allowing β-amylolytic attack without the control normally exerted by phosphorylation. Recent in vitro studies indicate that ESV1 can modulate the actions of GWD and PWD on starch-like surfaces (111). A second, similar protein, LIKE ESV (LESV), is not required for normal starch turnover, but overexpression results in a vast proliferation of starch granules that may suggest a role in the control of granule initiation (50).

4.1.3. Conversion of maltose to sucrose. Maltose and glucose produced by hydrolytic starch degradation at night are exported from the chloroplast by a maltose transporter, MALTOSE EXCESS 1 (MEX1), and a glucose transporter, plastidic glucose translocator (pGlcT), respectively (23, 127). MEX1 is essential for the normal conversion of starch to sucrose in *Arabidopsis* leaves: *mex1* mutants accumulate exceptionally high levels of maltose in the chloroplast and are defective in chloroplast ultrastructure, photosynthesis, and growth (106, 127, 169). Growth is unaffected in *pglcT* mutants, but *mex1 pglcT* double mutants—presumably blocked in both export routes—have an even more severe phenotype than the *mex1* mutant (23).

Exported maltose is metabolized by a glucosyl transferase, DPE2, which releases one glucose and transfers the other to an acceptor polysaccharide. The importance of DPE2 for conversion of starch to sucrose at night is revealed in the *dpe2* mutant, which—like the *mex1* mutant—accumulates exceptional levels of maltose and is compromised in metabolism and growth (22, 105). The acceptor in vivo is believed to be a heterogeneous cytosolic glycan (known as heteroglycan) composed of several different, variously linked sugars (53). Purified heteroglycan is a good substrate for recombinant DPE2, which can add glucose from maltose to several different terminal sugar residues, including mannose and xylose (52, 141, 168). Glucosylated heteroglycan in turn acts as a substrate for a cytosolic phosphorylase, PHS2, which generates glucose 1-phosphate from terminal glucose residues (53, 107). This glucose 1-phosphate and the glucose exported by pGlcT and released from maltose by DPE2 are the starting points for cytosolic sucrose synthesis.

Despite the fact that a large fraction of plant primary assimilate is metabolized by this pathway, its details remain obscure. Cytosolic heteroglycan is a poorly defined, low-abundance, complex polysaccharide with unknown biosynthetic and degradative pathways, and DPE2 is a multidomain enzyme for which there is limited structural and functional understanding (141, 168). Simplistically, one might suppose that conversion of maltose to sucrose could be achieved by initial hydrolysis of maltose to produce glucose. However, modeling shows that the DPE2/heteroglycan couple may form a glucosyl buffer between starch degradation and sucrose synthesis that allows for short-term mismatches between these two processes and hence more flexible tuning of nighttime metabolism than would be possible if maltose were simply hydrolyzed (141).

4.2. Control of Starch Mobilization in *Arabidopsis* Leaves

Most of our understanding of the control of starch mobilization comes from research in the past decade on leaves of *Arabidopsis* plants grown in highly controlled environments. Such studies have revealed an exquisite regulatory mechanism that operates at a systems level and involves the circadian clock (described in Section 4.2.1). However, it seems unlikely that these discoveries can be extrapolated directly to other circumstances in which starch is degraded in the plant—for example, in natural environments, in plant organs other than leaves, and in different species of plants. We discuss the evidence that the nature and control of starch degradation vary profoundly across the plant kingdom and highlight the need for more effort in these seriously underresearched areas.

4.2.1. Mechanisms in controlled environments. In *Arabidopsis* grown in carbon-limited conditions (light periods of 12 h or less), there are complex controls on both the fraction of photosynthate allocated to starch in the light and the rate of starch degradation in the dark. These

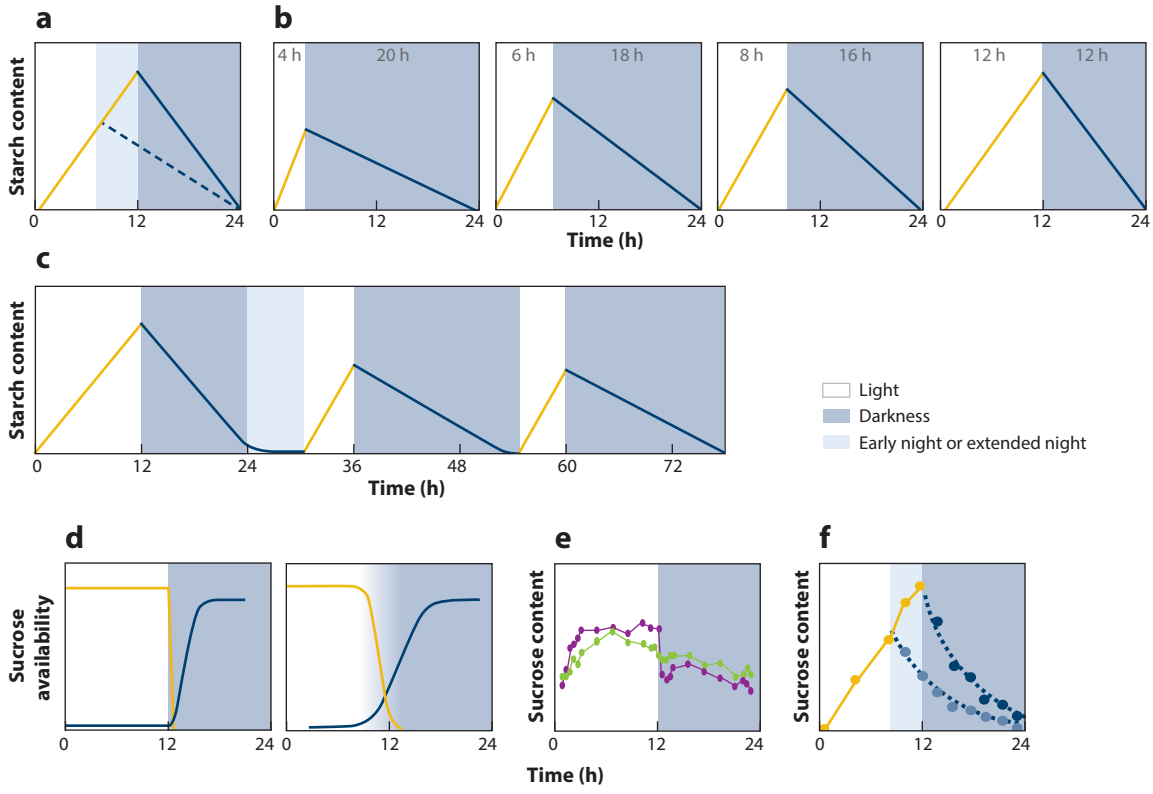

Figure 4

Diel changes in leaf carbohydrates in response to different day lengths. Blue regions represent darkness, ticks on *x* axis are at 12 h intervals and represent time after an initial dawn. (*a*) Diel changes in *Arabidopsis* leaf starch content in plants grown in 12 h light/12 h dark (*solid lines*) then subjected to a single early night (*light blue region*; *dashed line*). Panel *a* adapted from Reference 72. (*b*) Diel changes in *Arabidopsis* leaf starch content in plants grown in different photoperiods, from 4 h light/20 h dark up to 12 h light/12 h dark. Panel *b* adapted with permission from Reference 172. (*c*) The effect on *Arabidopsis* leaf starch content of an extended night (*light blue shading*) and subsequent short days/long nights. Panel *c* adapted with permission from Reference 67. (*d*) Representation of sucrose availability across the day-night transition from photosynthesis (*yellow line*) and starch degradation (*blue line*), in a square-wave light regime (*left*) and a natural light regime (*right*). (*e*) Comparison of the effect on *Arabidopsis* leaf sucrose content of a square-wave light regime (*purple*) and a natural light regime (*green*). Panel *e* adapted from Reference 3 (CC BY 4.0). (*f*) Diel sucrose content in barley leaves grown in 12 h light/12 h dark (*dark blue circles*) then subjected to a single early night (*light blue region and circles*). Dashed lines are fitted curves. Panel *f* adapted with permission from Reference 120.

controls make carbon available for growth during the day and the night, maximizing the fraction of assimilated carbon that can be allocated to growth over 24 h and avoiding periods of starvation that would inhibit growth. The shorter the day, the greater the fraction of photosynthate allocated to starch. At night, starch degradation is adjusted to the anticipated time of dawn to achieve a linear rate that consumes almost all of the starch by dawn (**Figure 4**). Remarkably, these controls permit about 80% of the net assimilated carbon to be allocated to rosette growth (biomass gain) across widely different day-night lengths (ranging from 12 h light/12 h dark to 4 h light/20 h dark) (172).

The adjustment of nighttime starch degradation to match the length of the night is remarkably robust. The appropriate rate is set regardless of long-term variation or immediate, unanticipated changes in the length of the light period (**Figure 4*a–c***) and the amount of starch present in the leaf (72, 150, 172). Correct adjustment of the rate occurs even in conditions of near starvation (117).

lhy: *late elongated hypocotyl*

cca1: *circadian clock associated1*

PRR7: *PSEUDO-RESPONSE REGULATOR7*

The rate is also unaffected by nighttime temperature over a wide range: plants grown at 24°C in the light had essentially the same rates of starch degradation during a 12-h night at 24°C, 16°C, and 12°C (134).

There is a wealth of evidence that the adjustment of the rate of starch degradation to the length of the night requires the circadian clock (72, 104). In the short-period clock mutant *late elongated hypocotyl/circadian clock associated1* (*lhy cca1*), which anticipates dawn well in advance of 24 h, the rate of degradation at night is adjusted to the anticipated rather than the real dawn, and starch reserves are depleted too early (72). Smaller defects in the timing of starch degradation occur in several other classic clock mutants with altered periods (55). Similarly, starch degradation in wild-type plants cannot be adjusted to dawn if plants are grown in day lengths other than 24 h—a hallmark of processes controlled by the circadian clock (72).

Situations in which the timing of depletion of starch reserves is not adjusted to the time of dawn illustrate the importance of such adjustment for plant growth. For example, in both the *lhy cca1* mutant grown in 24-h days and wild-type plants grown in 28-h days, the exhaustion of starch reserves before dawn leads to expression of so-called starvation genes and slowing or cessation of growth before the end of each night. As a consequence, these plants grow more slowly than those in which starch reserves last until dawn even though they receive the same amount of light over their lives (72).

How does the clock adjust the rate of starch degradation? The transcripts encoding many of the pathway enzymes undergo large diel fluctuations, peaking at the end of the day (166). However, in general, the enzymes themselves show little diel change in abundance and have long half-lives (e.g., 104, 164, 166, 201). Transcript-level fluctuations are thus unlikely to bring about rapid, short-term adjustments to flux through the pathway. Adjustments are probably at a posttranslational level, indirectly influenced by the circadian clock.

To gain insight into mechanisms that could link clock function to posttranslational adjustment of starch degradation, two general types of model [the arithmetic division (AD) model and the retrograde metabolic signaling (RMS) model] (117) have been developed. These have not yet led to identification of specific components of control mechanisms, but they provide hypotheses to guide further experimentation. The AD model can account for the rapid adjustment of the rate of degradation to unexpected changes in starch content and the length of the night (**Figure 4*a***). It is proposed that mechanisms in the leaf track both starch content and time remaining until dawn. During the night, arithmetic division of starch content by time until dawn then sets a rate of starch degradation that will result in exhaustion of reserves at dawn. In a simple iteration of the model, an entity *S* that represents starch content activates enzyme(s) of starch degradation at the granule surface, and an entity *T* that represents time remaining until dawn prevents this activation (149–151).

The RMS model assumes a need to maintain sugar homeostasis. This is achieved by the modulation by sugar signaling of the expression of circadian clock genes and hence the phase of the clock. The clock then sets rates of starch synthesis and degradation so that sugar homeostasis is maintained (196). Unlike the basic AD model, this model provides an integrated mechanism for control of both synthesis and degradation of starch. Evidence for such a mechanism comes from demonstrations that circadian clock genes are sensitive to changes in sugar status. For example, sugars inhibit expression of the clock gene *PSEUDO-RESPONSE REGULATOR7* (*PRR7*), which in turn alters expression of the CCA1 component of the central circadian oscillator and thus changes the phase of the clock (37, 57, 74, 75, 160).

Recent studies provide further insights into connections between the control of starch degradation, sugar status, and the circadian clock. For example, a single day of very low carbon assimilation resulted in large adjustments to the rate of nighttime starch degradation without significant

effects on the expression of clock genes (117), a result consistent with the AD rather than the RMS model. However, elevation at night of the sugar-signaling metabolite trehalose 6-phosphate reduced the rate of starch degradation (45, 112), indicating that high sucrose levels can modulate the mechanism that controls degradation. Whether this modulation occurs via a phase change in the clock is not yet clear.

The rate of leaf starch synthesis is subject to poorly understood controls that maximize daytime partitioning of assimilated carbon into starch when carbon availability at night is low. The effect of day length on partitioning of assimilate into starch is particularly profound (115). *Arabidopsis* plants partition 30–40% of their assimilate into starch following a short night, but up to 60% following a long night (67, 115) (**Figure 4*b*,*c***). It appears that long nights generate signals—including gibberellin signaling—that restrict growth and sucrose export from leaves during the following day (115, 127a). These restrictions cause a buildup of sucrose and related intermediates, leading to diversion of photoassimilate from sucrose to starch synthesis.

4.2.2. Additional complexity in natural environments. Most experiments on starch turnover in *Arabidopsis* leaves have used square-wave light regimes, in which starch degradation appears to be absent or minimal during the light period and is switched on only following the transition to darkness (51, 203). The abrupt light-dark transition causes a major hiatus in sugar supply: the supply of sucrose from photosynthesis stops immediately, but it takes an hour or more to establish a steady sucrose supply from starch degradation (**Figure 4*d*,*e***). Sucrose and sucrose phosphate levels drop abruptly after the transition, and there are transient spikes in levels of other primary metabolites (3) and a transient drop in the capacity for protein synthesis (133).

In natural light regimes, by contrast, light-dark and dark-light transitions occur gradually. Estimates of starch turnover reveal that as photosynthesis and hence starch synthesis decline toward dusk, starch degradation is progressively switched on. Loss of sucrose production from photosynthesis is thus compensated by increasing production of sucrose from starch degradation, and there is no perturbation of sucrose levels during the transition (3, 4, 51, 56, 152) (**Figure 4*d***). Preliminary evidence suggests that the circadian clock may gate the initiation of degradation so that it occurs in response to falling rates of photosynthesis at the end of the day but not at other times of the day (51).

4.3. Starch Turnover in Leaves of Other Species

Analyses of transcriptomes show that the genes encoding enzymes of starch turnover described for *Arabidopsis* are expressed in essentially all species examined so far. In some instances, the importance of these enzymes for leaf starch turnover has been confirmed through discovery of mutants with altered leaf starch contents (80, 100, 190). However, leaf starch turnover varies greatly among different groups of plants. In leaves of many grasses, diel starch turnover is small, but there is substantial diel turnover of sucrose (5, 21, 70, 120). In barley leaves, for example, much more carbon is accumulated as sucrose than as starch during the day. At night the sucrose content decays in an exponential fashion, at a rate that is sensitive to temperature (70, 120) (**Figure 4*f***). More research is required to elucidate the basis and implications for growth of these profound interspecific differences in leaf starch turnover.

4.4. Factors Triggering Leaf Starch Degradation

In addition to the diel controls of leaf starch synthesis and degradation described above, starch degradation can be triggered by acute environmental stresses during the day. The products of degradation—sugars and downstream metabolites—ameliorate the impact of the stress.

BLUS1: BLUE LIGHT SIGNALING1

HA1: H^+-ATPASE1

Conditions that trigger daytime degradation include cold shock, osmotic shock, and imposition of extreme photorespiratory conditions (90, 183, 197). In the case of acute osmotic stress, starch synthesis in *Arabidopsis* leaves is maintained but starch is simultaneously degraded, allowing increased export of sugars to the roots for osmotic adjustment (183). This response is mediated by transcriptional activation of the starch-degrading enzymes BAM1 and AMY3 via abscisic acid response pathways, and probably also by posttranslational enzyme modifications. BAM1, AMY3, and several other enzymes of starch degradation (LDA, GWD, and SEX4) can be activated through reduction of regulatory disulfide bonds—a feature more typical of chloroplastic enzymes that are activated in the light by the light-driven ferredoxin-thioredoxin system (68, 157, 165). The significance under normal conditions in vivo of reductive activation of enzymes of starch degradation remains to be discovered.

Starch degradation in stomatal guard cells is activated by light, so it commences at dawn rather than with the onset of darkness (81, 144). Blue light sensed by phototropins activates extremely rapid starch degradation catalyzed by BAM1 and AMY3, both of which are highly expressed in guard cells. Degradation products are presumably converted into sugars and/or organic acids and transported to the vacuole as part of the mechanism for increasing cell turgor, thus increasing the aperture of the pore. Activation of the degrading enzymes requires the blue-light signaling intermediate BLUE LIGHT SIGNALING1 (BLUS1; a protein kinase) and the activation of its target, the plasma-membrane proton pump H^+-ATPASE1 (HA1) (81). However, the link to the starch degradation machinery has yet to be elucidated.

4.5. Starch Turnover in Nonphotosynthetic Organs

Most nonphotosynthetic plant cells accumulate and degrade starch at some point in their histories. In *Arabidopsis*, starch occurs in tissues including the root columella, stem, pollen, petals, anthers, and young embryos and testas (19). Starch content in these tissues is affected in several of the starch mutants described above, indicating that components of the leaf pathways of starch metabolism are also important elsewhere in the plant (e.g., 1, 18, 34, 50). In at least some parts of the plant, the mutant phenotypes reveal that starch is continuously turned over (1, 50). Starch turnover may buffer short-term mismatches between the import and the consumption of carbohydrate in nonphotosynthetic organs, ensuring that carbohydrate is continuously available for maintenance and growth.

The physiological context and the pathway of starch degradation in the endosperm of cereal grains are radically different from those in *Arabidopsis*. Starch synthesis in endosperm amyloplasts of developing grain is similar in many respects to that in *Arabidopsis* leaves (except for the location of ADP-glucose synthesis; see Section 3.1). However, during grain maturation, the endosperm cells die, leaving the starch granules encapsulated in a matrix of cell walls.

During germination starch granules are hydrolyzed to glucose by only four enzyme activities. The primary hydrolytic enzyme is α-amylase, which is synthesized in and secreted from the living scutellum and aleurone cells surrounding the endosperm. In many cereals α-amylase enters starch granules via tiny pores and attacks the interior. It is an endoamylase, capable of attacking α-1,4 linkages throughout amylopectin molecules. The products of its action on starch polymers are glucose, maltose, and short oligosaccharides, many of which contain α-1,6 linkages (α-limit dextrins). The oligosaccharides can be further attacked and converted to glucose by β-amylase, maltase, and limit dextrinase. The latter two enzymes are synthesized in aleurone cells, whereas β-amylase accumulates in the endosperm during grain development and is activated following imbibition of the mature grain. Glucose derived from starch hydrolysis is taken up by the scutellum, for use in embryo growth (54).

The rate of conversion of starch to glucose in the endosperm of domesticated cereals is determined largely by the rate of synthesis and release of α-amylase from surrounding cells. Endosperm cell wall hydrolysis may also be important because it can affect the rate of α-amylase diffusion through the endosperm (2). Cereals have been bred for high grain starch contents and—in the case of malting barley—activities of hydrolytic enzymes that probably far exceed the requirements for germination. Embryo growth in barley, for example, is unaffected by loss or strong reductions in activity of either β-amylase (89) or maltase (167). Limitations on starch mobilization may be very different in the endosperm of wild grass species.

For many other starch-storing organs—for example, tubers and rhizomes, unripe fruits, the cambial rays of deciduous trees, and legume seeds—the pathways of starch turnover are not known, despite the likely importance of these reserves for plant performance. Nonetheless, transcript data indicate that the basic pathway of starch-to-sucrose conversion described for *Arabidopsis* leaves may be widely conserved (161, 188). More systematic studies of starch turnover in these organs are required—bringing together transcript levels, enzyme activities, metabolite and protein levels, and fluxes and taking into account growth rates.

SUMMARY POINTS

1. Starch granules are semicrystalline structures composed of α-1,4- and α-1,6-linked glucose polymers. All granules consist primarily of alternate crystalline and amorphous layers with a 9–10-nm repeat, formed by the branched polymer amylopectin. The layers may be organized into concentric growth rings hundreds of nanometers wide. Overall granule size and shape vary widely between species and organs.
2. The pathway of starch synthesis is well conserved across the plant kingdom. Inside plastids, ADP-glucose is the substrate for synthesis of the starch polymers by four or five different isoforms of starch synthase, acting at the surface of or within the growing granule. Branch points are introduced into amylopectin by starch-branching enzymes, then selectively removed by debranching enzymes to create a structure that can crystallize.
3. The branching pattern of amylopectin is determined by the concerted actions of isoforms of starch synthase and branching enzymes with distinct properties. In vivo, they may be organized into various complexes that modulate their activities.
4. Recent research has uncovered a suite of proteins essential for the normal initiation of starch granules in *Arabidopsis* leaves. These proteins—which include the starch synthase isoform SS4—are colocated in complexes found in discrete regions of the thylakoid membrane.
5. Starch reserves are mobilized to provide carbon for maintenance and growth when photosynthesis is absent or limited (e.g., at night, during germination, and following dormancy). The process of starch degradation is best understood in *Arabidopsis* leaves, where starch made during the day is mobilized during the following night.
6. In *Arabidopsis* chloroplasts, starch granules are degraded by the concerted actions of β-amylases, D-enzyme, and debranching enzymes to generate maltose and glucose, which are exported to the cytosol for sucrose synthesis. Degradation requires phosphorylation and dephosphorylation of the granule surface, which render the surface polymers accessible to the hydrolytic enzymes. Several other enzymes and proteins have been implicated in degradation, but their roles are not yet fully understood.

7. Nighttime starch degradation is controlled so that reserves are mobilized at a constant rate and are almost completely consumed at dawn. The rate of degradation is flexibly adjusted in response to variation in starch content and night length and is buffered against changes in night temperature. The rate is set in part by the circadian clock. The control mechanism is not fully understood, and various models have been proposed.
8. There is good evidence that both the nature and the control of the pathway of starch degradation differ profoundly between species and organs.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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