Biochemistry and Genetics of Starch Synthesis

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Abstract

Enormous progress has been made in understanding the genetics and biochemistry of starch synthesis in crop plants. Furthermore, starch remains at the very epicenter of the world's food and feed chains and has even now become one of the world's most important sources of biorenewable energy (biofuel). Yet, despite this remarkable progress and the obvious economic importance, very little has been achieved in terms of adding value to starch or increasing starch yield, particularly in cereal crops. Here, we review the genetics and biochemistry of starch synthesis in crop plants, particularly maize. With all this know-how in place and a chasm of opportunity ahead, the time is right to see science deliver progress into a new frontier. Thus, in our view the stage is set for a new era of changes in starch synthesis, delivering enhancements in functionality and yield.

INTRODUCTION

Globally, starch is an essential commodity that is valued for its uses in food, feed, fuel, and industry. Because starch provides more than 80% of the world's calories, it makes an unparalleled contribution to our food. Advances in genetics and biochemistry have led to significant discoveries in how starch is synthesized in crop plants. Furthermore, research has unraveled much of the biochemical and genetic basis of some useful natural genetic variations that affect starch deposition. Commercial exploitation of some of these variants includes types that cook to form clear sols rather than opaque gels (e.g., waxy corn, waxy rice, waxy wheat), others that are valued for imparting stickiness when cooked (e.g., indica- versus japonica-type rice) or are useful industrially (e.g., amylose extender corn), and finally others that accumulate less starch and more sugar (e.g., sweet peas, sweet corn, sweet potato). Thus, significant progress has been made in our understanding of the relationships between starch synthetic genes and enzymes and how these link to starch structure and functionality. For additional information, the reader is referred to previous reviews (Kossmann & Lloyd 2000, Nakamura 2002, Tomlinson & Denyer 2003, Ball & Morell 2003, Klucinec & Keeling 2005, Ball & Deschamps 2009).

This review seeks to pull together the disciplines of biology, biochemistry, and genetics in relation to the process of starch biosynthesis and storage in plants. The review is biased toward describing what is known about starch in cereal crops, particularly maize. First, we summarize the biological processes that come together to enable starch storage in plants. Second, we review the biochemistry of starch synthesis, focusing on the key enzymes involved in starch assembly. Next, we review what is known about the genetics of starch synthesis enzymes. Finally, we discuss how this multi-disciplinary progress is leading to future opportunities to increase crop yield and engineer crops with advanced starches having enhanced functionality and value.

Unique Glucan Biopolymer

Starch is a unique glucan biopolymer. It is found in higher plants, mosses, ferns, and some microorganisms. Starch serves as an important store of energy that is captured by plants using sunlight, water, carbon dioxide, and soil nutrients (**Figure 1**). Higher plants synthesize and store starch in

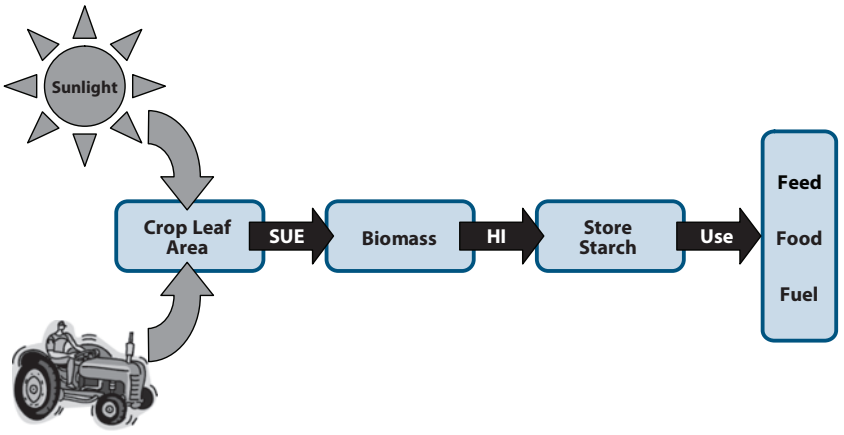


Figure 1

Process of glucan biopolymer storage. The diagram illustrates that the amount of biomass captured by leaves is dependent on sunlight utilization efficiency (SUE) and that this combined with harvest index (HI) gives rise to starch stored in grain or tubers.

the form of granules in storage tissues such as seeds and tubers and in a transitory form in leaves, roots, and stems. Starch is one of the main components of tuber crops such as potato, cassava, and yam as well as grain (seeds) harvested from cereal crops such as maize, wheat, barley, oats, sorghum, and rice. Starch is also an important component of many fruit crops such as melon, apple, pear, tomato, and banana. As well as having valuable functional properties (Klucinec & Keeling 2005), this glucan biopolymer represents a significant proportion by dry weight of starch crop yield.

Normal starch consists of semicrystalline granules, which, after extraction and drying, make a white powder. Starch granules are microscopic structures approximately 0.5–100 μm in diameter. In shape, they are spherical, elliptical, or polyhedral. The size and morphology of starch granules is characteristic of the organ and species in which they are produced (Jane et al. 1994). Starch granules appear similar in size and morphology with and without amylose and can be considered moderately inert with little capacity to hold water under most environmental conditions. Whether in grain or tubers or from processed isolated starch, these characteristics of starch granules make them ideal vessels for storage and shipping.

Glucan chain length: glucans are extended by starch synthase into linear α-1,4-glucan chains having a degree of polymerization (DP) that defines the chain length

Amylose and Amylopectin

Starch is composed of two polymers of glucose in the form of amylose and amylopectin. On a dry weight basis, the starches isolated from storage tissues such as potato tubers or cereal grain are normally made up of between 20–30% amylose and 70–80% amylopectin. Both of these glucan biopolymers are composed of clusters of an alternating pattern of linear α-1,4-glucan chains interspersed by regions of branch points owing to α-1,6-glucosidic linkages (see sidebar, Branched Chain Distribution). When the α-1,6-glucosidic linkages are infrequently dispersed in a polymer of approximately 100–10,000 glucosyl residues, the polymer is referred to as amylose. When the α-1,6-glucosidic linkages are more frequently and regularly dispersed in a polymer of approximately 10,000–100,000 glucosyl residues, the polymer is referred to as amylopectin. Amylopectin is required for normal starch granule formation, as evidenced by the fact that varied granule morphologies result from disruptions in amylopectin synthesis. Amylose content is not a requirement for normal granule formation, as starch granule morphology is similar even with widely varying amylose contents. These findings are supported by detailed structural analysis of starch granules, where it is clear that placement of branch linkages relative to one another is not random. Instead, these are clustered in a repeating manner, such that distinct regions of relatively high branch frequency alternate with other areas nearly devoid of branches. Additionally, the clustered linear chain regions have a distribution of chain lengths averaging approximately 12 units with an upper limit of 20–30 units. These architectural features are critical for starch function. The crystalline packing of glucosyl units within starch granules and the exclusion of water molecules allows storage of carbohydrates at much higher density than is possible in glycogen. Between species there is variation in the structure of amylopectin, the size and structure of amylose (Takeda et al. 1987, 1989), and the nature and amounts of proteins and lipids (Morrison 1988,

BRANCHED CHAIN DISTRIBUTION

Glucans that are branched by a branching enzyme form a non-linear polymer with a mixture of α-1,4-glucan chains interspersed by α-1,6-glucan branch points. The positioning of the branch points relative to other branch points defines a clustering of branches referred to here as the branched chain distribution.

Klucinec & Keeling 2005). More information on starch structure and functionality can be found in Delcour et al. (2010).

dap: days after pollination

Storage Organs

Fruits, seeds, and tubers represent remarkable storage organ adaptations in plants in order to store energy and nutrients. When present in these organs, starch accumulates during the process of storage organ development and is relatively stable for prolonged periods if kept dry. Remarkably, once wet, starch can be readily degraded to release its energy-rich store of sugars in the form of dextrins and glucose. In leaves, starch is stored transiently during daylight hours and degraded at night to support plant metabolism. Fruits are produced by plants during reproduction, giving rise to a storage organ composed of a fleshy fruit structure surrounding the seeds. Seeds are produced by plants during reproduction, giving rise to a storage organ composed of the embryo, endosperm, and seed coat or pericarp. In seeds, most of the starch is stored in the endosperm tissue, although some starch is stored (and later degraded during seed maturation) in the embryo and pericarp. Tubers are produced underground by plants as a modification of their root structures. Seeds become relatively dry during their maturation process (10–20% moisture), which contrasts with tubers that remain relatively high in water content (50–80% moisture). During the process of starch deposition in seeds or tubers, their moisture content is relatively similar (60–90% moisture). Fruits are typically sweet and vary widely in amounts of stored starch, with banana being most notable for its high starch content. In cereal crops like maize, rice, and wheat as well as in tuberous crops like potato, cassava, and yam, the starch-storage tissue represents approximately 70% of the dry weight of the seed or tuber and is composed primarily of starch (approximately 90% dry weight).

Cereal endosperm development has been described as composed of several steps, including a coenocytic phase, cellularization, differentiation, reserve synthesis, and maturation (Olsen 2001). Following pollination, endosperm nuclei undergo numerous divisions without any cellularization. This is referred to as the coenocytic phase, which is followed by cell wall formation from approximately 4 days after pollination (dap) until 10 dap. Cell division continues until approximately 20 dap. Differentiation is followed by the reserve synthesis phase, when most of the starch present in the endosperm is synthesized. This occurs from approximately 12 dap until 35 dap, after which time the seed starts to mature and dry-down.

Intracellular Compartmentation

In general terms, it is important to note that storage organs are composed of individual starch-storing cells (see sidebar, Transitory Starch Metabolism). Each cell contains several subcellular compartments including the nucleus, cytosol, mitochondria, vacuole, and plastid compartments. All plants accumulate starch in plastids, called chloroplasts, in leaves or amyloplasts in storage tissues. Sucrose, made in the leaves, is transported to the storage organ, where it is imported into the cytosolic compartment of each cell. A well-characterized pathway (**Figure 2**) of starch synthesis achieves the enzymatic conversion of sucrose to starch using enzymes that are encoded by nuclear genes. The first part of this pathway is localized in the cytosol, whereas the final steps are located in the amyloplast.

In the cytosol, the glucosyl and fructosyl moieties of sucrose are released by sucrose synthase (SUS) to form fructose and UDP-glucose that are next converted into hexose phosphates by the enzymes fructokinase (FRK) and UDP-glucose pyrophosphorylase (UGPP). The finding that SUS action may involve the formation of ADP-glucose as well as UDP-glucose (Baroja-Fernández et al.

TRANSITORY STARCH METABOLISM

Recently, transitory starch metabolism in plants has seen an exciting phase of research leading to many new discoveries (Smith et al. 2005, Fettke et al. 2009, Sulpice et al. 2009). During the day, starch and sucrose are synthesized together as the products of photosynthetic carbon assimilation in leaves. Sucrose is exported to nonphotosynthetic parts of the plant, and transitory starch accumulates in the chloroplasts. Degradation of transitory starch in plants results mainly in the formation of neutral sugars, such as glucose and maltose, that are transported into the cytosol via their respective translocators. The cytosolic metabolism of the neutral sugars includes the action of a hexokinase, a phosphoglucomutase, and a transglucosidase that utilizes high molecular weight glycans as a transient glucosyl acceptor or donor. Combining this new understanding with profiling studies has led to new hypotheses that describe variation in a regulatory network that balances growth with the carbon supply.

2003) requires further investigation. Early in seed development, invertase also cleaves sucrose into hexoses (Koch 2004). The hexose phosphates are readily interconverted by phosphoglucose isomerase (PGI) and phosphoglucomutase (PGM) in a process that includes some recycling through glycolysis and other metabolic pathways (Keeling et al. 1988, Glawischnig et al. 2002). Glucose-1-phosphate is next converted into ADP-glucose by the enzyme ADP-glucose pyrophosphorylase (AGPP), the first committed step in starch synthesis. In the endosperm of cereal crops, AGPP is located predominantly in the cytosol and ADP-glucose is transported directly into the amyloplasts. In contrast, in the storage organs of other starch-rich crops, AGPP appears to be located exclusively in amyloplasts and hexose phosphate is imported to support ADP-glucose synthesis inside the amyloplasts (Comparot-Moss & Denyer 2009). In a minor pathway, hexose phosphate also is transported in cereal endosperm and converted to ADP-glucose by a second AGPP located within amyloplasts. Multiple enzymes are required for conversion of ADP-glucose to starch (**Table 1**). These enzymes include, but may not be limited to, soluble starch synthases (SSs), granule bound starch synthase (GBSS), starch branching enzymes (SBE), and starch debranching enzymes (DBE) such as isoamylases (ISA) and pullulanase (PU). GBSSI is involved primarily in amylose biosynthesis, whereas SSs, SBEs, and DBEs are primarily responsible for amylopectin biosynthesis. Other enzymes reported to play a role in starch synthesis in at least some species include starch phosphorylase (SP) and disproportionating enzyme (DisPE).

Coordinated Expression

Enzymes in developing endosperm are coordinately expressed such that the temporal patterns of expression are similar and reveal a sharp increase and later gradual decrease in their activities during the linear phase of grain filling (**Figure 3**). This seemingly simple process is much more complex when viewed in the wider context of the functional genome as well as the complex array of amyloplast-specific proteins (DuPont 2008). This was best exemplified in a study of the maize endosperm proteome that revealed four general temporal expression patterns during the course of development (Mechin et al. 2007). Reserve synthesis occurred at 10–35 dap, when the carbohydrate, storage protein, and amino acid metabolism enzymes accumulated. The mechanisms controlling this coordinated expression are not completely understood, but are determined by transcriptional regulation of expression of the proteins that has begun to be studied using a transcriptome analysis of cDNA libraries (Lai et al. 2004), together with our increasing understanding of the roles played by transcription factors (Sun et al. 2003).

AGPP: ADP-glucose pyrophosphorylase

SS: starch synthase

SBE: starch branching enzyme

DBE: debranching enzyme

Table 1 List of starch synthesis enzymes, together with EC numbers, formal names, and isoforms together with structural match enzymes identified using PHYRE (Kelley & Sternberg 2009). Each structural match enzyme is listed with the PDB identifier in parentheses. The percentages represent the amino acid homology

Enzyme	EC number	Formal name	Class/Isoform	Structural match (PDB#%) with 100% fold recognition
Sucrose synthase	EC:2.4.1.13	NDP-glucose:D-fructose 2-α-D-glucosyltransferase	SUS-Sh1, SUS1, SUS2	Trehalose synthase (1uqt 11%)
UDP-glucose pyrophosphorylase	EC:2.7.7.9	UTP:α-D-glucose-1-phosphate uridylyltransferase	UGP1, UGP2	UGPP (2icy 14%)
Fructokinase	EC:2.7.1.4	ATP:D-fructose 6-phosphotransferase	FRK1, FRK2	Gluconokinase (2qcv 28%)
Phosphoglucose isomerase	EC:5.3.1.9	D-glucose-6-phosphate aldose-ketose-isomerase	PGI1	PGI (1gzd 45%)
Phosphoglucomutase	EC:5.4.2.2	α-D-glucose 1,6-phosphomutase	PGM1, PGM2	PGM (1kfi 53%)
Glucose phosphate transporter	No EC #	No formal name	GPT	Lactose permease (2cfq 6%)
ADP-glucose transporter	No EC #	No formal name	Bt1, Bt1-2	ADP/ATP carrier (1okc 21%)
ADP-glucose pyrophosphorylase	EC:2.7.7.27	ATP:α-D-glucose-1-phosphate adenylyltransferase	Bt2, Sh2, AGP1, AGP2	AGPP (1yp2 88%)
Starch synthase	EC:2.4.1.21	ADP-glucose:(1→4)-α-D-glucan 4-α-D-glucosyltransferase	GBSSI, SSI, SSII, SSIII, SSIV	Glycogen synthase (1rzu 30%)
Branching enzyme	EC:2.4.1.18	(1→4)-α-D-glucan:(1→4)-α-D-glucan 6-α-D-[(1→4)-α-D-glucano]-transferase	SBEI, SBEIIa, SBEIII	Branching enzyme (1m7x 23%)
Debranching enzyme	EC:3.2.1.68 and EC:3.2.1.41	glycogen α-1,6-glucanohydrolase and α-dextrin endo-1,6-α-glucosidase	ISA1, ISA2, ISA3, PU1	Isoamylase (1bf2 28%)
Starch phosphorylase	EC:2.4.1.1	(1→4)-α-D-glucan:phosphate α-D-glucosyltransferase	SP_H, SP_L	Glycogen phosphorylase (2gj4 46%)

*PU1 closest fold recognition is a pullulanase (2fh6), but still 100% to isoamylase (2gj4).
**SP_L closest fold recognition is a maltodextrin phosphorylase (1l5w).

Ancient Origins

Studies of the enzymes of glucan biosynthesis in plants and algae show that the processes have very ancient origins. For example, the glucan biosynthetic enzymes present in modern day crops are also found in ancient red and green algae (Ball & Deschamps 2009). Plastidial starch biosynthesis appears to have evolved when the enzymes for starch biosynthesis first appeared in the red and green algae more than 1500 million years ago (Deschamps et al. 2008a). Thus, starch synthesis that began in the cyanobacteria was transferred to plants with the endosymbiotic events that led to

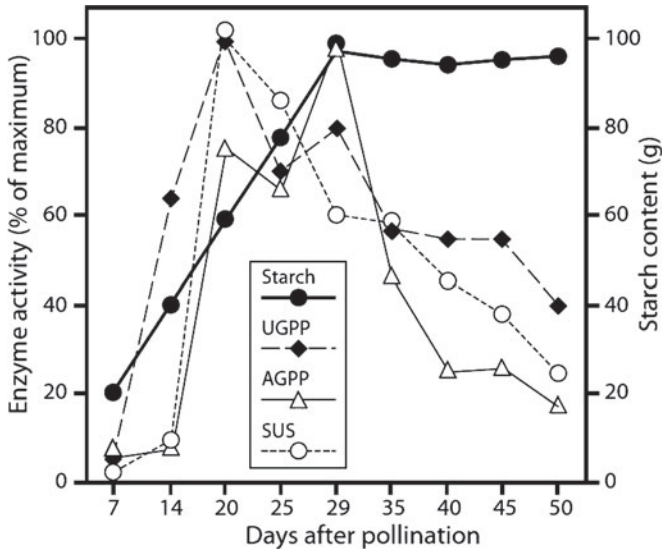


Figure 3

Starch content and enzyme activities in developing maize endosperm sampled after pollination until physiological maturity. The diagram indicates that activities of various enzymes of starch biosynthesis increase rapidly until approximately 20 days after pollination (dap). This coincides with a linear increase in starch content until 29 dap when starch accumulation ceases (Keeling 1999).

the formation of chloroplasts (Deschamps et al. 2008b). Remarkably, in cyanobacteria and modern crop plants the structures of these enzymes and their functional roles have been preserved over the eons of time, even to the extent that algal isoforms can substitute for crop plant isoforms (Sawada et al. 2009).

BIOCHEMISTRY

Recently, there has been considerable progress in using protein fold recognition (see sidebar, Protein Fold Recognition) algorithms to match specific proteins to known tertiary structures. This, combined with the dramatic increase in protein sequencing, has allowed a classification of enzymes into hierarchical biochemical databases composed of folds, superfamilies (clans), families, and protein domains (see sidebar, Protein Domains) within specific primary structures. These classifications provide invaluable insights into enzyme evolution and reveal structural similarities

PROTEIN FOLD RECOGNITION

Protein fold recognition uses the protein homology/analogy recognition engine (PHYRE). The models produced by PHYRE are based on finding a sequence alignment to a known structure, copying the coordinates, and relabeling the residues into a probability profile [or position-specific scoring matrices (PSSMs)] having a specific secondary structure according to the given sequence (based on the alignment). PHYRE then uses a profile-profile matching algorithm together with the predicted secondary structure to create a match to known structures. PHYRE can recognize similar secondary structures having only remotely homologous sequence alignments that cannot be found by conventional methods.

PROTEIN DOMAINS

Protein domains are three-dimensional structures that form part of protein sequence. Highly conserved domain structures can exist in many different yet related proteins. The Protein Families (Pfam) database (**http://pfam.janelia.org/**) is designed to be a comprehensive and accurate collection of protein domains and families. Pfam is based on UniProtKB and NCBI GenPept sequence databases.

that are not immediately obvious based solely on primary structure and homology. This section brings biochemical knowledge together with information from these sources, including the protein homology/analogY recognition engine (PHYRE) (Kelley & Sternberg 2009), the protein data bank (PDB), the structural classification of proteins (SCOP) database (Andreeva et al. 2004), the carbohydrate active enzymes (CAZY) database (Cantarel et al. 2009), and the protein families (PFAM) database (Finn et al. 2008). Additional information can be found in the enzyme (BRENDA) database (Chang et al. 2009).

Multiple enzymes are involved in the pathway of starch synthesis (**Figure 2**). Using the PHYRE recognition engine, we have summarized specifically identified protein homologs (**Table 1**). These protein homologs all had a 100% fold recognition yet widely varying (6% to 88%) primary sequence homology. This is because many of the enzymes involved in starch biosynthesis have not yet been crystallized and hence lack a known topology. Yet, structural comparisons made possible by analogy with known enzyme structures (e.g., using PHYRE) reveal their likely topology. We have summarized these structures in the following sections, together with specific domain information where it is available from SCOP, CAZY, and PFAM.

Sugar Phosphate Metabolism

Sucrose transported from leaves is catabolized in storage tissues into sugar phosphate intermediates using SUS, UGPP, FRK, PGI, and PGM. Some structural homology and similar topological features are shared by SUS and UGPP, allowing them to be classified as part of the glycosyl transferase (GT) superfamily (Liu & Mushegian 2003). The other sugar phosphate conversion enzymes are members of distinct families in the SCOP and PFAM databases.

SUS reversibly catalyzes the formation of sucrose from fructose and a nucleotide sugar. Within the GT superfamily, SUS is a member of the specific family denoted GT-5, and thus shares some structural similarities with SSs (see below). The enzyme is predicted to comprise two highly conserved regions incorporating the sucrose synthase domain (PF00862) and a glycos_transf_1 domain (PF00534), as illustrated by the known structure of trehalose synthase (**Figure 4**; **Table 2**). In plants, there are multiple SUS isoforms that differ in their timing of expression and/or tissue localization.

FRK irreversibly catalyzes the formation of fructose 6-phosphate from fructose and adenosine triphosphate (ATP). FRK belongs to the actin-like ATPase superfamily (CL0108), being a member of the hexokinase family of enzymes. Structurally, the functional enzyme has two highly conserved regions incorporating the hexokinase-1 and hexokinase-2 domains (PF00349 and PF03727) shown in the structural diagram (**Figure 5**) (Kuser et al. 2000). In plants, there are at least two different isoforms: FRK1 and FRK2.

GT: glycosyl transferase

ATP: adenosine triphosphate

PGI catalyzes the interconversion of fructose 6-phosphate and glucose 6-phosphate. PGI belongs to the SIS superfamily (CL0067), having a phosphosugar binding domain (Bateman 1999),

Table 2 List of enzymes, isoforms, genetic loci, and NCBI GenBank GeneID numbers for genes involved in the pathway of starch synthesis in maize endosperm. The letters in parentheses denote specific enzyme isoforms or genetic loci such as *Shrunken* (*Sh*), *Brittle* (*Bt*), *Waxy* (*Wx*), *Sugary* (*Su*), and *Dull* (*Du*). The order shown for each class/isoform is maintained for the genetic loci and GenBank ID numbers

Enzyme	Class/Isoforms	Genetic locus *	GenBank ID numbers
Sucrose synthase	SUS-Sh1, SUS1, SUS2	*Shrunken1* (*Sh1*), Sus1-1, n.d.	542365, 542247, 542091
UDP-glucose pyrophosphorylase	UGP1, UGP2	n.d., n.d.	100285949, n.d.
Fructokinase	FRK1, FRK2	n.d., n.d.	542107, 542108
Phosphoglucose isomerase	PGI1	n.d.	542313
Phosphoglucomutase	PGM1, PGM2	n.d., n.d.	542721, 542358
Glucose phosphate transporter	GPT1	n.d.	542599
ADP-glucose transporter	Bt1, Bt1-2	*Brittle1* (*Bt1*), *Bt1-2*	732804, 54651581ψ
ADP-glucose pyrophosphorylase	Bt2, Sh2, Agp1, Agp2	*Brittle2* (*Bt2*), *Shrunken2* (*Sh2*), n.d., n.d.	541902, 542761, 100101531, 542072
Starch synthase	GBSSI, SSI, SSIIa, SSIII, SSIV	*Waxy* (*Wx*), n.d., *Sugary2* (*Su2*), *Dull1* (*Du1*), n.d.	541854, 541669, 542481, 541657, 100170236
Branching enzyme	SBEI, SBEIIa, SBEIIb, SBEIII	*Sbe1*, *Sbe2a*, *Amylose Extender* (*Ae*), n.d.	542315, 542342, 542238, 100136878
Debranching enzyme	ISA1, ISA2, ISA3, PU1	*Sugary2* (*Su2*), n.d., n.d., zpu1	542318, 542679, 542095, 541711
Starch phosphorylase	SP_H, SP_L	n.d., *Shrunken4* (*Sh4*)ξ,	100285259, 100170240

*Gene loci are indicated when forward and/or reverse mutations are known that affect the indicated protein. ξ, *Sh4* controls expression of SP_L and may or may not be the structural gene. n.d., not yet determined. Ψ, ID numbers are from the NCBI Protein or Nucleotide Database.

and is a member of the PGI family of enzymes (Davies & Muirhead 2002). Structurally, the functional enzyme has a single highly conserved PGI region incorporating the PF00342 domain shown in the structural diagram (**Figure 5**). In plants, there are at least two different isoforms: PGI1 and PGI2.

PGM catalyzes the reversible isomerization of glucose 1-phosphate and glucose 6-phosphate. These enzymes constitute a family within the CL0108 superfamily. The enzyme structure exhibits multiple highly conserved regions incorporating the PGM1, PGM2, PGM3, and PGM4 domains (PF02878, PF02879, PF02880, and PF00408, respectively) shown in the structural diagram (**Figure 5**) (Liu et al. 1997). At least two different isoforms, designated PGM1 and PGM2, exist in plants.

Nucleotide Sugar Metabolism

Plants metabolize nucleotide sugars using UGPP and AGPP. These two enzymes catalyze the formation of glucose 1-phosphate and a nucleotide triphosphate from pyrophosphate and UDP-glucose or ADP-glucose, respectively. Both UGPP and AGPP are members of the GT-A superfamily of enzymes. Structurally, the functional UGPP and AGPP enzymes each have a single highly conserved N-terminal catalytic region, each incorporating a different nucleotidyl transferase domain (PF01704 and PF00483, respectively), together with a C-terminal domain made up of a parallel beta helix structure that is involved in cooperative allosteric regulation and subunit oligomerization (Jin et al. 2005, McCoy et al. 2007). These domains are shown in the structural

diagram (**Figure 6**). In its native state in plants, AGPP is highly regulated and forms a heterotetramer, being made up of two large and two small subunits that are highly homologous in both sequence and structure (Ballicora et al. 2004). Variants of AGPP have been identified with superior regulatory properties (e.g., turnover number in the presence or absence of 3-PGA, extent of 3-PGA activation, Pi inhibition, and heat stability) to those found in the maize endosperm (Boehlein et al. 2005).

GPT: glucose phosphate translocator

AGT: ADP-glucose translocator

Amyloplast Transporter Proteins

The glucose phosphate translocator (GPT) (Kammerer et al. 1998) functions to transport glucose-1-phosphate or glucose-6-phosphate produced in the cytosol into amyloplasts. The protein appears to be related to the major facilitator superfamily (CL0015), one of the two largest families of membrane transporters found in bacteria, archaea, and eukarya. Maize GPT is predicted to have the same general structure as a lactose permease protein (Mizra et al. 2006) shown in the structural diagram (**Figure 7**), which is an integral membrane protein with 12 predicted transmembrane regions. These proton symporters mediate the intake of a variety of sugars along with the uptake of hydrogen ions.

The ADP-glucose translocator (Cao et al. 1995, Leroch et al. 2005, Kirchberger et al. 2007) is a member of the mitochondrial ADP/ATP carrier family based on structural (PF00153) and phylogenetic evidence (Patron et al. 2004, Tjaden et al. 2004). ADP-glucose translocator (AGT) is located in the amyloplast membrane and can consist of up to three tandem repeats of a domain of approximately 100 residues, each domain containing two transmembrane regions. The closest match to this protein is an ADP/ATP carrier (Pebay-Peyroula et al. 2003) shown in the structural diagram (**Figure 7**). In maize, this protein is known as the BT1 protein after being identified from the maize *brittle1 (bt1)* mutant (Sullivan et al. 1991). Subsequent work showed that BT1 is required for ADP-glucose uptake into amyloplasts (Shannon et al. 1998).

α-1,4-Glucan Synthesis

Plants synthesize α-1,4-glucans using GT enzymes (EC:2.4.x.y), which perform one of the most commercially important biological reactions in nature, catalyzing the transfer of sugar moieties from an activated donor molecule to a specific acceptor molecule. In starch and glycogen biosynthesis, these enzymes are called starch synthase (SS), glycogen synthase (GS), and starch phosphorylase (SP). All these enzymes catalyze the formation of a polymer of α-1,4-glycosidic bonds using either ADP-glucose or glucose 1-phosphate as the glucosyl donor. ADP-glucose is the donor molecule used by SSs, whereas SP utilizes glucose-1-phosphate for this function. SS, GS, and SP enzymes retain the anomeric configuration of the glucosyl donor and are referred to as retaining GTs. Although all these enzymes have the same overall fold, and hence belong to the GT-B superfamily (CL0113), the SS and bacterial GS enzymes are classified as members of the GT5 family, the animal GS enzymes are classified as GT3, and the SP enzymes are classified as GT35 (Liu & Mushegian 2003). In plants, there are at least five independently-conserved classes of SS (GBSSI, SSI, SSII, SSIII, and SSIV) that are conserved in green algae and vascular plant species (Li et al. 2003, Leterrier et al. 2008).

Granule bound starch synthase (GBSS) is distinguished from other SSs because it is virtually entirely associated with starch granules. Based on studies of amylose-free mutants that lack the GBSSI protein, this enzyme class appears to be exclusively responsible for amylose synthesis in maize and other plant species (Denyer et al. 2001). Highly similar GBSS isoforms produced from different genes are present in endosperm and leaves in rice and presumably other plant species

(Vrinten & Nakamura 2000). Along with associating with starch granules, another key attribute of GBSSI is its ability to elongate a growing glucan chain processively, and its activity appears to be dependent on the presence of low concentrations of malto-oligosaccharides (Denyer et al. 1999), consistent with the proposed role of GBSSI in amylose synthesis. This means that the enzyme does not necessarily dissociate from the glucan chain after the addition of each glucose, but can remain associated with it to add further glucose units. Experiments with isolated granules show that GBSSI can elongate glucan chains within amylopectin, which explains why mutants lacking GBSSI have altered amylopectin structure as well as lack amylose (Yeh et al. 1981, Hizukuri et al. 1989, Reddy et al. 1993, Maddelein et al. 1994).

SS enzymes are also associated with starch granules, but in addition are also present in plastid stroma, leading to their classification as soluble starch synthases. SS catalyzes the transfer of glucose from ADP-glucose to an acceptor glucan chain by a distributive mechanism in which the enzyme dissociates from its substrate during each catalytic cycle (Denyer et al. 1999). Four classes of SS have been identified, mapped, and cloned from several species (Tomlinson & Denyer 2003, Ball & Morell 2003). These various enzyme classes appear to have different relative activities in different species and tissues even though they all appear to be present in all starch-synthesizing cells. SS is believed to be primarily responsible for amylopectin synthesis, and there is evidence that each class is responsible for synthesizing different chain lengths. Thus, based on biochemical studies of the isolated enzyme (Commuri & Keeling 2001), recombinant SS expressed in *Escherichia coli* (Guan & Keeling 1998), and structural studies of rice and *Arabidopsis* mutants (Nakamura 2002), SSI appears to be primarily responsible for synthesizing shorter chains of amylopectin (Delvallé et al. 2005). Studies of mutants and transgenic rice (Craig et al. 1998) suggest that SSII and SSIII isoforms appear to be involved in synthesizing the intermediate chains of amylopectin (Gao et al. 1998; Umemoto et al. 2002; Zhang et al. 2004, 2005, 2008b). The precise role of SSIV remains to be elucidated, although *Arabidopsis* mutants appear to be defective in granule initiation (Roldán et al. 2007). All of the genetic data cited here are consistent with evidence obtained from starch isolated from potatoes transformed with antisense constructs (Edwards et al. 1999, Fulton et al. 2002).

Sequence alignment comparisons show that all isoforms of SS contain a core or catalytic region of approximately 60 kDa, which is also found in GS (**Figure 8**). In addition, SSI, SSII, SSIII, and SSIV, but not GBSSI or GS, have additional sequence located N-terminal to the catalytic region; this region is sometimes referred to as the N-terminal extension. Studies of N-terminally-truncated SS enzymes have shown that the N-terminal extensions are not required for enzyme catalysis (Imparl-Radosevich et al. 1998, Edwards et al. 1999) or glucan affinity (Commuri & Keeling 2001), consistent with the proposal that they are responsible for the chain-length specificities of the enzymes or for enzyme-protein interactions (Hennen-Bierwagen et al. 2008).

SS, GS, and SP are members of the GT-B structural superfamily (Pfam clan CL0113). The GT-B structure is defined by two Rossman fold domains separated by a linker region forming a deep cleft between them (Franco & Rigden 2003). The two particular Rossman fold domains in SS and GS are identified in the Pfam database as glycos_transf_1 (PF00534; GT_1) and glyco_transf_5 (PF08323; GT_5). GT_1 is found in a wide range of GT-B superfamily enzymes such as those synthesizing starch, sucrose, sucrose P, trehalose, mannose, α-1,3-glucan, glycerolipids, lactose, and mannose P. Proteins containing GT_1 transfer sugars from UDP, ADP, GDP, or CMP to a variety of acceptor substrates, including glycogen, fructose-6-phosphate, and lipopolysaccharides. In contrast, the GT_5 domain is present only in plant SS and bacterial GS and some eukaryotic α-1,3-glucan synthases. In SS and GS, GT_5 occupies the N-terminal half of the catalytic region, and GT_1 is located in the C-terminal half (**Figure 8**). Sharing of GT_1 among diverse GT-B enzymes suggests this is the nucleotide binding domain, whereas the essentially SS/GS-specific GT_5 domain is likely to confer acceptor substrate specificity. Known structures

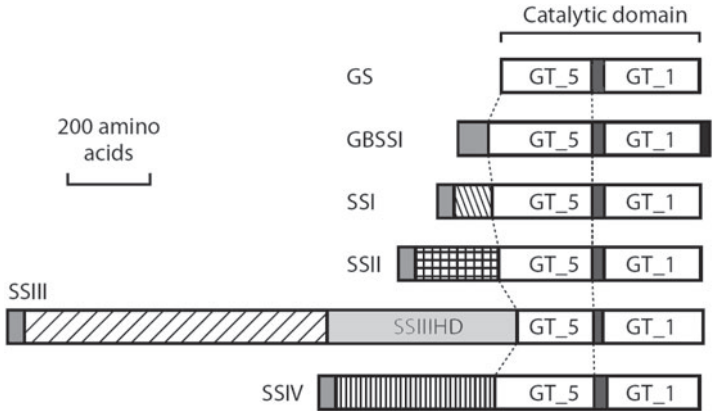


Figure 8

Primary structure comparison of maize starch synthases and glycogen synthase. The catalytic region includes conserved GT_5 and GT_1 domains separated by a short linker region. Gray boxes indicate known or predicted plastid transit peptides. Different patterned boxes indicate regions that show less than 15% identity when compared within SS class across species. The light gray box indicates the region of SSIII within the N-terminal extension (SSIIIHD) that shows greater than 40% identity compared across species. The dark gray box indicates a C-terminal extension present only in GBSS. Dotted lines indicate specific aligned sequences, such that the size differences of GT_5 result from internal insertions/deletions. The figure is drawn to scale.

of GT-B enzymes such as epimerases, sugar-phosphate synthases, acetyl-glucosamine transferases, and glucosyltransferases reveal a striking topological resemblance despite low sequence similarity (Lairson et al. 2008). Included in this group are an archaeal and a bacterial GS (Buschiazzo et al. 2004), whose structures reveal the interdomain crevice, typical of GT-B enzymes, which includes the catalytic center. Structural information is not yet available for the SS enzymes, although detailed bioinformatic analyses indicate with certainty that they exhibit structural similarities with GT-B superfamily members and bacterial GS in particular (**Figure 9**).

The only other conserved structural domain that has been recognized so far within the SSs occurs only in the SSIII class and is called the SSIII-specific domain (Pfam accession no. PF07036). Three tandem repeats of this domain are located immediately N-terminal to the catalytic region in most SSIII proteins. Bioinformatic and biochemical analyses indicate that the SSIII-specific domain has a starch binding function (Machovic & Janecek 2006, Palopoli et al. 2006) whereas other workers have suggested a regulatory function (Hennen-Bierwagen et al. 2008, Valdez et al. 2008, Hennen-Bierwagen et al. 2009).

SPs catalyze the formation of glucose 1-phosphate from inorganic pyrophosphate and α-1,4-glucans such as starch. SP from maize endosperm has been separated into different forms (Tsai & Nelson 1969), each with different properties or timing of expression. SPII and SPIII can initiate glucan chain synthesis without a primer and are present during the period of most rapid starch synthesis but not during germination. SPI is present during all stages of endosperm development and reappears during germination. Although the precise role of SP in starch biosynthesis remains to be fully elucidated, one proposal suggests it is involved in amylopectin chain length trimming (Yu et al. 2001). As a GT-B structural superfamily member, SP comprises an N-terminal and C-terminal Rossman fold domain with the catalytic site located in the center of a deep interdomain crevice (Watson et al. 1999). The C-terminal domain in SP shares significant similarity with the nucleotide-binding GT_1 domain of SS (Buschiazzo et al. 2004), although the two Rossman folds in SP are not separately designated in the Pfam database, and the two-domain structure is comprehensively recognized as the phosphorylase domain (PF00343).

The structural comparisons described above on the similarities between the GT enzymes and SS provide significant evidence that α-1,4-glucan chain extension by SS and SP enzymes involves the addition of an activated glucose to the nonreducing end of an acceptor glucan. Specifically, the crystal structures of MalP-maltooligosaccharide complexes are reported, revealing the natural glucan substrate aligned with specific glucan-binding sites. The results show a pentasaccharide bound across the catalytic site of MalP with sugars occupying subsites −1 to +4. In this configuration the phosphate group of G-1-P is poised to attack the glycosidic bond and promote phosphorolysis at the nonreducing end of the glucan chain (Watson et al. 1999, Geremia et al. 2002). Given that X-ray crystallographic studies of GS enzymes reveal sufficient structural similarities with SP (Buschiazzo et al. 2004), it is highly likely that the same mechanisms of glucan interaction occur in starch and glycogen biosynthesis. These findings are consistent with work from other laboratories, where enzyme catalytic data showed that SS enzymes act on glucan substrates by a distributive mechanism of action (Denyer et al. 1999) that can only be explained by a nonreducing end mechanism. Other work examined the action of SSII on a branched maltotriose (6‴-α-maltotriosyl-maltohexaose) and revealed a specific nonprocessive elongation of the nonreducing end of the shortest unit chain of the maltotriose chain (Damager et al. 2001). These findings are also consistent with the lack of evidence for any catalytically competent covalent glucan-enzyme intermediate for any retaining GT despite years of exhaustive studies (Lairson et al. 2008). Collectively, these findings are consistent with the proposal that the mechanism of action of GT enzymes involves a short-lived oxocarbenium ion-pair intermediate (Lairson et al. 2008), supporting a nonreducing end mechanism.

α-1,6-Glucan Linkages

Starch synthesis additionally requires the modification of α-1,4-glucans to introduce α-1,6-glucan linkages. These reactions are catalyzed by glycoside hydrolase (GH) enzymes (EC:3.2.1.z) classified in CAZY as members of the GH13 family of enzymes. Structurally, both SBE and DBE enzymes belong to the glycosyl hydrolase superfamily clan (CL0058). The functional BE enzymes appear to have multiple highly conserved regions incorporating two CBM48 carbohydrate binding modules (PF02922) as well as an α-amylase domain (PF00128) shown in the structural diagram (**Figure 10**) (Abad et al. 2002). In comparison, the functional DBE enzymes also have multiple highly conserved regions incorporating a single CBM48 carbohydrate binding module (PF02922) as well as an α-amylase domain (PF00128) (Katsuya et al. 1998). The structural similarity to amylases is consistent with mechanistic studies of SBE that revealed residues causing reductions in catalytic activity that are conserved in the α-amylase family of enzymes and suggests a common mechanism of action (Vu et al. 2008).

The SBEs may be found entrapped inside starch granules and hence appear to have a weak association with starch, similar to that seen with the SS enzymes (Mu-Forster et al. 1996). SBEs catalyze the hydrolysis of an α-1,4 linkage of a first glucan chain, with subsequent bonding of the cleaved portion of this glucan chain to an adjoining glucan chain via an α-1,6 linkage. There appear to be at least two major classes of highly conserved SBEs across plant species (SBEI and SBEII), which is consistent with the idea that SBEs have a distinct role in the mechanism of amylopectin synthesis. Furthermore, because amylose is lightly branched, the SBEs must also play some role in amylose synthesis. SBEIIb appears to be primarily responsible for transferring longer chains of amylopectin based on biochemical studies of the isolated enzyme (Takeda & Preiss 1993, Guan et al. 1997) and structural studies of the maize and rice mutant (Takeda et al. 1993, Stinard et al. 1993, Nishi et al. 2001). The N-terminal region of SBEs appears to be important for catalysis (Guan et al. 1997) and structural stability (Hamada et al. 2007), whereas both the N- and

C-terminus appear to play a role in determining substrate preference and chain length transfer (Kuriki et al. 1997). The role of SBEI and SBEIIa is less clear as mutants of SBEI in rice have reduced intermediate and long chains (Nakamura 2002), whereas in maize the chain lengths are unaffected in mutants of SBEI and SBEIIa (Blauth et al. 2001, 2002).

Four highly conserved DBE enzyme classes exist in the storage organs: three isoforms of isoamylase-type DBE (ISA) and one pullulanase-type DBE (PU). Like their well-characterized GH homologs in prokaryotes, plant DBEs are well known to catalyze hydrolysis of α-1,6 linkages in branched glucans (Rahman et al. 1998, Wu et al. 2002). The physiological role of this degradative function during starch biosynthesis is not at first obvious; however, genetic data (discussed below in the Genetics section) suggest that selective removal of some branch linkages is important for determining amylopectin structure and/or granule initiation frequency.

Interactions Among Starch Synthesis Enzymes

Although we know that a suite of multiple enzymes is required in the process of normal starch synthesis, the exact manner of their interactions during glucan polymer assembly has not been fully elucidated. One possible mechanism involves the formation of multi-enzyme complexes. This idea is supported by the observation of numerous pleiotropic effects on SS, SBE, and AGPP resulting from mutations in genes coding for specific enzymes of the pathway (Boyer & Preiss 1981, Singletary et al. 1997). The formation of multi-enzyme complexes is also supported by direct evidence for interactions between starch biosynthetic enzymes in wheat endosperm, where coimmunoprecipitation of SSI, SBEI, and SP was reported (Tetlow et al. 2004). Association of those proteins required phosphorylation at one or more serine residues in a target protein(s) that has not yet been identified. Subsequent characterization of high-molecular weight fractions isolated from developing maize and wheat endosperm revealed the existence of enzyme complexes containing SSI, SSIIa, SSIII, SBEIIa, and/or SBEIIb, in various combinations (Hennen-Bierwagen et al. 2008, Tetlow et al. 2008). In maize, essentially all of the SSIII is in a form that is greater than 670 kDa in apparent molecular mass, and the great majority of SSIIa is in an approximately 300 kDa form. Partial purification revealed that the SSIII-containing complex also comprises SSIIa, SBEIIa, and SBEIIb, whereas proteomic analysis identified additional proteins, including large and small subunits of AGPP and pyruvate phosphate dikinase (PPDK) (Hennen-Bierwagen et al. 2009). These findings raise the possibility of a broader metabolic significance of these enzyme complexes.

Recent proteomic analyses of maize endosperm have raised the hypothesis that PPDK is a key factor in regulating the division in carbon flux between protein and starch (Mechin et al. 2007). In photosynthetic tissues of C4 plants, PPDK produces phosphoenol pyruvate and inorganic pyrophosphate (PPi) from pyruvate, ATP, and phosphate. PPDK is also prevalent in developing endosperm tissue, where its function is not known. One proposal is that the PPi produced by PPDK negatively regulates AGPP through product inhibition, thus reducing the flux of glucosyl units from sucrose into ADP-glucose and subsequently starch (Mechin et al. 2007). Those glucosyl units presumably would be routed through other metabolic pathways to produce amino acid precursors and eventually more protein. This proposal originally was suggested to occur in the cytsosol because that is where the major AGPP is located, and because PPDK was thought to be located in that compartment as well. However, the studies of complexes isolated from amyloplasts revealed that PPDK is located in the plastid as well as the cytosol because it associates with known plastidial enzymes SSIII, SSIIa, and others (Hennen-Bierwagen et al. 2009). Strikingly, the same enzyme complexes also contain AGPP, so that the target enzyme and the proposed regulatory enzyme could interact through close physical associations. In this way, the plastidial AGPP might be regulated by PPDK, so that hexose phosphates transported to the amyloplast, or glucosyl units

in transported ADP-glucose, could be routed into amino acids rather than starch. Although the exact significance of these multi-enzyme complexes remains to be elucidated, these new findings seem likely to have profound importance. Thus, the high-molecular weight complexes may enable multi-enzyme interactions that determine the structure of the glucan polymer products and may also provide mechanisms for the coordinated regulation of storage tissue metabolism.

GENETICS

Across species, well-conserved families of genes encode the enzymes that accomplish starch biosynthesis (Ball & Morell 2003, Ball & Deschamps 2009). Thus, similar families of genes encode the same multiple classes of each enzyme across diverse plant species (e.g., SSI, SSII, etc. and BEI, BEII, etc.). This suggests that each enzyme class has a uniquely conserved role in the process of starch synthesis. This idea has been largely confirmed in numerous plant species by studies of genetic modifications in which particular classes have been overexpressed, reduced in expression, or eliminated (see below). As an introduction to the genetics of starch synthesis, below we list the enzymes, genetic loci, and GenBank gene identification numbers for genes involved in the pathway of starch synthesis in maize endosperm (**Table 2**). Although this review focuses particularly on those genes associated with maize endosperm, for many enzymes other loci exist owing to gene duplication events (Yu et al. 2005), which appears to be particularly the case for the plastid enzymes (Comparot-Moss & Denyer 2009). For example, distinct genes in maize code for multiple very closely related isoforms of the different classes of SS, SBE, DBE, and AGPP large and small subunits, some of which are expressed specifically in endosperm and others in leaves or embryo (e.g., SSIIa, SSIIb, SSIIc). Mutations in the genes coding for many of the starch biosynthetic enzymes are known, whereas in other instances the proteins have been identified based on protein purification and/or cDNA cloning, and genetic analysis is lacking. With the emergence of the maize genome sequence and the availability of a refined, targeted insertional mutagenesis approach (Ahern et al. 2009), mutations in each of these enzymes should be identified in the relatively near future. These genetic tools will be useful in better understanding the metabolic fluxes that lead to starch production in endosperm, analogous to the comprehensive metabolic analyses that have been possible in *Arabidopsis* and potato (Geigenberger et al. 2004, Smith & Stitt 2007).

Sugar Phosphate Synthesis Genes

Among the sugar phosphate synthesis genes, SUS (Chourey et al. 1998), FRK (Zhang et al. 2003), PGI (Lal & Sachs 1995), and PGM (Manjunath et al. 1998), only two forms of SUS have been characterized based on mutational analysis in maize. The gene *shrunken1* (*sh1*) encodes the SUS-SH1 class of sucrose synthase, and the *sus1* gene codes for the SUS1 class (**Table 2**). Known *sh1* mutations have only a modest effect on starch content (Huang et al. 1994), whereas *sus1-1* affects cell wall synthesis (Chourey et al. 1998). The remaining enzymes in this group that are expressed in endosperm have been identified based on cDNA cloning.

Nucleotide Sugar Metabolism Genes

UGPP (Abe et al. 2002) and AGPP (Hannah et al. 2001) genes have been mapped, cloned, and sequenced from several plant species, and specific forms appear to be expressed in the endosperm (**Table 2**). In rice, two UGPP genes have been identified with *Ugp1* mutants affected in pollen development (Chen et al. 2007). In maize, there are two well-characterized low-starch mutants affected in the *brittle2* (*bt2*) or *shrunken2* (*sh2*) genes known to encode the large and small subunits,

respectively, of the cytosolic AGPP (Hannah & James 2008). To date, there are no known mutants for the large and small subunits of the maize plastidial AGPP proteins encoded by the *Agp1* and *Agp2* cDNAs, respectively. An additional form of the plastidial small subunit could be produced by an alternative transcript from *bt2* that includes a transit peptide coding region (Rösti & Denyer 2007, Comparot-Moss & Denyer 2009). Interestingly, transcriptional and metabolic adjustments in *bt2* mutants (Cossegal et al. 2008) appear to trigger changes in transcripts for genes involved in carbohydrate or amino acid metabolic pathways.

Amyloplast Transporter Genes

GPT and AGT genes (Cao et al. 1995, Kammerer et al. 1998) have been characterized from several plant species, and specific forms appear to be expressed in the maize endosperm (**Table 2**). Although there are no known mutants for GPT, mutations of the *brittle1* locus are known to inactivate the ADP-glucose translocator. A functional *bt1* is required for an amyloplast ADP-glucose import process that supports starch synthesis as evidenced by a severe reduction in starch content and accumulation of ADP-glucose in mutant lines (Sullivan et al. 1991, Cao et al. 1995). More direct studies of maize *Bt1* expressed in *E. coli* reveal a redox-regulated protein with an ability to transport ADP-glucose in counter exchange with ADP (Kirchberger et al. 2007). The maize *Bt1* gene is expressed during starch synthesis. A close homolog is known based on a cDNA sequence, termed *Bt1-2*, that is more ubiquitously expressed (Kirchberger et al. 2007). Other homologs of this gene exist in various species, including dicotyledonous plants, where ADP-glucose import into plastids is thought not to occur, which appear to be involved in the export of plastidial adenine nucleotides. There is evidence for common origins by gene duplication followed by functional diversification (Comparot-Moss & Denyer 2009).

Genes that Control α-1,4-Glucan Synthesis

Genetic analysis has investigated the functions of each of the conserved classes of starch synthase in various starch storage tissues. In maize, at least one isoform from each of the GBSSI, SSI, SSII, SSIII, and SSIV classes is expressed in endosperm (**Table 2**). In some instances, specifically GBSSI and SSIII, separate genes appear to have arisen from duplication of certain chromosome segments that account for expression of highly related isoforms in leaf and/or other tissues.

The *Waxy* gene in maize endosperm, *Lam* gene in pea embryos, and *Amf* gene in potato tubers all control activity and expression of GBSSI (Shure et al. 1983, Klosgen et al. 1986, Hovenkamp-Hermelink et al. 1987, Hseih 1988, Denyer et al. 1995, Nakamura et al. 1995). The fact that all these mutations condition loss of both amylose and GBSSI activity provided a strong indication that this class of starch synthase produces the lightly branched amylose polymer. Positive correlation of GBSSI expression and amylose level was provided through genetic modification of amylose-free potato plants by transformation with GBSSI genes from other species. Expression of cassava GBSSI in the potato mutants, which are essentially devoid of amylose, resulted in 3.5–13% amylose in the transformants (Salehuzzaman et al. 1999). Similarly, amylose-free potatoes transformed with pea GBSSI isoforms resulted in potatoes with a small but significant increase (0.8% and 1%) in amylose content (Edwards et al. 2002).

The maize SSI (Knight et al. 1998) gene does not yet have any known mutant form. To date, the only reported storage tissue SSI mutant is in rice (Fujita et al. 2006). The SSI mutation had only a minor effect on starch content and quality, although the chain length distribution of amylopectin was altered such that short chains of DP 8–12 were deficient and intermediate chains of DP 16–19 were elevated relative to wild type. Similar results were observed in leaf starch from an SSI mutant of *Arabidopsis* (Delvallé et al. 2005).

The genes coding for SSIIa in storage starch tissues have been identified by mutations in multiple species including maize, wheat, barley, pea, and rice (Campbell et al. 1994, Craig et al. 1998, Yamamori 2000, Umemoto et al. 2002, Morell et al. 2003, Zhang et al. 2004). The relevant gene in maize is *sugary2* (Harn et al. 1998), which has been important in the food industry owing to the functional properties of starches produced in the absence of SSIIa. The effects of SSIIa deficiency are remarkably similar in all of these species. Amylopectin exhibits a deficiency in intermediate chains of DP12-25 and an increase in short chains of DP6-10. Amylose concentration within starch granules is increased significantly, up to 70% in the case of some barley mutants (Morell et al. 2003). Similar effects were observed in potato tubers when SSII expression was reduced by an antisense gene (Edwards et al. 1999, Lloyd et al. 1999) and in *Arabidopsis* leaves by mutation (Zhang et al. 2008b). This consistency of phenotype argues that the SS classes provide specific, selectable functions in the process of starch assembly, and this accounts for the conservation of each class throughout plant evolution.

The structural gene for SSIII in maize is known to be the *dull1* (*du1*) locus (Gao et al. 1998). Starch from *du1*-mutant plants is relatively lacking in longer amylopectin chains, with significant alterations in molecular architecture (Wang et al. 1993a,b; Gerard et al. 2009). In potato, the simultaneous antisense inhibition of SSII and SSIII resulted in a grossly modified amylopectin (Edwards et al. 1999, Lloyd et al. 1999), with yet further changes in structure if GBSSI, SSII, and SSIII are inhibited (Jobling et al. 2002).

Mutants lacking the putative SSIV isoform have not yet been reported. As mentioned in a previous section, *Arabidopsis* mutants lacking SSIV appear to be compromised in granule initiation (Roldán et al. 2007).

Two cDNAs coding for different SPs have been found in maize, one referred to as SP_L (low affinity for glycogen; also called SP1 or PHO1) and the other as SP_H (high affinity for glycogen; also called SP2 or PHO2), and summarized as GenBank accessions in **Table 2**. SP_L was identified as a soluble amyloplast protein based on a peptide sequence (Yu et al. 2001) and was also found in maize starch granules, again identified by peptide sequences (Grimaud et al. 2008). The maize *shrunken4* (*sh4*) gene controls expression of SP_L (Yu et al. 2001), although whether or not it codes for the enzyme remains to be determined. In rice, the SP_L protein, located within amyloplasts, is encoded by *pho1*; mutations in that gene cause increased abundance of short chains of DP6-12 and reduction in intermediate chains of DP13-22, and also abnormal granule size and morphology (Satoh et al. 2008).

Genes that Control α-1,6-Glucan Linkages

Maize endosperm contains both the SBEI and SBEII class of starch branching enzyme, the latter as two closely related isoforms designated SBEIIa and SBEIIb. All three of these enzymes are encoded by different genes, and mutations in each one have been characterized (**Table 2**). The gene coding for SBEIIb is *amylose extender* (*ae*) (Stinard et al. 1993, Kim et al. 1998), which when mutated results in severely altered amylopectin structure with very few short branches and highly elevated apparent amylose content of up to 50% (Garwood et al. 1976, Klucinec & Thompson 1998). The reason for the high apparent amylose is most likely that less amylopectin is produced, and the branch lengths in the altered amylopectin are so long that the material may appear as amylose in quantitative assays using iodine binding as the detection method. Thus, it appears that neither SBEI nor SBEIIa can provide the functions of SBEIIb in amylopectin biosynthesis in endosperm, even though the mRNAs encoding each of those enzymes are present during kernel development (Gao et al. 1997).

The genes coding for SBEI and SBEIIa (Fisher et al. 1995, Gao et al. 1997) have both been mutated by transposon insertions (Blauth et al. 2001, 2002). In neither instance was there a significant change in kernel phenotype, starch content, or starch structure. However, loss of SBEI did have a noticeable effect on amylopectin branching pattern in lines also lacking SBEIIb, indicating SBEI does, in fact, function in endosperm starch biosynthesis (Yao et al. 2004).

High-amylose starches have long been of interest owing to their functional properties, and naturally occurring high-amylose starch was reported more than 50 years ago. Although one might expect that high amylose might arise from simultaneous mutation of all the SBEs, kernels containing exclusively amylose as the reserve starch polysaccharide have never been found despite extensive research (Shi & Seib 1998). Furthermore, although the biochemical basis of maize starches having amylose contents above 50% clearly requires the *ae* mutant, the biochemical basis of the additional increases higher than 50% amylose is not clear at the present time (Sidebottom et al. 1998).

The genes coding for three evolutionarily conserved classes of isoamylase-type DBE and one pullulanase-type DBE have been identified by cDNA sequencing (James et al. 1995, Dinges et al. 2003), and mutations in maize are known that affect ISA1 and PU1 (**Table 2**). Genetic modifications in ISA1 activity in maize and rice result in significant changes in starch granule structure (Jane et al. 1994, Kubo et al. 1999). Similar results were observed using antisense technology to reduce ISAI activity in rice (Fujita et al. 2003). ISAI mutants in barley accumulate starch in compound instead of simple starch granules (Burton et al. 2002), where compound refers to amyloplasts in which many small granules have been initiated, and simple refers to amyloplasts containing one major granule. In potato, where antisense constructs for ISAI and ISAII were combined, the tubers accumulated large numbers of small granules (Burton et al. 2002, Bustos et al. 2004). From these observations, it has been proposed that isoamylase-type DBE activity suppresses initiation of granule formation (Burton et al. 2002, Bustos et al. 2004).

Another striking phenotype caused by mutation of ISA1, for example *sugary1* (*su1*) mutants of maize, is reduction in crystalline starch and accumulation of a soluble, glycogen-like polymer that is never observed in normal plants (James et al. 1995, Mouille et al. 1996, Kubo et al. 1999, Dinges et al. 2001, Burton et al. 2002, Fujita et al. 2003, Bustos et al. 2004). The structure of the residual amylopectin remaining in plants lacking ISA1 is altered from normal (Dinges et al. 2001, Delatte et al. 2005, Wattebled et al. 2005). These data suggest that DBEs function in starch production by the selective hydrolysis of certain branch linkages prior to crystallization of the molecule as granules form (Ball et al. 1996, Myers et al. 2000). The maize *sugary* mutants are important commercially because they are one of the main sources of sweet corn.

ISA2 is involved in starch production in leaves in the same way as ISA1, and the two proteins are required for activity of the ISA heteromeric enzyme (Delatte et al. 2005, Wattebled et al. 2005). The function of ISA2 in storage tissues may differ, however, as a maize mutant lacking this protein exhibits near-normal kernels (M. James & A. Myers, unpublished results). In leaves, ISA3 is involved in starch degradation (Smith et al. 2005), but the function of this enzyme in storage starch biosynthesis remains unresolved at the present time, as no mutants are known to exist. What little is known about the catalytic properties of the ISAs has led to the proposal that each isoform has retained a specific role (Hussain et al. 2003).

The pullulanase-type DBE of maize, ZPU1, is primarily involved in degradation of starch during kernel germination. Mutations in PUI have been identified (Dinges et al. 2003), but effects on starch content are minimal. ZPU1 also plays a role in starch biosynthesis because in combination with specific mutations of ISA1, loss of the pullulanase-type enzyme exacerbates the defect in storage starch biosynthesis (Beatty et al. 1999, Dinges et al. 2003).

FUTURE

In addition to improving our fundamental understanding of plant physiology, a major rationale for gathering the biochemical and genetic information regarding starch biosynthetic enzymes reviewed here is to utilize that information to improve the quality and quantity of starches that can be obtained from staple crops. The following sections address previous efforts and new opportunities to effect changes in yield and/or quality of storage starches.

Starch Functionality

As described above, maize, rice, and barley mutants have been useful for identifying enzymes involved in starch synthesis and for studying the roles of these enzymes in determining starch structure. Mutants initially classified into different phenotypes based primarily on appearance can now be categorized based on the enzymes encoded by the mutant loci (**Table 3**). Although studies of these mutants have revealed effects on carbohydrate composition and response to genetic background, allelic dosage, or interaction with other mutations, some of these mutants have useful functionality that is valued in industry (see review by Klucinec & Keeling 2005). For example, high amylose starches (BEII mutants) are valued in foods, offering improved product texture, adhesion to water-impermeable surfaces, or as a source of slowly digestible carbohydrate; whereas low amylose starches (GBSSI mutants) are valued as texturizers because of their improved transparency and better freeze-thaw stability after processing compared with normal starches. These low and high amylose traits can be readily transferred to other species using biotechnology (e.g., wheat, cassava, potato) and represent one of the more immediate benefits of all this knowledge. Yet other examples of enhanced starches include using SSII variants in barley and rice (Morell et al. 2003, Nakamura et al. 2005). Despite the improved functionality provided by enhanced starches, other

Table 3 Summary of individual isoforms of enzymes linked with specific maize mutants and the effects of these mutations on starch content (comparing mature kernels of mutant versus wild type as percent) and changes in starch structure due to the mutation

Enzyme	Class	Mutation	Starch content (Mutant versus WT)	Structural changes in mutant
Sucrose synthase	SuSy-SH1	sh1	73%[a], 73%[b], 88%[c]	None/minimal
AGPP	Cytosol. SS	bt2	19%[a], 17%[b], 25%[c],	None/minimal
AGPP	Cytosol. LS	sh2	20%[a], 34%[c]	None/minimal
AGT	AGT	bt1	14%[a], 30%[c]	None/minimal
Starch synthase	GBSSI	wx	88%[a]	Low-amylose
Starch synthase	SSI	-	-	-
Starch synthase	SSII	su2	50%[a]	Lacks intermediate chains in amylopectin
Starch synthase	SSIII	du1	53%[a], 84%[b]	Lacks longer chains in amylopectin
Starch synthase	SSIV	-	-	-
Branching enzyme	BEI	sbe1	None/minimal	None/minimal
Branching enzyme	BEIIb	ae	60%,[a] 70%,[b] 45%[c]	High-amylose, long chain amylopectin
Debranching enzyme	ISAI	su1	21%,[a] 57%,[b] 65%[c]	Compound granules, phytoglycogen
Debranching enzyme	ISAII	-	-	-
Debranching enzyme	ISAIII	-	-	-
Debranching enzyme	ZPUI	pu1	None/minimal	None/minimal

[a]Data from (Nelson 1982); [b](Singletary et al. 1997); [c](Doehlert & Kuo 1994), [d](Blauth et al. 2002), [e](Blauth et al. 2001), [f]Myers et al., unpublished, [g](Dinges et al. 2003).

product targets still remain, and novel starches still require additional modifications by physical processing, chemical substitution, and/or crosslinking in order to deliver an optimal functionality. This unfulfilled need has prompted continued genetic and biochemical research into ways of designing enhanced starches with yet further functionality.

Designing starches with enhanced functionality (e.g., crystallinity, gelation and pasting characteristics, flavor and starch granule morphology) requires a deep understanding of the individual and collective roles played by all enzymes involved in starch synthesis. More specifically, we have to understand the relationships between starch structure and starch functionality, which is an area that is still not well developed. In general terms, we do know that variation in GBSSI activity determines amylose content, and this has a major influence on starch functionality. Variation in amylopectin is more complex, being made up of the chain length profile and branching pattern that combine together to determine crystallinity. Studies of amylopectin chain lengths in mutants affecting SS (*waxy*, *dull1*, and *sugary2*), SBE (*amylose extender*), and ISA (*sugary1*) have revealed many alterations in amylopectin chain length, and mutant combinations provide even further variation (**Figure 11**). The high proportion of very short chains (below DP10) seen in maize SSII and SSIII mutants imparts a decreased tendency to retrograde compared with normal starch (Liu & Thompson 1998a,b). Similarly, reduced retrogradation was also observed in low amylose potatoes engineered to simultaneously eliminate SSII activity with applications for improved freeze-thaw tolerance (Jobling et al. 2002). Interestingly, observations in rice show that short chains of DP 6–12 correlate inversely with peak gelatinization temperature, whereas long chains correlate with peak viscosity and set-back temperature (Horibata et al. 2004). The inactivation of SSIII in the

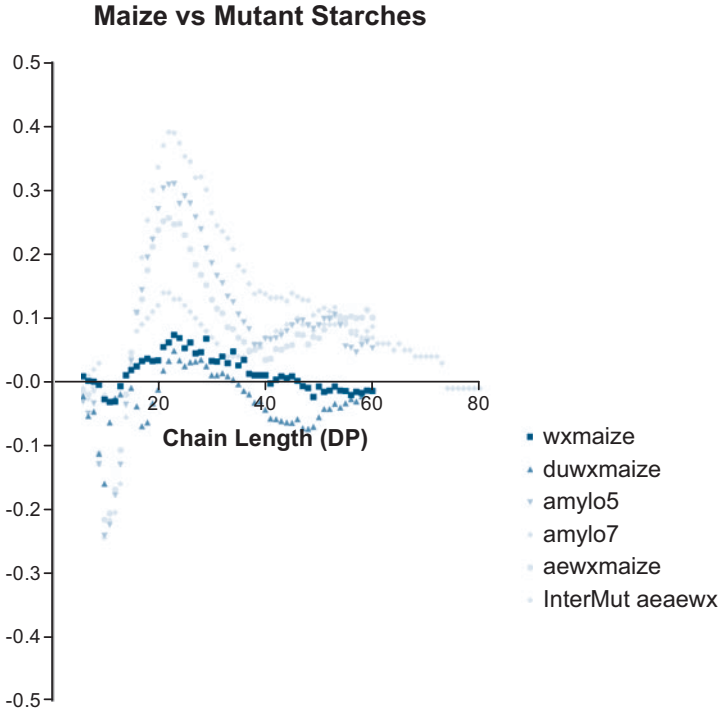


Figure 11

Difference plot showing amylopectin chain length differences among starches from mutant genotypes. The difference plot data are referenced against starch from wild-type maize (Keeling 1999).

dull mutant of maize results in a slight elevation in the chains with a DP less than 30–50 (Inouchi et al. 1987, Yuan et al. 1993, Shi & Seib 1995) and increased apparent amylose content to 30–40% (Helm et al. 1969). Furthermore, although high amylose starches have dramatic increases in long chain amylopectin (giving apparent amylose content greater than 50%) and bring valuable functionality such as improved texture, gelation, and adhesion, the biochemical and genetic mechanisms are not known.

As well as chain length distribution, another critical factor in structure/functionality relationships is the distribution of branch points within the amylopectin molecule. Unfortunately, there are no definitive ways of measuring the distance between branch points. Some information can be obtained from chain length determination after digestion with beta-amylase to create beta-limit dextrins (Yao et al. 2004). This reveals the distribution of lengths from the reducing end of each branch-bearing chain to the outermost branch point of that chain. Correlation of such information with functionalities can be informative. However, a big gap in our knowledge remains because of our inability to measure distance between branch points. Thus, it is important to recognize that more detailed studies are required to develop a much more comprehensive understanding of starch structure and functionality.

One long-term goal of this entire field of research is to enable a rational design of amylopectin structure to target specifically desired functionalities. The design parameters are chain length distribution, branch frequency, and branch location. In preceding sections of this review, we described studies of amylopectin chain length specificities, showing that individual SS enzymes have processive and distributive mechanisms of action, as well as specific yet overlapping chain length preferences. Overall, a model can be proposed in which the distinct chain length requirements and specificities of each different enzyme provide the ground rules for the discontinuous synthesis of amylopectin (Guan & Keeling 1998, Nakamura 2002). Thus, SSII competes with SSI for production of short to intermediate length chains, whereas SSIII competes with SSII for production of intermediate- to longer-length chains, but does not effectively compete with SSI. The SS enzymes all differ from GBSSI, whose unique processive capability enables its unique role in amylose synthesis. Studies of specific amino acids in determining the chain length preferences of rice SSIIa suggest that substitution of certain amino acids reduces SS activity while also affecting the capacity to synthesize *indica*-type amylopectin (Nakamura et al. 2005). These observations are consistent with other work showing changes in primer preferences using recombinant enzymes following site-directed mutagenesis (Nichols et al. 2000, Gao et al. 2004). Mechanistic studies of SBE and DBE have revealed a mechanism of action that is similar to the α-amylases combined with a domain-specific ability to recognize specific chain lengths to be transferred from one glucan helix to another. Collectively, and in combination with the topological studies of enzyme structure, this suggests that it may eventually be possible to engineer valuable changes in certain amino acids to change the specificities of SS, SBE, and DBE enzymes with respect to amylose and amylopectin chain length and branch point distribution. Taking this concept further, one can begin to envision broad genetic engineering efforts directed toward the various structural domains of these enzymes in order to change their functionality and deliver enhanced starch structures.

Starch Yield

The rapid deployment of transgenic technologies in the 1990s led to many attempts to increase the starch contents of cereal crops and potatoes by manipulation of enzymes involved in starch synthesis (see review by Smith 2008). Much of this work was conducted in industry and appears to have had limited success as evidenced by the lack of publications or press releases on product developments. The published academic works are consistent with this. Thus, although these early

efforts did meet with some initial success, more detailed studies of the starch-enhanced plants in varied growth conditions revealed limited utility. For example, because of its predicted role as a rate-limiting enzyme in starch biosynthesis, the most obvious enzyme to overexpress has been AGPP, with two genetic sources being studied most thoroughly, a mutant enzyme from *E. coli* (glgC16) and a mutant enzyme from maize (*Sh2-rev6*). First, maize lines expressing *Sh2-rev6* resulted in significantly heavier seeds than control lines (Giroux et al. 1996), but there was no increase in starch as a percentage of seed weight. Second, overexpressing the *Sh2-rev6* allele in the endosperm of wheat and rice gave an increase in seed and biomass yield, but no change in seed weight or starch content (Smidansky et al. 2002, 2003, 2007; Meyer et al. 2004). Third, field trials of wheat carrying the same *Sh2-rev6* allele revealed no increase in yield under conditions of normal interplant competition and rain-fed water supply (Meyer et al. 2007). Fourth, expression of glgC16 in maize gave no consistent change in seed weight or seed number (Sakulsingharoj et al. 2004), whereas the same gene in rice showed increased individual seed weight, although starch content was not measured (Wang et al. 2007). Collectively, these findings do not provide convincing evidence that AGPP represents a single rate-limiting step in the pathway of starch synthesis in cereal crops. Furthermore, the finding that seed number and biomass was sometimes increased (with no change in starch content) points to effects of AGPP on ovule initiation or pollination rather than specifically starch deposition during seed development.

Another approach to investigating the role of AGPP in determining starch yield employed gene dosage variation (Singletary et al. 1997). Cereal endosperm is particularly amenable to this type of study because of its triploid genetic makeup. Thus, the endosperm inherits two doses of each allele from the maternal parent and one dose of each allele from the paternal parent. By self pollinating or crossing mutants with wild type, it is possible to create a gene-dosage series of grain having 0, 1, 2, or 3 doses of the mutant allele, and previous studies showed a linear decrease in enzyme activity with increasing dosage of the mutant allele. The rate of starch accumulation, however, did not decrease linearly with decreasing AGPP activity, as would be expected in the simple case where that enzyme is rate limiting. In contrast, the duration of starch accumulation during endosperm development was affected by AGPP activity, even when one wild-type allele is still present (**Figure 12**). Considering these gene dosage data with the previously referenced transgenic data, a complex picture emerges for the role of AGPP in determination of starch yield.

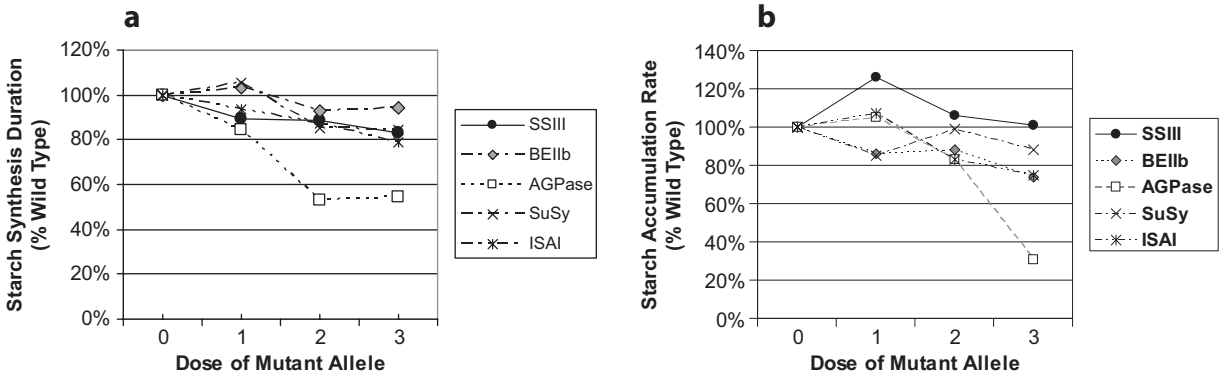


Figure 12

(*a*) Effects of mutant gene dosage on rate and duration of endosperm starch accumulation. The data show that AGPP (bt2) mutants severely affected starch accumulation rate and duration with three doses of the mutant allele. (*b*) Mutations affecting other enzymes had less severe but measurable effects on starch synthesis.

FLUX CONTROL COEFFICIENT THEOREM

The flux control coefficient (FCC) of an enzyme corresponds to the sensitivity of flux through a given pathway to changes in the activity of a specific enzyme. The theorem states that all the FCCs in a pathway must sum to unity. This suggests that instead of a single rate-limiting step in a pathway, there may be colimitation, where several enzymes contribute simultaneously to the control of flux through the network. In general, FCC research shows that pathways appear to display a shared control of flux.

Whether our knowledge of AGPP can be exploited to increase cereal yield in commercial crops remains to be determined.

Flux control coefficients (FCCs) (see sidebar, Flux Control Coefficient Theorem) provide a means of quantifying the role of each enzyme in a pathway on the rate of metabolic flux through that pathway (ap Rees & Hill 1994). Two key components of FCC analysis are that it should be based on only a small change in enzyme activity relative to normal, and all the FCCs in a pathway must sum to unity. Gene dosage variation provides one way of partially altering enzyme activity so that FCCs can be calculated. In the maize gene dosage study of starch synthesis (Singletary et al. 1997), the quantified FCCs using partial reductions in enzyme activity were relatively low for SUS (0.005), AGPP (0.045), and BE (0.137), whereas FCC could not be calculated for SS because mutants resulted in increased SS activity. However, an FCC value of 0.82 was obtained in other studies, revealing unusual sensitivity of SS enzymes to moderately elevated temperatures where SS enzymes become suboptimal above 25°C (Keeling et al. 1994). These data indicate SS as a key control point for metabolic flux into starch, and suggest them as targets for future strategies to increase starch yield. Interestingly, the temperature variation experienced during grain filling is within the range that affects SS because actual cob field temperatures exceed the SS optimum but almost never exceed 38°C (Keeling & Greaves 1990).

FCC: flux control coefficient

An advanced FCC analysis of starch biosynthesis combined with starch yield studies has been completed in potato (summarized in **Table 4**) (Geigenberger et al. 2004), albeit with the limitations that some of these studies were based on varieties or growth conditions that had low-starch yields

Table 4 FCC values and effects of overexpression of specific enzymes (except adenylate kinase, which was downregulated) on starch content in potato (Geigenberger et al. 2004). These data are complicated by relatively low potato tuber yields in greenhouse trials compared with the field

	Effects of enzymes on starch	
Enzyme	FCC	Starch WT%
SUS	+0.10	θ
Hexokinase	~0.00	θ
UGPP	~0.00	θ
PGM (cytosol)	+0.15	θ
PGM (plastid)	+0.23	θ
AGPP	+0.35	100%
SS	+0.12	θ
SBE	~0.00	θ
Adenylate kinase	−0.75	117%
NTT φ	+0.98	116% and 136%

θ, not measured. φ, data obtained from two separate studies.

(Zhang et al. 2008a). Low FCC values were found for SUS, hexokinase, UGPP, PGM, SS, and SBE. High FCC values were found for amyloplast transporter proteins and adenylate kinase, suggesting that they are likely to be key control points in the pathway. Relatively moderate FCC values were associated with AGPP, suggesting that this enzyme may also play a flux-limiting role. Initial tuber yield studies following expression of the glgC16 variant of AGPP showed an increase in starch yield (Stark et al. 1992), but these studies could not substantiated (Sweetlove et al. 1996). Further studies of yield in tubers expressing a nucleotide (adenylate) transporter (NTT) from *Arabidopsis* showed starch content per gram fresh weight increased by 16% and 36% (Tjaden et al. 1998, Geigenberger et al. 2001) and by 17% by downregulation of plastidial adenylate kinase, catalyzing the interconversion of adenine nucleotides (Regierer et al. 2002). In both cases, ADP-glucose levels were significantly elevated. Recently, transgenic potato plants simultaneously expressing a pea glucose-6-phosphate/phosphate translocator (GPT) and the *Arabidopsis* NTT exhibited up to a 44% increase in starch yield per plant. This was made up of an enhanced tuber yield of up to 19% concomitant with an increased starch content of up to 28% (Zhang et al. 2008a). These data suggest that starch formation within potato tuber amyloplasts is colimited by the energy import as well as carbon supply. Importantly, a similar scale of systematic pathway analysis and transformation needs to be done in cereal crops in order to identify the key enzymes exhibiting high FCC, regulation, and/or colimitation. Such studies will need to be combined with our advancing understanding of the biochemical functions of the recently identified multi-enzyme complexes and their possible roles in regulating metabolism in storage tissues.

SUMMARY POINTS

1. Over the past 20 years enormous progress has been made in our understanding of the genetics and biochemistry of starch synthesis, to the extent that we now know the structures of the enzymes and genes.
2. Although we have learned a lot about how carbon flux is regulated for potato, our knowledge is much less complete for cereals.
3. Despite this remarkable progress, very little has been achieved in terms of adding value to starch or increasing starch yield, particularly in cereal crops.

FUTURE ISSUES

1. What biochemical and genetic factors limit the accumulation of storage starch in cereal crops, and how might this be usefully engineered or improved in order to increase crop yield?
2. What structural changes might yet be made to amylopectin or amylose in storage starches in crops to enhance functionality and hence increase the real value of starch?
3. How is the biochemical pathway of storage starch synthesis regulated at a transcriptional level in relation to all reserve products accumulating in the developing endosperm?
4. How is the process of starch assembly regulated at an individual enzyme level, and how does this relate to the formation of enzyme-protein complexes?
5. What are the biochemical roles of the multi-enzyme starch synthesis complexes in storage tissues?

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.

LITERATURE CITED

Abad MC, Binderup K, Rios-Steiner J, Arni RK, Preiss J, et al. 2002. The x-ray crystallographic structure of *Escherichia coli* branching enzyme. *J. Biol. Chem.* 277:42164–70

Abe T, Niiyama H, Sasahara T. 2002. Cloning of cDNA for UDP-glucose pyrophosphorylase and the expression of mRNA in rice endosperm. *Theor. Appl. Genet.* 105:216–21

Ahern KR, Deewatthanawong P, Schares J, Muszynski M, Weeks R, et al. 2009. Regional mutagenesis using dissociation in maize. *Methods* 49:248–54

Andreeva A, Howorth D, Brenner SE, Hubbard TJP, Chothia C, et al. 2004. SCOP database in 2004: refinements integrate structure and sequence family data. *Nucleic Acids Res.* 32:D226–29

ap Rees T, Hill SA. 1994. Metabolic control analysis of plant metabolism. *Plant Cell Environ.* 17:587–99

Ball S, Guan HP, James M, Myers A, Keeling P, et al. 1996. From glycogen to amylopectin: A model for the biogenesis of the plant starch granule. *Cell* 86:349–52

Ball SG, Deschamps P. 2009. Starch metabolism. In *The Chlamydomonas Sourcebook*, ed. DB Stern, EHH Harris, pp. 2–40. Amsterdam: Elsevier. 2nd ed.

Ball SG, Morell MK. 2003. From bacterial glycogen to starch: Understanding the biogenesis of the plant starch granule. *Annu. Rev. Plant Biol.* 54:207–33

Ballicora MA, Iglesias A, Preiss J. 2004. ADP-glucose pyrophosphorylase: a regulatory enzyme for plant starch synthesis. *Photosynth. Res.* 79:1–24

Baroja-Fernández E, Muñoz FJ, Saikusa T, Rodríguez-López M, Akazawa T, Romero J. 2003. Sucrose synthase catalyzes the de novo production of ADPglucose linked to starch biosynthesis in heterotrophic tissues of plants. *Plant Cell Physiol.* 44:500–9

Bateman A. 1999. The SIS domain: a phosphosugar-binding domain. *Trends Biochem. Sci.* 24:94–95

Beatty MK, Rahman A, Cao HP, Woodman W, Lee M, et al. 1999. Purification and molecular genetic characterization of ZPU1, a pullulanase-type starch-debranching enzyme from maize. *Plant Physiol.* 119:255–66

Blauth SL, Kim KN, Klucinec JD, Shannon JC, Thompson DB, Guilitinan MJ. 2002. Identification of *Mutator* insertional mutants of starch-branching enzyme 1 (SBEI) in *Zea mays* L. *Plant Mol. Biol.* 48:287–97

Blauth SL, Yao Y, Klucinec JD, Shannon JC, Thompson DB, Guilitinan MJ. 2001. Identification of *Mutator* insertional mutants of starch-branching enzyme 2a in corn. *Plant Physiol.* 125:1396–405

Boehlein SK, Sewell AK, Cross J, Stewart JD, Hannah LC. 2005. Purification and characterization of adenosine diphosphate glucose pyrophosphorylase from maize/potato mosaics. *Plant Physiol.* 138:1552–62

Boyer CC, Preiss J. 1981. Evidence for independent genetic control of the multiple forms of maize endosperm branching enzymes and starch synthases. *Plant Physiol.* 67:1141–45

Burton RA, Jenner H, Carrangis L, Fahy B, Fincher GB, et al. 2002. Starch granule initiation and growth are altered in barley mutants that lack isoamylase activity. *Plant J.* 31:97–112

Buschiazzo A, Ugalde JE, Guerin ME, Shepard W, Ugalde RA, et al. 2004. Crystal structure of glycogen synthase: homologous enzymes catalyze glycogen synthesis and degradation. *EMBO J.* 23:3196–205

Bustos R, Fahy B, Hylton CM, Seale R, Nebane NM, et al. 2004. Starch granule initiation is controlled by a heteromultimeric isoamylase in potato tubers. *Proc. Natl. Acad. Sci. USA* 101:2215–20

Campbell MR, Pollak LM, White PJ. 1994. Dosage effect at the sugary-2 locus on maize starch structure and function. *Cereal Chem.* 71:464–68

Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V. 2009. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Res.* 37:D233–38

Cao HP, Sullivan TD, Boyer CD, Shannon JC. 1995. Btl, a structural gene for the major 39–44 kDa amyloplast membrane polypeptides. *Physiol. Plant.* 95:176–86

Chang A, Scheer M, Grote A, Schomburg I, Schomburg D. 2009. BRENDA, AMENDA and FRENDA the enzyme information system: new content and tools in 2009. *Nucleic Acids Res.* 37:D588–92

Chen R, Zhao X, Shao Z, Wei Z, Wang Y, et al. 2007. Rice UDP-glucose pyrophosphorylase1 is essential for pollen callose deposition and its cosuppression results in a new type of thermosensitive genic male sterility. *Plant Cell* 19:847–61

Chourey PS, Taliercio EW, Carlson SJ, Ruan YL. 1998. Genetic evidence that the two isozymes of sucrose synthase present in developing maize endosperm are critical, one for cell wall integrity and the other for starch biosynthesis. *Mol. Gen. Genet.* 259:88–96

Commuri PD, Keeling PL. 2001. Chain-length specificities of maize starch synthase I enzyme: studies of glucan affinity and catalytic properties. *Plant J.* 25:475–86

Comparot-Moss S, Denyer K. 2009. The evolution of the starch biosynthetic pathway in cereals and other grasses. *J. Exp. Bot.* 60:2481–92

Cossegal M, Chambrier P, Mbelo S, Balzergue S, Martin-Magniette ML, et al. 2008. Transcriptional and metabolic adjustments in ADP-glucose pyrophosphorylase-deficient bt2 maize kernels. *Plant Physiol.* 146:1553–70

Craig J, Lloyd JR, Tomlinson K, Barber L, Edwards A, et al. 1998. Mutations in the gene encoding starch synthase II profoundly alter amylopectin structure in pea embryos. *Plant Cell* 10:413–26

Damager I, Denyer K, Motawia MS, Møller BL, Blennow A. 2001. The action of starch synthase II on 6‴-α-maltotriosyl-maltohexaose comprising the branch point of amylopectin. *Eur. J. Biochem.* 268:4878–84

Davies C, Muirhead H. 2002. Crystal structure of phosphoglucose isomerase from pig muscle and its complex with 5-phosphoarabinonate. *Proteins* 49:577–79

Delatte T, Trevisan M, Parker ML, Zeeman SC. 2005. *Arabidopsis* mutants Atisa1 and Atisa2 have identical phenotypes and lack the same multimeric isoamylase, which influences the branch point distribution of amylopectin during starch synthesis. *Plant J.* 41:815–30

Delcour JA, Bruneel C, Derde LJ, Gomand SV, Pareyt B, et al. 2010. Fate of starch in food processing: from raw materials to final food products. *Annu. Rev. Food Sci. Technol.* 1:87–111

Delvallé D, Dumez S, Wattebled F, Roldán I, Planchot V, et al. 2005. Soluble starch synthase I: a major determinant for the synthesis of amylopectin in *Arabidopsis thaliana* leaves. *Plant J.* 43:398–412

Denyer K, Hylton CM, Jenner CF, Smith AM. 1995. Identification of multiple isoforms of soluble and granule bound starch synthase in developing wheat endosperm. *Planta* 196:256–65

Denyer K, Johnson P, Zeeman S, Smith AM. 2001. The control of amylose synthesis. *J. Plant Physiol.* 158:479–87

Denyer K, Waite D, Motawia S, Møller BL, Smith AM. 1999. Granule-bound starch synthase I in isolated starch granules elongates malto-oligosaccharides processively. *Biochem. J.* 340:183–91

Deschamps P, Colleoni C, Nakamura Y, Suzuki E, Putaux JL, et al. 2008a. Metabolic symbiosis and the birth of the plant kingdom. *Mol. Biol. Evol.* 25:536–48

Deschamps P, Haferkamp I, D'Hulst C, Neuhaus HE, Ball SG. 2008b. The relocation of starch metabolism to chloroplasts: when, why and how. *Trends Plant Sci.* 13:574–82

Dinges JR, Colleoni C, James MG, Myers AM. 2003. Purification and molecular genetic characterization of ZPU1, a pullulanase-type starch-debranching enzyme from maize. *Plant Cell* 15:666–80

Dinges JR, Colleoni C, Myers AM, James MG. 2001. Molecular structure of three mutations at the maize *sugary1* locus and their allele-specific phenotypic effects. *Plant Physiol.* 125:1406–18

Doehlert DC, Kuo TM. 1994. Gene expression in developing kernels of some endosperm mutants of maize. *Plant Cell Physiol.* 35:411–18

DuPont FM. 2008. Metabolic pathways of the wheat (*Triticum aestivum*) endosperm amyloplast revealed by proteomics. *BMC Plant Biol.* 8:39

Edwards A, Fulton DC, Hylton CM, Jobling SA, Gidley M, et al. 1999. A combined reduction in activity of starch synthases II and III of potato has novel effects on the starch of tubers. *Plant J.* 17:251–61

Edwards A, Vincken JP, Suurs LCJM, Visser RGF, Zeeman S, et al. 2002. Discrete forms of amylose are synthesized by isoforms of GBSSl in pea. *Plant Cell* 14:1767–85

Fettke J, Hejazi M, Smirnova J, Höchel E, Stage M, Steup M. 2009. Eukaryotic starch degradation: integration of plastidial and cytosolic pathways. *J. Exp. Bot.* 60:2907–22

Finn RD, Mistry J, Schuster-Böckler B, Griffiths-Jones S, Hollich V, et al. 2008. Pfam: clans, web tools and services. *Nucleic Acids Res.* 34:247–51

Fisher DK, Kim KN, Gao M, Boyer CD, Guiltinan MJ. 1995. A cDNA encoding starch branching enzyme I from maize endosperm. *Plant Physiol.* 108:1313–14

Franco OL, Rigden DJ. 2003. Fold recognition analysis of glycosyltransferase families: further members of structural superfamilies. *Glycobiology* 13:707–12

Fujita N, Kubo A, Suh D, Wong KS, Jane J-L, et al. 2003. Antisense inhibition of isoamylase alters the structure of amylopectin and the physicochemical properties of starch in rice endosperm. *Plant Cell Physiol.* 44:607–18

Fujita N, Yoshida M, Asakura N, Ohdan T, Miyao A, et al. 2006. Function and characterization of starch synthase I using mutants in rice. *Plant Physiol.* 140:1070–84

Fulton D, Edwards A, Pilling E, Robinson HL, Fahy B, et al. 2002. Role of granule bound starch synthase in determination of amylopectin structure and starch granule morphology in potato. *J. Biol. Chem.* 277:10834–41

Gao M, Fisher DK, Kim KN, Shannon JC, Guiltinan MJ. 1997. Independent genetic control of maize starch-branching enzymes IIa and IIb—Isolation and characterization of a *Sbe2a* cDNA. *Plant Physiol.* 114:69–78

Gao M, Wanat J, Stinard PS, James MG, Myers AM. 1998. Characterization of *dull1*, a maize gene coding for a novel starch synthase. *Plant Cell* 10:399–412

Gao Z, Keeling PL, Shibles R, Guan HP. 2004. Involvement of lysine-193 of the conserved "K-T-G-G" motif in the catalysis of maize starch synthase IIa. *Arch. Biochem. Biophys.* 427:1–7

Garwood DL, Shannon JC, Creech RG. 1976. Starches of endosperms possessing different alleles at the *amylose-extender* locus in *Zea mays* L. *Cereal Chem.* 53:355–64

Geigenberger P, Stamme C, Tjaden J, Schulz A, Quick PW, et al. 2001. Tuber physiology and properties of starch from tubers of transgenic potato plants with altered plastidic adenylate transporter activity. *Plant Physiol.* 125:1667–78

Geigenberger P, Stitt M, Fernie AR. 2004. Metabolic control analysis and regulation of the conversion of sucrose to starch in growing potato tubers. *Plant Cell Environ.* 27:655–73

Gerard C, Barron C, Colonna P, Planchot V. 2009. Amylose determination in genetically modified starches. *Carbohydr. Polym.* 44:19–27

Geremia S, Campagnolo M, Schinzel R, Johnson LN. 2002. Enzymatic catalysis in crystals of *Escherichia coli* maltodextrin phosphorylase. *J. Mol. Biol.* 322:413–23

Gibson RP, Tarling CA, Roberts S, Withers SG, Davies GJ. 2004. The donor subsite of trehalose-6-phosphate synthase: binary complexes with UDP-glucose and UDP-2-deoxy-2-fluoro-glucose at 2 A resolution. *J. Biol. Chem.* 279:1950–1955

Giroux MJ, Shaw J, Barry G, Cobb BG, Greene T, et al. 1996. A single gene mutation that increases maize seed weight. *Proc. Natl. Acad. Sci. USA* 93:5824–29

Glawischnig E, Gierl A, Tomas A, Bacher A, Eisenreich W. 2002. Starch biosynthesis and intermediary metabolism in maize kernels. Quantitative analysis of metabolite flux by nclear magnetic resonance. *Plant Physiol.* 130:1717–27

Grimaud F, Rogniaux H, James MG, Myers AM, Planchot V. 2008. Proteome and phosphoproteome analysis of starch granule-associated proteins from normal maize and mutants affected in starch biosynthesis. *J. Exp. Bot.* 59:3395–406

Guan H, Li P, Imparl-Radosevich J, Preiss J, Keeling P. 1997. Comparing the properties of *Escherichia coli* branching enzyme and maize branching enzyme. *Arch. Biochem. Biophys.* 342:92–98

Guan HP, Keeling PL. 1998. Starch biosynthesis: understanding the functions and interactions of multiple isozymes of starch synthase and branching enzyme. *Trends Glycosci. Glycotechnol.* 10:307–19

Hamada S, Ito H, Ueno H, Takeda Y, Matsui H. 2007. The N-terminal region of the starch-branching enzyme from *Phaseolus vulgaris* L. is essential for optimal catalysis and structural stability. *Phytochemistry* 68:1367–75

Hannah LC, James MG. 2008. The complexities of starch biosynthesis in cereal endosperms. *Curr. Opin. Biotechnol.* 19:160–65

Hannah LC, Shaw JR, Giroux MJ, Reyss A, Prioul JL, et al. 2001. Maize genes encoding the small subunit of ADP-glucose pyrophosphorylase. *Plant Physiol.* 127:173–83

Harn C, Knight M, Ramakrishnan A, Guan H, Keeling PL, et al. 1998. Isolation and characterization of the zSSIIa and zSSIIb starch synthase cDNA clones from maize endosperm. *Plant Mol. Biol.* 37:639–49

Helm JL, Fergason VL, Zuber MS. 1969. Interaction of dosage effects on amylose content of corn at the Du and Wx loci. *J. Hered.* 60:259–60

Hennen-Bierwagen TA, Lin Q, Grimaud F, Planchot V, Keeling PL, et al. 2009. Proteins from multiple metabolic pathways associate with starch biosynthetic enzymes in high molecular weight complexes: A model for regulation of carbon allocation in maize amyloplasts. *Plant Physiol.* 149:1541–59

Hennen-Bierwagen TA, Liu F, Marsh RS, Kim S, Gan Q, et al. 2008. Starch biosynthetic enzymes from developing *Zea mays* endosperm associate in multisubunit complexes. *Plant Physiol.* 146:1892–908

Hizukuri S, Takeda Y, Maruta N. 1989. Molecular structure of rice starches. *Carbohydr. Res.* 189:227–35

Horibata T, Nakamoto M, Fuwa H, Inouchi N. 2004. Structural and physicochemical characteristics of endosperm starches of rice cultivars recently bred in Japan. *J. Appl. Glycosci.* 51:239–346

Hovenkamp-Hermelink JHM, Jacobsen E, Ponstein AS, Visser RGH, Vos-Scheperkeuter GH, et al. 1987. Isolation of an amylose-free starch mutant of the potato (*Solanum tuberosum L.*). *Theor. Appl. Genet.* 75:217–21

Hseih JS. 1988. Genetic studies of the Wx gene of sorghum (*Sorghum bicolor* [L.] Moench). *Crop. Sci.* 14:643–46

Huang XF, Nguyen-Quoc B, Chourey PS, Yelle S. 1994. Complete nucleotide sequence of the maize (*Zea mays* L.) sucrose synthase 2 cDNA. *Plant Physiol.* 104:293–94

Hussain H, Mant A, Seale R, Zeeman S, Hinchliffe E, et al. 2003. Three isoforms of isoamylase contribute different catalytic properties for the debranching of potato glucans. *Plant Cell* 15:133–49

Imparl-Radosevich JM, Li P, Zhang L, McKean AL, Keeling PL, et al. 1998. Purification and characterization of maize starch synthase I and its truncated forms. *Arch. Biochem. Biophys.* 353:64–72

Inouchi N, Glover DV, Fuwa H. 1987. Chain length distribution of amylopectins of several single mutants and the normal counterpart, and sugary-1 phytoglycogen in maize (*Zea mays* L.). *Starch - Stärke* 39:259–66

James MG, Robertson DS, Myers AM. 1995. Characterization of the maize gene sugary1, a determinant of starch composition in kernels. *Plant Cell* 7:417–29

Jane J-L, Kasemsuwan T, Leas S, Ames I, Zobel HF, et al. 1994. Anthology of starch granule morphology by scanning electron microscopy. *Starch - Starke* 46:121–29

Jin X, Ballicora MA, Preiss J, Geiger JH. 2005. Crystal structure of potato tuber ADP-glucose pyrophosphorylase. *EMBO J.* 24:294–704

Jobling SA, Westcott RJ, Tayal A, Jeffcoat R, Schwall GP. 2002. Production of freeze-thaw stable potato starch by antisense inhibition of three starch synthase genes. *Nat. Biotechnol.* 20:295–99

Kammerer B, Fischer K, Hilpert B, Schubert S, Gutensohn M, et al. 1998. Molecular characterization of a carbon transporter in plastids from heterotrophic tissues: The glucose 6-phosphate/phosphate antiporter. *Plant Cell* 10:105–18

Katsuya Y, Mezaki Y, Kubota M, Matsuura Y. 1998. Three-dimensional structure of *Pseudomonas isoamylase* at 2.2 Å resolution. *J. Mol. Biol.* 281:885–97

Keeling PL. 1999. From enzyme activity to flux control: a quest to understand starch deposition in developing cereal grains. In *Plant Carbohydrate Biochemistry*, ed. J Bryant, MM Burrell, NJ Kruger, pp. 91–103. Oxford: BIOS Sci. Publ. Ltd.

Keeling PL, Banisadr R, Barone L, Wasserman BP, Singletary GW. 1994. Effect of temperature on enzymes in the pathway of starch biosynthesis in developing wheat and maize grain. *Aust. J. Plant Physiol.* 21:807–27

Keeling PL, Greaves JA. 1990. Effects of temperature stresses on corn: Opportunities for breeding and biotechnology. In *Proc. 45th Annu. Corn Sorghum Res. Conf.*, ed. D Wilkinson, pp. 29–42. Washington, DC: Am. Seed Trade Assoc.

Keeling PL, Woods JR, Tyson RH, Bridges IG. 1988. Starch biosynthesis in developing wheat grain: Evidence against the direct involvement of triose phosphates in the metabolic pathway. *Plant Physiol.* 87:311–19

Kelley LA, Sternberg MJE. 2009. Protein structure prediction on the Web: a case study using the PHYRE server. *Nat. Protocols* 4:363–71

Kim KN, Fisher DK, Gao M, Guiltinan MJ. 1998. Molecular cloning and characterization of the *amylose-extender* gene encoding starch branching enzyme IIB in maize. *Plant Mol. Biol.* 38:945–56

Kirchberger S, Leroch M, Huynen MA, Wahl M, Neuhaus HE, et al. 2007. Molecular and biochemical analysis of the plastidic ADP-glucose transporter (ZmBT1) from *Zea mays*. *J. Biol. Chem.* 282:22481–91

Klosgen RB, Gierl A, Schwarz-Sommer Z, Saedler H. 1986. Molecular analysis of the waxy locus of *Zea mays*. *Mol. Gen. Genet.* 203:237–44

Klucinec JD, Keeling PL. 2005. Genetic modifications of plant starches for food applications. In *Food Biotechnology*, ed. K Shetty, G Paliyath, A Pometto, RE Levine, pp. 675–708. Boca Raton, FL: CRC Press. 2nd ed.

Klucinec JD, Thompson DB. 1998. Fractionation of high-amylose maize starches by differential alcohol precipitation and chromatography of the fractions. *Cereal Chem.* 75:887–96

Knight ME, Harn C, Lilley CER, Guan HP, Singletary GW, et al. 1998. Molecular cloning of starch synthase I from maize (W64) endosperm and expression in *Escherichia coli*. *Plant J.* 14:613–22

Koch KE. 2004. Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Curr. Opin. Plant Biol.* 7:235–46

Kossmann J, Lloyd J. 2000. Understanding and influencing starch biochemistry. *Crit. Rev. Biochem. Mol. Biol.* 35:141–96

Kubo A, Fujita N, Harada K, Matsuda T, Satoh H, et al. 1999. The starch-debranching enzymes isoamylase and pullulanase are both involved in amylopectin biosynthesis in rice endosperm. *Plant Physiol.* 121:399–409

Kuriki T, Stewart DC, Preiss J. 1997. Construction of chimeric enzymes out of maize endosperm branching enzymes I and II: Activity and properties. *J. Biol. Chem.* 272:28999–9004

Kuser PR, Krauchenco S, Antunes OAC, Polikarpov I. 2000. The high resolution crystal structure of yeast hexokinase PII with the correct primary sequence provides new insights into its mechanism of action. *J. Biol. Chem.* 275:20814–21

Lai J, Dey N, Kim CS, Bharti AK, Rudd S, et al. 2004. Characterization of the maize endosperm transcriptome and its comparison to the rice genome. *Genome Res.* 14:1932–37

Lairson LL, Henrissat B, Davies GJ, Withers SG. 2008. Glycosyltransferases: Structures, functions, and mechanisms. *Annu. Rev. Biochem.* 77:521–55

Lal SK, Sachs MM. 1995. Cloning and characterization of an anaerobically induced cDNA encoding glucose-6-phosphate isomerase from maize. *Plant Physiol.* 108:1295–96

Leroch M, Kirchberger S, Haferkamp I, Wahl M, Neuhaus HE, Tjaden J. 2005. Identification and characterization of a novel plastidic adenine nucleotide uniporter from *Solanum tuberosum*. *J. Biol. Chem.* 280:17992–8000

Leterrier M, Holappa L, Broglie K, Beckles DM. 2008. Cloning, characterisation and comparative analysis of a starch synthase IV gene in wheat: functional and evolutionary implications. *BMC Plant Biol.* 8:98

Li Z, Sun F, Xu S, Chu X, Mukai Y, et al. 2003. The structural organisation of the gene encoding class II starch synthase of wheat and barley and the evolution of the genes encoding starch synthases in plants. *Funct. Integr. Genomics* 3:76–85

Liu J, Mushegian A. 2003. Three monophyletic superfamilies account for the majority of the known glycosyltransferases. *Protein Sci.* 12:1418–31

Liu Q, Thompson DB. 1998a. Effects of moisture content and different gelatinization heating temperatures on retrogradation of waxy-type maize starches. *Carbohydr. Res.* 314:221–35

Liu Q, Thompson DB. 1998b. Retrogradation of *du wx* and *su2 wx* maize starches after different gelatinization heat treatments. *Cereal Chem.* 75:868–74

Liu Y, Ray WJ, Baranidharan S. 1997. Structure of rabbit muscle phosphoglucomutase refined at 2.4 Å resolution. *Acta Crystallogr. D* 53:392–405

Lloyd JR, Landschütze V, Kossmann J. 1999. Simultaneous antisense inhibition of two starch-synthase isoforms in potato tubers leads to accumulation of grossly modified amylopectin. *Biochem. J.* 338:515–21

Machovic M, Janecek S. 2006. The evolution of putative starch-binding domains. *FEBS Lett.* 580:6349–56

Maddelein ML, Libessart N, Bellanger F, Delrue B, D'Hulst C, et al. 1994. Toward an understanding of the biogenesis of the starch granule. Determination of granule-bound and soluble starch synthase functions in amylopectin synthesis. *J. Biol. Chem.* 269:25150–57

Manjunath S, Lee CH, VanWinkle P, Bailey-Serres J. 1998. Molecular and biochemical characterization of cytosolic phosphoglucomutase in maize. Expression during development and in response to oxygen deprivation. *Plant Physiol.* 117:997–1006

McCoy JG, Bitto E, Bingman CA, Wesenberg GE, Bannen RM, et al. 2007. Structure and dynamics of UDP-glucose pyrophosphorylase from *Arabidopsis thaliana* with bound UDP-glucose and UTP. *J. Mol. Biol.* 366:830–41

Mechin V, Thévenot C, Le Guilloux M, Prioul JL, Damerval C. 2007. Developmental analysis of maize endosperm proteome suggests a pivotal role for pyruvate orthophosphate dikinase. *Plant Physiol.* 143:1203–19

Meyer FD, Smidansky ED, Beecher B, Greene TW, Giroux MJ. 2004. The maize Sh2r6hs ADP-glucose pyrophosphorylase (AGP) large subunit confers enhanced AGP properties in transgenic wheat (*Triticum aestivum*). *Plant Sci.* 167:899–911

Meyer FD, Talbert LE, Martin JM, Lanning SP, Greene TW, et al. 2007. Field evaluation of transgenic wheat expressing a modified ADP-glucose pyrophosphorylase large subunit. *Crop. Sci.* 47:336–42

Mizra O, Guan L, Verner G, Iwata S, Kaback HR. 2006. Structural evidence for induced fit and a mechanism for sugar/H^+symport in LacY. *EMBO J.* 25:1177–83

Morell MK, Kosar-Hashemi B, Cmiel M, Samuel MS, Chandler P, et al. 2003. Barley *sex6* mutants lack starch synthase IIa activity and contain a starch with novel properties. *Plant J.* 34:173–85

Morrison WR. 1988. Lipids in cereal starches: a review. *J. Cereal Sci.* 8:1–15

Mouille G, Maddelein ML, Libessart N, Talaga P, Decq A, et al. 1996. Preamylopectin processing: A mandatory step for starch biosynthesis in plants. *Plant Cell* 8:1353–66

Mu-Forster C, Huang R, Powers JR, Harriman RW, Knight M, et al. 1996. Physical association of starch biosynthetic enzymes with starch granules of maize endosperm. Granule-associated forms of starch synthase I and starch branching enzyme II. *Plant Physiol.* 111:821–29

Myers AM, Morell MK, James MG, Ball SG. 2000. Recent progress toward understanding biosynthesis of the amylopectin crystal. *Plant Physiol.* 122:989–97

Nakamura Y. 2002. Towards a better understanding of the metabolic system for amylopectin biosynthesis in plants: Rice endosperm as a model tissue. *Plant Cell Physiol.* 43:718–25

Nakamura Y, Francisco PB Jr, Hosaka Y, Sato A, Sawada T, et al. 2005. Essential amino acids of starch synthase IIa differentiate amylopectin structure and starch quality between japonica and indica rice varieties. *Plant Mol. Biol.* 58:213–27

Nakamura Y, Yamamori M, Hirano H, Hidaka S, Nagamura Y. 1995. Production of waxy (amylose-free) wheats. *Mol. Gen. Genet.* 248:253–59

Nelson OE. 1982. Genetic control of polysaccharide and storage protein synthesis in endosperms of barley, maize and sorghum. In *Advances in Cereal Science and Technology*, ed. Y Pomeranz, 3:41–71. St. Paul, MN: Am. Assoc. Cereal Chem.

Nichols DJ, Keeling PL, Spalding M, Guan HP. 2000. Involvement of conserved aspartate and glutamate residues in the catalysis and substrate binding of maize starch synthase. *Biochemistry* 39:7820–25

Nishi A, Nakamura Y, Tanaka N, Satoh H. 2001. Biochemical and genetic analysis of the effects of *amylose-extender* mutation in rice endosperm. *Plant Physiol.* 127:459–72

Olsen OA. 2001. Endosperm development: cellularization and cell fate specification. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52:233–67

Palopoli N, Busi MV, Fornasari MS, Gomez-Casati DF, Ugalde RA, et al. 2006. Starch-synthase III family encodes a tandem of three starch-binding domains. *Proteins* 65:27–31

Patron NJ, Greber B, Fahy BF, Laurie DA, Parker ML, et al. 2004. The lys5 mutations of barley reveal the nature and importance of plastidial ADP-Glc transporters for starch synthesis in cereal endosperm. *Plant Physiol.* 135:2088–97

Pebay-Peyroula E, Dahout-Gonzalez C, Kahn R, Trezeguet V, Lauquin GJM, et al. 2003. Structure of mitochondrial ADP/ATP carrier in complex with carboxyatractyloside. *Nature* 426:39–44

Rahman A, Wong KS, Jane JL, Myers AM, James MG. 1998. Characterization of SU1 isoamylase, a determinant of storage starch structure in maize. *Plant Physiol.* 117:425–35

Reddy RK, Ali ZS, Bhattacharya KR. 1993. The fine structure of rice-starch amylopectin and its relation to the texture of cooked rice. *Carbohydr. Polym.* 22:267–75

Regierer B, Fernie AR, Springer F, Perez-Melis A, Leisse A, et al. 2002. Starch content and yield increase as a result of altering adenylate pools in transgenic plants. *Nat. Biotechnol.* 20:1256–60

Roldán I, Wattebled F, Lucas MM, Delvallé D, Planchot V, et al. 2007. The phenotype of soluble starch synthase IV defective mutants of *Arabidopsis thaliana* suggests a novel function of elongation enzymes in the control of starch granule formation. *Plant J.* 49:492–504

Rösti S, Denyer K. 2007. Two paralogous genes encoding small subunits of ADP-glucose pyrophosphorylase in maize, Bt2 and L2, replace the single alternatively spliced gene found in other cereal species. *J. Mol. Evol.* 65:316–27

Sakulsingharoj C, Choi SB, Hwang SK, Edwards GE, Bork J, et al. 2004. Engineering starch biosynthesis for increasing rice seed weight: the role of the cytoplasmic ADP-glucose pyrophosphorylase. *Plant Sci.* 167:1323–33

Salehuzzaman SNIM, Vincken JP, Van de Wal M, Straatman-Engelen I, Jacobsen E, et al. 1999. Expression of a cassava granule-bound starch synthase gene in the amylose-free potato only partially restores amylose content. *Plant Cell Environ.* 22:1311–18

Satoh H, Shibahara K, Tokunaga T, Nishi A, Tasaki M, et al. 2008. Mutation of the plastidial alpha-glucan phosphorylase gene in rice affects the synthesis and structure of starch in the endosperm. *Plant Cell* 20:1833–49

Sawada T, Francisco PB Jr, Aihara S, Utsumi Y, Yoshida M, et al. 2009. Chlorella starch branching enzyme (BE) can complement the function of BEIIb in rice endosperm. *Plant Cell Physiol.* 50:1062–74

Shannon JC, Pien FM, Cao HP, Liu KC. 1998. Brittle-1, an adenylate translocator, facilitates transfer of extraplastidial synthesized ADP-glucose into amyloplasts of maize endosperms. *Plant Physiol.* 117:1235–52

Shi YC, Seib P. 1995. Fine structure of maize starches from four *wx*-containing genotypes of the W64A inbred line in relation to gelatinization and retrogradation. *Carbohydr. Polym.* 26:141–47

Shi YC, Seib P. 1998. Molecular structure of a low-amylopectin starch and other high-amylose maize starches. *J. Cereal Sci.* 27:289–99

Shure M, Wessler SR, Federoff N. 1983. Molecular identification and isolation of the waxy locus in maize. *Cell* 35:225–33

Sidebottom C, Kirkland M, Strongitharm B, Jeffcoat R. 1998. Characterization of the difference of starch branching enzyme activities in normal and low-amylopectin maize during kernel development. *J. Cereal Sci.* 27:279–87

Singletary GW, Banisadr R, Keeling PL. 1997. Influence of gene dosage on carbohydrate synthesis and enzymatic activities in endosperm of starch-deficient mutants of maize. *Plant Physiol.* 113:293–304

Smidansky ED, Clancy M, Meyer FD, Lanning SP, Blake NK, et al. 2002. Enhanced ADP-glucose pyrophosphorylase activity in wheat endosperm increases seed yield. *Proc. Natl. Acad. Sci. USA* 99:1724–29

Smidansky ED, Martin JM, Hannah LC, Fischer AM, Giroux MJ. 2003. Seed yield and plant biomass increases in rice are conferred by deregulation of endosperm ADP-glucose pyrophosphorylase. *Planta* 216:656–64

Smidansky ED, Meyer FD, Blakeslee B, Weglarz TE, Greene TW, Giroux MJ. 2007. Expression of a modified ADP-glucose pyrophosphorylase large subunit in wheat seeds stimulates photosynthesis and carbon metabolism. *Planta* 225:965–76

Smith AM. 2008. Prospects for increasing starch and sucrose yields for bioethanol production. *Plant J.* 54:546–58

Smith AM, Stitt M. 2007. Coordination of carbon supply and plant growth. *Plant Cell Environ.* 30:1126–49

Smith AM, Zeeman SC, Smith SM. 2005. Starch degradation. *Annu. Rev. Plant Biol.* 56:73–98

Stark DM, Timmerman KP, Barry GF, Preiss J, Kishore GM. 1992. Regulation of the amount of starch in plant tissues by ADP glucose pyrophosphorylase. *Science* 258:287–92

Stinard PS, Robertson DS, Schnable PS. 1993. Genetic isolation, cloning and analysis of a mutator-induced, dominant antimorph of the maize amylose extender1 locus. *Plant Cell* 5:1555–66

Sullivan TD, Strelow LI, Illingworth CA, Phillips RL, Nelson OE Jr. 1991. Analysis of maize brittle-1 alleles and a defective suppressor-mutator-induced mutable allele. *Plant Cell* 3:1337–48

Sulpice R, Pyl ET, Ishihara H, Trenkamp S, Steinfath M, et al. 2009. Starch as a major integrator in the regulation of plant growth. *Proc. Natl. Acad. Sci. USA* 106:10348–53

Sun C, Palmqvist S, Olsson H, Boren M, Ahlandsberg S, et al. 2003. A novel WRKY transcription factor, SUSIBA2, participates in sugar signaling in barley by binding to the sugar-responsive elements of the iso1 promoter. *Plant Cell* 15:2076–92

Sweetlove LJ, Burrell MM, ap Rees T. 1996. Starch metabolism in tubers of transgenic potato (*Solanum tuberosum*) with increased ADPglucose pyrophosphorylase. *Biochem. J.* 320:493–98

Takeda C, Takeda Y, Hizukuri S. 1989. Structure of amylomaize amylose. *Cereal Chem.* 66:22–25
Takeda Y, Guan HP, Preiss J. 1993. Branching of amylose by the branching isoenzymes of maize endosperm. *Carbohydr. Res.* 240:253–63
Takeda Y, Hizukuri S, Takeda C, Suzuki A. 1987. Structures of branched molecules of amyloses of various origins, and molar fractions of branched and unbranched molecules. *Carbohydr. Res.* 165:139–45
Takeda Y, Preiss J. 1993. Structures of B90 (sugary) and W64A (normal) maize starches. *Carbohydr. Res.* 240:265–75
Tetlow IJ, Beisel KG, Cameron S, Makhmoudova A, Liu F, et al. 2008. Analysis of protein complexes in wheat amyloplasts reveals functional interactions among starch biosynthetic enzymes. *Plant Physiol.* 146:1892–908
Tetlow IJ, Wait R, Lu Z, Akkasaeng R, Bowsher CG, et al. 2004. Protein phosphorylation in amyloplasts regulates starch branching enzyme activity and protein-protein interactions. *Plant Cell* 16:694–708
Tjaden J, Haferkamp I, Boxma B, Tielens AGM, Huynen MA, et al. 2004. A divergent ADP/ATP carrier in the hydrogenosomes of *Trichomonas gallinae* argues for an independent origin of these organelles. *Mol. Microbiol.* 51:1439–46
Tjaden J, Möhlmann T, Kampfenkel K, Henrichs G, Neuhaus HE. 1998. Altered plastidic ATP/ADP-transporter activity influences potato (*Solanum tuberosum* L.) tuber morphology, yield and composition of tuber starch. *Plant J.* 16:531–40
Tomlinson K, Denyer K. 2003. Starch synthesis in cereal grain. *Adv. Bot. Res.* 40:1–61
Tsai CY, Nelson OE. 1969. Two additional phosphorylases in developing maize seeds. *Plant Physiol.* 44:159–67
Umemoto T, Yano M, Satoh H, Short JM, Shamura A, et al. 2002. Mapping of a gene responsible for the difference in amylopectin structure between japonica-type and indica-type rice varieties. *Theor. Appl. Genet.* 104:1–8
Valdez HA, Busi MV, Wayllace NZ, Parisi G, Ugalde RA, et al. 2008. Role of the N-terminal starch-binding domains in the kinetic properties of starch synthase III from *Arabidopsis thaliana*. *Biochemistry* 47:3026–32
Vrinten PL, Nakamura T. 2000. Wheat granule-bound starch synthase I and II are encoded by separate genes that are expressed in different tissues. *Plant Physiol.* 122:255–63
Vu NT, Shimada H, Kakuta Y, Nakashima T, Ida H, et al. 2008. Biochemical and crystallographic characterization of the starch branching enzyme I (BEI) from *Oryza sativa* L. *Biosci. Biotechnol. Biochem.* 72:2858–66
Wang YJ, White P, Pollak L, Jane J. 1993a. Amylopectin and intermediate materials in starches from mutant genotypes of the Oh43 inbred line. *Cereal. Chem.* 70:521–25
Wang YJ, White P, Pollak L, Jane J. 1993b. Characterization of starch structures of 17 maize endosperm mutant genotypes with Oh43 inbred line background. *Cereal. Chem.* 70:171–79
Wang Z, Chen X, Wang J, Liu T, Liu Y, et al. 2007. Increasing maize seed weight by enhancing the cytoplasmic ADP-glucose pyrophosphorylase activity in transgenic maize plants. *Plant Cell Tissue Organ Cult.* 88:83–92
Watson KA, McCleverty C, Geremia S, Cottaz S, Driguez H, et al. 1999. Phosphorylase recognition and phosphorolysis of its oligosaccharide substrate: answers to a long outstanding question. *EMBO J.* 18:4619–32
Wattebled F, Dong Y, Dumez S, Delvallé D, Planchot V, et al. 2005. Mutants of *Arabidopsis* lacking a chloroplastic isoamylase accumulate phytoglycogen and an abnormal form of amylopectin. *Plant Physiol.* 138:184–95
Wu CY, Colleoni C, Myers AM, James MG. 2002. Enzymatic properties and regulation of ZPU1, the maize pullulanase-type starch debranching enzyme. *Arch. Biochem. Biophys.* 406:21–32
Yamamori M. 2000. Genetic elimination of a starch granule protein, SGP-1, of wheat generates an altered starch with apparent high amylose. *Theor. Appl. Genet.* 101:21–29
Yao Y, Thompson DB, Guiltinan MJ. 2004. Maize starch-branching enzyme isoforms and amylopectin structure. In the absence of starch-branching enzyme IIb, the further absence of starch-branching enzyme Ia leads to increased branching. *Plant Physiol.* 136:3515–23
Yeh JY, Garwood DL, Shannon J. 1981. Characterisation of starch from maize endosperm mutants. *Starch - Stärke* 33:222–30
Yu J, Wang J, Lin W, Li S, Li H, et al. 2005. The genomes of *Oryza sativa*: A history of duplications. *PLoS Biol.* 3:e38

Yu Y, Mu HH, Wasserman BP, Carman GM. 2001. Identification of the maize amyloplast stromal 112-kD protein as a plastidic starch phosphorylase. *Plant Physiol.* 125:351–59

Yuan RC, Thompson DB, Boyer CD. 1993. Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from 3 wx containing genotypes in 2 inbred lines. *Cereal Chem.* 70:81–89

Zhang L, Hausler R, Greiten C, Hajiresaei M-R, Haferkamp I, et al. 2008a. Overriding the co-limiting import of carbon and energy into tuber amyloplasts increases the starch content and yield of transgenic potato plants. *Plant Biotechnol. J.* 6:453–64

Zhang S, Nichols SE, Dong JG. 2003. Cloning and characterization of two fructokinases from maize. *Plant Sci.* 165:1051–58

Zhang X, Colleoni C, Ratushna V, Sirghie-Colleoni M, James M, et al. 2004. Molecular characterization demonstrates that the *Zea mays* gene *sugary2* codes for the starch synthase isoform SSIIa. *Plant Mol. Biol.* 54:865–79

Zhang X, Myers AM, James MG. 2005. Mutations affecting starch synthase III in *Arabidopsis* alter leaf starch structure and increase the rate of starch synthesis. *Plant Physiol.* 138:663–74

Zhang X, Szydlowski N, Delvallé D, D'Hulst C, James MG, et al. 2008b. Overlapping functions of the starch synthases SSII and SSIII in amylopectin biosynthesis in *Arabidopsis*. *BMC Plant Biol.* 8:96

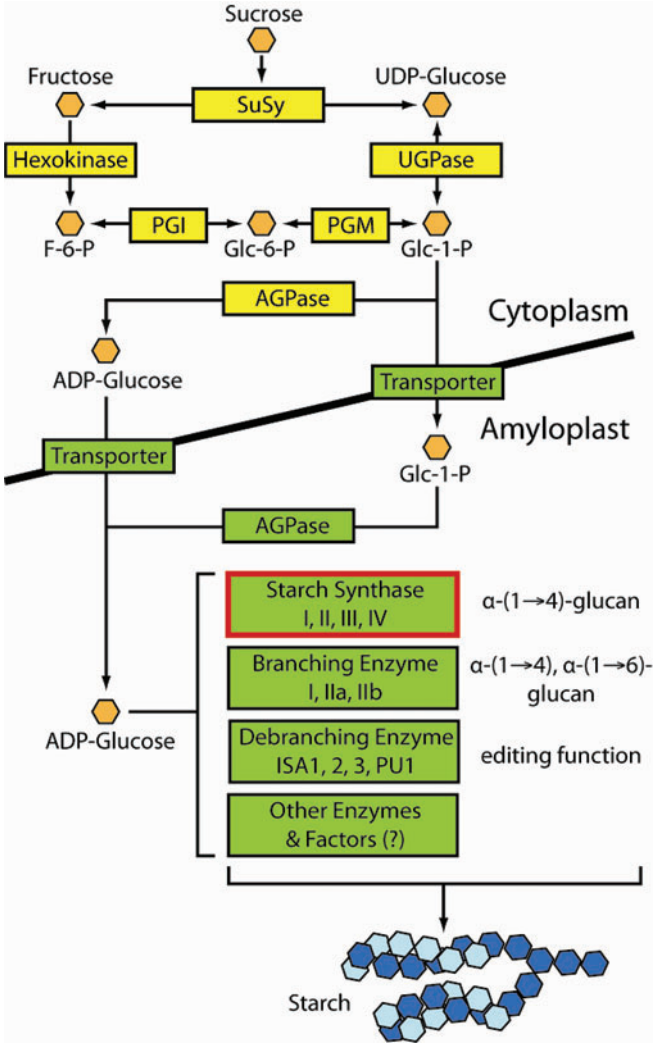
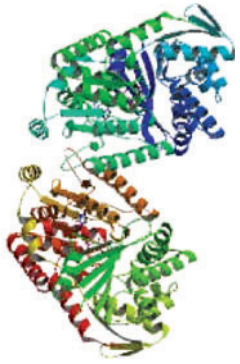


Figure 2

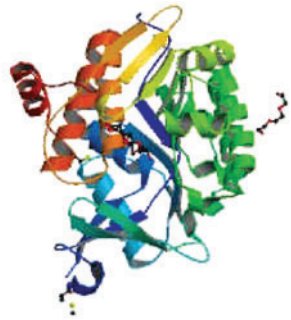
Pathway of amylopectin biosynthesis. The diagram indicates that SS, SBE, and DBE do not necessarily act in a linear biosynthetic pathway, but instead are likely to act simultaneously on a glucan precursor that matures when it transitions from the aqueous to the crystalline phase.



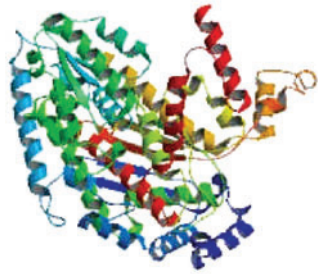
Trehalose synthase (ID: 1uqt)

Figure 4

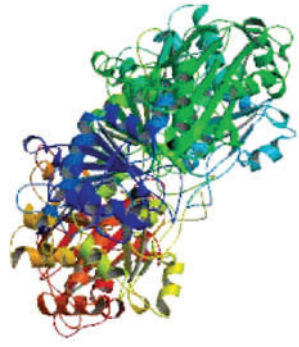
Structural comparison showing trehalose synthase having structural similarity to sucrose synthase (Gibson et al. 2004). Images from Protein Data Bank (**http://www.pdb.org**).



Glucokinase (ID: 2qcv)



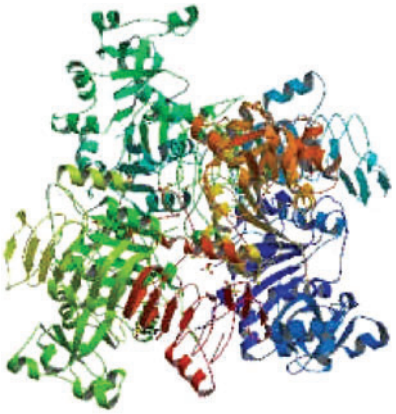
Phosphoglucose isomerase (ID: 1gzd)



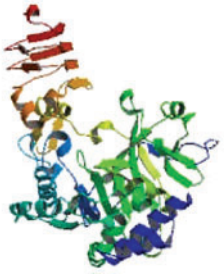
Phosphoglucomutase (ID: 1lxt)

Figure 5

Structural comparison showing glucokinase having structural similarity to fructokinase (Kuser et al. 2000), phosphoglucose isomerase (Davies & Muirhead 2002), and phosphoglucomutase (Liu et al. 1997). Images from Protein Data Bank (**http://www.pdb.org**).



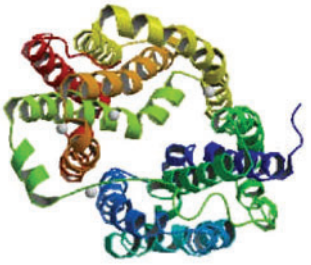
AGPP tetramer (ID: 1yp2)



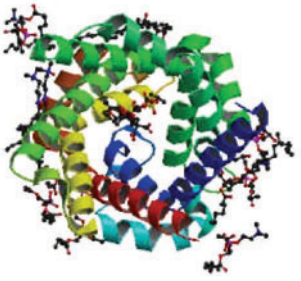
UGPP monomer (ID: 2icy)

Figure 6

Structural comparison showing AGPP tetramer (Jin et al. 2005) and UGPP monomer (McCoy et al. 2007). The N-terminal catalytic region includes conserved nucleotidyl transferase domains together with a parallel β-helix at the C-terminus of each protein subunit. Images from Protein Data Bank (**http://www.pdb.org**).



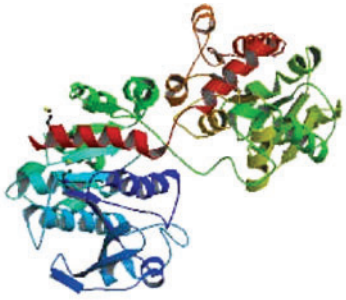
Lactose permease (ID: 2cfq)



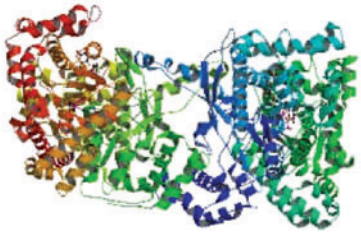
ADP/ATP carrier (ID: 1okc)

Figure 7

Structural comparison showing lactose permease having structural similarity to the glucose phosphate translocator (GPT) (Mizra et al. 2006) and the ADP/ATP carrier proteins with structural similarity to Brittle1 (Pebay-Peyroula et al. 2003). Images from Protein Data Bank (**http://www.pdb.org**).



Glycogen synthase (ID: 1rzv)



Maltodextrin phosphorylase (ID: 1qm5)

Figure 9

Structural comparison showing glycogen synthase (Buschiazzo et al. 2004) and maltodextrin phosphorylase (Watson et al. 1999). The deep interdomain crevice includes the catalytic center of these enzymes. Images from Protein Data Bank (**http://www.pdb.org**).

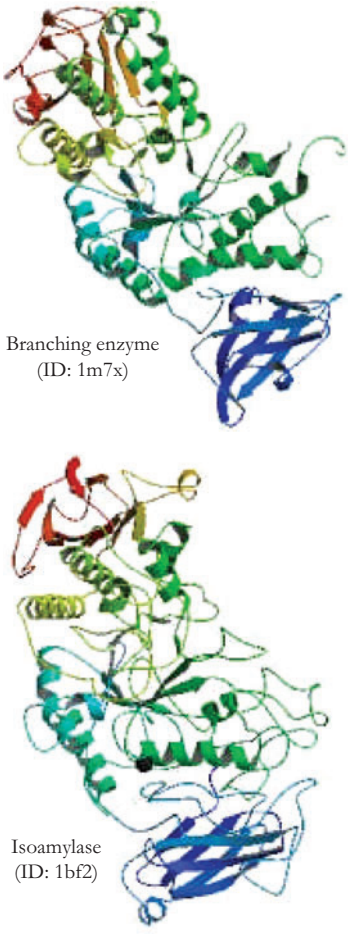


Figure 10

Structural comparison of a branching enzyme (Abad et al. 2002) and debranching enzyme (Katsuya et al. 1998). Images from Protein Data Bank (**http://www.pdb.org**).

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Errata

An online log of corrections to *Annual Review of Food Science and Technology* articles may be found at http://food.annualreviews.org