

# Starch synthesis in the cereal endosperm

Martha G James<sup>\*†</sup>, Kay Denyer<sup>‡</sup> and Alan M Myers<sup>\*§</sup>

The pathway of starch synthesis in the cereal endosperm is unique, and requires enzyme isoforms that are not present in other cereal tissues or non-cereal plants. Recent information on the functions of individual enzyme isoforms has provided insight into how the linear chains and branch linkages in cereal starch are synthesized and distributed. Genetic analyses have led to the formulation of models for the roles of de-branching enzymes in cereal starch production, and reveal pleiotropic effects that suggest that certain enzymes may be physically associated. For the first time, tools for global analyses of starch biosynthesis are available for cereal crops, and are heralded by the draft sequence of the rice genome.

## Addresses

<sup>\*</sup>Department of Biochemistry, Biophysics, and Molecular Biology, 1210 Molecular Biology Building, Iowa State University, Ames, Iowa 50011, USA

<sup>†</sup>e-mail: mgjames@iastate.edu

<sup>§</sup>e-mail: ammyers@iastate.edu

<sup>‡</sup>John Innes Centre, Norwich Research Park, Colney, Norfolk NR4 7UH, UK

e-mail: kay.denyer@bbsrc.ac.uk

**Current Opinion in Plant Biology** 2003, **6**:215–222

This review comes from a themed issue on  
Physiology and metabolism  
Edited by Alison Smith and Mary Lou Guerinot

1369-5266/03/\$ – see front matter  
© 2003 Elsevier Science Ltd. All rights reserved.

DOI 10.1016/S1369-5266(03)00042-6

## Abbreviations

<b>ADPG</b>	ADP-glucose
<b>ae</b>	amylose extender
<b>AGP</b>	ADP-glucose pyrophosphorylase
<b>BE</b>	branching enzyme
<b>DAF</b>	days after flowering
<b>DBE</b>	debranching enzyme
<b>DP</b>	degree of polymerization
<b>GBSSI</b>	granule-bound starch synthase I
<b>Pi</b>	orthophosphate
<b>SS</b>	starch synthase
<b>su1</b>	sugary1
<b>WSP</b>	water-soluble polysaccharide
<b>Wx</b>	waxy

## Introduction

Cereal crops accumulate starch in the seed endosperm as an energy reserve. This starch serves as the primary carbohydrate component in the diets of humans and livestock, and also has numerous important industrial applications. Starch comprises two D-glucose homopolymers, amylose and amylopectin. Amylose is essentially

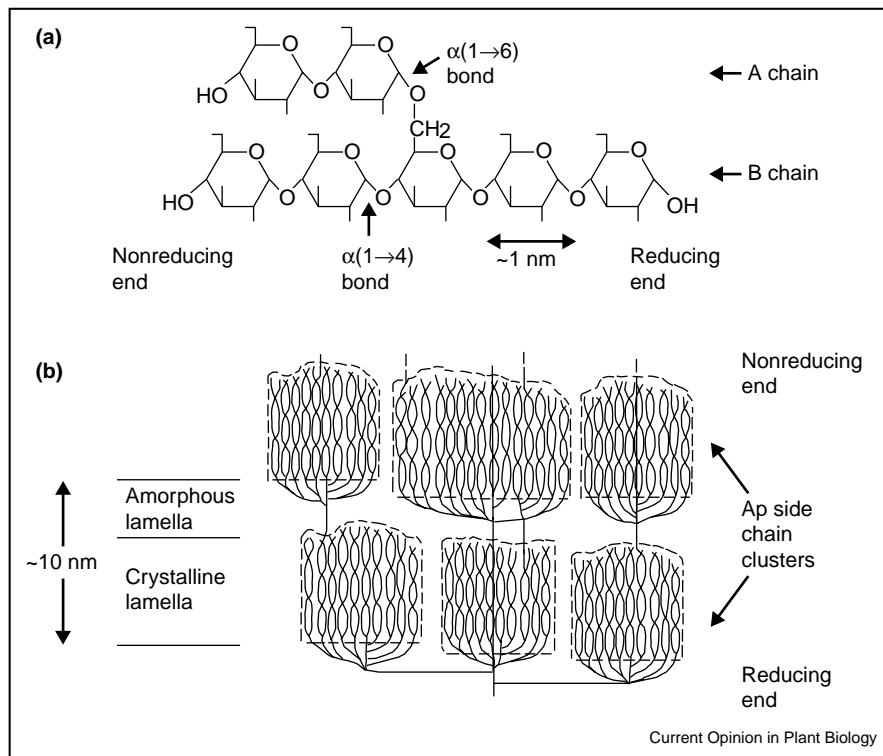
a linear molecule, in which glucosyl monomers are joined via  $\alpha$ -1,4 linkages. Amylopectin, the more abundant polymer in starch, contains linear chains of various lengths. Approximately 5% of the glucosyl units in amylopectin are joined via  $\alpha$ -1,6 linkages, which introduce chain branches. Amylopectin has a high degree of structural organization, as exemplified by the non-random distribution of linear chains and the clustered positioning of branch linkages. Regions of high-branch frequency alternate with regions that are devoid of branches, enabling intervening linear chains to align in parallel arrays of double helices (Figure 1; [1,2]). This conserved architecture is responsible for the semi-crystalline nature of starch granules, which allows the dense packaging of glucose units. A higher-order organization in amylopectin gives rise to two types of crystalline structure, A-type and B-type, which differ with respect to the symmetry and packing of short amylopectin chains [3,4]. Wildtype cereal starches are 100% A-type, in which double helices are arranged with a minimal amount of bound water.

This review covers recent advances in understanding the enzymatic activities necessary for starch synthesis and the determination of amylopectin structure, focusing particularly on new information on ADP-glucose pyrophosphorylase (AGP), starch synthase (SS), starch branching enzyme (BE) and starch debranching enzyme (DBE) [1,2,5<sup>\*</sup>]. Biochemical characterizations, expression analyses, and genetic and transgenic approaches have combined to provide insight into the roles of specific enzyme isoforms and factors that potentially regulate their activities. Furthermore, owing to the sequencing of the rice genome, genome-based approaches for the examination of the starch biosynthetic pathway in cereals are now available for the first time.

## AGP is uniquely extra-plastial in the cereal endosperm

AGP catalyzes the first reaction in starch synthesis, producing the activated glucosyl donor ADP-glucose (ADPG). AGP comprises two large subunits and two small subunits, each of which is encoded by distinct genes. The enzyme is now known to be largely extra-plastidial (i.e. 85–95% cytosolic) in cereal endosperm, but plastidial in other cereal tissues and in all tissues of non-cereal plants (Figure 2; [6–9,10<sup>\*\*</sup>,11]). Recent studies suggest that distinct cytosolic and plastidial forms of AGP that are encoded by separate large- and small-subunit genes exist in all cereal endosperms [6,9,10<sup>\*\*</sup>,12<sup>\*</sup>]. The AGP small subunit gene sequences from various eudicots and monocots differ primarily in exon 1 [13<sup>\*</sup>].

Figure 1



Diagrammatic representations of amylopectin structure. (a) The connections of glucosyl units by  $\alpha$ -1,4 and  $\alpha$ -1,6 glycoside linkages. (b) Cluster model of amylopectin structure. Solid lines represent glucan chains, dashed lines represent the boundaries of amylopectin side-chain clusters in which adjacent linear chains associate as double helices. Side chains are represented by amorphous structures. Modified from [2].

The diversity of exon 1 within the cereals suggests that cytosolic AGP may have evolved more than once.

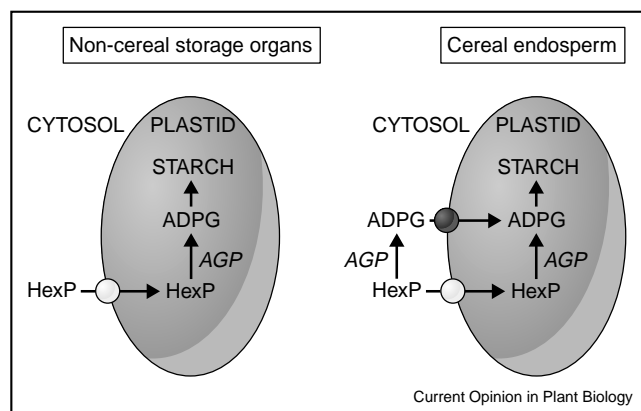
In cereal endosperm, ADPG synthesized in the cytosol is transported into plastids via a small inner envelope pro-

tein that, in maize, is encoded by the *Brittle1* gene [14]. This BT1 protein is capable of transporting ADPG from the cytosol into the plastid [15], most likely in exchange for AMP [16,17].

The cytosolic localization of AGP in cereal endosperm may have functional significance for partitioning large amounts of carbon into starch when sucrose is plentiful [10\*\*]. In plants that have exclusively plastidial AGP, the sucrose-to-starch pathway involves plastid import of hexose phosphates that can also be used in pathways other than starch synthesis. In cereals, however, carbon entering the plastid as ADPG is committed to starch synthesis and cannot be diverted into other metabolic pathways within the plastid.

The importance of the regulation of AGP in cereal endosperm is unclear. As in other plant organs, AGP in cereal endosperm is regulated positively by 3-phosphoglycerate (3-PGA) and negatively by orthophosphate (Pi) [18]. Evidence that this is important for the regulation of starch synthesis in cereals comes from studies of transgenic wheat containing maize AGP genes that contain point mutations that confer stability to subunit interactions and reduce Pi inhibition [19]. Analysis of a small

Figure 2



Schematic diagram depicting hexose transport from the cytosol into non-cereal or cereal plastids. The pathway to starch follows a similar route within plastids of both plant types.

number of these transgenic wheat plants showed they expressed a more stable and enzymatically active AGP that, in some cases, was associated with increased seed weight and total biomass. However, there is also evidence that the regulation of AGP in cereal endosperm is less important than in other organs such as leaves. First, AGP in cereal endosperm is less sensitive to 3-PGA and Pi than AGP in other tissues [20]. Second, AGP in cereal endosperm may be insensitive to redox regulation [21], unlike AGP from potato tubers, which is subject to powerful redox regulation [22].

### Individual starch synthase isoforms have unique roles in starch synthesis

SSs utilize ADPG to elongate linear chains by the formation of  $\alpha$ -1,4 linkages. Cereal endosperms contain at least five SS isoforms that are categorized according to conserved sequence relationships [23]. Four isoforms, termed SSI, SSIIa, SSIIb, and SSIII, are believed to have unique functions in amylopectin synthesis, although their precise roles have not been identified. A granule-bound isoform, GBSSI, which is encoded by the *Waxy* (*Wx*) locus in cereals, functions specifically to elongate amylose [24,25]. *wx* mutants typically lack amylose and have starches comprised solely of amylopectin. Although GBSSI activity and *Wx* gene dosage are linearly proportional, *Wx* dosage is not proportional to amylose content, as shown in early studies of maize [26] and more recent analyses of *Triticum monococcum*, a diploid wheat [27]. This suggests that amylose content may be responsive to factors besides GBSSI. Recent evidence suggests that such regulatory factors include the availability of small malto-oligosaccharides as primers or the availability of ADP-glucose as substrate [28].

Several *wx* mutants of barley synthesize small amounts of endosperm amylose, provoking an investigation of whether barley endosperm, like that of wheat, expresses two GBSSs. A second barley isoform, GBSSIIb, has been identified that shares 96.5% identity with wheat GBSSII and 65.3% identity with barley GBSSI [29]. As in wheat, however, this second isoform is not expressed primarily in endosperm but in tissues such as pericarp that accumulate starch transiently.

Elucidation of the roles of the soluble SS isoforms is essential to an understanding of amylopectin synthesis. A recent study of SSI glucan-binding affinities indicates that the entire carboxy-terminal region of this enzyme is required for starch binding, although the amino-terminus is not [30]. Furthermore, SSI binding affinity increases dramatically with the length of linear substrate chains, and is inversely proportional to the catalytic capability of the enzyme. These results, together with information from previous studies of altered amylopectin in maize SS mutants, suggest a model to account for the roles of different SSs in determining the distribution of amylo-

pectin chain lengths [31–33]. According to this model, SSI is primarily responsible for the synthesis of the shortest chains, that is, those with a degree of polymerization (DP) of 10 glucosyl units or less. Further extension to produce longer chains that extend between clusters is catalyzed by SSII and/or SSIII, and may involve the introduction of branches before extension can proceed [30].

Quantitative trait loci (QTL) mapping of the *japonica* and *indica* rice varieties, which differ in the functional and structural properties of their starch, supports this model [34]. In comparison with *indica* amylopectin, *japonica* amylopectin is enriched in chains that have a DP of 10 or less and has fewer intermediate chains (DP13–22). Umemoto and coworkers [34] established that the gene on chromosome 6 that encodes SSIIa is responsible for these varietal differences. They suggest that SSIIa plays a role in the elongation of short chains of DP <10 that leads to the production of intermediate chains, and that SSIIa activity is hindered in *japonica* rice. Because chains of DP13–22 typically form double helices, their reduced frequency in *japonica* may account for the functional differences between the starches of *japonica* and *indica*.

### Contributions of individual BE isoforms to the determination of starch structure

BEs generate  $\alpha$ -1,6 linkages by cleaving internal  $\alpha$ -1,4 bonds and transferring the released reducing ends to C6 hydroxyls. There are two classes of BE (BEI and BEII) that differ in terms of the lengths of chains transferred *in vitro*, with BEII transferring shorter chains than BEI [35,36]. In cereals, there are two closely related forms of BEII (BEIIa and BEIIb) [37,38]. These also differ in chain-length specificity *in vitro*, with BEIIb transferring shorter chains than BEIIa during extended incubation [37].

The temporal and spatial patterns of expression vary between BE isoforms. BEI and BEIIa are expressed in the endosperm and several other cereal tissues, whereas BEIIb is only expressed in the endosperm and reproductive tissues. In rice, BEIIa expression was detected earliest at 3 days after flowering (DAF) and maximally at 5–7 DAF [37]. BEIIa expression is also maximal at mid-development in wheat grains [38]. In contrast, BEI and BEIIb transcripts are most abundant later, at 7–10 DAF in rice [38].

There is good evidence that the different BE isoforms have distinct roles in endosperm starch synthesis. First, analysis of the amylopectin of BEI mutants reveals subtle deficiencies in intermediate and long chains. This suggests a role for BEI in the formation of chains of these lengths, and indicates that neither BEIIa nor BEIIb can compensate for the loss of BEI activity [5,39]. Second, the altered amylopectin structure in mutants that lack BEIIb suggests a unique role for this isoform. BEIIb

mutants of maize and rice were historically designated 'amylose-extender' (*ae*) because they have an apparent increase the relative proportion of amylose to amylopectin in the endosperm. Recently, however, rice *ae*; *wx* double mutants that lack amylose were shown to produce greatly elongated amylopectin chains that may previously have been mistaken for amylose [40\*\*]. Furthermore, the rice *ae* mutation has been shown to cause a 50% decrease in SSI in addition to eliminating BEIIb activity, suggesting that *in vivo* the BEIIb and SSI proteins may interact [40\*\*]. Mutational analysis of BEIIa genes in maize and rice, however, indicates that loss of this isoform affects neither the composition of endosperm starch nor the fine structure of amylopectin [5\*,41]. Therefore, either BEIIa does not have a critical role in amylopectin synthesis in the endosperm or other BE isoforms can compensate for its absence.

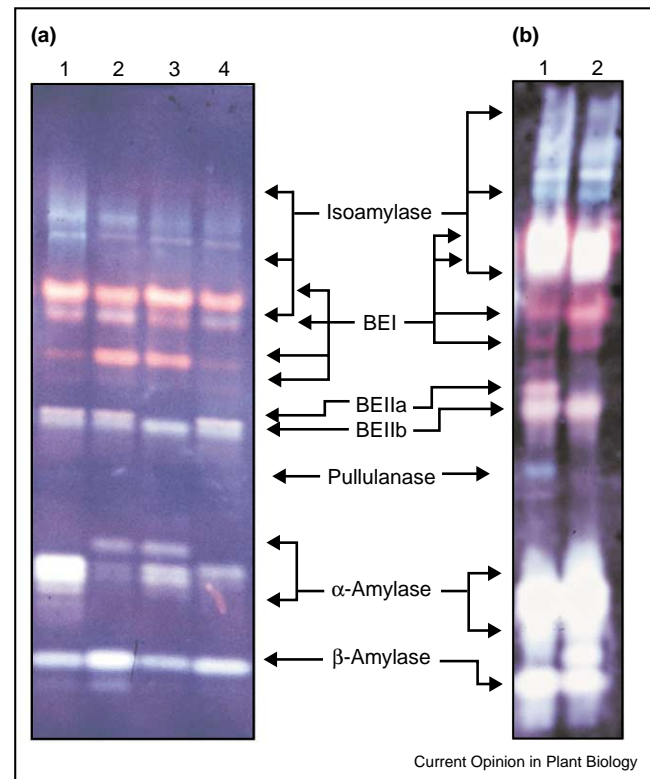
A model for the sequential action of BE isoforms derives from the heterologous expression in yeast of all three maize BEs, singly and in all combinations [42\*\*]. This model suggests that BEII may act before BEI on precursor polymers because both maize BEII isoforms complement the loss of glycogen BE and produce glucans with unique chain distributions and branch frequencies, but BEI has no effect unless both BEIIa and BEIIb are also present.

### Potential DBE functions

Mutations in many species indicate that starch synthesis involves DBEs in addition to SSI and BEs. Two DBE families exist in plants, isoamylase-type and pullulanase-type. Both types hydrolyze  $\alpha$ -1,6 linkages, but they differ in substrate specificity. Orthologous mutations of maize and rice (*sugary1* [*su1*]) and barley (*isa-1*) affect genes that encode an isoamylase-type DBE, and correlate with the accumulation of a polymeric water-soluble polysaccharide (WSP) termed phytoglycogen and reduced starch content [43–45,46\*\*]. The *su1* mutations also condition reduced pullulanase-type activity.

Many *su1* alleles are known that vary phenotypically. In maize, *su1*-allele-specific pleiotropic effects were observed on other enzymes, particularly BEIIa (Figure 3; [47\*\*]). The presence of a catalytically inactive SU1 eliminates several of these effects, indicating that the SU1 polypeptide has both enzymatic and non-enzymatic functions and suggesting that some starch biosynthetic enzymes may function in a complex. Dinges *et al.* [47\*\*] also found that a very small amount of SU1 activity is sufficient for the synthesis of near-normal quantities of amylopectin. In barley, allelic isoamylase-type DBE mutations (termed *isa1*) have been described recently [46\*\*]. *isa1* mutants synthesize phytoglycogen and have profoundly defective granule structure and initiation. Mutant granules are small, irregular in shape, often compound, and are produced during only one wave of granule initiation rather

Figure 3



Pleiotropic effects within DBE-deficient mutants are revealed by the activity gel analysis of the activities of starch-modifying enzymes. Total proteins from maize kernels harvested 20 days after pollination were separated on 15 cm native polyacrylamide gels, then electroblotted to starch-containing gels. The activities of starch-modifying enzymes were visualized by staining with an iodine solution. (a) Analysis of isoamylase-type DBE mutant alleles. 50  $\mu$ g protein from wildtype (lane 1) or homozygous mutant *su1* kernels (*su1-R4582::Mu1*, lane 2; *su1-starchy*, lane 3; and *su1-Ref*, lane 4) was analyzed. (b) Analysis of a pullulanase-type DBE mutant. 100  $\mu$ g protein from wildtype (lane 1) and homozygous *zpu1-204* kernels (lane 2) was analyzed. Specific isoform activities are indicated by arrows. BEIIa activity is missing in the *su1-starchy* and *zpu1-204* samples. Modified from [47\*\*,49].

than during the two waves that normally occur. Evidence to support a biosynthetic role for isoamylase comes from the pattern of expression for *iso1*, the wheat ortholog of *su1*. This cDNA is expressed maximally in developing wheat grains and not at all in mature grains [48].

The pullulanase-type enzyme, termed ZPU1 in maize, participates in starch degradation in the endosperm. In addition, it appears to provide a function that overlaps with that of the isoamylase-type DBE during biosynthesis [49]. Recent biochemical characterization of ZPU1 indicates it is an endo-acting enzyme that cleaves only very short branch chains, and is subject to activation by changes in redox status and inhibition in the presence of high sugar concentrations [50\*]. Sugar accumulation is an effect of *su1* mutations, and so this

may account for the secondary loss of pullulanase-type activity in *su1* mutants.

Although mutations that result in isoamylase-type DBE deficiencies can have dramatic effects on starch synthesis, the exact roles of isoamylase- and pullulanase-type DBEs in starch synthesis are not yet known. Several models could explain the function of DBE in starch synthesis and phytoglycogen accumulation. The glucan-trimming model suggests that DBEs directly participate in amylopectin synthesis, selectively removing branches that are inappropriately positioned [2,5]. Accordingly, DBE activity would be required for maintenance of the cluster structure of amylopectin, for the dense packing of linear chains, and for growing chains to crystallize onto the granule surface. The recent observation of numerous short chains on the surface of premature granules, which suggests an intermediary structure, is consistent with this model but does not provide direct support [51]. An alternative model suggests that DBEs eliminate soluble glucan from the stroma, thereby removing a substrate that competes for BE and SS binding [52]. DBE deficiency, therefore, would result in WSP accumulation at the expense of amylopectin. The observation of increased granule number in isoamylase-type DBE mutants could be explained by their more frequent nucleation of nascent granules from WSP, regardless of the origin of the soluble glucan. Alternatively, the data are also consistent with a function for DBEs in destroying a specific primer for granule initiation [46].

### Protein modifications that potentially regulate starch biosynthesis

Little is known about factors that regulate starch biosynthesis in cereals. Recent research suggests that some regulation occurs through protein modifications, which are also implicated in the control of other pathways. Reduction of the activity of SPK, a calcium-dependent protein kinase, in developing rice endosperm reduced the starch content and increased the sucrose content of the grain [53]. SPK phosphorylates sucrose synthase, the enzyme that provides substrate for AGP, and thus may regulate starch synthesis. For some enzymes, regulation of activity is a two-step process, involving protein phosphorylation followed by the formation of a complex with 14-3-3 proteins [54]. Sehnke and co-workers [55] found that reduction of the accumulation of granule-associated 14-3-3 proteins results in an increase in starch accumulation. One target for these 14-3-3 proteins is believed to be SSIII, which has a 14-3-3-binding motif. These findings suggest that phosphorylation and 14-3-3 binding serve to inactivate SSIII.

Some measure of coordination and regulation of starch biosynthetic enzymes is increasingly thought to occur through specific protein–protein interactions. Such interactions have long been suggested on the basis of observa-

tions of increased phenotypic severity in double mutants relative to single mutants of maize [56]. Furthermore, biochemical analyses of pleiotropic enzymatic effects of mutations, such as the observed reduction in SSI activity in *ae* rice grains, lend support to this hypothesis [40]. Recent analyses of an array of starch-metabolizing enzyme activities in maize DBE mutants identified allele-specific effects of *su1* and *zpu1* mutations on the activity of BEIIa in developing maize kernels [47,49]. In each case, the effect was a complete loss of the activity of BEIIa, but no change in the size or abundance of the BEIIa protein. This suggests that BEIIa functionality was lost in the mutants as the result of altered interactions with the DBEs.

### Conclusions and future directions

Our understanding of starch synthesis in cereal endosperms has advanced recently as we have gained insights into the specific functions of individual enzyme isoforms. A cytosolic form of AGP that is unique to cereals may serve to commit excess carbon to starch production. Roles for SSI, SSIIa, and all three BEs in chain-length determination and branch placement have been proposed. The pleiotropic effects of BE and DBE mutants complicate the analyses, and have led to additional hypotheses concerning their functions. Future research in this area will identify direct interactions among starch biosynthetic enzymes, as well as modifying factors that regulate enzyme activity.

Tools for genome-based analyses of starch biosynthesis are now available for cereal crops. Comparative genomics will allow us to distinguish components of the starch-synthesizing machinery that are conserved among all plant species from those that are unique to cereals, and to identify those components that differ between cereal species. This may eventually help to explain species differences in starch granule shape and size, and thus provide the potential for biotechnological advances.

The draft sequence of the rice genome [57,58,59] provides a foundation for characterizing other cereals, which is based on extensive genome synteny and sequence conservation, and enables expression patterns at the protein and transcript levels to be fully examined. Recently, a comprehensive proteomics study in rice analyzed tissue-specific expression in metabolic pathways [60]. This work showed that two small-subunit AGP isoforms are common to leaves and seeds, whereas two large-subunit isoforms are seed-specific and a third large-subunit isoform is present exclusively in leaves. Another study involved a microarray analysis of gene expression in developing maize endosperm in response to the *su1* mutation [61]. Few differences between the mutant and the wildtype were identified, supporting previous findings that the pleiotropic effects of *su1* are mediated post-transcriptionally [47,62]. This work emphasizes

the need to focus on protein interactions and modifications in the future. Other global analyses of starch synthesis have taken evolutionary approaches, providing, for example, an in-depth study of allele geneology at the *wx* locus in rice [63] or a comprehensive study of nucleotide diversity in the starch pathway of maize [64]. Both of these studies indicate that selective pressures for starch quality or yield act particularly strongly on genes that are involved in starch production. As bio-informatics resources become increasingly available, such genome-based approaches to starch metabolism promise to provide a more complete understanding of starch synthesis in the cereal endosperm.

## Acknowledgements

The authors thank Alison Smith for her helpful suggestions in the preparation of this article. We gratefully acknowledge the US Department of Agriculture, the US Department of Energy, and the National Science Foundation for providing research funding.

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
  - of outstanding interest
1. Smith AM: **The biosynthesis of starch granules.** *Biomacromolecules* 2001, **2**:335-341.
  2. Myers AM, Morell MK, James MG, Ball SG: **Recent progress toward understanding the amylopectin crystal.** *Plant Physiol* 2000, **122**:989-997.
  3. Imberty A, Buleon A, Tran V, Perez S: **Recent advances in knowledge of starch structure.** *Starch/Staerke* 1991, **43**:375-384.
  4. Gallant DJ, Bouchet B, Baldwin PM: **Microscopy of starch: evidence of a new level of granule organization.** *Carbohydrate Polymers* 1997, **32**:177-191.
  5. Nakamura Y: **Towards a better understanding of the metabolic system for amylopectin biosynthesis in plants: rice endosperm as a model tissue.** *Plant Cell Physiol* 2002, **43**:718-725.
- This review examines the contribution of each starch biosynthetic enzyme isoform toward determining the fine structure of amylopectin in rice. A model is presented that describes amylopectin synthesis in the endosperm as a two-step process. Branches are introduced and chains are elongated in the amorphous region to form a core for each new cluster, then branch formation and chain elongation proceeds in the crystalline region. As in the glucan-trimming model, ill-placed branches are cleared by DBEs.
6. Thorbjornsen T, Villand P, Kleczkowski LA, Olsen OA: **A single gene encodes two different transcripts for the ADP-glucose pyrophosphorylase small subunit from barley (*Hordeum vulgare*).** *Biochem J* 1996, **313**:149-154.
  7. Shannon JC, Pien FM, Liu KC: **Nucleotides and nucleotide sugars in developing maize endosperms (synthesis of ADP-glucose in *brittle-1*).** *Plant Physiol* 1996, **110**:835-843.
  8. Giroux MJ, Hannah LC: **ADP-glucose pyrophosphorylase in *shrunken-2* and *brittle-2* mutants of maize.** *Mol Gen Genet* 1994, **243**:400-408.
  9. Denyer K, Dunlap F, Thorbjornsen T, Keeling P, Smith AM: **The major form of ADP-glucose pyrophosphorylase in maize endosperm is extra-plastidial.** *Plant Physiol* 1996, **112**:779-785.
  10. Beckles DM, Smith AM, Rees T: **A cytosolic ADP-glucose pyrophosphorylase is a feature of graminaceous endosperms, but not of other starch-storing organs.** *Plant Physiol* 2001, **125**:818-827.
- A rapid screening method involving measurement of the ADPG to UDP-glucose ratio was used to infer the intracellular location of AGP. This study provides evidence that ADPG is synthesized primarily in the cytosol in the

endosperms of all grasses, and in the plastids in the starch-storing organs of other plant species.

11. Beckles DM, Craig J, Smith AM: **ADP-glucose pyrophosphorylase is located in the plastid in developing tomato fruit.** *Plant Physiol* 2001, **126**:261-266.
  12. Burton RA, Johnson PE, Beckles DM, Fincher GB, Jenner H, Naldrett MJ, Denyer K: **Characterization of the genes encoding the cytosolic and plastidial forms of ADP-glucose pyrophosphorylase in wheat endosperm.** *Plant Physiol* 2002, **130**:1464-1475.
- The authors show that cytosolic AGP accounts for most of the AGP activity in wheat endosperm. In wheat, as in barley, a gene that encodes the cytosolic AGP small subunit also produces a larger second transcript that is predicted to encode a transit peptide. The amino-acid sequence of the plastidial AGP small subunit does not, however, match that deduced for this second gene product. Thus, the gene encoding the plastidial AGP small subunit has not yet been identified.
13. Hannah LC, Shaw JR, Giroux MJ, Reyss A, Prioul JL, Bae JM, Lee JY: **Maize genes encoding the small subunit of ADP-glucose pyrophosphorylase.** *Plant Physiol* 2001, **127**:173-183.
- Sequence comparison of the AGP small subunit genes from eudicots and monocots is used to infer how these genes evolved. Exon 1 sequences that include subcellular-targeting information are particularly diverse among species, even within the monocots, suggesting they have independent and relatively recent origins. This is a thorough and insightful study, limited only by the lack of sequence information for species and organs other than the storage organs of the major crops.
14. Sullivan TD: **The maize *brittle1* gene encodes amyloplast membrane polypeptides.** *Planta* 1995, **196**:477-484.
  15. Shannon JC, Pein FM, Cao HP, Liu KC: **Brittle-1, an adenylate translocator, facilitates transfer of extraplastidial synthesized ADP-glucose into amyloplasts of maize endosperms.** *Plant Physiol* 1998, **117**:1235-1252.
  16. Mohlmann T, Tjaden J, Henrichs G, Quick WP, Hausler R, Neuhaus HE: **ADP-glucose drives starch synthesis in isolated maize endosperm amyloplasts: characterization of starch synthesis and transport properties across the amyloplast envelope.** *Biochem J* 1997, **324**:503-509.
  17. Emes MJ, Bowsher CG, Hedley C, Burrell MM, Scrase-Field ES, Tetlow IJ: **Starch synthesis and carbon partitioning in developing endosperm.** *J Exp Bot* 2003, **54**:569-575.
  18. Hannah LC: **Starch synthesis in the maize endosperm.** In *Advances in Cellular and Molecular Biology of Plants*, vol 4. Edited by Larkins BA, Vasil IK. Dordrecht, The Netherlands: Kluwer Academic Publishers; 1997:375-405.
  19. Smidansky ED, Clancy M, Meyer FD, Lanning SP, Blake NK, Talbert LE, Giroux MJ: **Enhanced ADP-glucose pyrophosphorylase activity in wheat endosperm increases seed yield.** *Proc Natl Acad Sci USA* 2002, **99**:1724-1729.
  20. Gomez-Casati D, Iglesias AA: **ADP-glucose pyrophosphorylase from wheat endosperm. Purification and characterization of an enzyme with novel regulatory properties.** *Planta* 2002, **214**:428-434.
  21. Ballicora MA, Frueauf JB, Fu YB, Schurmann P, Preiss J: **Activation of the potato tuber ADP-glucose pyrophosphorylase by thioredoxin.** *J Biol Chem* 2000, **275**:1315-1320.
  22. Tiesen A, Hendriks JHM, Stitt M, Branscheid A, Gibon Y, Farre EM, Geigenberger P: **Starch synthesis in potato tubers is regulated by post-translational redox modification of ADP-glucose pyrophosphorylase: a novel regulatory mechanism linking starch synthesis to sucrose supply.** *Plant Cell* 2002, **14**:2191-2213.
  23. Cao H, James MG, Myers AM: **Purification and characterization of soluble starch synthases from maize endosperm.** *Arch Biochem Biophys* 2000, **373**:135-146.
  24. Nelson OE, Rines HW: **The enzymatic deficiency in the waxy mutant of maize.** *Biochem Biophys Res Commun* 1962, **9**:297-300.
  25. Shure M, Wessler S, Federoff N: **Molecular identification and isolation of the *Waxy* locus in maize.** *Cell* 1983, **35**:225-233.
  26. Tsai CY: **The function of the waxy locus in starch synthesis in maize endosperm.** *Biochem Genet* 1974, **11**:83-96.

27. Fujita N, Hasegawa H, Taira T: **The isolation and characterization of a waxy mutant of diploid wheat (*Triticum monococcum* L.).** *Plant Sci* 2001, **160**:595-602.
28. Denyer K, Johnson P, Zeeman S, Smith AM: **The control of amylose synthesis.** *J Plant Physiol* 2001, **158**:479-487.
29. Patron NJ, Smith AM, Fahy BF, Hylton CM, Naldrett MJ, Rosnagel BG, Denyer K: **The altered pattern of amylose accumulation in the endosperm of low-amylose barley cultivars is attributable to a single mutant allele of granule-bound starch synthase I with a deletion in the 5'-non-coding region.** *Plant Physiol* 2002, **130**:190-198.
- Sequencing of the gene encoding a mutant form of GBSSI in several barley cultivars suggests that most waxy barleys carry the same mutation in GBSSI. This mutation likely arose in China in the 16th century. The mutation does not entirely eliminate the expression of GBSSI, but causes the expression of this enzyme to be restricted to the outer endosperm cells late in endosperm development.
30. Commuri PD, Keeling PL: **Chain-length specificities of maize starch synthase I enzyme: studies of glucan affinity and catalytic properties.** *Plant J* 2001, **25**:475-486.
- Investigation of the chain-length specificities of maize endosperm SSI shows that its preferred substrates are the shortest amylopectin chains. SSI tightly binds longer amylopectin chains, whereupon its catalytic activity diminishes or is lost. Thus, longer amylopectin chains are probably extended by isoforms other than SSI. This work strongly supports the idea that different SS isoforms perform specific roles in amylopectin synthesis.
31. Wang Y-J, White P, Pollack L, Jane J-L: **Characterization of starch structures of 17 maize endosperm mutant genotypes with Oh43 inbred line background.** *Cereal Chem* 1993, **70**:171-179.
32. Gao M, Wanat J, Stinard PS, James MG, Myers AM: **Characterization of *dull1*, a maize gene coding for a novel starch synthase.** *Plant Cell* 1998, **10**:399-412.
33. Harn C, Knight M, Ramakrishnan A, Guan H, Keeling PL, Wasserman BP: **Isolation and characterization of the zSSIa and zSSIb starch synthase cDNA clones from maize endosperm.** *Plant Mol Biol* 1998, **37**:639-649.
34. Umemoto T, Yano M, Satoh H, Shomura A, Nakamura Y: **Mapping of a gene responsible for the difference in amylopectin structure between *japonica*-type and *indica*-type rice varieties.** *Theor Appl Genet* 2002, **104**:1-8.
- Analysis of the biochemical and genetic differences between *japonica* and *indica* varieties of rice, and the physico-chemical properties of their starch granules, reveals the role of SSIa in elongating short- to intermediate-size chains in amylopectin. The strength of this study lies in the breadth of techniques brought to bear on a clearly defined question.
35. Takeda Y, Guan H-P, Preiss J: **Branching of amylose by the branching isoenzymes of maize endosperm.** *Carbohydr Res* 1993, **240**:253-263.
36. Guan H, Preiss J: **Differentiation of the properties of the branching isozymes from maize (*Zea mays*).** *Plant Physiol* 1993, **102**:1269-1273.
37. Mizuno K, Kobayashi E, Tachibana M, Kawasaki T, Fujimura T, Funane K, Kobayashi M, Baba T: **Characterization of an isoform of rice starch branching enzyme, RBE4, in developing seeds.** *Plant Cell Physiol* 2001, **42**:349-357.
- A BEIIa homologue (RBE4) that has been characterized in rice is expressed earlier than either BEIIb (RBE3) or BEI (RBE1) in the developing endosperm. Comparisons of the lengths of branch chains transferred by recombinant rice BEs showed that the two BEII forms are similar, primarily transferring chains of DP6, whereas BEI branch products are DP6-11. BEIIb also transfers shorter chains after extended incubation but BEIIa does not.
38. Rahman S, Regina A, Li Z, Mukai Y, Yamamoto M, Kosar-Hashemi B, Abrahams S, Morell MK: **Comparison of starch-branching enzyme genes reveals evolutionary relationships among isoforms. Characterization of a gene for starch-branching enzyme IIa from the wheat genome donor *Aegilops tauschii*.** *Plant Physiol* 2001, **125**:1314-1324.
39. Blauth SL, Kim KN, Klucinec J, Shannon JC, Thompson D, Guiltinan M: **Identification of *Mutator* insertional mutants of starch-branching enzyme 1 (*sbe1*) in *Zea mays* L.** *Plant Mol Biol* 2002, **48**:287-297.
40. Nishi A, Nakamura Y, Tanaka N, Satoh H: **Biochemical and genetic analysis of the effects of amylose-extender mutation in rice endosperm.** *Plant Physiol* 2001, **127**:459-472.
- Analysis of a rice double mutant that is deficient in BEIIb and GBSSI (and consequently lacks the amylose component of starch) reveals the role of BEIIb in amylopectin synthesis. The function of BEIIb in the transfer of short chains within amylopectin is not complemented by BEIIa or BEI, suggesting that each BE isoform has a specific role.
41. Blauth SL, Yao Y, Klucinec JD, Shannon JC, Thompson DB, Guiltinan MJ: **Identification of *Mutator* insertional mutants of starch-branching enzyme 2a in corn.** *Plant Physiol* 2001, **125**:1396-1405.
42. Seo BS, Kim S, Scott MP, Singletary GW, Wong KS, James MG, Myers AM: **Functional interactions between heterologously expressed starch-branching enzymes of maize and the glycogen synthases of Brewer's yeast.** *Plant Physiol* 2002, **128**:1189-1199.
- Heterologous expression of all three maize BEs in yeast, singly and in combination, shows that BEI functions only when expressed with both BEIIa and BEIIb. The activity of all three BEs produces a glucan that has significantly more branches than that produced when only BEIIa and BEIIb are functional. The authors predict that the BEs may act sequentially during glucan polymer construction, with the BEII isoforms producing precursor molecules that are suitable substrates for BEI activity.
43. Nakamura Y, Umemoto T, Takahata Y, Komae K, Amano E, Satoh H: **Changes in structure of starch and enzyme activities affected by sugary mutations in developing rice endosperm: possible role of starch debranching enzyme (R-enzyme) in amylopectin biosynthesis.** *Physiol Plant* 1996, **97**:491-498.
44. Pan D, Nelson OE: **A debranching enzyme deficiency in endosperms of the *sugary-1* mutants of maize.** *Plant Physiol* 1984, **74**:324-328.
45. James MG, Robertson DS, Myers AM: **Characterization of the maize gene *sugary1*, a determinant of starch composition in kernels.** *Plant Cell* 1995, **7**:417-429.
46. Burton RA, Jenner H, Carrangis L, Fahy B, Fincher GB, Hylton C, Laurie DA, Parker M, Waite D, van Wegen S *et al.*: **Starch granule initiation and growth are altered in barley mutants that lack isoamylase activity.** *Plant J* 2002, **31**:97-112.
- The authors of this paper suggest a new model for the role of the isoamylase-type DBE in the initiation of starch granules. Their model is based on the effects of barley *isa-1* mutations on the numbers of starch granules in endosperm plastids. The emphasis is shifted away from a role for isoamylase in polymer synthesis during granule assembly to a role in the early events of granule formation. According to this model, phytyloglycogen accumulation is relegated to a possible, but not an inevitable, consequence of isoamylase deficiency.
47. Dinges JR, Colleoni C, Myers AM, James MG: **Molecular structure of three mutations at the maize *sugary1* locus and their allele-specific phenotypic effects.** *Plant Physiol* 2001, **125**:1406-1418.
- Phenotypic differences among three alleles of *su1* in maize reveal allele-specific pleiotropic effects on enzymes other than the SU1 protein, and suggest that there are specific interactions between the SU1 protein and other components of the starch-biosynthetic machinery. This paper, and other work from the same laboratory, highlights the potential importance of protein-protein interactions in starch biosynthesis.
48. Genschel U, Abel G, Lorz H, Lutticke S: **The sugary-type isoamylase in wheat: tissue distribution and subcellular localisation.** *Planta* 2002, **214**:813-820.
49. Dinges JR, Colleoni C, James MG, Myers AM: **Mutational analysis of the pullulanase-type debranching enzyme in maize indicates multiple functions in starch metabolism.** *Plant Cell* 2003, **15**:666-680.
50. Wu C, Colleoni C, Myers AM, James MG: **Enzymatic properties and regulation of ZPU1, the maize pullulanase-type starch debranching enzyme.** *Arch Biochem Biophys* 2002, **406**:21-32.
- This biochemical characterization of the maize pullulanase-type DBE shows the enzyme to be endo-acting, to hydrolyze only very short chains, and to be subject to potential regulation by changes in redox status and sugar concentration in the endosperm.
51. Nielsen TH, Baunsgaard L, Blennow A: **Intermediary glucan structures formed during starch granule biosynthesis are enriched in short side chains, a dynamic pulse labeling approach.** *J Biol Chem* 2002, **277**:20249-20255.

In a new approach to the study of starch synthesis *in vivo*, this group used pulse labeling to reveal an intermediary starch form that is enriched in short chains of DP6–11 on the granule surface. The excessive branching is consistent with a role for DBEs in starch synthesis, but does not provide direct support for a particular model.

52. Zeeman SC, Northrop F, Smith AM, Rees T: **A starch-accumulating mutant of *Arabidopsis thaliana* deficient in a chloroplastic starch-hydrolysing enzyme.** *Plant J* 1998, **15**:357-365.
  53. Asano T, Kunieda N, Omura Y, Ibe H, Kawasaki T, Takano M, Sato M, Furuhashi H, Mujin T, Takaiwa F *et al.*: **Rice SPK, a calmodulin-like domain protein kinase, is required for storage product accumulation during seed development: phosphorylation of sucrose synthase is a possible factor.** *Plant Cell* 2002, **14**:619-628.
- Activation of sucrose synthase via phosphorylation is required for storage product synthesis in rice seeds. Absence of the protein kinase SPK, which is responsible for sucrose synthase phosphorylation, results in watery inviable seeds and represses the activity of several enzymes, including BEI.
54. Chung HJ, Sehnke PC, Ferl RJ: **The 14-3-3 proteins: cellular regulators of plant metabolism.** *Trends Plant Sci* 1999, **4**:367-371.
  55. Sehnke PC, Chung HJ, Wu K, Ferl RJ: **Regulation of starch accumulation by granule-associated plant 14-3-3 proteins.** *Proc Natl Acad Sci USA* 2001, **98**:765-770.
  56. Shannon JC, Garwood DL: **Genetics and physiology of starch development.** In *Starch; Chemistry and Technology*. Edited by Whistler RL, BeMiller JN, Paschall EF. Orlando, FL: Academic Press; 1984:25-68.
  57. Barry GF: **The use of the Monsanto draft rice genome sequence in research.** *Plant Physiol* 2001, **125**:1164-1165.
  58. Goff SA, Ricke D, Lan TH, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H *et al.*: **A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*).** *Science* 2002, **296**:92-100.
- This paper reports the draft sequence of the genome of the *japonica* subspecies of rice. This milestone event represents the first near-complete sequence of a cereal genome, and thus provides a foundation for acquiring important sequence information for other cereal crops that will facilitate our understanding of starch biosynthesis.
59. Yuan Q, Quackenbush J, Sultana R, Perlea M, Salzberg SL, Buell CR: **Rice bioinformatics. Analysis of rice sequence data and leveraging the data to other plant species.** *Plant Physiol* 2001, **125**:1166-1174.
  60. Koller A, Washburn MP, Lange BM, Andon NL, Deciu C, Haynes PA, Hays L, Schieltz D, Ulaszek R, Wei J *et al.*: **Proteomic survey of metabolic pathways in rice.** *Proc Natl Acad Sci USA* 2002, **99**:11969-11974.
- This group undertook the most complete proteomics analysis of rice to date. As part of this study, the tissue-specific expression of proteins that function in starch metabolism was examined, and new information was provided regarding tissue-specific forms of AGP.
61. Hunter BG, Beatty MK, Singletary GW, Hamaker BR, Dilkes BP, Larkins BA, Jung R: **Maize opaque endosperm mutations create extensive changes in patterns of gene expression.** *Plant Cell* 2002, **14**:2591-2612.
  62. Beatty MK, Rahman A, Cao H, Woodman W, Lee M, Myers AM, James MG: **Purification and molecular genetic characterization of ZPU1, a pullulanase-type starch debranching enzyme from maize.** *Plant Physiol* 1999, **119**:255-266.
  63. Olsen KM, Purugganan MD: **Molecular evidence on the origin and evolution of glutinous rice.** *Genetics* 2002, **162**:941-950.
  64. Whitt SR, Wilson LM, Tenailon MI, Gaut BS, Buckler ES: **Genetic diversity and selection in the maize starch pathway.** *Proc Natl Acad Sci USA* 2002, **99**:12959-12962.
- This study of nucleotide-sequence diversity in maize showed that genes in the starch biosynthetic pathway have dramatically lower diversity compared with random loci. This suggests that the starch pathway was critically important in maize evolution, exerting selective pressure for yield and/or grain quality.