Making starch

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Improvements in understanding the structure of the starch granule and the nature and roles of starch-synthesising enzymes have allowed detailed mechanisms of the synthesis of the amylopectin and amylose components of the granule to be suggested. However, none of these proposed mechanisms has yet been shown to operate *in vivo*. Several critical aspects of granule synthesis, including granule initiation and the formation of the growth rings, remain a mystery.

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Abbreviations
DBE debranching enzyme
GBSSI granule-bound starch synthase I
SBE starch-branching enzyme

Introduction

The possibility of designing starches for industrial use by genetic manipulation of starch synthesising enzymes in crop plants [1] has led to a recent surge of interest in the mechanisms by which starch granules are synthesised. Progress has been made in the last year in three related areas: elucidation of the structures of the starch polymers and the way in which they are organised to form a granule, discovery and characterisation of new isoforms of starch-synthesising enzymes and exploration of their roles *in vivo* through the use of mutant and transgenic plants, and the development of new models which attempt to integrate the properties and putative roles of the enzymes with the observed structure of the granule. I shall discuss these three areas, and then consider the major gaps remaining in understanding the synthesis of the granule.

The structure of the granule

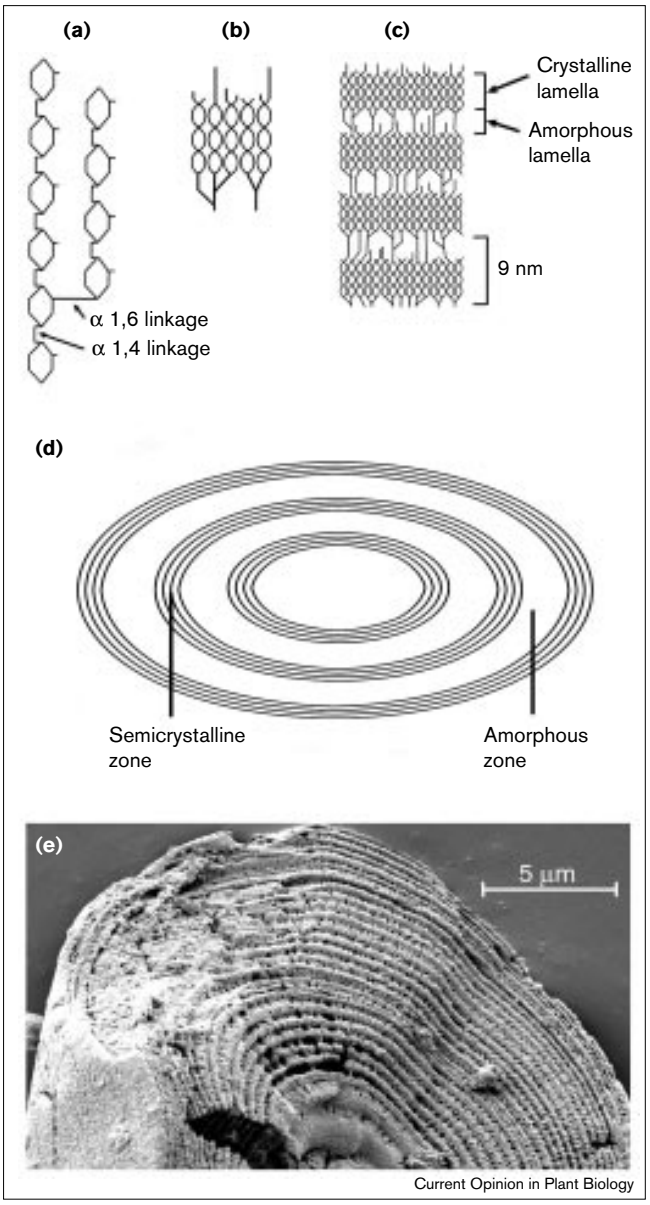
It is accepted that the unique polymodal distribution of chain lengths within the highly-branched, amylopectin component of the starch granule allows the chains to form double helices which can pack together in organised arrays. These arrays are the basis of the semi-crystalline nature of much of the matrix of the starch granule [2] (Figure 1). New methods of probing the organisation of polymers within the granule such as X-ray and neutron scatter, and advanced electron microscopy have suggested new layers of complexity, including the organisation of amylopectin into blocklets [3] and superhelices [4], and differences in structure between the core and the periphery of the granule [5•,6•,7]. This complexity potentially presents serious challenges to the bewildered biologist trying to explain synthesis of the granule. Genetics is of little help because the unambiguously-identified mutations affecting starch structure are in enzymes which synthesise or modify starch polymers (starch synthase, starch-branching enzyme [SBE] and debranching enzyme [DBE]) but are not obviously involved in their packing and higher-level organisation. An important development in explaining the structure of the granule has come with the suggestion that amylopectin may have the properties of a side-chain liquid crystal polymer [8•]. Such a polymer is expected to be self-organising, raising the possibility that the organisation of polymers within the granule requires primarily physical rather than biological explanations. In other words, no enzymes other than those involved in synthesising the polymers may be needed to organise the structure of the granule. The biologist is then required to explain the synthesis at the granule surface of a glucan with the appropriate polymodal distribution of chain lengths to function as a side-chain liquid crystal polymer. This problem is, of course, not independent of that of the physical organisation of a granule. The surface created by the physical organisation of newly-synthesised polymers may itself influence the activity of the polymer-synthesising enzymes.

An understanding of amylopectin synthesis requires accurate and sensitive methods for analysis of chain lengths of amylopectin. This has been facilitated by recent technical advances, among the most important of which are quantitative high-performance anion-exchange chromatography [9], and gel and capillary electrophoresis of chains tagged with a fluorophore [10•]. The power of these techniques is shown by, for example, their ability to reveal small differences in the chain-length profile of amylopectin from different storage organs [11] and from potatoes with different complements of isoforms of starch synthase [12••].

The starch-synthesising enzymes

A major part of the explanation of the specific chain-length profiles seen in the starch polymers is likely to lie in the existence of multiple, distinct isoforms of starch synthase and SBE. There have been valuable recent developments in understanding the nature, distribution and roles of particular classes of isoforms. The idea that plant organs possess isoforms of SBE which fall into two distinct classes (A and B) with different properties [13] has received further support. For example, the SBE activity of wheat endosperm has been attributed to two isoforms, conforming in sequence and properties to the definition of A and B isoforms [14]. Expression in *E. coli* of mutated forms of the A and B isoforms from maize endosperm has revealed that differences between these two classes in substrate preference and length of chains transferred are probably related to differences in amino-acid sequence at the amino and carboxyl termini [15]; however, the precise roles played by the two classes in amylopectin synthesis *in vivo* remains

Figure 1



The structure of the starch granule. **(a)** A portion of an amylopectin molecule containing a branch point. Two chains of α1,4-linked glucose units are joined by an α1,6 linkage. **(b,c)** Arrangement of chains of amylopectin in clusters. Within each cluster, adjacent chains arising from a branch point form double helices (b). The packing of the double helices in ordered arrays creates crystalline lamellae (c). These alternate with amorphous lamellae in which the branch points occur. The repeat structure has a periodicity of 9 nm. **(d)** The alternating crystalline and amorphous lamellae are concentrically arranged within the granule to give semicrystalline zones several hundreds of nanometres wide. These alternate with amorphous zones in which the organisation of amylopectin is much less ordered. One amorphous and one semicrystalline zone is known as a growth ring. **(e)** Scanning electron micrograph of an inner face of a starch granule from a potato tuber, after incubation with α-amylase to reveal growth rings. The α-amylase digests the amorphous zones, leaving the more resistant semicrystalline zones.

unclear. Studies of mutant and transgenic plants with reduced activity of one isoform of SBE are plagued by the possibility that differences in starch structure are caused by pleiotropic effects rather than a change in the nature of the SBE activity [16].

There has been rapid progress in understanding the nature and roles of isoforms of starch synthase. It is accepted that one class of isoform, granule-bound starch synthase I (GBSSI), is responsible for amylose synthesis (discussed below), and it is now apparent that starch-synthesising organs contain multiple isoforms of starch synthase responsible for amylopectin synthesis. The amylopectin-synthesising isoforms thus far described can be divided on the basis of their amino-acid sequences into three distinct classes, named SSI, SSII and SSIII. All three classes have been identified in potato tuber [17–20] and maize endosperm [21•,22•,23••], and they may be very widely distributed in higher plants [e.g. 24••,25,26]. Information about the roles of the isoforms *in vivo* is being derived from mutants lacking specific isoforms [23••,24••] and from transgenic plants [17–20]. The phenotypes of these plants appear to show that each class of isoform plays a distinct role in the synthesis of amylopectin, although data must be treated with caution because in some cases there are pleiotropic effects of the mutations on other enzymes of starch synthesis [27]. An example of an isoform with an apparently distinct role comes from study of the *rug5* mutant of pea. The mutation lies in the gene encoding SSII, an isoform which contributes 60% of the amylopectin-synthesising activity in the wild-type embryo. Loss of the SSII isoform results in reduced abundance of chains of intermediate length and increased abundance of very short chains of amylopectin in the mutant embryo [24••]. This indicates that SSII has a specific role in the synthesis of chains of intermediate length: other isoforms are unable to substitute for this role in its absence.

Recent work on potato reveals that the precise role played by an isoform of starch synthase is dependent not only on its intrinsic properties but also upon the genetic, developmental and perhaps environmental background in which it is being expressed. Study of transgenic tubers with reduced activities of either SSII or SSIII or both isoforms together has shown that the effects on amylopectin structure of reduction of one of these isoforms depends upon whether or not the activity of the other isoform has also been reduced [12••,28••]. This apparent synergy between the roles of the isoforms probably arises because the substrate of a particular isoform *in vivo* is the product of all of the other isoforms of starch synthase and of SBE present in the plastid. Changes in these other activities will alter the nature of the substrate and hence influence the product of the isoform. If this interpretation is correct, it will be extremely difficult to predict the contribution of a particular isoform of either starch synthase or starch branching enzyme to amylopectin synthesis *in vivo* simply from a knowledge of its properties in the test tube.

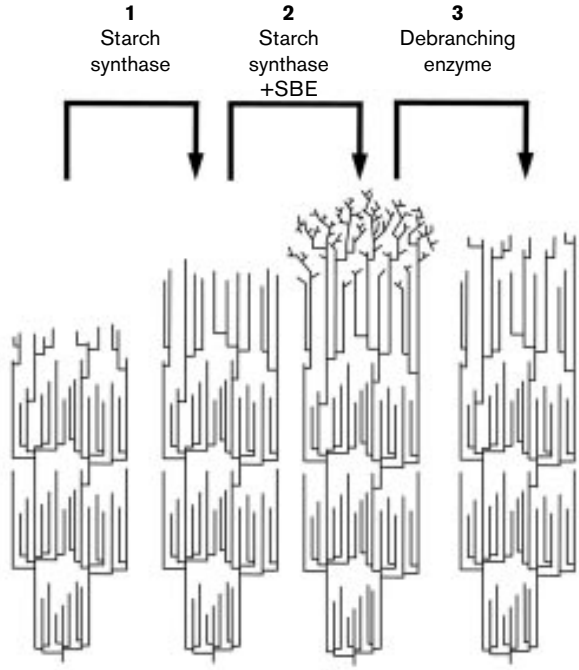
Figure 2

Models to explain the involvement of debranching enzyme in starch synthesis. **Top panel**: the preamylopectin trimming model [29] proposes a direct involvement of DBE in amylopectin synthesis. It is envisaged that a sequence of synthetic events at the surface of the granule creates a cluster within an amylopectin molecule, as follows: (1) short chains are elongated by starch synthase; (2) when chains reach a sufficient length to become substrates for SBE, a highly branched preamylopectin is formed; (3) selective trimming of this structure by DBE creates a bed of short chains from which the next round of synthesis can occur. When DBE activity is reduced or eliminated, preamylopectin accumulates. This highly-branched, soluble material is the phytoglycogen seen in the *su1*, *sta7* and *dbe1* mutants. **Bottom panel**: the soluble glucan recycling model [35••] proposes that DBE is only indirectly involved in starch synthesis. Amylopectin synthesis requires only starch synthase and starch-branching enzyme. In a normal plastid (top), small soluble malto-oligosaccharides in the stroma may be elongated by starch synthase, and then branched by SBE. Any glucans thus synthesised will be degraded by a suite of enzymes including DBE (dashed lines), preventing the accumulation of such products. In a plastid in which DBE activity is reduced or eliminated, this degradative mechanism is incomplete. Soluble branched glucans formed by starch synthase and SBE from malto-oligosaccharides can be further elaborated, providing more substrates for starch synthase and SBE, and leading to both an accumulation of phytoglycogen and a reduction in the rate of starch synthesis.

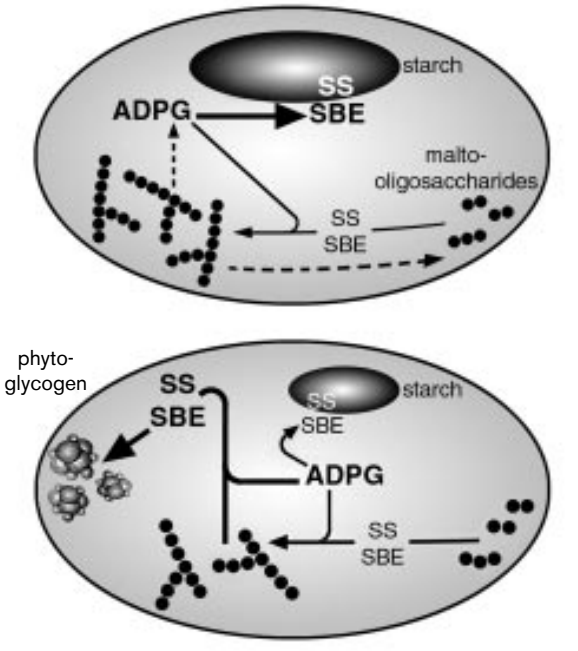
New models of amylopectin and amylose synthesis

Ideas and research on the synthesis of amylopectin have been strongly influenced in the last two years by the 'pre-amylopectin trimming' model of Ball and colleagues [29], in which not only starch synthase and SBE but also debranching enzyme (DBE) play direct roles. The general idea that DBE is involved in amylopectin synthesis arises from the observation that the endosperms of *sugary1* (*su1*) mutants of maize and rice, which are deficient in DBE activity, synthesise not only amylopectin but also a more highly-branched, soluble glucan called phytoglycogen [30–33]. Further support for an involvement of DBE in amylopectin synthesis has recently been provided by the discovery of DBE-deficient, phytoglycogen-accumulating mutants of *Chlamydomonas* (*sta7*) [34] and *Arabidopsis* (*dbe1*) [35••], by the demonstration that DBEs are probably of widespread occurrence in starch-synthesising organs [36], and by the discovery that a gene at the *su1* locus of maize actually encodes a DBE of the isoamylase class [31]. The preamylopectin trimming model proposes a specific, sequential course of events in which a highly-branched structure synthesised by starch synthase and SBE at the surface of the granule is 'trimmed' by DBE to provide the substrate for chain elongation by starch synthase (Figure 2). The model provides an explanation of the clustering of chains of amylopectin and hence, potentially, of the semi-crystalline ordering of the granule. This idea is tempting in that it integrates the synthesis of the amylopectin polymer with its organisation to form the granule, and provides an explanation for the accumulation of phytoglycogen in mutants deficient in DBE. However, proof that the trimming mechanism actually operates *in vivo* is still lacking.

Figure 2



Preamylopectin trimming model



Soluble glucan recycling model

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An alternative explanation for the accumulation of phytoglycogen in DBE-deficient cells has arisen from study of the *dbe1* mutant of *Arabidopsis*. Chloroplasts in the mutant accumulate both phytoglycogen and apparently normal amylopectin at the same time [35••]. This phenotype led Zeeman and colleagues to propose that DBE is not directly

involved in amylopectin synthesis. They suggested that DBE, together with other starch-metabolising enzymes, plays a scavenging role in the stroma, degrading any soluble glucans produced by the action of starch synthase and SBE on malto-oligosaccharides. In the absence of DBE, starch synthase and SBE will be able to elaborate branched, soluble glucans, resulting in phytoglycogen accumulation and reducing the amount of these enzymes available for the synthesis of amylopectin at the granule surface (Figure 2).

Both of these models for the involvement of DBE in starch synthesis are extremely difficult to test in a rigorous and direct way, and at present the question of whether DBE is directly involved in amylopectin synthesis remains open.

The mechanism of synthesis of the amylose component of the granule is also the subject of an unresolved debate. It is clear that this essentially linear polymer — which comprises 20–30% of the starch of storage organs — is synthesised via the GBSSI isoform of starch synthase [37], and that synthesis occurs within the amylopectin matrix of the granule [38]; however, the substrate for this synthesis is unknown. In isolated starch granules of pea, potato and *Chlamydomonas*, GBSSI can elongate both chains within the amylopectin matrix and exogenously-supplied, soluble malto-oligosaccharides which can diffuse into the granule [39,40••]. Both of these substrates can act as precursors of amylose *in vitro*. Synthesis of amylose within the matrix from malto-oligosaccharides has been demonstrated in isolated granules from higher plants and *Chlamydomonas* [39,40••]. Synthesis of amylose by cleavage of long chains of amylopectin within the granule matrix has thus far only been demonstrated during prolonged incubation of granules isolated from *Chlamydomonas* [40••]. Whether one or both (or indeed neither) of these routes to amylose actually operates *in vivo* has not been established for any starch-synthesising cell.

Unsolved problems

Four further aspects of the synthesis of the starch granule are central to a complete explanation of this process, but remain so poorly understood that robust models have not yet been proposed.

First, efforts to explain granule synthesis have concentrated on the organised, semi-crystalline part of the granule, and largely ignored the existence of amorphous zones within the granule. In the storage starch of higher plants, semi-crystalline zones alternate with amorphous zones, in which the conformation of amylopectin chains is not understood (Figure 1). What mechanisms determine the transition from organised to less-organised material and back on a periodic basis? The suggestion that the 'growth rings' are brought about by diurnal changes in supply of substrate for starch synthesis [41], which could influence structure through differential effects on isoforms of starch synthase [37], has not been rigorously tested.

Second, the role in starch synthesis of the phosphorylation of amylopectin observed in many species is unknown. Levels of phosphorylation range from about one phosphate per 300 residues in potato to only a few percent of this value in cereals [42•]. The fact that phosphorylation occurs concurrently with starch synthesis [43•], at a frequency directly related to the chain-length distribution of amylopectin [42•], suggests an intimate relationship between this process and polymer synthesis. The recent discovery of a protein which may be important in determining the level of phosphorylation in potato starch [44••] offers the prospect of further progress in this area.

Third, no roles have yet been discovered for the starch-catabolising enzymes usually present in the plastid during starch synthesis, for example D-enzyme, starch phosphorylase and limit-dextrinase [36,45,46]. Severe reduction of D-enzyme activity in potato tubers has no obvious effect on starch structure [45]. It is possible that combinations of these enzymes are involved in direct modification of newly-synthesised amylopectin, or in the more indirect cycling of soluble glucans proposed by Zeeman and colleagues from study of the *dbe1* mutant of *Arabidopsis* ([35••] Figure 2).

Finally, the process of the initiation of the granule remains to be elucidated. The synthesis of glycogen in animals requires a self-glucosylating protein, glycogenin, from which synthesis via glycogen synthase begins [47]. There is no evidence that the synthesis of either amylopectin or amylose requires such a protein during normal granule growth. Starch synthases can use malto-oligosaccharides as primers, and these are likely to be universally present in plastids. However, the initiation and organisation of the primary glucan chains from which granule growth proceeds might require such a protein, and self-glucosylating proteins which could be candidates for this role have been identified in plants [48]. At this stage, though, there is no good evidence to support any particular model for the initiation of the granule.

Conclusions

Further progress in this field is being promoted by the development of collaborations between biochemists and molecular biologists on the one hand and chemists and physicists on the other. Progress in the past has been impeded by lack of common goals between these groups. Physical and chemical analyses of starch were made on material from plants for which little or nothing was known of synthetic pathway, and often involved comparisons of starches from genetically very different or poorly-defined plants. Studies of starch synthesis concentrated on properties of the enzymes in solution, without reference to the granule itself. This picture is changing as the availability of plants deficient in specific enzymes on the biosynthetic pathway allows chemical and physical analysis of starch from genetically and biochemically closely-defined systems. Such multi-disciplinary studies will provide a deeper understanding of the mechanism of synthesis of the granule.

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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

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2. Jenkins PJ, Cameron RE, Donald AM: **A universal feature in the structure of starch granules from different botanical sources.** *Starch* 1993, **45**:417-420.

3. Gallant DJ, Bouchet B, Baldwin PM: **Microscopy of starch: evidence of a new level of granule organization.** *Carbohydr Polym* 1997, **32**:177-191.

4. Oostergetel GT, van Bruggen EJF: **The crystalline domains in potato starch granules are arranged in a helical fashion.** *Carbohydr Polym* 1993, **21**:7-12.

5. • Bogracheva TY, Morris VJ, Ring SG, Hedley CL: **The granular structure of C-type pea starch and its role in gelatinization.** *Biopolymers* 1998, **45**:323-332.

References [5•] and [6•] use sophisticated techniques to explore the internal structure of starch granules from pea embryos. These granules are known to contain two different sorts of amylopectin crystallites, A and B (hence the term C-type starch), which reflect two different types of packing of the double helices within clusters (see Figure 1 above). Whether A or B crystallites are formed is thought to be related to the average chain lengths of chains within amylopectin clusters: A crystallites are formed from shorter and B crystallites from longer chains. Both studies report that the inner part of the pea starch granule is enriched in B and the outer part in A crystallites. The implication is that chain lengths of amylopectin in the inner and outer part of the granule are different. This difference could result from the changes during embryo development in relative activities of different isoforms of starch synthase and SBE [13], and also from modification of amylopectin within the granule matrix (see [38] and discussion of [24••]). Although pea starch is unusual in containing both A and B crystallites, there is also evidence from potato of differences in polymer structure and composition between the core and the periphery of the granule [7]. The pea may be a good system in which to explore the origins of such differences.

6. • Buléon A, Gérard C, Riekel C, Vuong R, Chanzy H: **Details of the crystalline ultrastructure of C-starch granules revealed by syntchrotron microfocus mapping.** *Macromol* 1998, **31**:6605-6610.

See annotation for [5•].

7. Jane J, Shen JJ: **Internal structure of the potato starch granule revealed by chemical gelatinization.** *Carbohydr Res* 1993, **247**:279-290.

8. • Waigh TA, Perry P, Riekel C, Gidley MJ, Donald AM: **Chiral side-chain liquid-crystalline polymeric properties of starch.** *Macromolecules* 1998, **22**:7980-7984.

Donald and colleagues suggest that the structure of amylopectin is consistent with that of a side-chain liquid-crystal polymer. They point out that several important physical properties of starch can be explained on this basis. The suggestion would also legitimise the belief that physical rather than biological processes promote the organisation of newly-synthesised amylopectin at the granule surface. The full implications of this suggestion for the mechanism of synthesis of the granule have yet to be explored, but they may be considerable.

9. Wong KS, Jane J: **Quantitative analysis of debranched amylopectin by HPAEC-PAD with a postcolumn enzyme reactor.** *J Liq Chrom Rel Technol* 1997, **20**:297-310.

10. • Morell MK, Samuel MS, O'Shea MG: **Analysis of starch structure using fluorophore-assisted carbohydrate electrophoresis.** *Electrophoresis* 1998, **19**:2603-2611.

New methods for the separation and quantification of chains of amylopectin are described. Amylopectin is debranched with isoamylase, and chains are tagged with a fluorophore. Chains are then separated and quantified by gel or capillary electrophoresis coupled with fluorescence detection. The gel electrophoresis method uses a standard Applied Biosystems DNA sequencer and software. It has several potential advantages over the widely-used method involving anion-exchange chromatography coupled with pulsed amperometric detection. For example many samples can be analysed simultaneously, allowing a high level of replication and hence statistical analysis of differences between samples. This method is used in the work described in [12••].

11. McPherson AE, Jane J: **Physicochemical properties of selected root and tuber starches.** *Carbohydr Polym* 1999, in press.

12. •• Edwards A, Fulton DC, Hylton CM, Jobling SA, Gidley M, Rössner U, Martin C, Smith AM: **A combined reduction in activity of starch synthases II and III of potato has novel effects on the starch of tubers.** *Plant J* 1999, **17**:251-261.

The studies in [12••] and [28••] describe the structure and properties of amylopectin in tubers of transgenic potato plants with reduced activities of either the SSII or the SSIII isoform of starch synthase or of both isoforms together. Comparison of results from the three sorts of plant reveals that the effect upon amylopectin of reduction of a particular isoform depends upon the level of activity of the other isoform. This apparent synergy between the actions of the two isoforms is explained in terms of the complexity of the substrate presented to an isoform of starch synthase *in vivo*. The structure of the substrate, and hence the contribution of the isoform to its synthesis, will depend upon other starch synthesising and modifying enzymes present in the amyloplast, and this in turn will depend upon a host of genetic, environmental and developmental factors. The role of an isoform *in vivo* is thus determined in part by its intrinsic properties, and in part by the background in which it is being expressed. This is perhaps bad news for the cause of rational manipulation of starch structure, but at least it implies that almost any manipulation of combinations of starch synthesising enzyme may have surprising and potentially useful results. The two papers are also good illustrations of the use of chimeric constructs to express two antisense RNAs simultaneously.

13. Burton RA, Bewley JD, Smith AM, Bhattacharyya MK, Tatge H, Ring S, Bull V, Hamilton WDO, Martin C: **Starch branching enzymes belonging to distinct enzyme families are differentially expressed during pea embryo development.** *Plant J* 1995, **7**:3-17.

14. Morell MK, Blennow A, Kosar-Hashemi B, Samuel MS: **Differential expression and properties of starch branching enzyme isoforms in developing wheat endosperm.** *Plant Physiol* 1997, **113**:201-208.

15. Kuriki T, Stewart DC, Preiss J: **Construction of chimeric enzymes out of maize endosperm branching enzymes I and II: activity and properties.** *J Biol Chem* 1997, **46**:28999-29004.

16. Tomlinson KL, Lloyd JR, Smith AM: **Importance of isoforms of starch-branching enzyme in determining the structure of starch in pea leaves.** *Plant J* 1997, **11**:31-43.

17. Edwards A, Marshall J, Sidebottom C, Visser RGF, Smith AM, Martin C: **Biochemical and molecular characterisation of a novel starch synthase from potato tubers.** *Plant J* 1995, **8**:283-294.

18. Marshall J, Sidebottom C, Debet M, Martin C, Smith AM, Edwards A: **Identification of the major starch synthase in the soluble fraction of potato tubers.** *Plant Cell* 1996, **8**:1121-1135.

19. Abel GJW, Springer F, Willmitzer L, Kossmann J: **Cloning and functional analysis of a cDNA encoding a novel 139 kDa starch synthase from potato (*Solanum tuberosum* L.).** *Plant J* 1996, **10**:981-991.

20. Kossmann J, Abel GJW, Springer F, Lloyd JR, Willmitzer L: **Cloning and functional analysis of a cDNA encoding a starch synthase from potato (*Solanum tuberosum* L.) that is predominantly expressed in leaf tissue.** *Planta* 1999, in press.

21. • Knight ME, Harn C, Lilley CER, Guan HP, Singletary GW, Mu-Forster C, Wasserman BP, Keeling PL: **Molecular cloning of starch synthase I from maize (W64) endosperm and expression in *Escherichia coli*.** *Plant J* 1998, **14**:613-622.

[21•,22•,23••] describe the identification at a molecular level of the three isoforms of starch synthase likely to account for the amylopectin-synthesising activity of the maize endosperm. This paper describes an isoform of the SSI class first identified in rice [26] and recently found in potato, where it is expressed primarily in leaves [20].

22. • Harn C, Knight M, Ramakrishnan A, Guan HP, Keeling PL, Wasserman PL: **Isolation and characterization of the *zSSIIa* and *zSSIIb* starch synthase clones from maize endosperm.** *Plant Mol Biol* 1998, **37**:639-649.

See annotation for [21•]. Here cDNAs encoding two isoforms of the SSII class are described, only one of which (*zSSIIa*) is abundant in the endosperm. The *zSSIIa* gene is reported to map approximately to the *sugary2* (*su2*) locus, raising the possibility that loss of this isoform is the primary cause of the alterations in amylopectin structure seen in *su2* mutants. The *rug5* mutant of pea described in [24••] also lacks an isoform of the SSII class.

23. 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.
See annotation for [21•]. The gene at the *Dull1* locus of maize is shown to encode a starch synthase. The protein is more similar to the SSIII isoform of potato than to isoforms of the SSII and SSI classes, but a large amino-terminal portion of the protein is substantially different in sequence from any previously described isoforms. *dull1* mutations bring about large changes in the structure of amylopectin, hence this discovery provides further evidence that individual isoforms of starch synthase play specific roles in amylopectin synthesis. The precise contribution of the DU1 starch synthase is difficult to deduce because the mutation has pleiotropic effects on other enzymes of starch synthesis (see [27]). The authors suggest that at least some of these effects may be the consequence of destabilisation of a putative starch-synthesising complex of which the DU1 starch synthase is a component. They point out features of the amino-terminal region of the DU1 protein which might be involved in protein–protein interactions with SBE. This intriguing idea remains to be rigorously tested.

24. Craig J, Lloyd JR, Tomlinson K, Barber L, Edwards A, Wang TL,
•• Martin C, Hedley CL, Smith AM: **Mutations in the gene encoding starch synthase II profoundly alter amylopectin structure in pea embryos.** *Plant Cell* 1998, **10**:413-426.
Mutations at the *rug5* locus of pea are shown to lie in the gene encoding SSII, the isoform of starch synthase responsible for about 60% of the amylopectin-synthesising activity of the developing embryo. Although the mutations have little effect on the rate of starch synthesis during most of embryo development, they cause a decrease in amylopectin chains of intermediate length, thought to span two clusters (see Figure 1), and an increase in the very short chains. This indicates that SSII plays a specific role in the synthesis of chains of intermediate length in the wild-type embryo. There is also an increase in very long chains of amylopectin. It is suggested that this may be attributable to an increased specific activity of GBSSI in the mutant. This is consistent with a growing body of evidence, not discussed in this review, that GBSSI is not only responsible for amylose synthesis but also contributes to the synthesis of long chains in the amylopectin fraction of the granule matrix (see [39,40••]).

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•• **inhibition of two starch synthase isoforms in potato tubers leads to accumulation of grossly modified amylopectin.** *Biochem J* 1999, **338**:515-521.
See annotation for [12••].

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A mutant of *Arabidopsis* is shown to accumulate most of its glucan as phytoglycogen rather than starch, and to lack a DBE of the isoamylase class. It is, therefore, comparable with the *su1* mutants of cereals and the *sta7* mutant of *Chlamydomonas*, described in [30–34]. Detailed analysis shows that individual chloroplasts accumulate simultaneously both phytoglycogen and starch granules containing amylopectin very similar to that in wild-type leaves. The authors explain this phenotype by proposing that DBE is one of a suite of enzymes required to prevent the production of highly-branched soluble glucans from malto-oligosaccharides at the expense of amylopectin synthesis at the granule surface. This proposal for the role of DBE in starch sythesis differs radically from that put forward in [29]. Neither of these proposals has yet been rigorously tested.

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Using starch granules isolated from the unicellular alga *Chlamydomonas*, the authors show that amylopectin within the granule matrix can act as a substrate for amylose synthesis via the granule-bound starch synthase. During prolonged incubations of the granules, long chains which have been elongated by the granule-bound enzyme are cleaved from amylopectin within the matrix to produce amylose. The mechanism of cleavage is presently unknown. Models are proposed in which the cleavage occurs via either the granule-bound enzyme itself, utilising a putative hydrolytic domain, or the chain-transfer reaction catalysed by a granule-bound starch-branching enzyme. The authors suggest that this amylopectin-based mechanism is a more likely route of amylose synthesis in *Chlamydomonas* than elongation of soluble malto-oligosaccharides shown to give rise to amylose in isolated granules from pea, potato [39] and *Chlamydomonas* (this reference). Neither of these routes has yet been shown to operate *in vivo*.

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References [42•] and [43•] enforce the idea that the phosphorylation of amylopectin occurs via a common mechanism during the synthesis of amylopectin in a very wide range of species, and that the mechanism of phosphorylation is intimately related to the process of amylopectin synthesis. Here, a detailed analysis of phosphorylated chains in starches from several species reveals a strong relationship between the chain length profile of amylopectin and the degree of phosphorylation.

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See annotation for [42•]. Supply of radiolabelled glucose 6-phosphate to amyloplasts isolated from potato tubers reveals that the phosphate group of the hexose phosphate is incorporated into amylopectin. This may provide a valuable system on which to study the mechanism of phosphorylation.

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