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# Transitory and storage starch metabolism: two sides of the same coin?

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The industrially important polymer, starch, is manufactured through a complex process involving multiple isoforms of several different enzymes. These contribute to alter the structure of starch which, in turn, affects its downstream industrial use. This review compares recent advances in our knowledge of starch metabolism in leaves and storage organs. Starch granule initiation and formation, enzyme complexes involved in starch metabolism and control of flux in starch synthesis and degradation are examined.

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The polyglucan starch is a major industrial product isolated from the storage organs of a number of plant species. It is utilized in diverse industrial applications, such as a food thickener, but also acts as a feedstock for bioethanol production as well as in the brewing industry [1]. It consists of two fractions, the branched amylopectin and essentially linear amylose. Our current model of starch synthesis indicates that one of the earliest steps is the production of a glycogen like molecule, named phytoglycogen. Similar to the amylopectin fraction of starch, phytoglycogen contains short α1,4 glucan chains linked together by α1,6 branch points. The polymer is formed from ADP-glucose by a combination of starch branching enzymes (SBE) and starch synthases (SS), of which multiple isoforms exist in plants. It is then thought that debranching enzymes (isoamylases and pullulanase) remove excess branch points while other degradative enzymes (α-amylase and β-amylase) trim the chains, leading to the formation of amylopectin [1,2]. This aggregates with amylose in insoluble granules within plastids. In terms of its degradation the initial step is the phosphorylation of amylopectin chains by two enzymes, the glucan, water, and phosphoglucan, water dikinases (GWD and PWD) leading to the partial solubilization of the granule surface. Amylolytic enzymes can then release phosphorylated malto-oligosaccharides which need to be dephosphorylated by two specific phosphatases, SEX4 and Like Sex Four-2 (LSF2) allowing their further mobilization [3,4].

Because of its industrial relevance, early work on starch metabolism often concentrated on the effects of genetic alterations in mutant and transgenic plants on storage starch in seeds and tubers. Since the sequencing of the Arabidopsis genome and subsequent production of easily identifiable insertion mutants, much progress has been made in studying transitory starch (starch that is manufactured and degraded in one day/night cycle) metabolism in leaves of this plant. The question remains however, how applicable that research is to understanding starch metabolism in commercially important non-photosynthetic storage organs. This review examines recent advances in our understanding of starch metabolism and tries to identify gaps in knowledge between the formation of transitory and storage starches.

## Starch granule initiation and formation

The first step in forming a starch granule has been debated for many years, mainly as it is not clear whether starch synthases form polyglucans spontaneously from ADP-glucose, or whether a separate protein primer is necessary. Mutants in two Arabidopsis SS isoforms (SS3 and SS4) appear to indicate that the former possibility is most likely correct as *ss4* mutants reduce granule initiation as demonstrated by reduced starch contents and the presence of single enlarged starch granules in their chloroplasts, rather than the many smaller ones found in WT controls [5]. Double mutants lacking both SS4 and SS3 contain almost no starch, indicating functional overlap between these isoforms in granule initiation [6]. Recently this process has been studied in more depth in an *ss4* mutant. This study concluded that SS4 was indeed an initiator of granule synthesis, but that when chloroplast volume was increased the number of granules formed also increased [7]. The question remains as to whether a similar mechanism exists in storage organs. It has been found that a rice double mutant lacking both SS1 and SS3a was sterile, most likely as it could not manufacture starch [8], which may indicate that these isoforms play a similar role in rice to SS3 and SS4 in Arabidopsis leaves (Figure 1).

From these data it seems clear that starch synthase isoforms are important in initiating granule production, and that this can have a knock-on effect on granule size, but are there other mechanisms that can influence granule formation? It seems unlikely that the space available within plastids is important as mutants affecting chloroplast size did not affect starch granule size, but rather number produced [7,9]. Recently two proteins which influence starch formation in rice endosperm were identified [10••,11••]. Starch granules in this tissue are classified as being compound as they are composed of many smaller granules that are assembled (but not fused) together. Mutation in the SUBSTANDARD STARCH GRAIN4 protein leads to enlarged compound granules and reduced total starch. This mutation also altered the shape of starch granules in rice pollen and leaves, which were more spherical and less elongated [10••]. The precise role of this protein is unknown, but the most similar Arabidopsis protein is thought to be involved in embryo development [12]. Mutations in the second protein, known as FLOURY ENDOSPERM6 (FLO6) [11••], result in disruption of the compound granules within endosperm and reduced starch amounts and an alteration in the physical properties of the starch. This protein has been shown to bind to starch granules through a starch binding domain that it contains and was shown to interact directly with an isoamylase1 (ISA1) type debranching enzyme. This makes sense as repression of ISA1 in other species affects both amylopectin accumulation and starch granule size [13,14]. In this case the FLO6 protein is assumed not to have a catalytic activity, but to chaperone ISA1 to the surface of starch granules. Unfortunately, its role was only examined in endosperm, and mutations in orthologous genes in other species have not been reported (Figure 1).

## Interactions between starch biosynthetic enzymes

There has often been an assumption that the enzymes involved in synthesizing the complex starch polymer would interact with each other in some way to accomplish this. This could either be in the form of one enzyme producing a specific substrate that would be utilized preferentially by another enzyme (e.g. a polyglucan chain of a specific length), or by direct physical interaction (as between FLO6 and ISA1 mentioned above or the many protein complexes described in diverse species [15]). Such interactions are beginning to be unraveled through the production of mutant plants lacking multiple proteins involved in starch synthesis and degradation. Two recent examples of this come from demonstrations that starch synthases and isoamylases interact with each other. Work in maize endosperm [16] demonstrated that mutations in ISA2 will lead to accumulation of phytoglycogen, but only when combined with mutations in SS3. This indicates that SS3 and ISA2 suppress phytoglycogen accumulation in maize endosperm. Similar experiments have also been performed in Arabidopsis [17]. From this it was concluded that specific starch synthase isoforms are important for production of chains that are long enough to crystallize, an important pre-requisite for starch formation (Figure 1).

Interactions of starch synthase and branching enzymes have also recently been examined. Analysis of rice mutants lacking specific branching enzymes alongside mutations in SS1 demonstrated that SS1 acts on the products that are manufactured by a specific branching enzyme (BE2b). This leads to alterations in starch structure that affect the affinity of other starch biosynthetic enzymes for the granule, meaning that the increase in amylose and alteration is starch structure in this mutant is not only due to the lack of BE2b, but also due to a large number of downstream alterations [18]. A second SS isoform (SS3a) has also been shown to interact with BE2b as a double mutant lacking both enzymes contains more amylose than either of the single mutants [19]. Interestingly this was shown to lead to an increase in the amylose forming granule bound SS, something also observed in a high amylose barley *amo1* mutant, where the *SS3a* gene is very tightly linked to the *amo1* locus [20] In a complementary study combinations of recombinant Arabidopsis SS (SS1, SS2, SS3 and SS4) and SBE (SBE2 and SBE3) proteins demonstrated that all SS isoforms interacted with all SBE's. They further showed that when these recombinant enzymes were combined with ADP-glucose, the only combination that produced glucan similar in structure to amylopectin was that when SS1 was combined with either SBE2 or SBE3 [21].

## Control of the rate of starch turnover

The rate of transitory starch turnover in Arabidopsis leaves is exquisitely regulated to maximize plant productivity. For example, Arabidopsis triple mutants lacking an α-amylase (Amy3), limit dextrinase (LDA) and isoamylase (ISA3) are completely repressed in starch degradation. In the various single and double mutants produced as part of this study it was found that plant growth inversely correlated with the rate of starch degradation [22•]. The mechanisms for this decrease in growth are beginning to be understood [3]. It seems likely that in many cases it is due to sugar starvation at night, and recent evidence has indicated that this can lead to down-regulation of gibberellin synthesis [23•]. In the specific case of the *ss4* mutant, the plants accumulate ADP-glucose to such an extent that it probably leads to phosphate limitation of photosynthesis [24].

Because of its importance, regulation of starch turnover will likely include many post-translational mechanisms [15,18,25]. Researchers have often examined the role of ADP-glucose pyrophosphorylase (AGPase) as this is the first committed step in the starch biosynthetic pathway. It has been known for decades that this enzyme is allosterically regulated by Pi and 3-phosphoglyceric acid, but