Starch is the primary storage carbohydrate in most photosynthetic organisms and allows the accumulation of carbon and energy in form of an insoluble and semi-crystalline particle. In the last decades large progress, especially in the model plant Arabidopsis thaliana, was made in understanding the structure and metabolism of starch and its conjunction. The process underlying the initiation of starch granules remains obscure, although this is a fundamental process and seems to be strongly regulated, as in Arabidopsis leaves the starch granule number per chloroplast is fixed with 5-7. Several single, double, and triple mutants were reported in the last years that showed massively alterations in the starch granule number per chloroplast and allowed further insights in this important process. This mini review provides an overview of the current knowledge of processes involved in the initiation and formation of starch granules. We discuss the central role of starch synthase 4 and further proteins for starch genesis and affecting metabolic factors.

Keywords: starch biosynthesis, starch granule biogenesis, starch synthase, plastidial phosphorylase, malto-oligosaccharides
in two steps. We define starch initiation in a strict sense as a process in which a primer-like structure/initiation complex is generated, that can in principle be further used for polymerization reactions. This also includes the possibility that no starch granules are detectable since the primer-like structure/initiation complex is not further synthesized. The term starch formation indicate when these potential primer-like structures/initiation complexes are used to form starch granules, and so starch granules are detectable in chloroplasts.

IMPORTANT ROLE OF STARCH SYNTHASE 4

Starch synthases (SSs) are the enzymes responsible for elongation of amylose and amylopectin chains. These enzymes transfer glucosyl residue from ADPglucose to the non-reducing end of a preexisting glucan. It was discussed whether SSs are able to synthesize glucan chains without a glucan primer (Szydlowski et al., 2009; D’Hulst and Mérida, 2012). However, using heterologously expressed and purified SSs from Arabidopsis a primer was always required, that contained at least two glucosyl residues. Thus, maltose but not glucose was elongated by the SSs (Brust et al., 2013).

Analyses of SS mutants revealed that SSs have overlapping and in parts distinct functions during starch synthesis (Delvallé et al., 2005; Zhang et al., 2005, 2008; Roldán et al., 2009, 2011). SS4 seems to be most important for formation of starch granules. Thus, the ss4 mutant reveals mostly one starch granule per chloroplast (Roldán et al., 2007). However, other SS isoforms may partially substitute the SS4 function and so additional loss of SS3 further reduces the starch granule number in chloroplasts (Szydlowski et al., 2009; D’Hulst and Mérida, 2012). Interestingly, the number of starch granules in ss4 is still flexible as the number shows variability in regards to growth conditions/developmental stage and enzymatic setup (see below). Thus, immature leaves of ss4 have no detectable starch granules in contrast to mature leaves (Crumpton-Taylor et al., 2013). The number of starch granules per chloroplast depends of the length of the light phase as under continuous light the starch granule number increases to up to three (Malinova et al., 2017). Furthermore, the additional lack of α-amylase 3 (Seung et al., 2016) and an increased capacity for glucan synthesis via expression of a bacterial glycogen synthase (Crumpton-Taylor et al., 2013) result in a higher number of starch granules per chloroplast. An over expression of SS4 in Arabidopsis causes an elevated in an increased starch content, although an increase in starch granule number per chloroplast was not reported (Gamez-Arjona et al., 2011).

All five SSs in Arabidopsis share a core sequence of approximately 60 kDa at the C-terminus responsible for the catalytic activity. The N-terminal sequences are largely different. Interestingly, SS3 and SS4 possess a large N-terminal extension. The N-terminal region of SS4 is predicted to contain two long coiled-coil domains (Leterrier et al., 2008; D’Hulst and Mérida, 2012) and was reported to enable the association with the thylakoid membrane and interaction with thylakoid- and plastoglobule-bound fibrillin proteins (Gamez-Arjona et al., 2014; Raynaud et al., 2016). Furthermore, a subchloroplastidial localization of SS4 peripheral to starch granules was shown (Gamez-Arjona et al., 2014). Expression of the N-terminal region of SS4 in ss4 did not result in alterations of the starch granule number. However, the expression of the C-terminal catalytically active region of SS4 in ss4 resulted in an increased number of starch granules per chloroplast (Lu et al., 2018). It was shown that SS4 forms dimers necessary for its enzymatic activity and dimerization is mediated by a region located between the coiled-coil domain and the glycosyltransferase domain (Raynaud et al., 2016).

Summarizing, SS4 has impact on formation of starch granules and seems to be important for the generation of the regular number of granules per chloroplast. However, the observed reduced number of starch granules by the lack of SS4 can be in parts reversed by metabolic factors (see below).

ADDITIONAL PROTEINS INFLUENCING THE STARCH GRANULE NUMBER

Recently, a double mutant lacking the plastidial phosphorylase (PHS1; Zeeman et al., 2004) and the disproportionating enzyme 2 (DPE2; Chia et al., 2004; Lu and Sharkey, 2004; Fettke et al., 2006) was published (Malinova et al., 2014). Both parental lines have 4-7 starch granules per chloroplast similar to wild type. Interestingly, dpe2/phs1 revealed mostly one starch granule per chloroplast when grown under 12 h light and 12 h dark conditions, similar to ss4. However, expression of SS4 was unaltered in this mutant (Malinova et al., 2014). The triple mutant dpe2/phs1/ss4 also has only one starch granule per chloroplast when grown under 12 h light and 12 h dark (Malinova et al., 2017). More interestingly, when the light/dark regime was altered ss4, dpe2/phs1, and the triple mutant plants revealed variability in the starch granule number per chloroplast. An elongated light phase and continuous light resulted in an increased number of starch granules per chloroplast in all mutants but with different quantities. In ss4 the number increased up to three granules whereas dpe2/phs1 showed with 5-7 the wild type number of starch granules per chloroplast. The dpe2/phs1/ss4 revealed an intermediate number of starch granules with 1-4 granules per chloroplast (Malinova et al., 2017). The observed increased number of starch granules in dpe2/phs1 similar to wild type was supposed to be linked to the missing dark phase and the lack of starch degradation (Malinova et al., 2014). To test whether ongoing starch degradation in dpe2/phs1 is necessary for the observed reduction of the starch granule number a triple mutant lacking the glucan, water dikinase (GWD; sex1-8; Ritte et al., 2002; Mahlow et al., 2014) in the background of dpe2/phs1 was generated. GWD is involved in the phosphorylation/dephosphorylation cycle at the starch granule surface and is the key enzyme of the initiation of starch degradation (Hejazi et al., 2012; Mahlow et al., 2016). However, sex1-8 did not reveal a reduced starch granule number per chloroplast (Mahlow et al., 2014). Similarly, dpe2/phs1/sex1-8 revealed 5-7 starch granules even when plants were grown at 12 h light and 12 h dark conditions (Malinova and Fettke, 2017).
Thus, it was concluded that ongoing starch degradation is essential for the observed reduced number of starch granules in dpe2/phs1 (Malinova and Fettke, 2017).

Recently, three new starch granule bound proteins were discovered sharing high homology and building up a new protein family, PROTEIN TARGETING TO STARCH (PTST; Seung et al., 2015, 2017). All three proteins contain conserved coiled coil domains and a CBM48. While PTST1 was shown to play an essential role in amylase synthesis by localizing granule bound SSs to starch granules (Seung et al., 2015), PTST2 and PTST3 were demonstrated to be involved in alteration of the number of starch granules per chloroplast (Seung et al., 2017). In ptst2 a reduced starch granule number was observed with mostly one starch granule per chloroplast. PTST3 has partially redundant function to PTST2 but reduction of granule number was less pronounced in ptst3 compared with ptst2. Furthermore, it was shown that an overexpression of PTST2 in wild type background resulted in an increased number of small granules. In contrast, an overexpression of PTST2 in ss4 background resulted in a further reduction of the starch granule number per chloroplast. Interestingly, PTST2 was shown to interact with SS4 and longer soluble maltodextrins (Seung et al., 2017).

METABOLIC IMPLICATIONS OF VARIABILITY IN STARCH GRANULE NUMBER PER CHLOROPLAST

For ss4 as well as for ptst2 and ptst2/ptst3 an accumulation of ADPglucose was reported (Crumpton-Taylor et al., 2013; Ragel et al., 2013; Seung et al., 2017). The highest ADPglucose accumulation was observed for ss4 (with ~100-fold increase compared to wild type). ptst2/ptst3 revealed also a high accumulation of ADPglucose (with ~30-fold compared to wild type) that was twice of that of ptst2 (Seung et al., 2017). This accumulation of ADPglucose, the substrate of SSs, is thought to show that the SS4 acts upstream of all other SSs and that the other SSs have limited activity when SS4 is lacking. However, ss4/ss3 showed a similar high accumulation of ADPglucose as ss4 (with ~100-fold increase compared to wild type). dpe2/phs1 and especially dpe2/phs1/ss4 also revealed mostly one starch granule per chloroplast but showed only a very moderate increase in ADPglucose content (two- and fourfold, respectively, compared to wild type). Furthermore, dpe2/ss4 and phs1/ss4, each having one starch granule per chloroplast, revealed totally different ADPglucose values (dpe2/ss4: approximately twofold, phs1/ss4: 250-fold increase compared to wild type). Under continuous light, an increase in the granule number per chloroplast was observed for ss4, dpe2/phs1, and dpe2/phs1/ss4. But these observations were not accompanied by a reduction in the ADPglucose amounts (Malinova et al., 2017). Thus, a simple direct connection of a high ADPglucose content and the lack of SS4 or a reduced granule number is not given.

Another factor influencing the starch granule number is probably the availability of soluble maltodextrins as glucan primers for the initiation of a starch granule. The generation and elongation of soluble maltodextrins is proposed to be important during granule initiation (Nakamura, 2015). It was postulated that longer soluble maltodextrins (e.g., maltodecaose), long enough to produce a helical secondary structure, are bound by PTST2. PTST2 further interacts with SS4 and initiate the synthesis of starch granules (Seung et al., 2017). Interestingly, increased levels of soluble glucans were detected in ss4 (Crumpton-Taylor et al., 2013; Malinova et al., 2017). Most of
These glucans were rather shorter with a degree of polymerization (DP) less than six. In dpe2/phs1 and dpe2/phys1/ss4 a massive increase in these short glucans was observed (Malinova et al., 2014, 2017). The origin of the chloroplastidial maltodextrins necessary for initiation of starch granules is unknown so far. However, the chloroplastidial maltodextrin pool is certainly also influenced by starch degradation processes.

PHS1 could be involved in metabolism of the chloroplastidial maltodextrins and therefore in starch synthesis. Several indications for a principle involvement of PHS1 in starch synthesis were published. For Chlamydomonas reinhardtii, potato tubers, and for rice endosperm it was shown that the plastidial phosphorylase is involved in starch synthesis (Dauvillé et al., 2006; Orawetz et al., 2016) and in granule initiation (Satoh et al., 2008). Interestingly, the alteration of starch quantities and qualities in rice and potato mutants were exclusively detected when plants were grown under low temperature conditions. It was speculated that at low temperatures SSs cannot sufficiently substitute the lack of the plastidial phosphorylase (Fettke et al., 2012b; Orawetz et al., 2016). Furthermore, for endosperm starch evidences exist for a protein–protein interaction of the plastidial phosphorylase with branching enzyme and disproportionating enzyme 1 (DPE1) and production of longer soluble maltodextrins (Tetlow et al., 2004; Hwang et al., 2016; Nakamura et al., 2017).
Interestingly, in Arabidopsis PHS1 interacts with SS4 (Figure 1; Qasim, 2018).

The reduced starch granule number per chloroplast observed in dpe2/phs1 is not detected in the phs1. The single mutant revealed a starch granule number similar to wild type (Malinova et al., 2014, 2017). It could be speculated that other proteins compensate the lack of PHS1 but if in addition the starch turn-over is altered an effect on starch initiation could be observed. A balance between generation and degradation of maltodextrins could be important. In this regards, it can be supposed, that an additional lack of degradation enzymes such as α-amylase 3 (AMY3; Seung et al., 2016) and DPE2 (Malinova et al., 2014, 2017) influence this balance. An increase of the starch granule number per chloroplast was detected in the ss4/amy3 mutant, which point to a degradation of glucans in ss4 and this degradation generates primers that can be used by other SSSs. In this context it can also be seen that an introduction of the glycogen synthase from Agrobacterium tumefaciens (able to initiate and elongate glucan chains using ADPglucose as substrate; Ugalde et al., 2003) in ss4 resulted in an increase in starch granule number per chloroplast by generating more of such primers (Crumpton-Taylor et al., 2013; Lu et al., 2018). In agreement with the observed binding of longer soluble maltodextrins by PTST2, it could be speculated that soluble maltodextrins longer than DP10 are involved in the starch granule initiation, as in dpe2/phs1 no limitation for maltodextrins shorter than DP10 were observed (Malinova et al., 2014). Similarly, when in the background of dpe2/phs1 the starch degradation was blocked by the additional lack of GWD, this glucan balance was again influenced and resulted in an increased number of starch granules with 5-7 granules per chloroplast similar to wild type (Malinova and Fettke, 2017). However, the additional lack of GWD in ss4 has no effect on the starch granule number (Crumpton-Taylor et al., 2013). Interestingly, phs1/mex1 double mutant lacking PHS1 and in addition the maltose exporter 1 [essential for export of the starch degradation product maltose (Nittylä et al., 2004) and thus it acts upstream to DPE2 (Fettke et al., 2012a)] in contrast to dpe2/phs1 has a similar to wild type starch granule number per chloroplast. In regards to the soluble maltodextrins both mutants are different too, thus only a small increase in soluble maltodextrins (length up to DP10) was observed in mex1/phs1 compared to wild type (Malinova et al., 2014). A further rather indirect indication of this balanced maltodextrin system is related to the observed dependency of the length of the light and dark phase on the starch granule number in ss4, dpe2/phs1, and dpe2/phs1/ss4 (Malinova et al., 2017).

Summarizing, as depicted in Figure 2, in a strict sense, the initiation of starch granules seems to be linked to maltodextrins serving as glucosyl acceptors. Increasing evidences reveal that these maltodextrin primers are influenced by the overall glucan metabolism in chloroplast. Thus, proteins such as cytosolic DPE2, PHS1, PTST2 and 3 are directly and/or indirectly involved in this process and so in starch granule initiation. Furthermore, this would also imply that the observed alteration of the starch granule number in dpe2/phs1 as well as in pts2 and pts3 are upstream of the action of SS4. In agreement to this dpe2/phs1 and dpe2/phs1/ss4 contained mostly one starch granule. The double mutant revealed higher variability in the starch granule number in connection with metabolic alterations than the triple mutant. Thus, generation of the glucan primer seems more variable than formation of the starch granules regulated via SS4. Unfortunately, for both ptst2 and ptst3 such metabolic data are currently missing. However, the increase of the starch granule number when PTST2 is overexpressed in wild type but not when overexpressed in the ss4 mutant is also in agreement with this assumption. Likewise, the observed increase of the starch granule number per chloroplast in ss4 by expression of the glycogen synthase is conforming.

**IMPACT OF A REDUCED STARCH GRANULE NUMBER ON GRANULE MORPHOLOGY**

Starch isolated from mutants with reduced number of starch granules per chloroplast reveal distinct alterations in the starch granule morphology. Starch granules from Arabidopsis leaves are thin and discoid. In contrast, starch from mutants lacking SS4 or DPE2/PHS1 show big and spherical starch granules (Crumpton-Taylor et al., 2013; Malinova et al., 2014, 2017).

It was reported that the N-terminal domain of SS4 influences the morphology of starch granules (Lu et al., 2018). Furthermore, the lack of PTST2 and PTST3 revealed single and spherical starch granules (Seung et al., 2017). Very interesting in this regards is again the dpe2/ptst1 mutant. When one starch granule per chloroplast was observed (under 12 h light/ 12 h dark regime) the granule was large and spherical, whereas when 5-7 starch granules were monitored (under continuous light) the starch granule morphology was similar to wild type (Malinova et al., 2017). It seems that the generation of a reduced number of starch granules number per chloroplasts is connected with formation of spherical rather than thin and flattened starch granules. However, also other starch-related mutants reveal alterations in morphology albeit these are less pronounced (SEX4; Kötting et al., 2009; LSF1; Comparot-Moss et al., 2010; GWD; Mahlow et al., 2014).

**CONCLUSION**

In the last years we got more insights into starch granule biosynthesis. The appearance of starch granules is a rather complex mechanism influenced by various factors. According to this, it is very important to distinguish between the possibility to initiate starch and the formation of starch granules. It is quite clear that SSSs, or more precisely SS4, is important in the formation of the typical number of starch granules per chloroplast in Arabidopsis (5-7, Crumpton-Taylor et al., 2012). However, the lack of SS4 is not limiting the formation of starch granules. The formation of starch granules occur independent of SS4 in several mutants. Starch isolated from these mutants analyzed so far did not reveal large inner structural alterations, although the starch morphology reveals considerable differences.
Thus, following the starch initiation, the process of starch granule formation is rather similar in these mutants and point to the overlapping functions of the further SSs. Interestingly, the majority of mutants affected in the starch granule number display mostly one starch granule per chloroplast (Figure 2). The initiation of starch seems to depend on a chloroplastidial maltodextrin metabolism. At least PTST2 and PHS1 seem to be involved in this process. As this maltodextrin metabolism can be influenced by the ongoing starch turn-over and distinct enzymes of the starch metabolism (e.g., AMY3) the involvement of further proteins/enzymes is highly likely. In yeast cells, starch-like synthesis was recreated by introduction of Arabidopsis SSs without plant enzymes related to synthesis of glucans with polymodal chain length distribution similar to amylopectin. 

**REFERENCES**

Ball, S., Colleoni, C., Cencil, I., Raj, I. N., and Tirtiaux, C. (2011). The evolution of glycozen and starch metabolism in eukaryotes gives molecular clues to understanding the establishment of plastid endosymbiosis. J. Exp. Bot. 62, 1776–1801. doi: 10.1093/jxb/erq411

Brust, H., Lehmann, T., D’Hulst, C., and Fettke, J. (2014). Analysis of the functional interaction of Arabidopsis starch synthase and branching enzyme isoforms reveals that the cooperative action of SS4 and BEs results in glucans with polymodal chain length distribution similar to amylopectin. *PLoS One* 9:e102364. doi: 10.1371/journal.pone.0102364

Brust, H., Orzechowski, S., Fettke, J., and Steup, M. (2013). Starch synthesizing reactions and paths: *in vitro* and *in vivo* studies. *J. Appl. Glycosci.* 60, 3–20. doi: 10.5458/jag.jag.IAG-2012_018

Chia, T., Thorneycroft, D., Chapple, A., Messerli, G., Chen, J., Zeeman, S. C., et al. (2006). Plastidial phosphorylase is required for normal starch synthesis in *Chlamydomonas reinhardtii*. *Plant J.* 43, 398–412. doi: 10.1111/j.1365-313X.2006.02632.x

Crumpton-Taylor, M., Grandison, S., Png, K. M. Y., Bushby, A. J., and Smith, A. M. (2012). Control of starch granule numbers in Arabidopsis chloroplasts. *Plant Physiol.* 158, 202–217. doi: 10.1104/pp.111.186957

Fettke, J., Chia, T., Eckermann, N., Smith, A. M., and Steup, M. (2006). A transglucosidase necessary for starch degradation and maltose metabolism in leaves at night acts on cytosolic heteroglycans (SHG). *Plant J.* 46, 668–684. doi: 10.1111/j.1365-313X.2006.02732.x

Hejazi, M., Hetke, J., and Steup, M. (2012). Starch phosphorylation and dephosphorylation: the consecutive action of starch-related dikinases and phosphatases, in "Essential Reviews in Experimental Biology: Starch: Origins, Structure and Metabolism," Vol. 5, ed. I. J. Tetlow (London: SEB), 279–308.

Hwang, S. K., Koper, K., Satoh, H., and Okita, T. W. (2016). Rice endosperm starch phosphorylase (Pho1) assembles with disproportionating enzyme (Dpe1) to form a protein complex that enhances synthesis of malto-oligosaccharides. *J. Biol. Chem.* 291, 19994–20007. doi: 10.1074/jbc.M116.735449

Kötting, O., Santelia, D., Edner, C., Eiche, S., Marthaler, T., Gentry, M. S., et al. (2009). STARCH-EXCESS 4 is a laforin-like phosphoglucomutase required for starch degradation in *Arabidopsis thaliana*. *Plant Cell* 21, 334–346. doi: 10.1105/tpc.108.064360

Leterrier, M., Holappa, L. D., Broglie, K. E., and Beckler, D. M. (2008). Cloning, characterisation and comparative analysis of a starch synthase IV gene in wheat: functional and evolutionary implications. BMC *Plant Biol.* 8, 98–119. doi: 10.1186/1471-2229-8-98

Lu, K. J., Pfister, B., Jenny, C., Eiche, S., and Zeeman, S. C. (2018). Distinct functions of starch synthase 4 domains in starch granule formation. *Plant Physiol.* 176, 566–581. doi: 10.1104/pp.17.01008

Lu, Y., and Sharkey, T. D. (2004). The role of amyloamylase in maltose metabolism in the cytosol of photosynthetic cells. *Planta* 218, 466–473. doi: 10.1007/s00425-003-1127-z

Mahto, S., Hejazi, M., Kuhnert, F., Garz, A., Brust, H., Baumann, O., et al. (2014). Phosphorylation of transitory starch by a glucan, water dikinase during starch turnover affects the surface properties and morphology of starch granules. *New Phytol.* 203, 495–507. doi: 10.1111/nph.12801

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Malinova, I., Alseekh, S., Feil, R., Fernie, A. R., Baumann, O., Schöttler, M. A., et al. (2017). Reduced starch granule number per chloroplast in the dp2e/ps1 mutant is dependent on initiation of starch degradation. PLoS One 12:e0187985. doi: 10.1371/journal.pone.0187985

Malinova, I., Mahlow, S., Alseekh, S., Orawetz, T., Fernie, A. R., Baumann, O., et al. (2014). Double knockout mutants of Arabidopsis thaliana grown under normal conditions reveal that the plastidial phosphorylase isozyme (PHS1) participates in transitory starch metabolism. Plant Physiol. 164, 907–921. doi: 10.1104/pp.113.227843

Nakamura, Y. (2015). “Initiation process of starch biosynthesis,” in Starch: Metabolism and Structure, ed. Y. Nakamura (Tokyo: Springer), 315–332.

Nakamura, Y., Ono, M., Sawada, T., Crofts, N., Fujita, N., and Steup, M. (2017). Characterization of the functional interactions of plastidial starch phosphorylase and starch branching enzymes from rice endosperm during reserve starch biosynthesis. Plant Sci. 264, 83–95. doi: 10.1016/j.plantsci.2017.09.002

Niittylä, T., Messerli, G., Trevisan, M., Chen, J., Smith, A. M., and Zeeman, S. C. (2004). A previously unknown maltose transporter essential for starch degradation in leaves. Science 303, 87–89. doi: 10.1126/science.1091811

Orawetz, T., Malinova, I., Orzechowski, S., and Fettke, J. (2016). Reduction of the plastidial phosphorylase in potato (Solanum tuberosum L.) reveals impact on storage starch structure during growth at low temperature. Plant Physiol. Biochem. 100, 141–149. doi: 10.1016/j.plaphy.2016.01.013

Pfister, B., Sanchez-Ferrer, A., Diaz, A., Lu, K. J., Otto, C., Holler, M., et al. (2016). Recreating the synthesis of starch granules in yeast. eLife 5:e15552. doi: 10.7554/eLife.15552

Qasim, H. M. (2018). Protein Interactions During Starch Granule Initiation in Arabidopsis thaliana. Ph.D. thesis report. Potsdam: University of Potsdam.

Ragel, I., Parodi, A. J., Ugalde, J. E., and Ugalde, R. A. (2003). De novo synthesis of amylopectin in potato (Solanum tuberosum L.) reveals impact on starch granule initiation in Arabidopsis. Plant Physiol. Biochem. 41, 863–875. doi: 10.1016/S0981-9429(03)00033-9

Raynaud, S., Ragel, P., Rojas, T., and Merida, A. (2016). The N-terminal part of Arabidopsis thaliana starch synthase 4 determines the localization and activity of the enzyme. J. Biol. Chem. 291, 10759–10771. doi: 10.1074/jbc.M115.698332

Ritte, G., Lloyd, J. R., Eckermann, N., Rottmann, A., Kossmann, J., and Steup, M. (2002). The starch-related R1 protein is an alpha-glucan water dikinase. Proc. Natl. Acad. Sci. U.S.A. 99, 7166–7171. doi: 10.1073/pnas.062053099

Roldán, I., Lucas, M. M., Delvalle, D., Planchot, V., Jimenez, S., Perez, R., 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, 504–510. doi: 10.1111/j.1365-313X.2006.02998.x

Sato, H., Shibahara, K., Tokunaga, T., Nishi, A., Tasaki, M., Hwang, S. K., et al. (2008). Mutation of the plastidal alpha-glucan phosphorylase gene in rice affects the synthesis and structure of starch in the endosperm. Plant Cell 20, 1833–1849. doi: 10.1105/tpc.107.054007

Seung, D., Boudet, J., Monroe, J., Schreier, T. B., David, L. C., Abt, M., et al. (2017). Homologs of PROTEIN TARGETING TO STARCH control starch granule initiation in Arabidopsis leaves. Plant Cell 29, 1657–1677. doi: 10.1105/tpc.17.00222

Seung, D., Lu, K. J., Sététier, M., Streb, S., and Zeeman, S. C. (2016). Degradation of glucan primers in the absence of starch synthase 4 disrupts starch granule initiation in Arabidopsis. J. Biol. Chem. 291, 20718–20728. doi: 10.1074/jbc.M116.730648

Seung, D., Soyk, S., Coiro, M., Maier, B. A., Eicke, S., and Zeeman, S. C. (2015). PROTEIN TARGETING TO STARCH is required for localizing GRANULE-BOUND STARCH SYNTHASE to starch granules and for normal amylose synthesis in Arabidopsis. PLoS Biol. 13:e1002080. doi: 10.1371/journal.pbio.1002080

Szydlowski, N., Ragel, P., Hennen-Bierwagen, T. A., Planchot, V. R., Myers, A. M., Merida, A., et al. (2011). Integrated functions among multiple starch syntheses determine both amylopectin chain length and branch linkage location in Arabidopsis leaf starch. J. Exp. Bot. 62, 4547–4559. doi: 10.1093/jxb/err172

Szydlowski, N., Ragel, P., Raynaud, S., Lucas, M. M., Roldan, I., Montero, M., et al. (2009). Starch granule initiation in Arabidopsis requires the presence of either class IV or class III starch syntheses. Plant Cell 21, 2443–2457. doi: 10.1105/tpc.109.066522

Telfow, J. I., Wait, R., Lu, Z., Akkasaeng, R., Bowsher, C. G., Esposito, S., et al. (2004). Protein phosphorylation in amyloplasts regulates starch branching enzyme activity and protein-protein interactions. Plant Cell 16, 694–708. doi: 10.1105/tpc.017400

Ugalde, J. E., Parodi, A. J., and Ugalde, R. A. (2003). De novo synthesis of bacterial glycosgen: Agrobacterium tumefaciens glycogen synthase is involved in glucan initiation and elongation. Proc. Natl. Acad. Sci. U.S.A. 100, 10659–10663. doi: 10.1073/pnas.1534787100

Zeeman, S. C., Thornycroft, D., Schupp, N., Chapple, A., Weck, M., Dunstan, H., et al. (2004). Plastidial alpha-glucan phosphorylase is not required for starch degradation in Arabidopsis leaves but has a role in the tolerance of abiotic stress. Plant Physiol. 135, 849–858. doi: 10.1104/pp.103.032681

Zhang, X., Myers, A. M., and James, M. G. (2005). Mutations affecting starch synthase III in Arabidopsis alter leaf starch structure and increase the rate of starch synthesis. Plant Physiol. 138, 663–674. doi: 10.1104/pp.105.060319

Zhang, X., Szydlowski, N., Delvalle, D., D’Hulst, C., James, M., and Myers, A. (2008). Overlapping functions of the starch synthases SSII and SSIII in amylopectin biosynthesis in Arabidopsis. BMC Plant Biol. 8:96. doi: 10.1186/1471-2229-8-96

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