Regulation of Starch Metabolism in Arabidopsis Leaves

Starch is the main form by which plants store carbohydrate and is a major photosynthetic product in many species. Despite the importance of starch, there is still much more that needs to be learned about its synthesis and subsequent degradation. An article by Smith et al. (2004) applied the power of genomics to help shed light on these complex processes. Their article, titled ''Diurnal Changes in the Transcriptome Encoding Enzymes of Starch Metabolism Provide Evidence for Both Transcriptional and Posttranscriptional Regulation of Starch Metabolism in Arabidopsis Leaves,'' appeared in our September 2004 issue and as of October 2006 had been cited 29 times according to Thompson ISI (Thompson ISI Web of Science, http://www.isinet.com).

BACKGROUND

Starch can be found in large amounts in fruits, seeds, rhizomes, and tubers, as well as photosynthetic tissues. After cellulose, starch is the most abundant carbohydrate in many species of plant (Esau, 1977). In photosynthetic tissues, transitory starch accumulates in the light and up to half of the assimilated carbon can be stored as starch inside the chloroplast. In the dark, the transitory starch is degraded into Glu and maltose, and then exported to the cytosol to be used either for Suc synthesis or as an energy source. Sugars derived from photosynthesis are transported via the phloem to sink organs where storage starch can be synthesized and stored.

Starch molecules are polymers of Glu in two configurations: amylose, which is mostly unbranched, and amylopectin, which is branched. Amylose is predominately made up of α-1,4-linked glucosyl moieties, while amylopectin contains both α-1,4 and α-1,6 linkages. The α-1,6 linkages cause the molecule to be branched. The branches of amylopectin molecules pack together into ordered arrays to form semicrystalline starch granules.

Starch synthesis begins with the synthesis of ADP-Glu from Glu-1-P and ATP via ADP-Glu pyrophosphorylase. Starch synthases use ADP-Glu as a substrate. Granule-bound starch synthase, the enzyme responsible for amylose synthesis, is located within the starch granules, while other isoforms of starch synthase, together with starch-branching enzymes, synthesize amylopectin (for review, see Ball and Morell, 2003). Debranching enzymes have also been shown to play a role in amylopectin synthesis, although the exact role is not certain. Plants with mutations in genes encoding the isoamylase class of debranching enzymes accumulate a soluble α-1,4, α-1,6 linked glucan called phytoglycogen in place of some or all of their starch (Delatte et al., 2006; for review, see Ball and Morell, 2003).

The majority of the early work identifying the components of starch degradation was done in vitro. However, recent studies utilizing mutant and knockout plants have led to the revision of much of what was previously known about starch degradation. Many of the enzymes identified as belonging to the degradation pathway have multiple isoforms, which can be either plastidic or cytosolic (for review, see Lloyd et al., 2005; Smith et al., 2005).

In leaves, starch degradation starts with the phosphorylation of a small portion of Glu residues of amylopectin by glucan-water dikinase (GWD). In potato (*Solanum tuberosum*) plants expressing an antisense construct of GWD and in Arabidopsis (*Arabidopsis thaliana*) GWD-deficient *sex1* mutants, there is a dramatic excess of starch in the leaves, indicating the importance of GWD in starch degradation. A second dikinase, phosphoglucan-water dikinase, identified from Arabidopsis leaves, is also required for normal rates of starch degradation. Despite its importance, the significance of glucan phosphorylation is unknown, other than it is a prerequisite for normal rates of starch degradation by other enzymes. One hypothesis is that it makes the granule matrix more accessible to starch-degrading enzymes (for review, see Lloyd et al., 2005).

Although α-amylase has been demonstrated to play an important role in starch degradation in cereal endosperm and in rice (*Oryza sativa*) leaves (Asatsuma et al., 2005), it does not appear to have an essential role in Arabidopsis leaves (Yu et al., 2005). The nightly increase in leaves of levels of maltose, the main product of β-amylase, provides circumstantial evidence for an important role for β-amylase in leaves. Direct evidence for an important role for β-amylase in potato and Arabidopsis leaves has come from RNAi lines and mutants lacking specific β-amylase isoforms, which have a starch-excess phenotype (for review, see Lu and Sharkey, 2006).

Amylases, though able to cleave the α-1,4 linkage in amylopectin molecules, are not capable of cleaving the α-1,6 linkage. Debranching enzymes, as their name implies, are capable of cleaving the α-1,6 linkages. There are two classes of debranching enzymes; isoamylases and pullulanases (limit dextrinases). In Arabidopsis leaves, isoamylase3 is probably the major debranching enzyme involved in starch degradation at night and may work directly on granular starch (Delatte et al., 2006). The linear malto-oligosaccharides resulting from the action of debranching enzymes are catabolized by β-amylase, most likely in conjunction with disproportionating enzyme (DPE1; for review, see Lloyd et al., 2005).

The major exports from the plastid as a result of starch degradation are maltose (produced by the action of β-amylase) and Glu (produced by the action of DPE1). The maltose transporter MEX1 has been

www.plantphysiol.org/cgi/doi/10.1104/pp.104.900209

identified in Arabidopsis and its role has been supported by mutant studies (Niittylä et al., 2004). In the cytosol, the transglucosidase DPE2 is believed to be involved in the further metabolism of maltose as *dpe2* mutants accumulate maltose and are inhibited in starch degradation (Chia et al., 2004; Lu and Sharkey, 2004). A plastidic Glu transporter has also been identified, but there are no mutant or transgenic studies supporting its importance (for review, see Lloyd et al., 2005). Indirect evidence from potato supports the importance of Glu export from plastids. Plants that did not express the transporter degraded starch much more slowly than control plants.

WHAT WAS SHOWN

Arabidopsis L. Heynth plants were grown in 12 h light/dark and leaves were harvested for RNA at 11 time points during a 24-h period for array analysis. These time points corresponded to those that have been previously used for metabolic data sampling and focused on transition times from light to dark or dark to light. Forty-eight genes were selected for particular attention due to their known or hypothesized association with starch synthesis, metabolism, or transport of intermediates across the chloroplast membrane.

The results of this study highlight the complexity of starch synthesis and degradation and how much is still unknown about the process. It might be anticipated that enzymes that function in the same pathway would have similar diurnal patterns of gene transcription. However, for many of the genes, this was not the case. A lack of coordination between changes in transcript levels and in enzyme levels was also observed. This highlights the importance of posttranscriptional regulation for regulating both the amount of the enzymes and their activities.

While relatively little change occurred in transcript levels for the majority of the genes involved in starch synthesis during the 24-h period, the transcript level of both granule-bound starch synthase and starch synthase II increased dramatically during the dark-to-light transition. It was hypothesized that the diel change in expression of these two enzymes could be due to their location within the starch granule; as the granule is degraded, the proteins are lost at night, necessitating resynthesis of the proteins.

Transcripts encoding many of the enzymes of starch degradation showed a common pattern of change over 24 h. Levels fell progressively during the dark and increased in the latter part of the light period. However, levels of some of the proteins were shown not to change substantially, suggesting an important role for post-transcriptional regulation. Among those genes whose levels decreased during the dark period and increased rapidly during the light, there was a group of genes that appeared to be coordinately regulated. Nevertheless, these apparently coordinately regulated genes do not encode the complete pathway of starch degradation. Mutant studies have demonstrated that some of the encoded enzymes are not strictly necessary for starch degradation, while some enzymes that have been shown to be necessary (for example, β-amylase) have completely different patterns of transcript change.

The entire transcriptome was searched for other transcripts with expression patterns similar to the coordinately expressed set, revealing a small number of genes of unknown function containing putative plastid transit peptide sequences. The potential involvement of these enzymes in starch degradation is currently under investigation.

THE IMPACT

The regulation of starch-degradation genes was also the topic of a paper by Lu et al. (2005). In this study, they looked at the influence of both daylength and circadian rhythm on starch degradation and maltose metabolism. They found that starch was degraded faster in plants grown in long days, while maltose metabolism was under circadian control. However, the transcripts for the enzymes involved in starch degradation and maltose metabolism had a strong diurnal rhythm. As was also noted by Smith et al. (2004), no change in the levels of many of the enzymes was observed further, suggesting this to be due to post-transcriptional regulation. Lu et al. (2005) also found the same set of genes to be under circadian control (DPE2, AtPHS2, and SEX1). However, the magnitude of the oscillations was greater than observed by Smith et al. (2004). They postulated this difference could be due to a difference in light regimes.

The studies by Smith et al. (2004) and Lu et al. (2005) demonstrated that some of the genes encoding enzymes of starch metabolism are under circadian or diurnal regulation with no corresponding change in the level of the enzymes. While phosphorylation has shown to play an important role in the regulation of starch synthesis enzymes (Tetlow et al., 2004) not much is not known about the regulation of starch degradation. Recent studies by Niittylä et al. (2006) and Kerk et al. (2006) describe a chloroplast-localized dual-specificity protein phosphatase (DSP), offering an interesting option for the regulation of starch degradation. DSPs regulate protein kinase signaling cascades and are present in plants, animals, and fungi. They are potentially able to phosphorylate Ser/Thr or Tyr residues and contain a carbohydrate-binding domain. An earlier survey of the Arabidopsis genome found 18 putative DSPs, the majority of which had no obvious function (Kerk et al., 2002). The carbohydrate-binding domains of these DSPs were compared using multiple-sequence alignment with genes from plants, animals, and fungi also containing this domain, revealing that conserved residues in this domain are maintained across species, suggesting that the carbohydrate-binding domain is both "phylogenetically dispersed and ancient" (p. 400; Kerk et al., 2006). One isoform, encoded by At3g52180, was further characterized and found to contain a chloroplast-targeting

domain, in addition to having a gene expression pattern consistent with the transcripts of key starch-degrading enzymes. The functionality of its carbohydrate-binding domain was tested and demonstrated to bind not only purified starch, but also amylose, amylopectin, and pullulan. Smith et al. (2004) showed that At3g52180 has a diurnal pattern of transcript abundance almost identical to that of the coordinately regulated set of genes involved in starch degradation. Following on from this, Niittylä et al. (2006) recognized that a previously discovered mutation causing reduced starch degradation (at the SEX4 locus) mapped to this position. They were able to show that *sex4* mutations eliminate the function of the protein phosphatase encoded by At3g52180. This enzyme is thus required for normal starch degradation. It could potentially activate a protein kinase, thereby modulating the activity of enzymes involved in starch degradation.

CONCLUSION

The study by Smith et al. (2004) highlights the complex nature of the control of starch metabolism and degradation. Unexpectedly, this and subsequent studies (i.e. Lu et al., 2005) did not find a general diurnal coordination of gene expression for starch synthesis, and found incomplete coordination for starch degradation. Additionally, they found that the levels of enzymes of starch degradation and synthesis did not change in parallel with gene expression, suggesting the importance of posttranscriptional regulation of this process. Ongoing work on the regulation of starch synthesis and degradation is expected to shed more light onto these processes.

LITERATURE CITED

Asatsuma S, Sawada C, Itoh K, Okito M, Kitajima A, Mitsui T (2005) Involvement of alpha-amylase I-1 in starch degradation in rice chloroplasts. Plant Cell Physiol **46:** 858–869

Ball SG, Morell MK (2003) From bacterial glycogen to starch: understanding the biogenesis of the plant starch granule. Annu Rev Plant Biol **54:** 207–233

Chia T, Thorneycroft D, Chapple A, Messerli G, Chen J, Zeeman SC, Smith SM, Smith AM (2004) A cytosolic glucosyltransferase is required for conversion of starch to sucrose in Arabidopsis leaves at night. Plant J **37:** 853–863

Delatte T, Umhang M, Trevisan M, Eicke S, Thorneycroft D, Smith SM, Zeeman SC (2006) Evidence for distinct mechanisms of starch granule breakdown in plants. J Biol Chem **281:** 12050–12059

Esau K (1977) Anatomy of Seed Plants, Ed 2. John Wiley & Sons, New York

Kerk D, Bulgrien J, Smith DW, Barsam B, Veretnik S, Gribskov M (2002) The complement of protein phosphatase catalytic subunits encoded in the genome of Arabidopsis. Plant Physiol **129:** 908–925

Kerk D, Conley TR, Rodriguez FA, Tran HT, Nimick M, Muench DG, Moorhead GBG (2006) A chloroplast-localized dual-specificity protein phosphatase in Arabidopsis contains a phylogenetically dispersed and ancient carbohydrate-binding domain, which binds the polysaccharide starch. Plant J **46:** 400–413

Lloyd JR, Kossmann J, Ritte G (2005) Leaf starch degradation comes out of the shadows. Trends Plant Sci **10:** 130–137

Lu Y, Gehan JP, Sharkey TD (2005) Daylength and circadian effects on starch degradation and maltose metabolism. Plant Physiol **138:** 2280–2291

Lu Y, Sharkey TD (2004) The role of amylomaltase in maltose metabolism in the cytosol of photosynthetic cells. Planta **218:** 466–473

Lu Y, Sharkey TD (2006) The importance of maltose in transitory starch breakdown. Plant Cell Environ **29:** 353–366

Niittylä T, Comparot-Moss S, Lue W-L, Messerli G, Trevisan M, Seymour MDJ, Gatehouse JA, Villadsen D, Smith SM, Chen J, et al (2006) Similar protein phosphatases control starch metabolism in plants and glycogen metabolism in mammals. J Biol Chem **281:** 11815–11818

Niittylä T, Messerli G, Trevisan M, Chen J, Smith AM, Zeeman SC (2004) A previously unknown maltose transporter essential for starch degradation in leaves. Science **303:** 87–89

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

Smith SM, Fulton DC, Chia T, Thorneycroft D, Chapple A, Dunstan H, Hylton C, Zeeman SC, Smith AM (2004) Diurnal changes in the transcriptome encoding enzymes of starch metabolism provide evidence for both transcriptional and posttranscriptional regulation of starch metabolism in Arabidopsis leaves. Plant Physiol **136:** 2687–2699

Tetlow IJ, Wait R, Lu Z, Akkasaeng R, Bowsher CG, Esposito S, Kosar-Hashemi B, Morell MK, Emes MJ (2004) Protein phosphorylation in amyloplasts regulates starch branching enzyme activity and protein-protein interactions. Plant Cell **16:** 694–708

Yu TS, Zeeman SC, Thorneycroft D, Fulton DC, Dunstan H, Lue WL, Hegemann B, Tung SY, Umemoto T, Chapple A, et al (2005) alpha-Amylase is not required for breakdown of transitory starch in Arabidopsis leaves. J Biol Chem **280:** 9773–9779

Aleel K. Grennan
University of Illinois
Urbana, IL 61801