Review

Plant α-amylases: functions and roles in carbohydrate metabolism

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Abstract: Plants store carbon predominantly as starch, and the metabolism of this polysaccharide is of importance to all life. In the past ten years the processes of starch synthesis have been well studied, however only recently has the same amount of attention been directed towards starch degradation, which previously had been focussed almost exclusively on degradation in germinating cereal grains. Studies of the degradation of diurnal leaf starch have revealed a number of surprises, such as the importance of starch phosphorylation in initiating degradation, and shown that starch degradation in leaves differs significantly from that in cereal grains. We have previously shown that plants contain three distinct α-amylase families. T-DNA knockouts of each α-amylase family member in *Arabidopsis* had no effect on diurnal leaf starch degradation, raising questions about the function of α-amylases in leaves. Here we describe the known aspects of these three families and suggest specific roles for each in carbohydrate metabolism.

Key words: Starch degradation, α-amylase, subcellular localisation, cytosolic polysaccharide.

Abbreviations: GWD, glucan water dikinase; PWD, phosphoglucan water dikinase; DPE, disproportionating enzyme.

Introduction

Starch is the main carbon-storage molecule of plants and is the main source of energy for animals, including humans. Starch is produced in leaves during the day from the products of photosynthesis, and accumulates in the chloroplast until the end of the day, forming insoluble granules. As night falls, net accumulation gives way to net degradation of starch, and the carbohydrate is exported from the plastid, predominantly as maltose, and to a lesser extent as glucose (Niittyla et al., 2004). In the cytosol, maltose is converted to hexose phosphate, and either consumed by the glycolytic pathway or synthesised into sucrose for export from the cell. The sucrose is carried from photosynthetic tissues (source tissues), through the phloem, to other tissues of the plant (sink tissues). Sink tissues may be rapidly growing tissues, such as meristems and young leaves, which catabolise the sucrose to produce energy, or storage organs, such as roots, tubers, bark and fruit, which resynthesise starch in the plastid. Storage starch is very important to plants, and is broken down following specific seasonal or developmental cues, such as the beginning of spring in roots and bark, and the onset of ripening in many fruit.

Even though plant starch is of such great importance to life on Earth, very little is known about its degradation *in planta*. Until recently, most research has concentrated upon the degradation of starch within the endosperm of germinating grains, particularly in barley and rice, all of which are monocotyledons and members of the Poaceae. During germination, the embryo triggers starch degradation by releasing gibberellins, which stimulate cells of the aleurone layer to secrete degradative enzymes into the endosperm (Beck & Ziegler, 1989). A number of enzymes vital to starch degradation have been identified from this system. One of the most important enzymes is α-amylase, an endohydrolase that is able to rapidly degrade the starch into soluble substrates for other enzymes to attack (Beck & Ziegler, 1989). However, the circumstances of starch degradation in endosperm are unlike those in other plant tissues, as the majority of enzymes involved in breakdown of endosperm starch are secreted enzymes, acting in an acellular matrix rather than within a living organelle.

More recent understanding of starch breakdown in plants has come from work in model and commercially useful non-cereal plants, particularly potato (*Solanum tuberosum*) and *Arabidopsis thaliana* mutants (for recent reviews, see Zeeman et al., 2004a; Tetlow et al., 2004; and see also the final section "Proposed roles for

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plant α-amylases in carbohydrate metabolism"). Several mutants have a phenotype termed "starch excess", where starch is retained in leaf chloroplasts and mobilisation is either retarded or blocked. Proteins shown to be causal in this include: glucan water dikinase (GWD; formerly designated as R1 and SEX1; Lorberth et al., 1998; Yu et al., 2001; Ritte et al., 2002); starch debranching enzyme (Dinges et al., 2003); disproportionating enzymes (DPE; Critchley et al., 2001; Chia et al., 2004; Lu & Sharkey, 2004); β-amylase (Scheidig et al., 2002); phosphoglucan water dikinase (PWD) enzyme (Baunsgaard et al., 2005; Kötting et al., 2005); and the plastid maltose transporter (Niittyla et al., 2004). In contrast, disruption of the plastidial α-glucan phosphorylase of *Arabidopsis* had no effect on diurnal starch degradation (Zeeman et al., 2004b), despite historical implication in starch breakdown (Beck & Ziegler, 1989).

The GWD enzymes add phosphate groups to starch; a reduction in the amount of enzyme leads to a decrease in starch phosphate and an increase in starch in leaves and tubers (Lorberth et al., 1998). During transitory starch degradation, GWD binds to starch granules and increases the rate of starch phosphorylation (Ritte et al., 2000; 2004). In contrast, PWD acts only on starch that has been phosphorylated by GWD, and mutant *Arabidopsis* plants lacking PWD activity display a starch-excess phenotype, although it is not as severe as that seen in *sex1* plants (Kötting et al., 2005). Despite the apparent similarity in activity for GWD and PWD, the enzymes display only 19% identity at the amino acid level. Maize plants deficient in pullulanase-type debranching enzyme show a reduced rate of starch degradation at night (Dinges et al., 2003), as do *Arabidopsis* deficient in either the plastidial or cytosolic isoforms of DPE (Critchley et al., 2001; Chia et al., 2004; Lu & Sharkey, 2004). Down regulation of a chloroplastic β-amylase also gave reduced night starch degradation in potato leaves and hence a starch excess phenotype at the end of the night (Scheidig et al., 2002). Down regulation of the maltose transporter of *Arabidopsis* caused accumulation of maltose in the plastid and reduced night starch degradation (Niittyla et al., 2004). Interestingly only two of the above studies include organs where starch is stored in non-photosynthetic tissues, and the enzymes and regulation involved may well be very different in these tissues, compared to photosynthetic tissues. For example, it has been argued that both species and organs may differ in the manner of starch degradation (Fettke et al., 2004; Lloyd et al., 2004; Zeeman et al., 2004a) and there have been several records of pleiotropic effects on starch degrading enzymes in response to modification of one starch associated enzyme (Dinges et al., 2003; Zeeman et al., 2004b).

Although α-amylase has been shown to be very important in initiating starch degradation in cereal grains (Beck & Ziegler, 1989), very little is known about

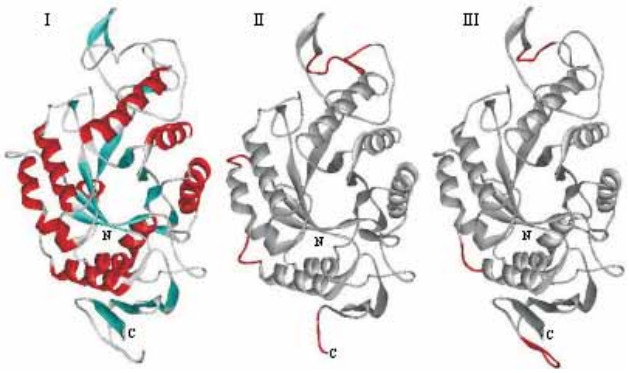

Fig. 1. Predicted tertiary structures for the three α-amylases of *Arabidopsis*. I, family one α-amylase, displaying secondary structure elements: α-helices in red, β-strands in blue and loops in white. The N- and C-terminus of each structure is indicated. Domain A is the central "barrel" of the structure, i.e. the $(\beta/\alpha)_8$-barrel, with domain B above and domain C below. The catalytic site is found in the centre of the barrel, on the far face of the enzyme. II and III, predicted structures of family two and family three α-amylases, respectively, in the same orientation as family one: grey shows regions of structural similarity to family one, red parts of each molecule display structural divergences. Domain C of family two did not model fully, and neither did the large N-terminal domain of family three.

α-amylases in other tissues and other plants. In *Arabidopsis* there are three α-amylase genes (Stanley et al., 2002). The three α-amylases are predicted to be directed to different cellular compartments: apoplast and other extracellular compartments (family one), cytosol (family two) and plastid (family three), and genes encoding members of each family were shown to be present in representatives of monocotyledons, dicotyledons and gymnosperms. The gene structures of the three families are different, with no single intron position conserved in all three families (Stanley et al., 2002). The recently sequenced unicellular alga *Ostreococcus tauri* also contains three distinct α-amylase genes (Ral et al., 2004), which correspond to the three families described in higher plants (D. Stanley, unpublished data).

All three *Arabidopsis* α-amylase genes (and all the other plant genes isolated to date) have the characteristic features of all α-amylases (Stanley et al., 2002), such as the three canonical domains (A, B and C), a $(\beta/\alpha)_8$-barrel structure (Svensson et al., 2002), and amino acids considered to be indicative of true α-amylases: His122, Asp206, Phe/Tyr207, Ala/Val208, Lys/Arg209, Gly210, Glu230, His296, Asp297 (numbering as in Taka-amylase A; MacGregor et al., 2001). Only two proteins of family two from monocotyledons differ, by substituting Ala/Val208 with Thr. The most significant differences to the α-amylase structure are found in domain B, which is shorter in families two and three (Stanley et al., 2002; Fig. 1).

Based on the knowledge from cereal grains and bacteria, it could be expected that each α-amylase protein participates in degradation of starch in its compart-

ment. However, granular starch is insoluble and normally found only in plastids, and while other polymers containing glucose have been demonstrated in the cytosol, plastid and extracellular matrix, these are either soluble polyglycans or heteroglycans (Yang & Steup, 1990; Zeeman et al., 1998b; Fettke et al., 2004). α-Amylases have previously been implicated in the break down of diurnal starch; the *Arabidopsis sex4* mutant displays a classic starch excess phenotype, and was shown to be deficient in a plastidial α-amylase activity (Zeeman et al., 1998a), however the mutation that leads to this phenotype is not in the gene encoding the plastidial α-amylase of *Arabidopsis* (Yu et al., 2005). Knockouts of the other two α-amylase gene families also did not show any impairment in the degradation of diurnal leaf starch. This suggests that what we have learnt from the cereal grains in plants and from bacterial α-amylases in terms of function may not be relevant in normal plastidial starch metabolism, and that α-amylases may not be involved in diurnal leaf starch degradation. It also suggests that we have much to learn about metabolism of glycan polymers in other plant cell compartments. It is therefore pertinent to define what we do know about each of these three α-amylase gene families, and the next three sections will draw together what has been discovered about each family.

Family one α-amylases

This family is characterised by having a predicted secretory signal peptide and all the well-characterised cereal grain α-amylases fall within it (Stanley et al., 2002). The pivotal role of this family in degradation of starch in cereal grain endosperms is well established, and indeed for many years these enzymes were assumed to represent all plant α-amylases. Secretion of the enzymes from the cells of the aleurone layers is well established in cereal grains, and there is evidence that a similar process takes place in at least some dicotyledonous seeds (Beck & Ziegler, 1989; Nakajima et al., 2004). Predicted secretory signal peptides for two rice family one genes, Amy3 and Amy8, fused to GFP and GUS, respectively, and as part of the full length protein for Amy3, have recently been reported to localise to both plastids and the cell wall in tobacco leaves, putting doubt on direct predictability of the α-amylase presequences (Chen et al., 2004). In contrast, Chan et al. (1994) showed only secretion with GUS fusions to the same rice signal peptide (Amy8) using cultured rice cells. When the predicted signal peptide of the *Arabidopsis* family one member was fused to GFP and expressed in *Arabidopsis* and *Nicotiana benthamiana*, no evidence was found for either secretion or sequestration in the plastid (Stanley, 2004). The contradictory results of these three studies suggest either a problem with the secretion of fusion proteins in transgenic plants, or genuine differences in targeting of α-amylases between species. Apoplastic α-amylase activity has been detected in leaves of *Arabidopsis* (Lin et al., 1988), pea (Beers & Duke, 1988), and tobacco (Heitz et al., 1991).

Crystal structures have been solved for two barley isoforms (AMY1 and AMY2; Kadziola et al., 1994; Robert et al., 2003), and differences between these two enzymes include different affinities in binding to starch granules, sensitivity to inhibitors, stability in acid, affinity for calcium ions, sensitivity to EDTA and substrate specificity (Rodenburg et al., 1994). At least 10 isoforms encoded by different genes have been identified in rice (Ranjhan et al., 1991), with gene intron/exon structure preserved except for loss of the second of the three introns in one subfamily (Huang et al., 1990). A fourth intron was identified in the family one gene of *Arabidopsis* (Stanley et al., 2002), which has not been seen in other family one α-amylases. Monocotyledonous family one genes are expressed in different spatial and temporal patterns, sometimes in response to hormones or metabolic signals (Yu et al., 1996; Sugimoto et al., 1998), and have been detected in leaves and flowers as well as grains (Jacobsen et al., 1986; Kaneko et al., 2004). A number of regulatory elements and the transcription factors that bind to them have been identified (Hwang et al., 1998; Chen et al., 2002). Posttranscriptional regulation of protein synthesis, and variations in transcript stability have also been reported to modulate α-amylase mRNA levels (Chan & Yu, 1998).

Family one α-amylases from dicotyledons and non-angiosperm plants are much less well understood. Gene orthologues have been isolated from *Vigna mungo* (Yamauchi & Minamikawa, 1990), *Ipomoea nil* (Nakayama et al., 2002), *Pinus taeda*, apple, and *Arabidopsis* (Stanley et al., 2002). *Arabidopsis* has a single family one α-amylase gene and apple has two. *I. nil* is predicted to also have two, but information from other species is not available. Family one genes are expressed in developing and germinating seeds of *V. mungo* (Yamauchi et al., 1994) and *I. nil* (Nakajima et al., 2004). In the latter, the mRNA level showed increased expression in the seed coat following anthesis, with treatment with gibberellins, and was also found to be expressed in leaves, flower buds and stems. The family one α-amylase gene of *Arabidopsis* shows little change in expression in leaves during the diurnal cycle (Smith et al., 2004). There is also evidence for appearance of a secreted α-amylase in response to pathogen attack - viral challenge in tobacco cell walls (Heitz et al., 1991) and the hypersensitive response in *Arabidopsis* (Monroe et al., 2003). In addition, public databases (e.g. GenBank; Benson et al., 2004) contain ESTs from family one genes, derived from several species (including grape, capsicum, potato, and *Medicago truncatula*) that have been challenged with diseases. The induced α-amylases could mobilise starch from the dead cells, perhaps inhibiting pathogens by altering osmolarity in the environment, or by signalling neighbouring cells to activate pathogen responses. ESTs and genes have also

been identified from stress treatments (*Mesembryanthemum crystallinum*, sugar beet, and sugar cane), flowers (poplar, cotton, *Arabidopsis*, and apple) and maturing or ripening fruit (banana, peach, mandarin, and apple).

Unfortunately, except for the secreted α-amylases from cereal grains (Rodenburg et al., 1994; Svensson et al., 2002) and *in vitro* expression of the *I. nil* gene (Nakayama et al., 2002), there is little data on *in vivo* or *in vitro* activity or specificity of the enzymes from this family, including ability to attack raw starch granules. This is because in most instances enzyme activity has been measured on extracts that would include enzymes from all possible compartments, or the gene has not been expressed *in vitro*.

Family two α-amylases

This family is characterised by having no predicted targeting peptide and therefore is thought to localise to the cytoplasm. Analysis of its gene structure suggested that it was the closest of the three families to an ancestral plant α-amylase (Stanley et al., 2002). Both family two genes from apple have a single intron in the 5' UTR of the gene, and in one of the genes the intron undergoes alternate splicing using two possible acceptor splice sites (Wegrzyn et al., 2000; Stanley et al., 2002). Comparison of cDNA and genomic sequences of the family two gene of *Arabidopsis*, reveals that the 5' UTR intron is conserved in this species and spliced following transcription. Since protein targeting signals are difficult to predict, the first 37 amino acids of an apple family two α-amylase was fused to GFP and examined in transgenic *Arabidopsis* and *N. benthamiana*; the localisation of the fusion protein was cytosolic, as predicted (Stanley, 2004). Family two α-amylases have been identified from monocotyledons, dicotyledons and gymnosperms (Stanley et al., 2002).

The existence of a cytosolic α-amylase raises a question regarding potential substrates for the enzyme. Many starch-degrading enzymes have cytosolic isoforms, including starch phosphorylase, DPE, and β-amylase. Traditional thinking is that starch is synthesised only in the plastid, however, the cytosolic starch phosphorylase of *Pisum sativum* has been shown to interact with a soluble, high molecular weight glycan that is present in the cytosol (Yang & Steup, 1990; Fettke et al., 2004). The glycan was divided into several subfractions, each of which contains a high proportion of arabinogalactan-like linkages, plus some minor components, including α-glucosyl linkages (Fettke et al., 2004). The role of this soluble heteroglycan in plant carbohydrate metabolism has not been determined, however, it has been proposed that it may act as a buffer between plastidial starch degradation and cytosolic hexose phosphate metabolism (Chia et al., 2004).

In *Arabidopsis* leaves, the family two α-amylase gene reaches maximal expression in the morning, during the first few hours of light, and drops again before the onset of night (Smith et al., 2004). This suggests that the enzyme may be most active when the plastidial starch reserves of leaf cells are most depleted. In apple, the expression of a family two gene increased significantly when fruit were placed at 0.5 ℃, with maximal expression reached after 8 days (Wegrzyn et al., 2000). Public databases also contain many ESTs from this family, including expression in meristems, storage tissues, such as bulbs, tubers and fruit pericarps, flowers and roots. ESTs were identified across a range of species in response to interactive signals such as insect feeding, nodule symbiosis, oligogalacturonide treatment, drought and low temperature.

Analysis of a family two α-amylase from apple, expressed as recombinant protein in *E. coli*, shows that the enzyme is able to degrade soluble and insoluble starch, is not affected by the presence of EDTA, and is more active at alkaline pH (7.7–9.2) than acidic pH (Stanley, 2004). This contrasts strongly with the secreted barley α-amylases, which are most active at acidic pH (Søgaard et al., 1993). Similar characteristics, including an alkaline pH optimum, were also identified in an α-amylase purified from potato tubers (Witt & Sauter, 1996), although results suggested that this enzyme was at least partly localised to the plastid.

Family three α-amylases

Family three α-amylases are characterised by a large N-terminal domain, typically 400-500 amino acids in length (approximately doubling the size of the α-amylase protein from 45 kDa to 90 kDa), which contains a predicted chloroplast transit peptide (Stanley et al., 2002), and shares features with the N-terminal domain of GWD proteins, such as predicted starch-binding motifs (Stanley, 2004, Yu et al., 2005). The transit peptide and N-terminal region of one of the two apple genes was able to direct GFP to the plastids of transformed *N. benthamiana* and *Arabidopsis* (Stanley, 2004), and the *Arabidopsis* protein was shown to immunolocalise to leaf chloroplasts (Yu et al., 2005). As with the other α-amylase families, family three genes have been identified in numerous plant species, including apple, *Arabidopsis*, rice, kiwifruit, and loblolly pine (Stanley et al., 2002; Stanley, 2004).

The *Arabidopsis* family three α-amylase gene undergoes diurnal regulation in leaves, showing maximal expression at the end of the light period and minimal expression at the end of the dark period (Schaffer et al., 2001; Smith et al., 2004, Yu et al., 2005). This pattern of expression contrasts with starch concentrations in leaves, which reach a maximum at the end of the light period and decrease during night. The same expression pattern was found for genes required for normal starch degradation in *Arabidopsis* leaves, including DPEs, GWD and PWD (Smith et al., 2004), strongly suggesting a role for family three α-amylases in diur-

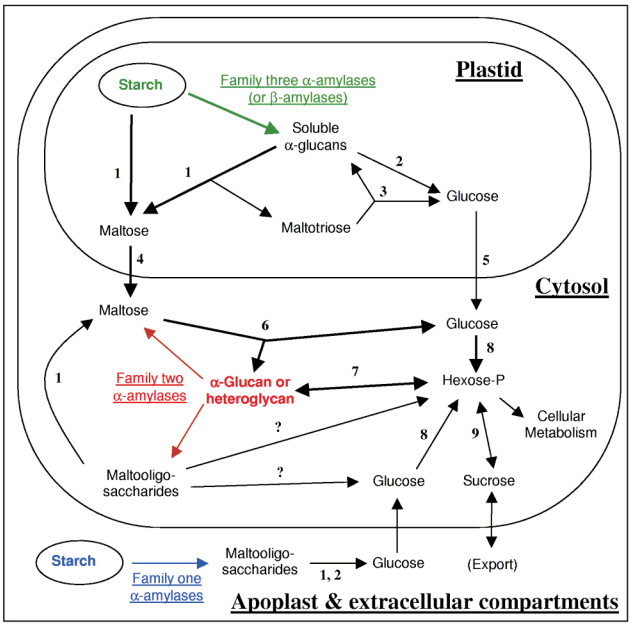

Fig. 2. Proposed roles of plant α-amylases in starch metabolism. In the plastid, family three α-amylases (green) could initiate starch granule breakdown, producing soluble substrates for other starch degrading enzymes such as β-amylases/debranching enzymes (1), and α-glucosidases (2). Alternatively this process may occur by the action of β-amylases alone (Scheidig et al., 2002). DPE1 (3) is responsible for recycling maltotriose into the α-glucan pool, with glucose as a by-product. The majority of carbohydrate is removed from the plastid as maltose, via the maltose transporter (4), with some glucose exported via the glucose transporter (5). The maltose is transferred to a putative cytosolic α-glucan or heteroglycan via DPE2 (6), with glucose again produced. The cytosolic α-glucan/heteroglycan is the substrate of a cytosolic α-glucan phosphorylase (7), which produces glucose phosphate. Hexose phosphate is also produced by the action of hexokinase (8) upon cytosolic glucose, and is used in cellular metabolism, or is converted to sucrose by the action of sucrose-phosphate synthase and sucrose-phosphate phosphatase (9), and exported from the cell. Family two α-amylases (red) probably interact with the putative cytosolic α-glucan or heteroglycan, producing maltose or maltooligosaccharides, which could in turn act as substrate for other enzymes, including cytosolic β-amylases. Family one α-amylases (blue) break down extracellular starch into maltooligosaccharides, which are further catabolised by other starch degrading enzymes, producing glucose. Adapted and modified from Chia et al., (2004).

nal starch breakdown. In apple, ESTs representing a family three α-amylase are particularly abundant in libraries constructed from developing and ripening fruit tissue (D. Stanley & E. MacRae, unpublished data). ESTs have also been identified from public databases, especially from leaves, as well as from shoots, roots and flowers. Recombinant family three α-amylases, from apple (Stanley, 2004) and *Arabidopsis* (Yu et al., 2005), were able to degrade starch. The apple recombinant protein degraded amylopectin in native PAGE gels, and reconstituted *Arabidopsis* recombinant protein (both the C terminal domain alone and the full-length protein without the leader sequence) degraded soluble starch to form malto-oligosaccharides and glucose, thus confirming predicted endo-amylolytic activity. Plastidial α-amylase activity has been extracted from isolated plastids from several tissues. In *Arabidopsis* one form of plastidial activity was isolated with an apparent pH optimum around 6, and displayed higher reactivity with β-limit dextrins or amylopectin than amylose, yielding maltose and glucose (Lin et al., 1988). In both *Arabidopsis* and pea (Ziegler, 1988), plastidial α-amylase activity was a small fraction of the total leaf amylase activity. The *sex4* mutation of *Arabidopsis* affects the abundance of a plastidial α-amylase, but the mutation maps to a different chromosome from the family three α-amylase gene (Zeeman et al., 1998a; Yu et al., 2005). This suggests that the mutation may affect some regulator of the α-amylase, such as a transcription factor, or a protein that interacts with the α-amylase.

Enzyme activity

It is worth pointing out that the plant literature is full of measurements of α-amylase activity on crude extracts from whole tissues using a range of substrates. This has now confused interpretation in many studies because differently localized enzymes with different efficiencies and kinetics have been combined in the analysis. In most instances an acidic pH has been used with soluble starch as a substrate. Generally, it would appear that the enzyme under study has been either the cytosolic or secretory α-amylase, as most reports characterise the enzyme as having a molecular weight of around 45 kDa. In some instances, native activity gels have been used, demonstrating that there are indeed different α-amylases present, and in others, a range of substrates have been tested. It is imperative that several members of each family are expressed *in vitro* and thoroughly examined for properties. In addition, localisation of the proteins and kinetic characteristics of proteins purified from plant extracts should be determined and compared if we are to gain real insight into the role that each plays in the plant.

Proposed roles for plant α-amylases in carbohydrate metabolism

The three families of α-amylase genes must each fulfil a distinct role in plants, although the relative involvement of α-amylases and other enzymes in starch degradation will probably vary between plant species. Figure 2 shows a proposed pathway of starch breakdown in plants, including possible roles for α-amylases.

Family one α-amylases are involved in the breakdown of extracellular starch, for example in the endosperm of cereal grains and dicotyledonous seeds (Beck & Ziegler, 1989; Nakajima et al., 2004), and probably in diseased tissue where cell death has occurred (Heitz et al., 1991; Monroe et al., 2003). Secreted α-amylases could also be important for pollen

tube germination and growth during invasion of the stigma surface (Edlund et al., 2004).

The family two α-amylases are cytosolic enzymes that presumably degrade either a hypothetical cytosolic α-glucan, or a heteroglycan, such as that described by Yang & Steup (1990) and Fettke et al. (2004). This polysaccharide may act as a buffer between the processes of starch degradation and hexose phosphate metabolism (Chia et al., 2004). It is possible that family two α-amylases is involved in general stress responses. For example, cold acclimation in plants includes an increase in soluble sugars and hexose-phosphates in the cytosol (Ristic & Ashworth, 1993; Hurry et al., 1995), and could lead to a transitory increase in cytosolic α-glucan. Family two α-amylases, at least one of which is up-regulated in response to cold (Wegrzyn et al., 2000), may be responsible for degrading this cytosolic α-glucan, maintaining a high concentration of sugar within the cytosol, without recourse to transport of carbon across plastid membranes. Modification of the lipid component of membranes, and hence membrane protein function, is a well-documented response to low temperature.

Although the family three α-amylase of *Arabidopsis* does not appear to be necessary for diurnal starch degradation, its strong diurnal regulation of expression in leaves (Smith et al., 2004) suggests a role in this process, which is yet to be elucidated. Family three α-amylases may also be responsible for degrading plastid-bound starch in starch storage tissues and in leaves of other plant species, perhaps where starch phosphorylation is low or not detectable (Blennow et al., 2002). The enzyme could rapidly produce soluble α-glucans from plastidial starch, which could be further degraded by β-amylases and debranching enzymes. However the β-amylases are responsible for the production of maltose, which is the main carbohydrate exported from the plastid (Niittyla et al., 2004), and a plastid-localised β-amylase of potato is capable of attacking native starch granules (Scheidig et al., 2002). This suggests that β-amylases, combined with debranching enzymes, are probably responsible for some or all of the transitory starch degrading activity in potato and *Arabidopsis* leaves.

It is becoming increasingly clear that starch degradation in plants is an extremely complicated process. For example, it has become clear that phosphorylation is vital for both diurnal starch and storage starch degradation (Reimann et al., 2004; Ritte et al., 2004). How this affects starch structure, and the interaction of different enzymes with starch, is not yet clear, nor is it clear whether phosphorylation is required for degradation in other tissues. The conserved N-terminal domains of GWDs and family three α-amylases may be involved in enzyme regulation and the control of starch breakdown. It is almost certain that further surprises will be uncovered as research into this area continues.

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