Chapter 7
Starch Degradation

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Abstract Degradation of starch (and of glycogen as well) converts carbohydrates accumulated as metabolically inert storage products back into forms that are usable for various biosynthetic and catabolic routes. Starch and glycogen share their basic biochemistry, but distinct physicochemical and enzymatic differences exist. Structural (dis)similarities of hydroinsoluble starch and hydrosoluble glycogen are briefly discussed. Various types of starch-degrading enzymes and their mode of action are presented. Features frequently observed in starch-degrading enzymes, such as carbohydrate-binding modules and secondary binding sites, are discussed including kinetic implications. Approaches to identify proteins functional in vivo starch degradation and their limitations are discussed. Three types of in vivo starch degradation are distinguished: degradation of transitory starch, mobilization of reserve starch in dead tissue, and that in living cells. Most of the current biochemical knowledge of starch degradation relates to mobilisation of transitory starch. We discuss this process in the context of cellular organisation and location/distribution of starch granules within the cell. Transitory starch degradation is initiated at the granule surface and includes local transitions from a hydroinsoluble ordered to a soluble state. Transition is facilitated by iterating cycles of phosphorylating and dephosphorylating reactions that act on starch-related glucosyl residues. In the

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stroma, four main products are synthesised by highly interconnected paths and are then exported into the cytosol. Plastidial transporters and cytosolic downstream processes are presented which link starch degradation to biosynthetic or degradative routes all originating from the cytosol. Finally, current views on plastidial transitory starch degradation as controlled by the cytosol are discussed.

Keywords Amylose • Amylopectin • Glycogen • Carbohydrate-active enzymes • Carbohydrate-binding module (CBM) • Reserve starch • Secondary binding site (SBS) • Starch degradation • Starch modification • Transitory starch

7.1 Introduction

Higher plants often withdraw reduced carbon compounds from ongoing metabolic processes by accumulating a storage carbohydrate that is both osmotically inert and metabolically inactive. As a highly efficient form to store carbon, capacity to metabolise starch is more than a billion years old going back to the establishment of chloroplast-containing cells. By starch degradation, reduced carbon is converted back into a metabolically active state which can easily be utilised by many paths of the plant. For photoautotrophic organisms, the day–night cycle periodically causes interruptions of photosynthesis and large alterations of the intracellular carbon fluxes. It usually is associated with changes in the environmental temperature. Under natural conditions, length of light and dark periods generally varies throughout the year.

Environmental variations, such as changes in temperature, act also on heterotrophic tissues, organs, or plants which indirectly depend on the carbon fixation performed by photoautotrophic cells. They may, however, not be directly exposed to the light and dark periods. Seedlings completely rely on the degradation of storage products that have been accumulated by the mother plant until they are photosynthesis competent and the environmental conditions permit photoautotrophic growth. As starch is degraded under variable external conditions, the entire process must be precisely regulated to reliably provide reduced carbon to living cells and to prevent carbon starvation. Starch degradation is also performed by many non-plant organisms, such as bacteria or animals. Although the basic enzymology of non-plant starch degradation is similar, it deviates in several aspects from that of plants and is not considered here.

Compared to other biopolymers, chemical features of starch are simple as it is essentially built of a single monosaccharyl type, i.e. the α-D-glucosyl moiety, and contains only two types of interglucose linkages, namely α-1,4- and α-1,6-bonds. Furthermore, glucosyl residues are covalently modified with a very low frequency. Despite its chemical simplicity, the physical order of the glucosyl moieties and of the α-glucan chains within native starch is complex and has not yet been fully elaborated. To the best of our knowledge, no cell-free synthesis of starch-like particles has yet been reported.

The structural complexity of native starch is mirrored by both the complex starch-related biochemistry and the multiple control of the expression of starch-related genes, all of which are located in the nucleus. Turnover of plastidial starch is assumed to be based on a close collaboration of 30–40 (iso)enzymes directly or indirectly involved in forming or cleaving interglucose bonds (Deschamps et al. 2008), but these numbers are likely to underestimate the biochemical complexity. Heteromeric protein complexes are functional that enable the plastid to temporarily and locally combine several enzyme activities (Hussain et al. 2003; Bustos et al. 2004; Delatte et al. 2005; Kubo et al. 2010; Emes and Tetlow 2012; Sundberg et al. 2013; Sim et al. 2014; see Chap. 8 this volume). Mechanisms of forming and targeting the complexes to distinct areas of starch granules as well as their disassembly are largely unknown. Repeatedly, 14-3-3 proteins have been reported to be directly involved in the regulation of starch metabolism (for details see Denison et al. 2011), but their action is incompletely understood. Additional plastidial or extraplastidial proteins essentially lacking catalytic activity affect starch metabolism (Zeng et al. 2007; Fulton et al. 2008; Isshiki et al. 2008; Lohmeyer-Vogel et al. 2008; Li et al. 2009; Fu and Xue 2010; Yin and Xue 2012). Some starch-regulating genes encode transcription factors, such as β-amylase-like proteins (Reinhold et al. 2011; Soyk et al. 2014) and the starch biosynthesis regulator1 (RSR1) which indirectly affects starch properties by controlling the expression of genes encoding reserve starch biosynthetic enzymes (Fu and Xue 2010). Finally, some structural features of native starch may spontaneously arise due to self-organisation of α-glucan chains, but, possibly, proteins facilitate this process (Hejazi et al. 2010; Regina et al. 2012).

During the last decades, starch and starch-related biochemistry have attracted interests from a wide range of disciplines including applied sciences (see also Chaps. 10, 11, and 12 this volume), non-linear optics and molecular medicine. Novel enzymes and transporters have been discovered now considered to be essential for starch metabolism. Metabolic routes that for decades were presented in textbooks had to be refined.

Carbon fluxes towards and from starch certainly affect the carbon status of a cell. A disturbed leaf starch turnover often results in reduced size of the entire plant, and essentially all processes controlling plant growth and development are, via sugar sensing, directly or indirectly linked to starch turnover (Moghaddam and Van den Ende 2012; Sparks et al. 2013; Ruan 2014; Häusler et al. 2014; Lastdrager et al. 2014; Pfister et al. 2014; Schmitz et al. 2014). In addition, transitory starch metabolism is also closely coupled to processes previously thought to be unrelated to starch, such as polysome loading and protein biosynthesis (Pal et al. 2013).

Rates of assimilatory starch synthesis and degradation are also controlled by the circadian clock. Transitory starch degradation is carefully balanced to prevent carbon starvation but also to ensure that more than 90 % of the carbon stored is mobilised even when length of and temperature during the dark period vary (Graf et al. 2010; Graf and Smith 2011; Paparelli et al. 2013; see below).

In autotrophic and heterotrophic tissues, starch granule formation is closely linked to plastid division (Yun and Kawagoe 2010; Crumpton-Taylor et al. 2013). Under some conditions, however, starch-derived compounds destabilise

chloroplasts and, finally, lead to lysis of the entire organelle (see below). Because of these manifold interactions, it is increasingly difficult to strictly distinguish starch-related genes or gene products and those unrelated to starch.

Recently, starch metabolism has been widely accepted as an area that permits to successfully study early evolutionary processes leading to the formation of a photosynthesis-competent eukaryote. Metabolism of starch is thought to originate from that of glycogen functional in the host. Transition from glycogen to starch was, however, a multistep process and some recent algal groups appear to represent intermediate stages of this transition (Ball et al. 2011; Ball 2012; Cenci et al. 2014; see also Chap. 4 this volume).

Last, but not least, fundamental enzymatic processes leading to accumulation and mobilisation of storage carbohydrates appear to be similar in plants and animals. Mammalian mutations that cause disturbed glycogen metabolism are usually associated with several severe diseases designated as glycogen storage diseases (GSDs). Therefore, seemingly very distant areas of life sciences, i.e. molecular medicine and plant biochemistry, have started to closely interact (Gentry et al. 2007, 2013; Nitschke et al. 2013).

Several comprehensive reviews discussing various aspects of starch metabolism in plants have been published in the last years (Fettke et al. 2009; Keeling and Myers 2010; Kötting et al. 2010; Zeeman et al. 2010; Stitt and Zeeman 2012; Sonnewald and Kossmann 2013; Zhang and Wing 2013; Zhou et al. 2013; Lloyd and Kossmann 2015). Here we present some recent results, views and developments in the field of starch degradation in higher plants. Because of space limitation, the review is largely restricted to plants that perform C3 photosynthesis and utilize starch as their dominant carbon store.

7.2 (Bio)Chemical and Structural Properties of Starch and Glycogen

In the following, basic information on internal structure and morphology of native starch particles is given (see also Chaps. 1, 2, 3, and 13 this volume). We then discuss both similarities and differences in starch and glycogen, the common storage polysaccharide in most heterotrophic organisms, and briefly mention wild-type higher plants that simultaneously form starch and glycogen.

7.2.1 Starch and Glycogen Share Chemical and Functional Features but Differ in Some Physicochemical and Biochemical Properties

Starch is deposited as hydroinsoluble, highly dense particles designated as granules. The internal structure of starch particles seems to be evolutionarily conserved

(Buléon et al. 1998; Zeeman et al. 2010; Bertoft 2013). By contrast, both size (diameters ranging from 0.1 to 100 μm) and morphology of native starch vary largely, depending on plant species and organ (Jane et al. 1994; Cenci et al. 2014). In many species and/or tissues, the entire starch particle population consists of a single granule type. In other cases, such as the endosperm of wheat, barley, rye and triticale, the same cell, however, forms several granule populations (designated as A, B and C type; please note that this classification is unrelated to the starch allomorph) that differ in size, time of appearance and biochemical features (Stoddard 1999; Peng et al. 2000). Furthermore, up to several dozen small non-fusing particles (each of which is polyhedral and possesses a diameter of 3–8 μm) may form a functional unit designated as compound starch (Jane et al. 1994). As an example, the rice endosperm massively accumulates this starch type.

Given that starch structure and biochemistry actually permit an essentially unlimited particle growth, it is unexpected that in higher plants granule populations largely differ in average size and size distribution. For various reasons, cells appear to arrest granule growth at largely different biosynthetic stages (see also Huang et al. 2014; Mahlow et al. 2014). Recently described proteins, such as SSG4 and FLO6, are likely involved in defining starch particle sizes (Matsushima et al. 2014; Peng et al. 2014), but plastidial and/or cellular mechanisms are unknown that permit sensing and controlling of granular volume.

Typically, starch granules are composed of two types of polyglucan molecules designated as amylopectin and amylose. The former is dominant in terms of both quantity and relevance for the internal granule structure (see below), but amylose might stabilise structural elements formed by amylopectin (Bertoft 2013; see Chaps. 1 and 3 this volume). Amylopectin and amylose are disperse α-glucans; the average size of amylopectin usually exceeds that of amylose. Several methods are used to distinguish and/or to physically separate both polyglucan types, including the absorption spectrum of the iodine-starch complexes, gel filtration or treatment of solubilised starch with butanol-isoamyl alcohol. It is, however, uncertain whether these methods permit a reliable distinction and/or separation of the two types of α-glucans if, due to genetic reasons, starch is massively altered (Vilaplana et al. 2012).

In amylose, amylopectin and glycogen, α-1,4-interglucose bonds are the dominant interglucose linkages. They result in elongation and formation of a left-handed helical structure of α-glucans or glucan chains. Helices of at least 18 α-1,4-interlinked glucosyl residues appear to selectively react with iodine (Bailey and Whelan 1961). Because of its short helical regions, glycogen is poorly stained by iodine.

In the three α-glucan types mentioned above, branching is formed by α-1,6-interglucose bonds which account for less than 10 % of the total interglucose linkages. By each branching, the number of nonreducing end(s) per polyglucan molecule increases by one, but the single reducing terminus remains unchanged.

The term 'amylose' designates a heterogeneous mixture of α-glucan molecules possessing a wide range of degrees of polymerisation (DP). Based on the total number of interglucose linkages, amylose contains less α-1,6-interglucose bonds (not exceeding 1 % of the total interglucose bonds) than amylopectin or lacks

branchings completely (see also Chap. 2 this volume). In aqueous solution, amylose forms a flexible random coil including left-handed helical segments that tend to be favoured at low hydration levels. The crystalline structure of isolated amylose has been determined, but it is strongly affected by chain length distribution and other parameters (Nishiyama et al. 2010; Putaux et al. 2011; Roblin et al. 2013). In addition, non-amylose compounds, such as lipids, strongly interact with amylose (López et al. 2012). As inside the starch granule, neighbouring compounds may exert similar effects, the in vivo structure(s) of amylose remains uncertain. Amylose appears to be unevenly distributed within the granule (Pérez and Bertoft 2010; Buléon et al. 2014).

In amylopectin, approximately 5–7 % of all interglucose bonds are branchings. Due to clustering, vicinal α-glucan side chains are capable of forming parallel double helices which then are arranged in a defined physical order yielding the A-type or the B-type starch allomorph.

While the actual amylopectin structure(s) remains hypothetical, it is widely accepted that several structural features of native starch granules are determined by intra- and/or intermolecular organisation of amylopectin (for details see Pérez and Bertoft 2010; Bertoft 2013; see also Chap. 1 this volume).

In higher plants, metabolism and function of plastidial starch appear to be related to those of cytosolic glycogen which in most heterotrophic eukaryotic cells serves as storage polysaccharide. The overall branching frequency of mammalian glycogen is in a similar range as that of amylopectin (Ball et al. 2011), but branching points are more evenly distributed in glycogen than in amylopectin (Roach et al. 2012; Cenci et al. 2014). Probably interconnected with the more even distribution of branchings, two major physicochemical differences exist between starch particles and glycogen molecules: Glycogen is typically hydrosoluble but, when hydroinsoluble, is associated with severe diseases such as epilepsy (Minassian et al. 1998; Pederson et al. 2013; Gentry et al. 2013). For steric reasons glycogen is predicted to have an upper size limit (approximately 40 nm diameter; Meléndez-Hevia et al. 1993). Likewise, empirically determined diameters of glycogen (β-particles) rarely exceed 45 nm. Larger glycogen particles (α-particles) are exclusively formed by closely associated (but not covalently linked) β-particles (Ryu et al. 2009; Sullivan et al. 2014).

Eukaryotic biosynthesis of starch and glycogen largely relies on the same two reaction types, chain elongation and branching. Chains are elongated by repetitive glucosyl transfers from a donor molecule to the nonreducing end(s) of a given α-glucan forming additional α-1,4-interglucose bonds. ADP-glucose is the most common glucosyl donor for starch biosynthesis. Eukaryotic glycogen formation relies on UDP-glucose, whereas prokaryotes often use ADP-glucose. In all cases, glucosyl transfer reactions result in a strictly directional chain growth (for details see Fujita and Nakamura 2012; Roach et al. 2012) and massively alter size (DP), but the molar amounts of α-glucans remain unchanged. Formation of α-1,6-interglucose bonds causes branching of chains and does not alter molar amounts nor the total DP of α-glucans as glucan chains act both as glucanosyl donor and acceptor.

In amylopectin, clustering of branchings is essential for double-helix formation. According to the model of 'glucan trimming' (or of 'preamylopectin processing'),

clustering is achieved by hydrolysing excess branches mediated by a single or several isoamylase complex(es) that selectively or preferentially act(s) during biosynthesis of amylopectin (Ball and Morell 2003; Fujita et al. 2003, 2009; Kubo et al 2005; Sim et al. 2014; see also Chap. 5 this volume). Recent studies performed with double or multiple knockout mutants from *Arabidopsis* suggest that starch granule biosynthesis requires a carefully balanced ratio of chain elongation and branching reactions. Disturbance of this balance appears to favour amylolysis of α-glucan chains and/or formation of a plastidial hydrosoluble glycogen-like polyglucan, designated as phytoglycogen (Wattebled et al. 2005). In *Arabidopsis* leaves, the amount of the latter storage carbohydrate undergoes dial fluctuations, but its turnover does not allow normal growth of the entire plant (Streb et al. 2008, 2012; Pfister et al. 2014). Glycogen, by contrast, appears to be formed without any trimming.

7.2.2 Starch and Glycogen-Like Polysaccharides Formed Within a Single Organism

Only few higher plant wild-type species, such as *Cecropia peltata* (Bischof et al. 2013) and *Ryparosa kurrangii* (Webber et al. 2007), synthesise both starch and a glycogen-like hydrosoluble α-polyglucan. Both carbohydrate stores are formed in plastids but are functionally diverse and physically separated as they reside in different cell types. Starch is the general carbon storage product of mesophyll cells. By contrast, glycogen-like α-glucans are restricted to plastids of some highly specialised organs/tissues collectively designated as food bodies. They form a heterogeneous group of multicellular systems occurring in those plants only that mutualistically interact with ants. Until now, only glycogen-like polysaccharides from *Cecropia peltata* have been characterised in more detail (Bischof et al. 2013).

Mesophyll cells of some higher plant mutants, however, form both starch and a hydrosoluble glycogen-like α-polyglucan designated as phytoglycogen. In *Arabidopsis* the two carbohydrate stores are synthesised when isoamylase isozyme 1 (ISA1) involved in preamylopectin processing is lacking (Zeeman et al. 1998; Delatte et al. 2005; Wattebled et al. 2008). This phenotypical feature points again to a similar enzymatic apparatus required for starch and glycogen metabolism.

7.2.3 Major Starch Pools in Higher Plants

Higher plants usually contain several major starch pools that are functionally divers and interconnected by whole-plant partitioning of carbohydrates (Fig. 7.1). Biosynthesis of transitory (or assimilatory) starch is locally and temporarily immediately linked to photosynthesis as it proceeds in the stroma of photosynthesising chloroplasts. In the subsequent dark phase, transitory starch is often degraded.

a Transitory starch

Biosynthesis

Degradation

CO_2

Calvin cycle

Transitory starch

Sucrose

Photosynthesis-competent tissues

b Reserve starch biosynthesis

Reserve starch

Storage tissues

c Reserve starch degradation (dead tissue)

Growing tissue

Aleurone layer

Dead tissue

d Reserve starch degradation (living tissue)

Fig. 7.1 Major starch pools in higher plants
(**a**) Transitory (synonymous with assimilatory) starch is formed by photosynthesising chloroplasts and is restricted to source organs. Biosynthesis is driven by intermediates of the Calvin cycle and occurs only during illumination. In darkness, typically most of the transitory starch is degraded and converted to transport metabolites, such as sucrose. Metabolism of assimilatory starch, therefore, consists of iterating phases of biosynthesis and degradation

Thus, leaves often perform many diel cycles of biosynthesis and degradation of transitory starch in the same organelle. Utilisation of transitory starch is essentially restricted to the individual plant that forms it. In terms of evolution, transitory starch constitutes the by far oldest starch pool (Ball et al. 2011; Facchinelli et al. 2013; Cenci et al. 2014; Nougué et al. 2014).

Another major proportion of the reduced carbon gained by photosynthesis is either temporarily stored in the vacuole or used to synthesise transport metabolites which, in many plant species, largely consist of sucrose. Transport metabolites move from mesophyll cells of source organs into the phloem and, via long distance transport, are then approaching sink organs which often massively accumulate another starch pool designated as reserve starch (Turgeon and Wolf 2009; De Schepper et al. 2013). During illumination, biosynthesis of reserve starch is mainly driven by intermediates linked to the reductive pentose phosphate cycle in source organs. During darkness, reserve starch biosynthesis relies on the nocturnal conversion of transitory starch to sucrose or to other transport metabolites. Unlike the metabolically more active transitory starch, reserve starch is often continuously formed over several weeks. The amount of reserve starch per plant frequently exceeds that of transitory starch. Reserve starch is essential for growth and development of plant seedlings during those periods of time in which photosynthetic carbon fixation is impossible. It is, however, also relevant for various non-plant uses, such as food of animals or humans and as starting material for various technological applications including energy conversion (Zeeman et al. 2010). In addition, minor starch pools largely unrelated to the central carbon metabolism exist in higher plants and as such will not be considered here.

While reserve starch is consistently synthesised in living cells of sink organs, its mobilisation separates two major subgroups as starch can be degraded in either living cells or dead tissue (Fig. 7.1). Starch metabolism differs in both subgroups. Reserve starch degradation in living cells occurs in differentiated storage organs, such as potato tubers, yam roots, cotyledons or the endosperm of many dicots, as well as in more specialised duration organs, such as the turions from Lemnaceae. In all these cases, starch is degraded over several days or weeks following transition of the organ or of parts of it from sink to source. Several cycles of biosynthesis and

Fig. 7.1 (continued) (**b**) Reserve starch formed in nongreen plastids of sink organs is continuously synthesised over an extended period of time which largely exceeds a single light period. It utilises transport metabolites derived from either Calvin cycle intermediates or from starch. Reserve starch biosynthesis often is a major determinant of the strength of the sink. In terms of quantity, reserve starch frequently exceeds that of transitory starch. Size, shape and allomorph of the reserve starch granules often differ from those of the assimilatory starch synthesised by source organs of the same plant

(**c**) Following an extended period of reserve starch biosynthesis, the starch-containing tissue may undergo apoptosis as typical for cereal seeds. Degradation products obtained by lysis of the entire tissue are imported by living cells

(**d**) Reserve starch is degraded in living cells permitting a limited number of starch synthesising and degrading phases

degradation can occur within the same tissue, although the turnover is slow and the turnover number is low as compared to assimilatory starch (see Sect. 7.4.1.2).

In the other subgroup, typically represented by the endosperm of cereal seeds, reserve starch is degraded following a period of desiccation. During this period the starch-storing tissues or organs undergo one of the various selective apoptosis programmes of higher plants. Subsequently, starch is mobilised extracellularly. Degrading enzymes have either been deposited during starch biosynthesis within the granules in a catalytically inactive state or are synthesised at the onset of starch degradation in surrounding living cells and are then exported to the dead tissue. Reserve starch mobilisation is closely associated with the enzymatic degradation of the entire starch-storing tissue including lysis of proteins and cell wall materials. Products derived from the various degradation processes are resorbed by vicinal living cells or tissues and are then recycled for further uses until the seedling reaches photosynthesis competence. In this subgroup of starch degradation, only the onset of reserve starch degradation (which is closely associated with seed germination) can be controlled and, subsequently, rates of degradation can be modulated. Another period of starch synthesis, however, cannot be initiated once desiccation occurred (see Sect. 7.4.1.1).

7.3 Current Knowledge of Starch-Degrading Pathways

During the last decades, the view on in vivo starch-degrading paths has been largely altered. In most cases progress achieved relates to transitory starch, whereas biochemical knowledge of reserve starch degradation is less advanced (Vriet et al. 2010; Tanackovic et al. 2014). In the following we discuss both advantages and limitations of the genetic approaches used and summarise the current knowledge as to how starch-degrading enzymes act under in vitro and in vivo conditions.

7.3.1 Identification of Starch-Degrading Gene Products by Using Knockout Mutants

When grown under normal conditions, mutants of higher and lower plants that are impaired in transitory starch degradation often gradually accumulate more starch than the wild-type resulting in a starch-excess phenotype. Furthermore, this phenotype is often retained following prolonged darkness. Time dependence and degree of starch excess, however, may largely vary depending on the plant species and the gene affected. The macroscopic phenotype of these mutants can qualitatively be tested by iodine staining of decolorised leaves or cells, and therefore, a large number of potentially relevant lines can be screened within a short period of time. After selecting the respective mutants, the affected gene locus is determined. In the past, this approach led to the discovery of novel starch-related proteins that

are functional inside the plastid, in the cytosol (see below) or even in the nucleus (Reinhold et al. 2011). Furthermore, novel transporters of the inner plastidial envelope membrane were identified that mediate the export of starch-derived maltose or glucose (Niittylä et al. 2004; Cho et al. 2011). In addition, bioinformatic approaches were used to search for novel genes that possess sequence similarities to identified starch-related genes or even to non-plant genes. By using these tools, genes may, however, be identified whose products have a dual or multiple function also acting in other paths, such as starch biosynthesis, or even in routes unrelated to starch metabolism. Detailed biochemical analyses, therefore, are required to fully determine the function(s) of a given gene product.

The procedures outlined above may, however, fail to detect any starch-excess phenotype when single knockout mutants can compensate the loss of function by altering carbon fluxes. For compensation, either existing paths are used or novel routes are established. In either case, the phenotype of the respective mutant will be close to or even indiscernible from that of the wild-type. In the undisturbed (i.e. wild-type) plant the gene product of interest may, however, participate in the analysed path. Similarly, contradicting phenotypical features of mutants from different plant species that are affected in essentially the same gene are ambiguous as they may reflect either species-dependent differences in the undisturbed metabolic paths or, alternatively, different abilities of compensating a loss of function (Asatsuma et al. 2005; Yu et al. 2005). Furthermore, compensating degradative paths may possess an overlapping but not identical temperature dependence as compared to the blocked route as it has been observed in starch biosynthesis (Satoh et al. 2008; Fettke et al. 2012b). If so, screening conditions may strongly affect identification of mutants. Finally, in *Arabidopsis thaliana* a single constitutive knockout of a starch-related gene can be associated with altered expression levels of more than 100 other genes, and therefore, the resulting phenotype may be caused by complex alterations of gene expression rather than by the loss of function of a single gene product (Comparat-Moss et al. 2010).

Starch metabolism (as well as that of glycogen; Wilson et al. 2010; Gentry et al. 2013) tends to be closely integrated into the entire biology of the respective cell and organ. Thus, a distinct block in starch degradation may even result in signalling processes that finally lead to disintegration of the entire chloroplast. If so, older leaves gradually loose photosynthetic capacity containing less starch and chlorophyll as compared to young leaves. Chloroplasts are disintegrated in several mutants impaired in transitory starch degradation but remain functional when essentially starch-free mutants are used as genetic background (Stettler et al. 2009; Cho et al. 2011; Malinova et al. 2014). Chloroplast disintegration appears to be related to the large group of sugar-mediated signalling effects (for review see Eveland and Jackson 2012; Ruan 2014), but neither the chemical nature of the signalling molecule(s) nor the subcellular site(s) of biosynthesis has been determined. Furthermore, it is unclear whether the various metabolic blocks all give rise to the same signal(s) or various mechanistically different but phenotypically similar cellular responses exist. In any case, chloroplast disintegration massively alters central carbon metabolism and may also significantly

affect screening procedures. Mutants containing inducible RNAi constructs may offer some advantages as compared to constitutive knockout lines (Weise et al. 2012; Martins et al. 2013).

7.3.2 How to Define 'Starch-Degrading Enzymes'?

Frequently, the term 'starch-degrading enzymes' lacks any precise definition. In a strict sense, it may designate enzymes using native starch as carbohydrate substrate, but for several reasons, this definition is not meaningful. Enzymes acting on starch granules often are capable to utilise solubilised α-glucans as well (and, possibly, even at higher rates; see below). Furthermore, structural alterations at the surface of native starch may favour local accessibility of degrading enzymes, but these processes are often not fully understood. The term 'starch' itself is ambiguous as it frequently designates both native particles and granule-derived hydrosoluble α-glucans containing both amylose and amylopectin. Finally, isolation of starch granules intends to retain in vivo structures of the particles, but minor artificial changes occurring unnoticed cannot be excluded.

For practical reasons, we use here a wider definition of 'starch-degrading enzymes'. We define plastidial and cytosolic enzymes as starch-degrading when they are likely to operate in starch-degrading paths and in vitro act on starch or on α-glucans (including glycogen and maltodextrins) that are chemically similar to starch. Most of these enzymes cleave interglucose bonds. We do, however, include those enzymes that do not act on interglucose bonds per se but are functionally related to their cleavage by other enzymes. This definition does not, however, imply that starch-like α-glucans act as in vivo substrate of the respective enzyme. Under in vivo and/or in vitro conditions, the catalytic action of a 'starch-degrading enzyme' may even lead to an increase in the DP of an α-glucan. Furthermore, 'starch-degrading enzymes' may also be involved in other processes.

7.3.3 Cleavage of Interglucose Bonds by Starch-Degrading Enzymes from Higher Plants

Interglucose bonds are cleaved by transferases that depending on the carbohydrate moiety transferred, the reaction mechanism used, the target bond cleaved and chemical features of the acceptor can be grouped into various enzyme types, each of which may be represented by several isozymes. We include enzymes mediating 'disproportionating reactions' that, within a single catalytic cycle, cleave and form interglucose linkages (Fig. 7.2).

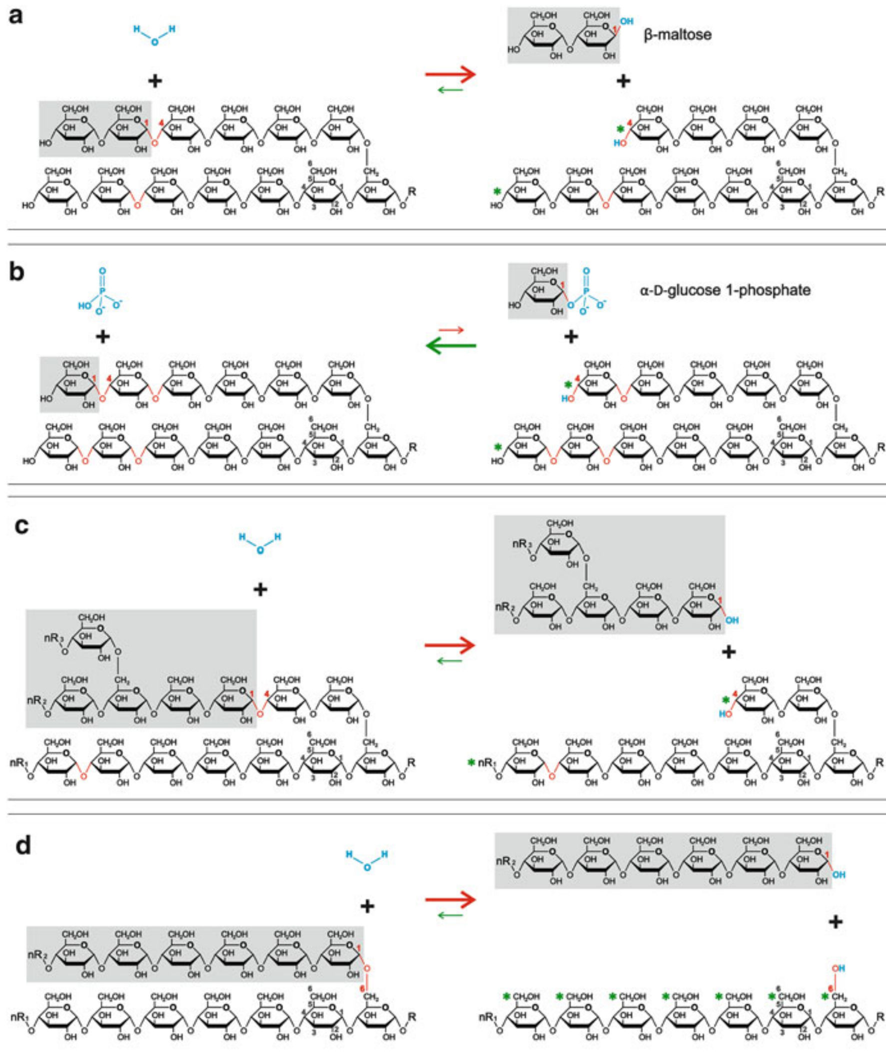

Fig. 7.2 Enzymatic actions of starch-degrading enzymes on interglucose bonds of α-glucans
For the sake of clarity, not all non-reducing ends of α-glucan chains have been labelled. (**a**) *Directed action of β-amylases*. On the *left*, an A-type (*upper row*) and a B-type (*lower row*) α-glucan chain of a branched α-glucan are shown together with a water molecule (*blue*). The A-type chain is linked to the B-type chain by a single α,1-6-interglucose bond but does not carry any further branching. Both a single β-maltosyl residue and a free β-maltose are marked in grey. An α-1,4-interglucose bond of the B chain (*lower row*) can also act as target of a hydrolytic action of β-amylases (marked in *red*). Vicinity of target bonds relative to branching point is shown randomly. Although thermodynamically favoured, hydrolytic reactions are reversible. For condensation, the nonreducing end of the A chain (*upper row* on the *left* side) and of the B chain (both marked with a *green asterisks*) potentially acts as a β-maltosyl acceptor. Carbon atoms 1 and 4 are marked in some glucosyl residues. R: glucanyl residue containing the reducing end of a chain. Directed hydrolysis is given by a *red arrow*. Condensation is indicated by a *smaller arrow* in *green*

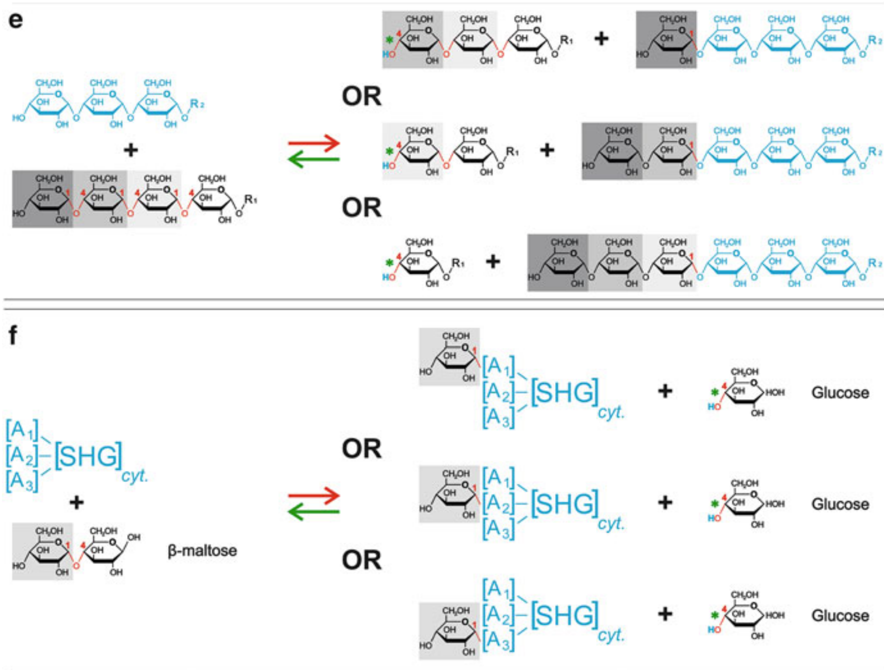

Fig. 7.2 (continued) (**b**) *Directed action of phosphorylases*. On the *left*, an A-type and a B-type chain (as in Fig. 7.2a) are shown. Orthophosphate (*left side*) and α-D-glucose 1-phosphate (*right side*) are also given. A single glucosyl transferred from the nonreducing end of the A chain to orthophosphate yielding α-D-glucose 1-phosphate is marked in *grey*. As a potential target for an enzymatic action, the terminal α-1,4-interglucose bond of the B chain is also given in *red*. Furthermore, two other interglucose bonds are labelled in *red* as potential targets for subsequent phosphorolytic actions. The phosphorolytic reaction is indicated by a *red arrow*. In the reverse reaction, the nonreducing end (*green asterisks*) of the A and the B chain acts as glucosyl acceptor. The glucan synthesising reaction is indicated by a *larger arrow* (*green*) as V_{max} exceeds that of phosphorolysis. Vicinity of the target bonds relative to the branching point is shown randomly. In some glucosyl residues, carbon atoms 1 and 4 are indicated. R: a glucanyl residue containing the reducing end

(**c**) *Non-directed action of α-amylases*. On the *left*, a branched α-glucan and a water molecule (*blue*) are shown. The α-1,4-interglucose bond to be hydrolytically cleaved (B chain; *left side middle row*) is labelled in *red* and the α-glucanyl residue to be liberated by endohydrolysis of a single α-1,4 interglucose is marked in *grey*. On the *right*, the α-glucan liberated (consisting of both an A and a B chain) is marked in *grey*. This glucan enters the pool of branched hydrosoluble α-glucans. A further interglucose bond of the B chain (*red*) is a target for another action of α-amylases. Vicinity of target bonds relative to the branching points is shown randomly. Hydrolysis is thermodynamically favoured and indicated by a *red arrow*. For the reverse reaction (condensation; *small green arrow*), a potential acceptor site for the glucanyl transfer is also marked (*Green asterisks*). In some glucosyl residues, carbon atoms 1 and 4 are labelled. R: glucanyl residue containing the reducing end (C chain). nR1, nR2 and nR3: glucanyl residues containing the nonreducing end of the C, B and A chains, respectively

7.3.3.1 Exo-hydrolases (β-Amylases)

The quantitatively most prominent exo-hydrolases acting on α-glucans are β-amylases (glycoside hydrolase family 14; GH14) which typically transfer maltosyl residues from the nonreducing end of a linear α-glucan or of an α-glucan chain to water following an inverting (i.e. single replacement) mechanism. Thereby, the anomeric carbon is converted from α- to β-configuration and β-maltose is released (Fig. 7.2a). Subsequently, the same α-glucan or glucan chain may act further as maltosyl donor until a minimum distance to either the reducing end or to a branching point is reached. Although additional reaction types have been reported for β-amylases (Hehre et al. 1979, 1986; Mikami et al. 1994; Fazedas et al. 2013), formation of β-maltose appears to be their main starch-related activity. This concurs with the starch-excess phenotype of transgenic potato plants lacking a functional plastidial β-amylase (Scheidig et al. 2002). β-Amylases completely convert linear α-glucans to maltose (plus a maltotriose if the initial DP equals $2n-1$), but branched α-glucans are incompletely degraded as exo-hydrolases cannot bypass any

Fig. 7.2 (continued) (**d**) *Direct debranching of α-glucans*. Following hydrolysis of a single α-1,6-interglucose bond, the A chain (*grey*; *left*) is released as a linear α-glucan whose free carbon atom 1 (reducing end) is in the α-configuration and the α-glucan released enters the pool of hydrosoluble α-glucans. Direct debranching is thermodynamically favoured and given as *red arrow*. Due to the reversibility of enzyme-mediated reactions, the reverse reaction cannot be excluded (*small green arrow*). For this direction, potential acceptor sites are marked (*right side* carbon atoms 6; *Green asterisks*). In some glucosyl residues, carbon atoms 1 and 6 are marked. R: glucanyl residue containing chain C and the reducing end. nR1: glucanyl residue of the B chain containing the nonreducing end. nR2: glucanyl residue of the A chain containing the nonreducing end
(**e**) *'Disproportionating reactions' as mediated by the plastidial disproportionating enzyme 1 (DPE1)*. For the DPE1-mediated reactions, two linear oligoglucans are substrates. One is donor (*left side lower row*) and the other one is acceptor (*left side upper row*; *blue*) for a transfer of a carbohydrate residue consisting of one to three glucosyl moieties. Depending on the size of the α-glucanyl residue, transferred rates of the transfer differ largely. When acting on two maltotriose molecules, the enzyme forms maltopentaose plus glucose or maltotetraose plus α-maltose. Both transfer reactions are easily reversible as indicated by two *arrows* equal in size. Due to multiple and easily reversible transfer reactions, DPE1 generates a polydisperse α-glucan mixture. Both the sum of the interglucose bonds and the total amount of glucosyl moieties distributed in the reaction mixture remain constant
(**f**) *'Disproportionating reactions' as mediated by the cytosolic disproportionating enzyme 2 (DPE2)*. A disaccharide, typically β-maltose, acts as donor (*left side lower row*) and cytosolic heteroglycans (SHG_{cyt}; *left side*; marked as *blue*) as acceptor of a glucosyl residue (marked in *grey*). Heteroglycans are branched glycans and carry several nonreducing ends as potential acceptor sites. Some details of the glucosyl transfer are still hypothetical. Three sites (A_1 to A_3) are shown but neither the number of sites nor the selecting mechanism(s) is known. The acceptor sites may be formed by terminal glucosyl and/or by other terminal sugar residues. The interglucose bond in β-maltose that is cleaved when the disaccharide acts as glucosyl donor is marked in *red*. In some glucosyl moieties, carbon atoms 1 and 4 are labelled. Several reversible transfer reactions are possible that all mediate the formation of glucosylated heteroglycans and release free glucose (*right side*). Glucosyl transfer is easily reversible as indicated by the two *arrows* equal in size. For further information, see text

branching point. Typically, plants contain a relatively high number of β-amylase isozymes that differ in kinetic features and in vivo are functionally diverse since they possess various subcellular locations (for review see Fettke et al. 2012a). As β-amylases from plants act on single helices, they prefer hydrosoluble rather than highly ordered and hydroinsoluble glucans (Edner et al. 2007; Hejazi et al. 2008).

7.3.3.2 Phosphorylases

In a functional state, phosphorylases (GH13) are oligomeric proteins selectively acting on nonreducing ends of α-glucans or glucan chains. Due to reversibility of the glucosyl transfer, they mediate both the directed glucan elongation and the directed phosphorolytic degradation. Phosphorolysis is restricted to the terminal interglucose linkage at the nonreducing end (Fig. 7.2b). Terminal glucosyl residues are transferred to orthophosphate yielding glucose 1-phosphate (G1P). Arsenate can replace orthophosphate, but transfer rates are much lower and, unlike G1P, the arsenate ester formed is spontaneously hydrolysed to arsenate and glucose. In the elongating reaction, phosphorylases use the nonreducing chain end as glucosyl acceptor and glucose 1-phosphate as donor. The in vitro determined V_{max} values of elongation are usually higher than those of phosphorolysis. Unless a product of the transfer reactions is immediately removed, α-glucan chains are simultaneously elongated and shortened under a wide range of G1P to orthophosphate ratios. The ratio of both reactions, however, varies depending on the respective G1P/P_i levels. Phosphorylases cannot bypass branchings. Furthermore, maltodextrins with a DP less than four usually cannot further be phosphorolytically (or arsenolytically) degraded. For the plastidial phosphorylase isozyme (Pho1 or, in *Arabidopsis*, PHS1), maltotetraose is an efficient competitive inhibitor of phosphorolysis (Steup and Schächtele 1981).

Pho1 from rice endosperm appears to efficiently elongate small maltodextrins even at high orthophosphate levels as P_i seems to selectively inhibit phosphorolysis (Hwang et al. 2010). The plastidial phosphorylase preferentially uses single-stranded α-glucans or α-glucan chains as substrate. Under in vivo conditions, the enzyme likely acts in both starch synthesising and degrading paths (Satoh et al. 2008; Fettke et al. 2012b; Malinova et al. 2014; see below).

In higher plants, plastidial phosphorylase isozymes (Pho1/PHS1) possess a large insertion (approximately 80 amino acid residues) located between the N- and the C-terminal domain containing a high proportion of charged amino acids (Albrecht et al. 2001).

Cytosolic phosphorylase isozymes (Pho2/PHS2) as well as glycogen phosphorylases do not contain this insertion. Likewise, maltodextrin phosphorylase from *E. coli* (which preferentially acts on oligoglucans as does Pho1/PHS1) lacks the insertion. As opposed to Pho1/PHS1, Pho2/PHS2 exhibits high affinity towards branched polyglucans, such as amylopectin and glycogen, but also to the glucose-containing cytosolic heteroglycans; see below. Non-covalent binding to these glycans occurs even in the absence of P_i or G1P, therefore permitting an easy distinction of the two isozyme types by affinity electrophoresis.

As revealed by carbohydrate microarrays, the cytosolic phosphorylase isozyme (Pho2/PHS2) mediates the glucosyl transfer from G1P to various glycans, such as cytosolic heteroglycans, carrageen, rhamnogalacturan I and II as well as xyloglucans. G1P-dependent labelling pattern observed with recombinant PHS2 was very similar to that obtained with DPE2 in the presence of ^{14}C-maltose. This similarity suggests that in vivo both glucosyl transferases utilise the same glycans (Ruzanski et al. 2013; see below). It does, however, not support the common designation of Pho2/PHS2 as being an isozyme of the α-glucan phosphorylase.

Multiple phosphorylation of the plastidial and the cytosolic phosphorylases is known to occur, but functional implications of the covalent modifications are largely unknown (Walley et al. 2013; see below).

7.3.3.3 Endo-hydrolases (α-Amylases)

The second large group of starch-related hydrolases are α-amylases (GH13) which, unlike β-amylases, cleave internal α-1,4-interglucose bonds and, depending on the structure of the α-glucan substrate, release either linear or branched α-glucans (Fig. 7.2c). When acting on branched substrates, target bonds exist at either site of the α-1,6-bond, provided a minimum distance is given in either direction. Furthermore, α-amylases use the mechanistically more sophisticated retaining (double-replacement) mechanism, and, following hydrolytic cleavage of the target bond, the free carbon atom 1 of the glucan released has α-configuration (Fig. 7.2c). α-Glucans liberated by α-amylases appear to largely lack terminal branchings (Kainuma and French 1970).

Similar to β-amylases, additional activities of endo-hydrolases have been reported (Kim et al. 1999; Qian et al. 2001), but hydrolysis of internal interglucose bonds appears to be the main catalytic activity. Endo-hydrolases from plants act preferentially on single helical and hydrosoluble carbohydrates.

7.3.3.4 Debranching Enzymes (DBEs)

All plant debranching enzymes (GH13) hydrolyse only α-1,6-interglucose bonds following the direct debranching mode and using the double-replacement mechanism. Thus, the reducing end of the α-glucan liberated retains the α-configuration (Fig. 7.2d). Based on sequence similarities and substrate specificities, plant DBEs can be classified into two groups: isoamylases (ISAs) and limited dextrinases (LDs or pullulanases). Pullulan consists of α-1,4-maltotriosyl units linked via α-1,6-interglucose bonds. Under in vitro conditions, LDs (but not by ISAs) are capable of hydrolysing the latter bonds (for details see Zeeman et al. 2010).

In *Arabidopsis*, the ISA type is represented by three genes (designated as *AtISA1* to *AtISA3*), but LD exists as a single gene product. Presumably, both ISA3 and LD act exclusively in the starch-degrading path exerting nonredundant functions (Fig. 7.2d; Delatte et al. 2006). Depending on the botanical source, ISA1 and/or

ISA2 form homomeric or heteromeric complexes apparently functional only in preamylopectin processing (Facon et al. 2013). In rice endosperm, the starch-related FLO6 protein appears to be a scaffolding protein which simultaneously binds to both native starch and ISA1 thereby locating ISA1 to the granule surface (Peng et al. 2014).

7.3.3.5 Disproportionating Enzymes (DPE1 and DPE2)

Some starch-degrading enzymes that, presumably, act selectively on hydrosoluble oligoglucans cleave α-1,4-intersugar bonds (thereby diminishing the DP of the sugar donor) and transfer the carbohydrate moiety to another glycan acting as acceptor (whose DP increases). Formally, the reaction resembles the 'disproportionation' of oxidation states and enzymes mediating this type of carbohydrate transfer are called 'disproportionating' enzymes. DPE1 and DPE2 are located in the plastidial stroma and in the cytosol, respectively. DPE1 acts on maltodextrins and generates neutral sugars to be exported into the cytosol. DPE2 is essential for the cytosolic metabolism of β-maltose.

DPE1 (4-α-glucanotransferase) catalyses readily reversible reactions according to the equation $G_n + G_m \leftrightarrow G_{n-q} + G_{m+q}$ where q (equalling 1, 2, 3) designates the number of glucosyl moieties transferred in a single reaction (Fig. 7.2e). Due to the multiplicity of transfer reactions, DPE1 generates a mixture of linear α-glucans (Kartal et al. 2011).

The cytosolic 'disproportionating enzyme 2' (DPE2) also mediates diversification of α-glucans or glycans but deviates from DPE1in two aspects: first, DPE2 preferentially uses high molecular weight glycans as one of the substrates and, second, it transfers a single glucosyl residue (presumably that containing the nonreducing end; Steichen et al. 2008) from β-maltose as glucosyl donor and cleaves the interglucose bond of the disaccharide (Dumez et al. 2006; Fig. 7.2f). Under in vitro conditions, DPE2 efficiently transfers the glucosyl residue to glycogen (Chia et al. 2004; Fettke et al. 2006) which acts as non-physiological substitute of cytosolic heteroglycans. The latter are common in photoautotrophic and in heterotrophic organs of higher plants (Malinova et al. 2013; for details see Fettke et al. 2012a). Cytosolic heteroglycans (often designated as water-soluble heteroglycan [SHG] subfraction I) are highly branched glycans whose apparent size ranges up to approximately 70 kDa. In glycosidic linkage pattern and monosaccharide composition, cytosolic heteroglycans are similar to apoplastic arabinogalactans, but, unlike the latter, they often do not interact with the synthetic β-glucosyl Yariv reagent. Interestingly, cytosolic heteroglycans are also efficiently used as glucosyl donor or acceptor by another cytoplasmic transferase from plants, Pho2 (or, in *Arabidopsis thaliana*, PHS2; Ruzanski et al. 2013). By contrast, neither the plastidial phosphorylase isozyme, Pho1/PHS1, nor the main muscle glycogen phosphorylase from mammals can utilise cytosolic heteroglycans as substrate (Fettke et al. 2004, 2005a, b).

For the reverse in vitro glucosyl transfer from glycogen, DPE2 uses free D-glucose (yielding maltose) but also several other glucosyl acceptors, such as D-xylose, D-allose, D-mannose and *N*-acetyl-D-glucosamine. As various monosaccharides are used as glucosyl acceptors, DPE2 forms several glucose-containing disaccharides. In this respect, DPE is similar to the prokaryotic MalQ, a transferase essential to the bacterial metabolism of maltose (Dippel and Boos 2005; Fettke et al. 2006; Park et al. 2011; Ruzanski et al. 2013).

7.3.4 Frequently Observed Features of Starch-Degrading Enzymes

In the following section, we discuss some features that are frequently found in starch-related enzymes but also in enzymes acting on cell wall materials. In these cases, many target bonds exist that are thermodynamically similar (Goldberg et al. 1991) and, in principle, can be enzymatically cleaved. A relatively low proportion of the linkages, however, are actually used by the degrading enzymes.

7.3.4.1 Multi-Chain and Single-Chain Attack

Assuming a single starch-degrading enzyme molecule is surrounded by various hydrosoluble α-glucans, the enzyme can cleave a single interglucose bond in one glucan and, subsequently, act on another glucan molecule (Fig. 7.3a). This mode is designated as multi-chain attack as the enzyme apparently randomly selects a target bond and, following a single reaction, interacts with another carbohydrate molecule. On average, this mode of degradation results in a decreasing DP of all substrate molecules. Starch-degrading enzymes can, however, follow another mode of action, designated as single-chain attack or processivity, and repeatedly act on interglucose bonds of the same substrate molecule (Fig. 7.3b). Processive enzymes catalyse essentially the same reaction utilising a series of substrate molecules originating from a single polymeric molecule and, typically, use the series of substrates with essentially the same efficiency. All substrates of a series differ in size but are structurally and chemically very similar. During the repetitive action, the enzymes remain in close vicinity to the substrate and, except the first and the last cycle, do not associate or dissociate. Dissociation of the enzyme–glucan complex terminates a series of catalytic cycles. Depending on the respective processive enzyme and conditions of catalysis, the number of repetitive cycles varies and, therefore, the term ‘processive enzyme’ covers a wide range of modes of action. If an extended series of catalytic cycles is performed at the same α-glucan chain, intermediate DPs may represent only a minor (and difficult to detect) proportion of substrate molecules and the α-glucan mixture is dominated by two extremes which are close to the starting

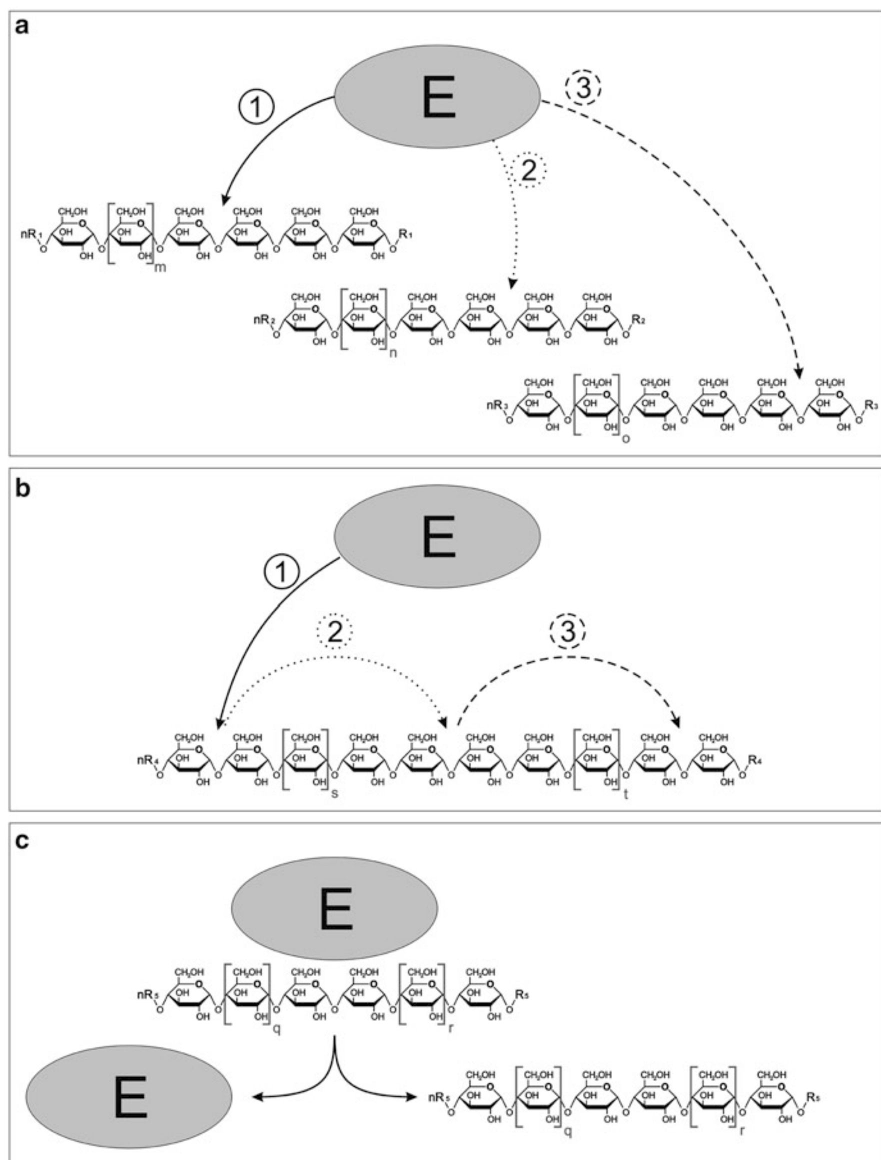

Fig. 7.3 Interaction of starch-degrading enzymes with soluble α-glucans
(**a**) *Multiple attack of a hydrolysing enzyme* (*E*). The enzyme (E) hydrolyses an interglucose bond in one α-glucan molecule molecule (1). Following hydrolysis, it dissociates and interacts with another glucan molecule (2). Finally, it hydrolyses an interglucose bond of the third glucan molecule (3). R_1, R_2, R_3 means a α-glucanyl residue containing the reducing chain end of the α-glucan molecule 1, 2, 3. nR_1, nR_2, nR_3 means a α-glucanyl residue containing the nonreducing end of molecule 1, 2, 3. m, n, o means the number of glucosyl residues in a part (as indicated) of a given α-glucan chain

distribution and near to the final sizes. Depending on the state of degradation, the ratio between both extremes varies.

Several β- and α-amylases from higher plants appear to start catalysis by following a more random approach (i.e. multi-chain attack) and sooner or later largely use the processive mode of action. The precise mode of degradation strongly depends, however, on the biological source of the hydrolase and the experimental conditions, such as temperature and substrate level (Kramhøft et al. 2005; Ishikawa et al. 2007; Nielsen et al. 2012).

Modes of the multi-chain and single-chain attack have, however, been defined using sufficiently diluted hydrosoluble α-glucans. Under these conditions, all interglucose bonds are easily accessible to the enzymes. By contrast, in the hydroinsoluble starch granule, many target bonds are not accessible due to the compact structure of the particle. Degradation of native starch is more complex as it requires additional processes that precede enzymatic hydrolysis of interglucose bonds (see below).

7.3.4.2 Productive and Non-productive Enzyme-Glucan Complexes

Starch-degrading enzymes may also bind to α-glucans resulting in so-called non-productive complexes as they do not lead to catalysis. These complexes are formed by binding of all glucosyl residues of the α-glucan (chain) together with the respective target linkage to subsites of the enzyme that are located at only one side of the catalytic site which, therefore, lack access to interglucose bond to be cleaved (Fig. 7.3c). Formation of non-productive complexes often leads to in a time-dependent decrease of the respective apparent V_{max} values although catalytic activity of the enzyme is fully retained.

7.3.4.3 Non-catalytic Carbohydrate-Binding Sites

Starch-degrading enzymes often posses domains unrelated to catalysis but capable of selectively and non-covalently binding carbohydrate substrate(s). Two types of non-catalytic carbohydrate-binding sites are known: **c**arbohydrate-**b**inding **m**odules

Fig. 7.3 (continued) (**b**) *Single attack of a hydrolysing enzyme* (*E*). The enzyme (E) binds to a α-glucan molecule and, subsequently, hydrolyses sequentially several interglucose bonds before dissociating from the carbohydrate substrate. In this example, three target interglucose bonds (indicated by 1, 2 and 3) are given. R4: α-glucanyl residue of this glucan molecule containing the reducing end. nR4: α-glucanyl residue of the same glucan molecule containing the nonreducing end.
(**c**) *Non-productive enzyme-glucan complex and its dissociation*. Due to the mode of binding of a hydrolase (E) to an α-glucan, the catalytic site has no access to an interglucose bond to be hydrolysed. The non-productive complex is retained or dissociates without hydrolysing an interglucose bond. *p* and *r* are the number of glucosyl moieties as indicated. R_5 and nR_5: α-glucanyl residue of the same α-glucan molecule containing the reducing and nonreducing ends, respectively

(CBMs) and **s**econdary **b**inding **s**ites (SBSs; Boraston et al. 2004; Kiessling et al. 2008; Cuyvers et al. 2012). Both types of binding sites support the catalytic action of the respective enzyme by effects such as proximity and targeting. The entire protein–carbohydrate complex is based on multiple interactions between various sites of the protein and those of the bound glycan, but the action of the non-catalytic site(s) may be dominant. Multiple interactions of the entire complex are often designated as avidity or functional affinity (Kiessling et al. 2008). When homomeric or heteromeric protein complexes interact with glycans (see Chap. 8 this volume), multiple binding to the carbohydrate is also often observed.

7.3.4.3.1 Carbohydrate-Binding Modules (CBMs)

Starch-degrading enzymes are often modular proteins. A module is defined as a structural and functional unit of a monomeric protein. In addition to the catalytic module, starch-degrading enzymes frequently possess a single non-catalytic CBM or several CBM copies placed in either the N- or the C-terminal domain. The term CBM covers a contiguous amino acid sequence whose size ranges from 30 to approximately 200 residues. CBMs are often (but not always; Fontes and Gilbert 2010; Atmodjo et al. 2013) directed against the same carbohydrate used by the catalytic module of the respective enzyme. The CBM domain tends to fold into a functional three-dimensional structure even in the absence of the residual sequence of the protein (Luís et al. 2013).

CBMs are often distant from and connected to the catalytic site(s) by a flexible linker sequence (Kiessling et al. 2008; Guillén et al. 2010) which also may participate in carbohydrate binding (Payne et al. 2013). Carbohydrate targets of CBMs are, in principle, distinct monosaccharyl or oligosaccharyl residues (Cantarel et al. 2009). Occasionally, CBMs are found in separate proteins apparently lacking any catalytic domain (for details see Guillén et al. 2010; see also below).

Depending on the physicochemical state, oligoglycans or oligoglycan chains that non-covalently interact with CBMs possess either a low or a significant conformational flexibility. The latter is frequently caused by rotation about glycosidic linkages provided the target carbohydrate is soluble (Kiessling et al. 2008). By contrast, highly ordered and hydroinsoluble glycans possess often little flexibility.

Within the binding sites, distinct aromatic amino acids (such as tryptophan or tyrosine residues) often undergo stacking interactions with a distinct heterocyclic sugar moiety, but other amino acid residues may also contribute to carbohydrate binding (Guillén et al. 2010).

Typically, CBMs tend to favour the action of the respective enzyme by bringing (and retaining) the catalytic domain in close vicinity to the carbohydrate substrate. When acting on highly ordered glycans, binding of CBMs per se may even result in structural alterations (designated as substrate disruption) that locally convert the carbohydrate into a structure favouring subsequent enzymatic catalysis.

7.3.4.3.2 Secondary Bindings Sites (SBSs)

In the families of glycoside hydrolases (GHs) including glycosidases and trans-glycosidases (Cantarel et al. 2009), non-catalytic carbohydrate binding can also occur by sites that are physically not distant from the catalytic site but are located close to the surface of the structural unit that comprises the active site. These sites are designated as **s**econdary **b**inding **s**ites (SBSs). As opposed to CBMs (which originally were defined as **c**ellulose-**b**inding **m**odules), most SBSs have been discovered in starch-active enzymes (Nielsen et al. 2009; Meekins et al. 2013, 2014), but they also exist in carbohydrate-active enzymes unrelated to starch (Cuyvers et al. 2012). Carbohydrate-active enzymes frequently possess more than a single SBS per polypeptide, and a single monomer may contain both SBSs and CBDs (for details see Cuyvers et al. 2012).

Typically, SBSs are situated on the surface of the module containing the catalytic site and, therefore, are more fixed than CBMs (Cuyvers et al. 2012). Similar to CBMs, aromatic amino acid residues appear to be of special relevance for the selective binding of sugar moieties.

Several functions have been ascribed to SBSs, such as targeting of the enzyme to its substrate, assisting catalysis by substrate loading into the active-site groove, facilitating catalysis by disrupting the structure of target carbohydrates and retaining the enzyme in close contact to the carbohydrate substrate for subsequent reactions. These functions largely overlap those of CBMs. Due to their surface-near location, SBSs may also be involved in the interaction of enzymes with highly ordered carbohydrates (Cuyvers et al. 2012; see below).

7.3.4.4 Starch-Degrading Enzymes Acting on Starch Granules

Any starch-degrading path starts at the surface of the starch particle, but subsequent reactions may either be also restricted to the periphery of the starch particle or may form additional surfaces leading into interior parts of the granule. In any case, the process takes place in a nonhomogeneous system. Understanding of the initial reactions of starch degradation is, however, impeded as detailed structural information on the granule surface is largely lacking. In a formal sense, enzymatic deconstruction of other hydroinsoluble polysaccharides, such as cellulose and chitin, poses similar problems. Microbial deconstructions of cellulose and chitin are, however, extracellular processes, whereas starch is largely degraded within living plant cells. Efficient cellulolysis and chitinolysis by microbial enzymes require a close collaboration of processive and non-processive glycoside hydrolases (Ragauskas et al. 2006; Gilbert 2010; Payne et al. 2012; Shang et al. 2013). It is unknown whether a similar synergistic process is functional in starch degradation.

7.3.4.5 Kinetic Implications for Starch-Degrading Enzymes

Due to features of the enzyme itself, to the mode of enzymatic action or to properties of the carbohydrate substrates, actions of starch-degrading enzymes can often not been described by using the classical concept developed by Michaelis–Menten. For each of the three cases, an example is discussed in more detail.

In the classical theory of Michaelis–Menten (and in some more advanced versions as well), the population of enzyme molecules is assumed to consist either of two states, a catalytically active and a nonactive state. Except at saturating substrate levels, the ratio between both states is determined by the actual substrate concentration and the affinity of the enzyme to the respective substrate. At limiting substrate levels, distinct ratios between the two states of a given enzyme exist that are essentially constant as long as steady state is maintained. Following each catalytic cycle, an enzyme molecule either undergoes a further catalytic action or change from the active state into an inactive state which is caused by the actual lack of substrate for the respective enzyme molecule. To maintain steady state, in this case an enzyme molecule previously catalytically inactive must now participate in catalysis.

If, however, the enzyme itself exhibits heterogeneity in its kinetic properties, the simple approach outlined above cannot be applied. Enzyme heterogeneity can be caused by several processes, such as the reversible formation of protein complexes (that, in some cases, may even include α-glucans) or by covalent modifications of a single enzyme. As an example, the mammalian muscle glycogen synthase possesses at least nine phosphorylation sites selectively used by distinct protein kinases. Some esterification sites significantly alter the kinetic properties of the enzyme, but others affect mainly subcellular location and targeting of the glycogen synthase (for review see Palm et al. 2013).

Plant genomes encode considerably more protein kinases than mammalian genomes (Zulawski et al. 2013). Both phosphorylation sites of starch-degrading enzymes and starch-related protein kinases have not been completely identified. Furthermore, functional implications of covalent modification of starch-degrading enzymes are largely unknown. As a monomeric protein that is independently phosphorylated at n sites exists in up to 2^n phosphorylation states, even a few esterification sites may cause a large kinetic diversity (Salazar and Höfer 2009).

Recent studies have empirically confirmed a large number of phosphorylation sites in plant proteins (Baginsky 2009; Walley et al. 2013; van Wijk et al. 2014). In *Arabidopsis* leaves, several starch-related enzymes and transporters are known to be phosphorylated (Heazlewood et al. 2009; Reiland et al. 2009; see Fig. 7.4).

Starch-related heteromeric protein complexes tend to organise carbohydrate substrate(s) by forming a distinct microenvironment and, therefore, do not act in a homogeneous system. Similarly, processive enzymes often remain in close vicinity to the carbohydrate substrate (for discussion see Nishimo et al. 2004).

Furthermore, starch-degrading enzymes utilising granules as substrate are surface-active enzymes and have no access to compounds inside the dense starch particle irrespective of their chemical similarity or identity. Conventional plots of the reaction velocity versus the entire substrate content of reaction mixtures use virtual substrate levels that, to a large extent, are not relevant to the enzyme. Finally,

due to the large size of native starch, enzyme–substrate complexes are formed only by movement and binding of the enzyme to the surface of starch particles.

7.4 In Vivo Starch Degradation

In living cells, starch degradation consists of both plastidial and cytosolic routes, but autophagy-related starch-degrading processes cannot be excluded (Weidberg et al. 2011). In animals, defects in lysosomal α-glucosidases severely affect glycogen metabolism, and, at least in the long term, lysosomal glycogen degradation or a lysosomal glycogen-related degradative process appears to be essential (glycogen storage disease type II; Roach et al. 2012; Zirin et al. 2013). Autophagy-related glycogen degradation seems also to exist in yeast (see Roach et al. 2012). More than 30 autophagy-related genes (designated as *ATGs*; Mizushima et al. 2011) have been identified in *Arabidopsis thaliana*. Some *AtATGs* are constitutively expressed, but transcript levels are elevated under stress conditions (Liu and Bassham 2012). It is unknown whether in higher plants starch-related autophagy is restricted to stress situations (which in some starch-related mutants may permanently be effective) or also occurs in non-stressed plants. In some autophagy-deficient *Arabidopsis* mutants, transitory starch degradation appears not to be significantly affected (Izumi et al. 2013).

In living cells of higher plants, the widely accepted paths of starch degradation cover several processes that at the level of a single chloroplast usually occur more or less simultaneously rather than strictly sequentially:

- Cleavage of α-1,4-interglucose bonds
- Cleavage of α-1,6-interglucose bonds
- Disproportionating reactions
- Transport of products of the plastidial starch degradation into the cytosol
- Conversion of starch-derived sugars into intermediates of established cytosolic paths of the central carbon metabolism including various biosynthetic routes

7.4.1 Reserve Starch Degradation

Reserve starch metabolism occurs in vegetative storage organs, ranging from potato tubers, roots to more specialised organs like turions of Lemnaceae, as well as in seeds from monocotyledons or dicotelydons. Seeds that essentially retain large quantities of water and their metabolic activities almost unchanged have been designated as recalcitrant, but those that develop desiccation tolerance were named orthodox seeds (Roberts 1973). For the latter, desiccation is essential for the subsequent germination. Dry seeds, however, still contain some water and actually represent a low-hydrated state permitting some metabolic activities (Weitbrecht et al. 2011).

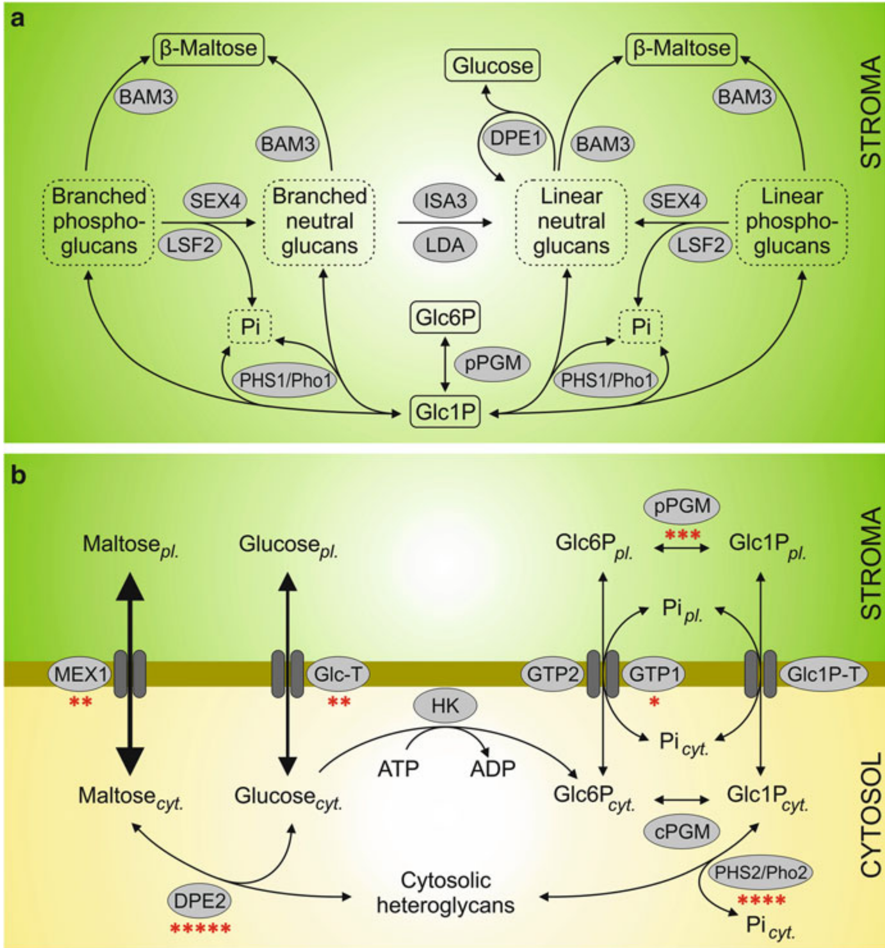

Fig. 7.4 Stromal and cytosolic paths of starch-derived hydrosoluble α-glucans
(**a**) *Plastidial routes*. Hydrolytic enzymes acting on assimilatory starch granules release four groups of α-glucans (branched phosphoglucans, branched neutral glucans, linear neutral glucans and linear phosphoglucans; all marked by *broken lines*). Each group consists of several types of molecules as DP, position of the branching point(s), number and location of monophosphate ester(s) are not defined. In addition, β-amylases (in *Arabidopsis* largely AtBAM3; At4g17090) liberate β-maltose directly from the starch granule surface and the disaccharide released also enters the plastidial β-maltose pool. Phosphorylated α-glucans are converted to neutral oligoglucans by SEX4 (in *Arabidopsis* At3g52180) and/or **L**ike **S**ex **F**our **2** (LSF2; At3g10490). Branched glucans are linearised by isoamylase3 (in *Arabidopsis* AtISA3; At4g09020) and/or limit dextrinase (AtLDA; At5g04360) which both convert side chains into free linear α-glucans. For clarity, actions of α-amylase3 (AMY3; At1g69830) have been omitted. The order of glucan dephosphorylation and debranching is not defined. Depending on DP and structure of the respective α-glucans, they may act as carbohydrate substrate of the plastidial phosphorylase (Pho1/PHS1; At3g29320) yielding glucose 1-phosphate (Glc1P) and/or of BAM3 (At4g17090) forming β-maltose. In addition, linear neutral glucans can undergo disproportionating reactions mediated by DPE1 (At5g64860) which forms α-glucose. Glc1P can be converted to glucose 6-phosphate (Glc6P)

Depending on the botanical source, the relative reserve starch content varies largely. In dry cereal seeds reserve starch can account for approximately 75 % of the grain dry weight, but in roots it is often much lower. The general biochemistry of reserve starch mobilisation appears to be similar, but contributions of distinct enzymes and/or isozymes may significantly vary. Large differences exist in the control of reserve starch degradation and its integration into the entire biology of the respective tissue.

Starch degradation is initiated by various mechanisms, such as carbon or nutrient starvation signals, a given hormone (or a combination of hormones) and/or light. As a common theme, reserve starch degradation appears to be initiated only following a major delay after the period of accumulation. Starch-storing cells may undergo an extended period of desiccation during which cells remain living or die. Desiccation does, however, not occur in other starch-storing organs, such as potato tubers. Thus, reserve starch degradation proceeds in a highly heterogeneous group of biological systems and is integrated into largely differing cellular contexts.

7.4.1.1 Reserve Starch Degradation in Dead Tissue

Typically, germinating cereal seeds degrade starch in dead tissue (Bewley 1997; Sreenivasulu and Wobus 2013). When the dry seeds are subjected to an extended period of time (often designated as after-ripening), dormancy is finally lost and the seeds massively take up water (phase I according to Bewley 1997). This process is driven by the very low matrix potential of the seed and occurs also in dead tissues. During phase I, solutes and metabolites leak into the surrounding medium. Loss of solutes is, however, terminated by rapid reorganisation of cellular membranes

Fig. 7.4 (continued) by the plastidial phosphoglucomutase isozyme (pPGM; At5g51820). The two neutral sugars and the two glucose monophosphates are final products of the plastidial starch-degrading path. Export of β-maltose and/or glucose cannot by fully compensated by other routes (Niittylä et al. 2004; Cho et al. 2011; see text)

(**b**) *Export of starch-derived products into the cytosol and their conversion into glycolytic and/or biosynthetic intermediates*. The neutral disaccharide, β-maltose and free glucose are exported into the cytosol via the transporters of the inner plastidial envelope, MEX1 (At5g17520) and Glc-T (At5g16150), respectively. Glucose 6-phosphate (Glc6P) can be exported into the cytosol in a counter-exchange to an anionic compound via a hexose phosphate transporter (such as At5g54800). (such as At5g54800; GTP1). For GTP1, phosphorylation has been empirically observed as indicated. The *Arabidopsis* genome contains a second GTP encoding gene (At1g61800; GTP2). Expression of this gene is highly variable. So far, no phosphorylation site(s) has/have been empirically confirmed for the gene product. A similar exchange has been shown for the transport of glucose 1-phosphate (Glc1P), but the transporter protein has not been identified. Pi_{cyt} and Pi_{pl}: cytosolic and plastidial orthophosphate pool, respectively. HK: cytosolic hexokinase activity (possibly AtHXK1; At4g29130). As the hexokinase protein has not unequivocally been identified, no phosphorylation sites are given. cPGM and pPGM: the cytosolic (PGM2; At1g70703) and the plastidial (PGM1; At5g51820) phosphoglucomutase isozyme, respectively. Empirically confirmed phosphorylation sites from *Arabidopsis thaliana* are marked by *red asterisks* (Data are taken from PhosphAt; see Sonnewald and Kossmann 2013; van Wijk et al. 2014)

(Bewley 1997). In phase I, protein biosynthesis largely relies on extant mRNAs. Phase II is characterised by a cessating water uptake but also by many intracellular processes that occur during this period, such as translation of newly synthesised mRNAs and of mitochondria. Phase III which actually initiates the postgermination period is characterised by massive mobilisation of storage products, such as starch, cell wall and storage proteins, taking place in the dead endosperm (Tan-Wilson and Wilson 2012). Degradation products are imported by living cells and used for biosynthetic processes and cell divisions (Bewley 1997).

Control of mobilisation of storage products is complex and not yet fully elucidated. It includes sugar and nutrient signalling and the action of antagonistic hormones, especially gibberellic acid (GA; Sreenivasulu et al. 2008; Yamaguchi 2008) and abscisic acid (ABA; Weitbrecht et al. 2011; Sun 2011; Zi et al. 2014), and downstream-acting proteins, such as a unique group of nuclear transcriptional regulators that repress GA responses and inhibit plant growth (DELLA proteins; for review see Claeys et al. 2014) and phytochrome-interacting factors (PIFs; Leivar and Quail 2011; Sun et al. 2012).

α-Amylase isozymes (and many other gene products as well) are massively synthesised in aleurone cells surrounding the starchy endosperm and are secreted into the latter. In germinating barley seeds, two classes of α-amylases (designated as HvAMY1 and HvAMY2) are synthesised, which are both more closely related to the extraplastidial α-amylase AMY1 from *Arabidopsis* rather than to the chloroplastic hydrolase, AMY3. HvAMY2 strongly interacts with native starch, whereas HvAMY1 preferentially hydrolyses linear maltodextrins, suggesting that *in planta* the two classes exert a sequential mode of action (Zeeman et al. 2010). Following the addition of GA to barley and rice aleurone cells, up to 1,300 genes are upregulated, which encode many hydrolases but also other proteins that are functionally diverse (Chen and An 2006; Tsuji et al. 2006). In barley and rice, two MYB transcription factors (designated as MYBS1 and MYBGA) appear to be of crucial importance in integrating signalling paths that are initiated by sugar and/or nutrient starvation and by GA (Hong et al. 2012). MYBGA is a GA-inducible transcription factor that binds to promotor regions of genes encoding α-amylase isozymes (and of other hydrolases as well) and thereby activates gene expression (Tsuji et al. 2006). MYBS1 is a sugar-repressible transcription factor that binds to the same promotors under sugar starvation (Lu et al. 2007).

In principle, the highly complex control of gene expression appears to balance the availability of sugars and nutrient (as formed by hydrolysis of the entire endosperm) and the varying needs of the living heterotrophic cells of the cereal seed but prevent starvation.

β-Amylases are possibly involved in cereal reserve starch degradation, but the exoamylases are deposited in an inactive state during starch granule biosynthesis (Zeeman et al. 2010). Structural features of the inhibition of the β-amylases have been elucidated (Rejek et al. 2011). As in barley and rye mutants that essentially or

completely lack β-amylases, germination appears to proceed largely unaffected and similar to the wild-type (Daussant et al. 1981; Kreis et al. 1987; Kihara et al. 1999), the actual in vivo function of the exoamylases is unclear. Possibly, they mainly act as reserve protein. An α-glucosidase (maltase) is likely to exert a dual function in cereal endosperm as it converts short dextrins to glucose and also acts directly on the starch granule surface (Zeeman et al. 2010).

Extracellular cereal reserve starch degradation apparently lacks cycles of phosphorylation and dephosphorylation of amylopectin which is an important process in turnover of assimilatory starch (see below). The genes of α-glucan phosphorylating and dephosphorylating enzymes are present in cereals (Zeeman et al. 2010), but their contribution to reserve starch metabolism remains unclear. In transgenic barley, varying levels of cereal endosperm starch phosphorylation do affect degradation during germination (Shaik et al. 2014).

During degradation of cereal reserve starch, morphology of the granules usually undergoes large alterations as hydrolysis appears to be initiated at specific sites resulting in pitting and increasing roughness of the entire surface. Hydrolysis proceeds then into interior parts of the particle, whereas other parts of the granule appear to be more resistant to hydrolysis (Shaik et al. 2014). Similar effects are observed during in vitro amylolysis of reserve starch (Blazek and Copeland 2010). Neither kinetic nor structural implications of this mode of degradation are fully understood.

7.4.1.2 Reserve Starch Degradation in Living Tissue

Although highly heterogeneous, both biochemistry and compartmentation of this group appear to be more similar to that of assimilatory starch. For living tissues degrading reserve starch, intact compartmentation is often assumed rather than proven. Starch from potato tubers is highly phosphorylated and glucosyl moieties are esterified during both starch biosynthesis and degradation (for details see Hejazi et al. 2012). Likewise, potato tubers possess both plastidial and cytosolic phosphorylase isozymes as well as cytosolic heteroglycans (see below; Fettke et al. 2008). These glycans and the disproportionating isozyme 2 (DPE2) exist also in roots of *Arabidopsis* (Malinova et al. 2011).

As starch-degrading enzymes and starch-related glycans are similar to those of leaves, reserve starch in living heterotrophic cells appears to be degraded by routes similar to those of photoautotrophic cells. Detailed biochemical analyses are, however, largely lacking. Furthermore, starch-rich heterotrophic tissues may degrade reserve starch not in a synchronised manner. If so, tissue samples consist of cells representing various states of starch metabolism and the metabolic heterogeneity would not favour detailed biochemical analyses.

7.4.2 Transitory Starch Degradation

As opposed to reserve starch, transitory starch is degraded without massive de novo biosynthesis of enzyme cleaving interglucose bonds. Furthermore, it is has not been demonstrated that catalytically inactive β-amylase(s) is included in transitory starch granules as it has been documented for cereal endosperm starch (see above).

Importantly, under a wide range of external conditions (such as length and temperature of the dark period; for leaf starch degradation under elevated night temperatures, see Glaubitz et al. 2014), assimilatory starch turnover during the entire light–dark period remains largely unchanged. As under natural conditions, increasing length of the light period is associated with decreasing length of darkness, an essentially unchanged starch turnover requires that average rates of starch synthesis are diminished and those of degradation are enhanced (Sulpice et al. 2014). In this section, we first consider biochemical processes occurring inside and outside the chloroplasts (Sect. 7.4.2.1) and then discuss regulation of transitory starch degradation (Sect. 7.4.2.2).

The organisation of a typical mesophyll cell certainly affects starch degradation. A single mesophyll cell from *Arabidopsis* leaves contains more than 100 chloroplasts but a single cytosolic compartment. Based on current knowledge, essentially all chloroplasts of a given cell degrade starch. Thus, a metabolic path consisting of both plastidial and cytosolic reactions is likely to include both multiparallel plastidial paths and a reaction sequence in a single compartment, i.e. the cytosol. This type of organisation does not favour any feedback inhibition of starch degradation unless the multiple carbon fluxes that enter the cytosol are integrated.

Within a single chloroplast, the number of starch granules ranges from approximately 5 to 7 starch granules. At the end of the light phase, both the size of the chloroplasts and the volume of the individual starch granules in a given mesophyll cell vary significantly in mature *Arabidopsis* leaves. The number of granules correlates with the volume of the respective chloroplast, but at the end of the dark period, this correlation is not observed (Crumpton-Taylor et al. 2012, 2013; Ingkasuwan et al. 2012; Ragel et al. 2013).

As in mature *Arabidopsis* leaves the number of starch granules per chloroplasts remains largely constant over the entire light–dark cycle, starch is turned over largely (or exclusively) by alterations of starch granule size. By contrast, de novo biosynthesis of granules contributes (if any) little to the light-dependent starch accumulation. Diminished granule volume is a major factor leading to decreasing chloroplast volume during darkness. Presumably, within a single chloroplast degradation is simultaneously initiated in several starch granules (and also at different sites of each starch particle). Imaging of *Arabidopsis* leaf tissue does not indicate a strictly sequential degradation of the granules (Crumpton-Taylor et al. 2012).

As opposed to mature leaves, growing tissues contain dividing chloroplasts and the number of starch granules per organ increases. In *Arabidopsis*, the soluble starch synthase isozyme 4 is likely essential for the de novo biosynthesis of starch granules

under these conditions (Crumpton-Taylor et al. 2013), but it is unknown whether the number of starch granules remains unchanged during darkness. Single cell analyses revealed that starch turnover includes stochasticity which is also inherent to gene expression and partitioning of gene products (for details see Garz et al. 2012).

7.4.2.1 Chloroplast Path of Transitory Starch Degradation

Plastidial degradation of transitory starch includes processes at the starch granule consisting of transition from a highly ordered and less hydrated to a less ordered and more hydrated state as well as those that are distant from the granule and take place in the hydrosoluble phase, i.e. the chloroplast stroma. Formation of a few intermediates that are exported into the cytosol terminates the plastidial path of starch degradation.

7.4.2.1.1 Starch-Phosphorylating Enzymes

For a detailed discussion of starch-phosphorylating enzymes, the reader is referred to Chap. 13. Here only some more general comments are given.

In living plant cells, phase transition at the surface of starch granules often proceeds by covalent starch modifications of very few glucosyl residues (by far less than 1 %) that are phosphorylated at carbon atoms 6, 3, and (at least in some cases) 2. Esterification at C6 and C3 is mediated by two starch-related dikinases (for details see Ritte et al. 2002; Ritte et al. 2004; Hejazi et al. 2012), but the enzyme(s) phosphorylating at C2 is unknown. Starch-related dikinases act in both the A- and B-type allomorph of starches of starch-like particles (Hejazi et al. 2009). Interestingly, the few red algae (such as *Galdieria sulphuraria*) forming glycogen rather than starch lack genes encoding starch-related dikinases (Shimonaga et al. 2008; Schönknecht et al. 2013). Therefore, highly ordered glucans as present in starch strictly correlate with the occurrence of starch-related dikinases.

All three monophosphorylation sites mentioned above are used in mammalian glycogen as well, but none of the phosphorylating enzyme activities have unequivocally been determined. As opposed to starch, glucosyl 6-phosphate residues are not the dominant phosphorylation sites in mammalian glycogen (Tagliabracci et al. 2011; Chikwana et al. 2013; Nitschke et al. 2013; DePaoli-Roach et al. 2015).

Although insignificant in terms of quantity, esterification appears to be sufficient to favour the action of enzymes that hydrolyse interglucose bonds (see below; Edner et al. 2007). Introduction of monophosphate esters into starch also occurs during biosynthesis (Nielsen et al. 1994; Ritte et al. 2004). *Arabidopsis* mutants containing a lower GWD content are compromised in both accumulation and degradation of starch, but the dikinase exerts little control over the total leaf starch content during darkness (Skeffington et al. 2014). GWD-deficient *Arabidopsis* mutants synthesise starch granules that differ from that of the wild-type in morphological and surface-near (bio)chemical features. By contrast, total side chain patterns obtained

for solubilised granules are similar (Mahlow et al. 2014). Thus, mutants lacking functional GWD deviate from the wild-type in both starch-related metabolic paths and some properties of the granule itself.

7.4.2.1.2 Starch-Dephosphorylating Enzymes

Seemingly paradoxical, the normal turnover of transitory starch requires both formation and hydrolysis of monophosphate esters. The directed hydrolytic degradation of α-glucan chains cannot bypass monophosphate esters (Takeda and Hizukuri 1981). Thus, α-1,4-interglucose bonds can be completely hydrolysed only if starch-related monophosphate esters are removed and de-esterification follows the phosphorylation-dependent local alterations at the granule surface.

In this review, we discuss the three putative phosphatases designated as SEX4 [*At3g52180*], **L**ike **S**ex **F**our 1 (LSF1) [*At3g01510*] and **L**ike **S**ex **F**our 2 (LSF2) [*At3g10940*], all of which reside in the plastidial compartment having direct access to both starch and starch-derived α-glucans (Silver et al. 2014). Due to the action of the putative phosphatases, esterification of starch is, to a large extent, transient (Hejazi et al. 2012; Fettke et al. 2012a), and therefore, the intramolecular phosphate patterns as revealed by carbohydrate analyses are determined by both phosphorylating and dephosphorylating activities not necessarily reflecting activity of starch-related dikinases.

The three putative phosphatases are members of the large group of phosphotyrosine phosphatases (PTPs) forming the subgroup of dual-specificity phosphatases (DSPs). The latter have been studied mainly in mammals as some DSPs, if not functional, cause severe diseases. Unlike other protein phosphatases, DSPs are capable of hydrolysing phosphate esters at both serine/threonine and tyrosine residues, and some DSPs act also on nonprotein substrates, such as phospholipids and/or phosphorylated polyglucans (Pulido and Hooft van Huijsduijnen 2008).

SEX4

SEX4 is common in higher plants. The SEX4 gene exhibits a conserved exon/intron structure and its expression is closely related to that of the two starch-related dikinases, GWP and PWD (Ma et al. 2014). The precursor sequence of AtSEX4 contains an N-terminal transit peptide, the DSP domain, and possesses a C-terminal CBM (now classified as CBM48) which is closely related to CBM20 of a starch-related dikinase, PWD (Christiansen et al. 2009). The mature SEX4 protein from *Arabidopsis thaliana* comprises 379 amino acid residues.

Originally, SEX4 was thought to primarily act on phosphoproteins but also being able to bind to starch. Because of its sequence-deduced interaction with SNF1-related protein kinases via a kinase interaction sequence (KIS), SEX4 from *Arabidopsis thaliana* was first named PTPKIS1 (Fordham-Skelton et al. 2002; Kerk et al. 2006). At approximately the same time, this protein (designated as DSP4) was reported to be redox regulated. During illumination, it was found to be associated

with starch granules but to dissociate during night. DSP4-deficient *Arabidopsis* mutants exhibited high leaf starch levels (see below) and enlarged starch granules (Sokolov et al. 2006; see also Silver et al. 2013). It remains, however, unclear how the diel intraplastidial partitioning of DSP4/SEX4 fits proposed in vivo functions.

When chemically mutagenised *Arabidopsis* lines were screened for a starch-excess phenotype, the same locus encoding the so-called SEX4 was independently identified, but the product of the gene identified lacked any known function in starch metabolism. Later studies clearly showed that the protein is capable of dephosphorylating α-polyglucans, such as amylopectins (Gentry et al. 2007; Niittylä et al. 2006). The designation SEX4 is now widely accepted.

When acting as α-glucan phosphatase in vitro, its substrate selectivity is lower than that of the starch-related dikinases. SEX4 hydrolyses both C6 and C3 monophosphates and acts on prephosphorylated starch granules (Kötting et al. 2009). It utilises prephosphorylated maltodextrins in both the insoluble and the soluble state. Dephosphorylation of the latter is almost complete, whereas insoluble maltodextrins retain approximately 50 % of the phosphate esters. This difference, however, is likely due to a structural reorganisation of the insoluble maltodextrins rather than an enzymatic property of SEX4 itself. Unlike crystalline maltodextrins, hydrosoluble neutral α-glucans act as inhibitors of the SEX4 phosphatase activity (Hejazi et al. 2010). These results suggest (but do not prove) that the *in planta* substrate of SEX4 is particulate starch.

SEX4-deficient *Arabidopsis* mutants gradually accumulate more starch as compared to the wild-type leading to elevated starch levels throughout the light–dark cycle. In absolute values, nocturnal leaf starch degradation is slower than in the wild-type. Furthermore, the AtSEX4-deficient mutant accumulates soluble phospho-oligoglucans which are undetectable in wild-type leaves. This phenotypical feature is by far more selective for SEX4 than the starch-excess (see below). The phospho-oligoglucans are likely derived from transitory starch as they are not detectable in essentially starch-free double mutants deficient in both SEX4 and the plastidial phosphoglucomutase (*pgm*). Levels of the soluble phosphoglucans are lowered (but still much higher compared to the wild-type) in double mutants lacking AtSEX4 in an *α-amylase3* (*amy3*) or *isoamylase3* (*iso3*) background. Phospho-oligoglucans remain, however, undetectable when GWD plus SEX4 are nonfunctional (Kötting et al. 2009) confirming that *in planta* AtSEX4 acts downstream of AtGWD. In summary, there is good evidence that SEX4 acts as α-glucan phosphatase, but it is still uncertain whether it also functions as phosphoprotein phosphatase.

Recently, AtSEX4 has been crystallised and the three-dimensional structure has been resolved at high resolution (Vander Kooi et al. 2010; Meekins et al. 2014). Four important results of the structural studies are mentioned. Firstly, the entire SEX4 protein possesses a highly compact structure with extensive interactions between three domains, i.e. the DSP, the CBM and a previously unrecognised C-terminal domain. Secondly, the DSP and the CBM domains directly interact and together form a continuous pocket that runs the length of the protein incorporating both catalytic and glucan binding functions. Thirdly, the C-terminal domain folds into the core of the phosphatase domain and is essential for protein stability (Vander

Kooi et al. 2010). Fourthly, AtSEX4 appears to interact with a phosphorylated α-glucan in two steps: Interaction is initiated by the CBM of AtSEX4 which non-covalently binds both to neutral and phosphorylated α-glucans (or α-glucan chains). Subsequently, DSP (which contributes little to the overall binding of the enzyme to α-glucans) positions the target carbohydrate at the active site in a way that the phosphate ester at C6 preferential is preferentially hydrolysed. In the DSP domain, two amino acid residues were identified which contribute to the preferential action of AtSEX4. Site-directed mutagenesis of these two residues increases hydrolysis of C3 monophosphate esters (Meekins et al. 2014).

Interestingly, SEX4 is common in chlorophytes but absent in prokaryotes and glycogen metabolising eukaryotes. Mammals express a DSP called laforin as, if not functional, it is associated with the Lafora disease (Minassian et al. 1998). This is a recessively inherited form of epilepsy and, probably, one of the most severe rare diseases. Patients suffering Lafora disease accumulate poorly branched but highly phosphorylated glycogen-like intracellular inclusions which are designated as Lafora bodies (Turnbull et al. 2010). Laforin is reported to be highly conserved in vertebrates but is rarely found in invertebrates (Genry and Pace 2009). The three-dimensional crystal structure of laforin has been elaborated only recently (Raththalaga et al. 2015; Sankhala et al. 2015). Structural and functional (dis)similarities of SEX4 and laforin are complex: SEX4 and laforin are certainly not orthologues as they differ in the intramolecular order of the DSP motive and the CBM (Gentry et al. 2009) and, therefore, appear to originate from independent domain fusions. In vitro, both phosphatases dephosphorylate α-glucans, such as amylopectin (Hejazi et al. 2010; Kötting et al. 2009; Worby et al. 2006; Niittylä et al. 2006; Gentry et al. 2007). Under in vivo conditions, laforin and SEX4 share functional similarities as wild-type human laforin largely complements the SEX4-deficient *Arabidopsis* mutant (Niittylä et al. 2006; Gentry et al. 2007). Laforin-deficient mice (that accumulate Lafora bodies) were, however, complemented by a mutated laforin lacking any noticeable phosphatase activity, and no Lafora bodies were observed (Gayarre et al. 2014; but see also Agualdo et al. 2010; Rao et al. 2010). These results appear to argue against the assumption that dephosphorylation of glycogen is crucial in preventing the pathogenic process. True orthologues of laforin (but no SEX4) have been reported for red algae which accumulate starch outside the chloroplast (Deschamps et al. 2008; Collén et al. 2013).

Like Sex Four 1 (LSF1)

Similarly to SEX4, LSF1 possesses a C-terminal CBM and a DSP motive that is oriented towards the N-terminal domain. In both regions, sequence similarity to SEX4 is relatively high. The sequence of LSF1 differs from that of the SEX4 and of LSF2 mainly by the approximately 200 amino acid residues large N-terminal extension whose function is unknown. The extension is also found in LSF1 proteins from other higher plant species. In lower plants, such as green and red algae, LFS1 appears to be absent (Comparat-Moss et al. 2010).

Based on the phenotpye of LSF1-deficient *Arabidopsis* mutants, leaves possess an elevated starch level but, as opposed to *AtSEX4*, no elevated phospho-oligoglucan levels. Double mutants lacking both AtSEX4 and AtLSF1 have a more severe leaf starch-excess phenotype than each parental single knockout mutant (Comparat-Moss et al. 2010). Thus, AtSEX4 and AtLSF1 are likely to exert non-identical starch-related functions. Until now, however, no enzymatic activity of plant-derived or recombinant AtLSF1 has been observed. It has, therefore, been hypothesised that the actual function of the LSF1 protein is non-catalytic, such as mediating the interaction of another protein (or other proteins) with the surface of starch granules (Comparat-Moss et al. 2010), but no target protein of AtLSF1 has been identified so far.

Like Sex Four 2 (LSF2)

Among the three plastidial DSPs in *Arabidopsis*, AtLSF2 is the smallest protein. It lacks any CBM but possesses three secondary binding sites (SBSs) which appear to functionally replace CBMs (Meekins et al. 2013). LSF2 differs from LSF1 in three aspects: Firstly, it is widely distributed in lower and higher plants. Secondly, the protein possesses a phosphatase activity using hydrosoluble phospho-oligoglucans, amylopectin and hydroinsoluble starch particles as substrates, but, unlike SEX4, it selectively hydrolyses starch-related C3 monophosphate esters. Thirdly, AtLSF2-deficient *Arabidopsis* mutants do not possess a starch-excess phenotype but possess elevated starch-related C3 monophosphate levels (Santelia et al. 2011). AtLSF2-deficient mutants do not contain a high level of phospho-oligoglucans. Constitutive double knockout *Arabidopsis* mutants deficient in both AtSEX4 and AtLSF2 have higher leaf starch content than the parental single knockout lines and are more strongly compromised in growth. Despite the massive starch-excess phenotype of the double mutant, leaf starch can be degraded at night with an absolute rate similar to that of the wild-type (Santelia et al. 2011).

Recently, the structure of AtLSF2 has been determined following crystallisation both in the presence and the absence of phosphomaltohexaose (Meekins et al. 2013). Based on this study, AtLFS2 acts on a phosphorylated single glucan chain. The phosphatase possesses an extended active-site channel which binds maltohexaose that selectively is phosphorylated at C3. Five evolutionary conserved aromatic amino acid residues interact with the glucosyl residues and thereby orient the phosphate ester towards the catalytically active site. In a series of mutated AtLSF2 molecules, single aromatic amino acids were replaced by alanine. These replacements severely affected dephosphorylation of the phosphohexaose but had little effect on the hydrolysis of the frequently used non-physiological substrate, *p*-nitrophenyl phosphate. Thus, the aromatic residues close to the active site are involved in binding the phosphorylated carbohydrate substrates. In addition, AtLSF2 possesses two other SBSs that are more distant to the catalytic site and, possibly, mediate the (simultaneous) interaction with other α-glucan chains (Meekins et al. 2013). It is, however, uncertain whether LSF2 exclusively acts on

the granule surface, on the pool of hydrosoluble phosphoglucans or on both types of substrates.

Finally, *Arabidopsis* plants can largely compensate the PWD/LSF2-dependent path of starch degradation. Mutants lacking functional PWD are capable to degrade leaf starch, although at a lower rate, and growth is only slightly reduced (for details see Hejazi et al. 2012). Likewise, mutants deficient in functional AtLSF2 possess a wild-type level of starch (Santelia et al. 2011). As revealed by the LSF2-deficient double mutants, the phenotype, however, severely deviates from the wild-type if an additional metabolic block is introduced (see above). In this case, the capacity of metabolic compensation is strongly diminished.

7.4.2.1.3 Metabolism of Hydrosoluble Starch-Derived Compounds

Neutral and phosphorylated α-glucans released from the starch granule surface (see above) are further metabolised in the stromal space. They undergo a complex net of reactions which ensures metabolic flexibility (but do not favour any efficient feedback inhibition) and in Fig. 7.4a are grouped as branched phosphoglucans, branched neutral glucans, linear neutral glucans and linear phosphoglucans. Each group consists of molecules differing in various features such as DP, the position of the branching point and/or phosphate esters. In addition, β-amylase activity releases β-maltose from the surface of the starch granules which can be directly exported to the cytosol (see below). Depending on DP and structure, the constituents of the four (phospho)oligoglucan groups are further depolymerised by hydrolytic or phosphorolytic enzymes. Furthermore, they can be hydrolysed by the plastidial α-amylase isozyme, AMY3, provided the DP and position of any phosphate ester and/or branching permits endohydrolysis. For the sake of clarity, α-amylolysis is not included in Fig. 7.4a.

Phosphorolysis is likely restricted to the pools of hydrosoluble (phospho-) oligoglucans rather than on particulate starch. At the molecular level, phosphorolysis of linear or branched oligoglucans cannot be separated from the reverse reaction, i.e. the chain elongation, and therefore, the plastidial phosphorylase (Pho1 or, in *Arabidopsis thaliana*, PHS1) diversifies the oligoglucan pools as does DPE1 when acting on the pool of linear dextrins. Finally, two neutral sugars (maltose and glucose) and two monophosphate esters of glucose (glucose 6-phosphate and glucose 1-phosphate) are formed as end products of the plastidial starch degradation (for details see Fig. 7.4a). In terms of quantity, the two neutral sugars are dominant, but the ratio between maltose and glucose is flexible.

7.4.2.1.4 Transport of Starch-Derived Products into the Cytosol

In wild-type plants, most of the starch-derived carbon is exported into the cytosol as maltose. The disaccharide is also the main compound released by darkened isolated chloroplasts (Weise et al. 2004). The maltose transporter, MEX1, has been identified

in higher and lower plants (Niittylä et al. 2004). Leaves from *Arabidopsis* mutants lacking functional AtMEX1 have highly elevated maltose levels, accumulate more starch and are compromised in growth as compared to the wild-type. Furthermore, in these mutants leaf sucrose content decreases during darkness (indicating a decreased nocturnal starch-sucrose conversion) and the gene encoding the plastidial glucose transporter (At5g16150; see below) is more strongly expressed (Cho et al. 2011). Expression of *MEX1* has been reported to be strongly affected by changes in temperature (Purdy et al. 2013).

Several lines of evidence suggest that β-maltose is the metabolically active anomeric form during starch degradation. Firstly, in leaves from *Arabidopsis thaliana* and *Phaseolus vulgaris*, the level of the α-anomer remains equal in the light and in the dark period, but β-maltose is high during darkness and low during illumination. Secondly, *Arabidopsis* mutants that accumulate only tiny amounts of starch possess very low β-maltose levels throughout the light–dark cycle. Thirdly, in wild-type plants the nocturnal β-maltose level is high in chloroplasts but low in the cytosol favouring the export of this anomer during starch degradation, but α-maltose does not form any gradient between both compartments throughout the light–dark cycle (Weise et al. 2005).

The recently identified glucose transporter is highly expressed in leaves (Cho et al. 2011). As opposed to AtMEX1-deficient lines, *Arabidopsis* mutants lacking a functional glucose transporter do not strongly deviate from the wild-type control in leaf starch content and growth suggesting compensation by other routes. Double mutants deficient in both functional maltose and glucose transporter are extremely compromised in growth. Leaves possess less chlorophyll and diminished photosynthetic capacity as compared to the wild-type. Mesophyll chloroplasts are largely deformed and subjected to degradation. Thus, the extremely reduced growth appears to be due to both disturbed starch utilisation and diminished photosynthetic activity. Growth of the double mutant is almost completely restored in the presence of exogenous sugars (Cho et al. 2011) which also prevent chlorophyll degradation.

Both massively retarded growth and chloroplast disintegration have also been reported for an *Arabidopsis* double mutant deficient in both DPE1 and MEX1 (Niittylä et al. 2004). Likewise, in two PHS1-related double mutants, *phs1/dpe2* and *phs1/mex1*, the nocturnal starch degradation is associated with a strongly impaired growth and premature chloroplast disintegration, but none of the single knockout parental lines exhibit this phenotype. Under continuous illumination, neither double mutant exhibits any of these phenotypical features (Malinova et al. 2014). These data strongly suggest that the plastidial phosphorylase isozyme, Pho1/PHS1, mediates distinct reactions during the nocturnal degradation of transitory starch.

During starch degradation, plastidial glucose monophosphate esters are exported predominantly in counter-exchange with orthophosphate. The glucose 6-phosphate/orthophosphate transporter (GTP1) has first been identified in heterotrophic tissues. In addition to orthophosphate, the transporter also utilises phosphoglycerate or triose phosphates but neither fructose 6-phosphate nor glucose 1-phosphate (Kammerer et al. 1998).

When potato tuber discs are incubated with ^{14}C-labelled glucose 1-phosphate, the uptake of label is not affected by external glucose 6-phosphate, and therefore, an additional transporter is postulated to exist. Glucose 1-phosphate is imported at a rate exceeding that of glucose and sucrose. Furthermore, imported glucose 1-phosphate appears to directly act as glucosyl donor for the ^{14}C incorporation into native starch granules (Fettke et al. 2010). By contrast, in chloroplasts isolated from *Arabidopsis* leaves, ^{14}C-glucose 1-phosphate is efficiently taken up but is then almost completely converted to ADP-glucose before the glucosyl moiety is transferred to starch (Fettke et al. 2011).

7.4.2.1.5 Cytosolic Metabolism of Starch-Derived Compounds

A mesophyll cell of higher plants typically contains a large number of chloroplasts considerably varying in size (Crumpton-Taylor et al. 2012) but a single cytosolic compartment. The export of starch-derived compounds into the cytosol links multiparallel processes to a single carbon flux. Feedback inhibitions are difficult to construct unless the cytosol monitors the carbon status of the entire cell by integrating the various fluxes from all chloroplasts. In the cytosol, major starch-derived compounds undergo a complex and highly interconnected process before they enter distinct catabolic or biosynthetic paths including cellular respiration and synthesis of cell wall materials or of sucrose.

An enzyme essential for the cytoplasmic metabolism of maltose is the disproportionating isozyme 2 (DPE2). Transcript levels decrease during dark but increase during illumination. The amount of the DPE2 protein, however, remains essentially unchanged throughout the light–dark cycle (Smith et al. 2004). Post-translational regulations are likely to occur (see Fig. 7.4b). The DPE2 monomer possesses two vicinal copies of a putative CBM20 close to the N-terminus. Truncated forms of AtDPE2 lacking CBM20s retain the disproportionating activity and exhibit a much higher affinity towards maltodextrins, but binding to high molecular weight glucans is largely diminished. Most of the DPE2 sequence is covered by the GH77 domain which, however, is interrupted by a more than 170 amino acid residue large insertion. The two copies of CBM20, the GH77 domain and the large insertion are conserved in plants (and in some non-plant organisms as well). By contrast, DPE1 and the bacterial MalQ (see below) contain an uninterrupted GH77 domain but lack any CBM20 copy (Steichen et al. 2008).

Arabidopsis mutants lacking functional AtDPE2 possess a starch-excess phenotype, reduced growth and up to two orders of magnitude higher maltose levels (Chia et al. 2004). DPE2 selectively acts on β-maltose (Dumez et al. 2006).

In an easily reversible reaction, DPE2 transfers a glucosyl residue from β-maltose to one of the nonreducing ends of a high molecular weight carbohydrate and releases the residual glucosyl moiety as free glucose (Chia et al. 2004). In vitro, glycogen is often used as glucosyl acceptor substituting the in vivo substrate, the cytosolic heteroglycans (often designated as water-soluble heteroglycan subfraction I; see

Fig. 7.2f). They are highly branched and dynamic polyglycans with a relatively wide size distribution (up to 70 kDa). Their monosaccharide pattern consists of arabinose, galactose, glucose, xylose and mannose residues resembling that of apoplastic arabinogalactans. Cytosolic heteroglycans from intact plant organs do, however, not generally interact with the synthetic β-glucosyl Yariv reagent (for review see Fettke et al. 2012a).

During the light–dark cycle, both the glucosyl content and the size distribution of the cytosolic heteroglycans alter (Fettke et al. 2012a, b). Furthermore, the glycans are not used as glucosyl acceptors by the plastidial phosphorylase isozyme (Pho1 or, in *Arabidopsis thaliana*, PHS1) nor by the main mammalian muscle glycogen phosphorylase. By contrast, the cytosolic phosphorylase isozyme (Pho2/PHS2) efficiently utilises the cytosolic heteroglycans both as glucosyl acceptor and donor. To obtain evidence under in vivo conditions, tuber slices from plants that over- or underexpress the cytosolic phosphorylase isozyme were incubated with ^{14}C-labelled glucose 1-phosphate. Incorporation of ^{14}C-labelled glucosyl residues into the cytosolic heteroglycans reflects the level of the Pho2 protein (Fettke et al. 2008). Interestingly, the DPE2-deficient *Arabidopsis* mutant possesses a several times higher level of the cytosolic phosphorylase (PHS2; Chia et al. 2004).

The flux of carbon from starch to the cytosolic heteroglycans was demonstrated using *Arabidopsis* mesophyll protoplasts photosynthetically prelabelled with ^{14}C. Following the transfer to darkness, a transient increase in the labelled glucosyl content of the heteroglycans was observed in wild-type protoplasts but not in those prepared from dpe2-deficient mutants (Malinova et al. 2013).

To some extent, DPE2 can be functionally replaced by MalQ from *E. coli*. In some *Arabidopsis* lines expressing this transferase in a DPE2-deficient background, transitory starch amounts, maltose levels and growth of the entire plants are more close to the wild-type. Likewise, expression of the cytosolic phosphorylase isozyme (AtPHS2) is lower as compared to the DPE2-deficient control. Other *Arabidopsis* lines, however, strongly resemble the phenotype of the genetic background, i.e. DPE2 deficiency. As opposed to DPE2, MalQ has a high affinity towards maltodextrins but is essentially unable to interact with the cytosolic heteroglycans (Ruzanski et al. 2013; Smirnova 2013).

Glucose directly exported from the chloroplasts or released from maltose by the DPE2-mediated glucosyl transfer is phosphorylated by the cytosolic hexokinase activity. Possibly, this activity is largely or exclusively due to a distinct cytosolic isozyme (in *Arabidopsis thaliana* AtHXI1; At4g29130). As the enzyme has not unequivocally been identified (for discussion see Claessen and Rivoal 2007; Häusler et al. 2014), no phosphorylation sites are given in Fig. 7.4b. In any case, glucose exported to the cytosol can be converted to glucose 6-phosphate and join the cytosolic pools of glucose monophosphates that are linked to the plastidial ones by the respective transporters. Thus, several routes lead to the two cytosolic glucose monophosphate pools. Glucose 6-phosphate can enter the glycolytic path but may also be converted to glucose 1-phosphate which is linked to various biosynthetic routes, such as the formation of cell wall materials or sucrose. In addition, it may also act as glucosyl donor for a transfer to the cytosolic heteroglycans (Fig. 7.4b).

7.4.2.2 Regulation of Transitory Starch Degradation

In intact plants, leaf starch is degraded under varying external conditions, such as length of and temperature during the dark period (Graf et al. 2010; Pyl et al. 2012). Irrespective of the actual growth conditions, transitory starch is almost completely mobilised at the end of the night to permit growth to be continued throughout the light and dark phase but preventing carbon starvation. It is obvious that transitory starch-degrading path(s) cannot be regulated by varying the activity of a single starch-related enzyme. Rather a complex and highly flexible regulatory system is required that includes monitoring of the actual availability of carbohydrate(s) and the subsequent balancing of starch mobilisation. Because of the organisation of mesophyll cells, it is likely that regulation of starch degradation acts (at least) on the level of the entire mesophyll cell rather than on that of individual chloroplasts. Importantly, transitory starch appears to be selectively controlled by this postulated regulatory system which prevents carbon starvation. Neither soluble low molecular weight carbohydrates nor hydrosoluble α-glucans, such as phytoglycogen, are continuously metabolised over the entire dark period.

Currently, the cellular control of transitory starch degradation is largely unknown. During recent years, however, two elements have been identified that are likely involved in the complex regulatory system, the circadian clock and trehalose 6-phosphate. Constituents of the circadian clock that control starch metabolism have been recently discussed (Graf et al. 2010; Fettke et al. 2012a).

The other compound presumably involved in the regulation of transitory starch metabolism is trehalose 6-phosphate (Tre6P). Trehalose(phosphate) is a disaccharide composed of two α-D-glucosyl residues linked via a α-1,1-bond and, therefore, lacking any reducing terminus. Typically, higher plants contain sucrose in at least hundred-fold higher molar concentration as compared to trehalose (Carillo et al. 2013). Likewise, illuminated *Arabidopsis* rosettes convert newly fixed carbon approximately four orders of magnitude faster to sucrose than to trehalose (Szecowka et al. 2013) indicating that in plants (as opposed to bacteria, fungi and invertebrates) trehalose has little relevance as carbon store, osmolyte or stress protectant as all these functions require significant amounts of the disaccharide. Despite its low abundance, trehalose-based signals are now considered to effectively control several areas of plant metabolism and development (Paul et al. 2008).

Recently, strong evidence has been provided that Tre6P acts as the actual signal metabolite. By contrast, the neutral disaccharide, trehalose, appears to be an intermediate in the degradation of the metabolic signal, Tre6P (for details see Lunn et al. 2014). Tre6P is synthesised from UDP-glucose and glucose 6-phosphate by trehalose-phosphate synthase (TPS) and dephosphorylated to yield the neutral disaccharide, trehalose, by trehalose-phosphate phosphatase (TPP). Both enzymes are widely distributed in plants. In the *Arabidopsis* genome, 11 and 10 genes putatively encoding TPSs and TPPs, respectively, have been identified (Lunn 2007): Similar isozyme numbers have also been determined in other higher plant species (Henry et al. 2014).

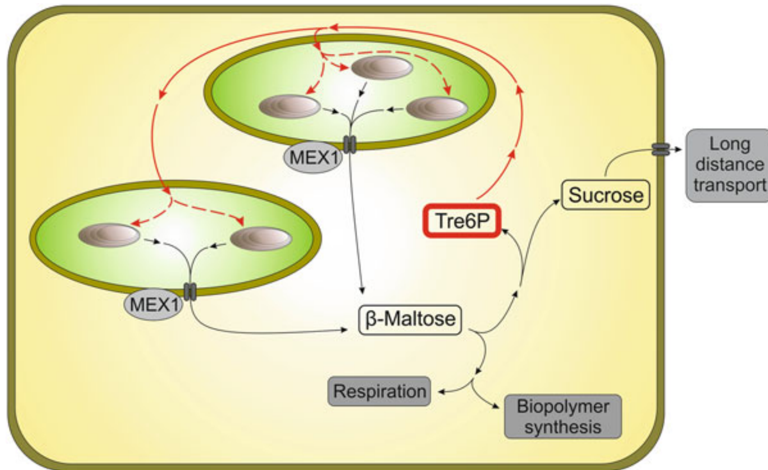

Fig. 7.5 Hypothetical regulation of transitory starch degradation
This scheme integrates some in vivo effects observed by altering Tre6P levels at night on transitory starch degradation into a hypothetical scheme of regulation of starch degradation. Two chloroplasts and the cytosol of a mesophyll cell are given. Only the quantitatively dominant product of starch degradation, β-maltose, is shown which enters the cytosol. Metabolic paths are given in *black*. Hypothetical regulatory processes are marked as closed (cytosolic) or broken (plastidial) *red line*. *MEX1* maltose transporter, *Tre6* trehalose 6-phosphate

For several reasons, Tre6P appears to be the most promising candidate controlling transitory starch degradation (Fig. 7.5). Tre6P and sucrose levels are closely related. Carbon-starved *Arabidopsis* seedlings have very low Tre6P and sucrose levels. Following the application of sucrose, Tre6P concentrations in the seedlings increase in parallel to those of sucrose, but Tre6P levels correlate to a lesser extent if other sugars are added (Lunn et al. 2014; Yadav et al. 2014). In the starch-deficient *Arabidopsis* mutant lacking a functional plastidial phosphoglucomutase (*pgm*), Tre6P levels are lower as compared to the wild-type at the end of the night but increase more strongly during light (Lunn et al. 2014). Using *Arabidopsis* lines that contain an inducible TPS gene, Tre6P contents could be experimentally altered during either the light or the dark period. In the latter case, a two- to threefold increase in Tre6P is associated with rapidly diminished rate of starch degradation and an inhibition of the nocturnal increase in maltose levels (Martins et al. 2013) suggesting that starch degradation is inhibited upstream the release of maltose. As revealed by nonaqueous localisation, Tre6P was recovered predominantly or exclusively in the cytosol. It seems, therefore, likely that Tre6P indirectly controls plastidial starch degradation. Although many steps of the Tre6P-dependent effects mentioned above are unknown, we propose a hypothetical scheme of the potential negative control that is exerted by the cytosol over plastidial starch degradation (Fig. 7.5).

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References

Aguado C, Sarkar S, Korolchuk VI et al (2010) Laforin, the most common protein mutated in Lafora disease, regulates autophagy. Hum Mol Genet 19:2867–2876

Albrecht T, Koch A, Lode A et al (2001) Plastidic (Pho1-type) phosphorylase isoforms in potato (*Solanum tuberosum* L.) plants: expression analysis and immunochemical characterization. Planta 213:602–613

Asatsuma S, Sawada C, Itoh K et al (2005) Involvement of α-amylase I-1 in starch degradation in rice chloroplasts. Plant Cell Physiol 46:858–869

Atmodjo MA, Hao Z, Mohnen D (2013) Evolving views of pectin biosynthesis. Annu Rev Plant Biol 64:747–779

Baginsky S (2009) Plant proteomics: concepts, applications, and novel strategies for data interpretation. Mass Spectrom Rev 28:93–120

Bailey JM, Whelan WJ (1961) Physical properties of starch I. Relationship between iodine stain and chain length. J Biol Chem 236:969–972

Ball S (2012) Evolution of the starch pathway. In: Tetlow IA (ed) Starch: origins, structure and metabolism, vol 5, Essential reviews in experimental biology. Society for Experimental Biology, London, pp 29–54

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

Ball S, Colleoni C, Cenci U et al (2011) The evolution of glycogen and starch metabolism gives molecular clues to understand the establishment of plastid endosymbiosis. J Exp Bot 62:1775–1801

Bertoft E (2013) On the building block and backbone concepts of amylopectin structure. Cereal Chem 90:294–311

Bewley DJ (1997) Seed germination and dormancy. Plant Cell 9:1055–1066

Bischof S, Umhang M, Eicke S et al (2013) *Cecropia peltata* accumulates starch or soluble glycogen by differentially regulation starch biosynthetic genes. Plant Cell 25:1400–1415

Blazek J, Copeland L (2010) Amylolysis of wheat starches. II. Degradation patterns of native starch granules with varying functional properties. J Cereal Sci 52:295–302

Boraston AB, Bolam DN, Gilbert HJ et al (2004) Carbohydrate-binding modules: fine-tuning polysaccharide recognition. Biochem J 382:769–781

Buléon A, Colonna P, Planchot V et al (1998) Starch granules: structure and biosynthesis. Intern J Biolo Macromol 23:85–112

Buléon A, Cotte M, Putaux J-L et al (2014) Tracking sulfur and phosphorus within single starch granules using synchrotron X-ray microfluorescence mapping. Biochim Biophys Acta 1840:113–119

Bustos R, Fahy B, Hylton CM et al (2004) Starch granule initiation is controlled by heteromultimeric isoamylase in potato tubers. Proc Natl Acad Sci U S A 101:2215–2220

Cantarel BL, Coutinho PM, Rancurel C et al (2009) The carbohydrate-active enzymes database (CAZy): an expert resource for glycogenomics. Nucleic Acids Res 37:D233–D238

Carillo P, Feil R, Gibon Y et al (2013) A fluorometric assay for trehalose in the picomole range. Plant Methods 9:21

Cenci U, Nitschke F, Steup M et al (2014) Transition from glycogen to starch metabolism in archaeplastida. Trends Plant Sci 19:18–28

Chen K, An Y-QC (2006) Transcriptional responses to gibberellin and abscisic acid in barley aleurone. J Integr Plant Biol 48:591–612

Chia T, Thorneycroft D, Chapple A et al (2004) A cytosolic glucosyl transferase is required for conversion of starch to sucrose in *Arabidopsis* leaves at night. Plant J 37:853–863

Chikwana VM, Khanna M, Baskaran S et al (2013) Structural basis for 2′-phosphate incorporation into glycogen by glycogen synthase. Proc Natl Acad Sci U S A 110:20976–20981

Cho M-H, Lim H, Shin DH et al (2011) Role of the plastidic glucose transporter in the export of starch degradation products from the chloroplasts in *Arabidopsis thaliana*. New Phytol 190:101–112

Christiansen C, Hachem NA, Glaring MA et al (2009) A CBM20 low-affinity starch-binding domains from glucan, water dikinase. FEBS Lett 583:1159–1163

Claessen É, Rivoal J (2007) Isoenzymes of plant hexokinase: occurrence, properties and functions. Phytochemistry 68:709–713

Claeys H, De Bodt S, Inzé D (2014) Gibberellins and DELLAs: central nodes in growth regulatory networks. Trends Plant Sci 19:231–239

Collén J, Porcel B, Carré W et al (2013) Genome structure and metabolic features in the red seaweed *Chondrus crispus* shed light on evolution of the Archaeplastida. Proc Natl Acad Sci U S A 110:5247–5252

Comparat-Moss S, Kötting O, Stettler M et al (2010) A putative phosphatase, LSF1, is required for normal starch turnover in *Arabidopsis* leaves. Plant Physiol 152:685–697

Crumpton-Taylor M, Grandison S, Png KMY et al (2012) Control of starch granule number in Arabidopsis chloroplasts. Plant Physiol 158:905–916

Crumpton-Taylor M, Pike M, Lu K-J et al (2013) Starch synthase 4 is essential for coordination of starch granule formation with chloroplast division during Arabidopsis leaf expansion. New Phytol 200:1064–1074

Cuyvers S, Dornez E, Delcour JA et al (2012) Occurrence and functional significance of secondary carbohydrate binding sites in glycoside hydrolases. Crit Rev Biotechnol 32:93–107

Daussant J, Zbaszyniak B, Sadowski J et al (1981) Cereal β-amylase: immunochemical study on two enzyme-deficient inbred lines of rye. Planta 151:176–179

De Schepper V, De Swaef T, Bauweraerts I et al (2013) Phloem transport: a review of mechanisms and controls. J Exp Bot 64:4839–4850

Delatte T, Trevisan M, Parker M et al (2005) Arabidopsis mutants Atisa1 and Atisa2 have identical phenotypes and lack the same multimeric isoamylase, which influences the branch point distribution of amylopectin during starch synthesis. Plant J 41:815–830

Delatte T, Umhang M, Trevisan M et al (2006) Evidence for distinct mechanisms of starch granule breakdown. J Biol Chem 281:12050–12059

Denison FC, Paul A-L, Zupanska AK et al (2011) 14-3-3 proteins in plant physiology. Semin Cell Dev Biol 22:720–727

DePaoli A, Contreras CJ, Segvich DM et al (2015) Glycogen phosphomonoester distribution in mouse models of the progressive myoclonic epilepsy, Lafora disease. J Biol Chem 290:841–850

Deschamps P, Colleoni C, Nakamura Y et al (2008) Metabolic symbiosis and the birth of the plant kingdom. Mol Biol Evol 25:536–548

Dippel R, Boos W (2005) The maltodextrin system of *Escherichia coli*. Metabolism and transport. J Bacteriol 187:8322–8331

Dumez S, Wattebled F, Dauvillee D et al (2006) Mutants of *Arabidopsis* lacking starch branching enzyme II substitute plastidial starch synthesis by cytoplasmic maltose accumulation. Plant Cell 18:2694–2709

Edner C, Li J, Albrecht T, Mahlow S et al (2007) Glucan, water dikinase activity stimulates breakdown of starch granules by plastidial β-amylases. Plant Physiol 145:17–28

Emes MJ, Tetlow IJ (2012) The role of heteromeric protein complexes in starch synthesis. In: Tetlow IJ (ed) Starch: origins, structure and metabolism, Essential reviews in experimental biology. Society for Experimental Biology, London, pp 255–278

Eveland AL, Jackson DP (2012) Sugars, signalling, and plant development. J Exp Bot 63:3367–3377

Facchinelli F, Colleoni C, Ball SG et al (2013) Chlamydia, cyanobion, or host: who was on top in the ménage à trios? Trends Plant Sci 18:673–679

Facon M, Lin Q, Azzaz AM et al (2013) Distinct functional properties if isoamylase-type starch debranching enzymes in monocot and dicot leaves. Plant Physiol 163:1363–1375

Fazedas E, Szabó K, Kandra L et al (2013) Unexpected mode of action of sweet potato β-amylase on maltooligomers. Biochim Biophys Acta 1834:1976–1981

Fettke J, Eckermann N, Poeste S et al (2004) The glycan substrate of the cytosolic (pho2) phosphorylase isozyme from *Pisum sativum* L.: identification, linkage analysis and subcellular localization. Plant J 39:933–946

Fettke J, Eckermann N, Tiessen A et al (2005a) Identification, subcellular localization and biochemical characterization of water-soluble heteroglycans (SHG) in leaves of *Arabidopsis thaliana* L.: distinct SHG reside in the cytosol and in the apoplast. Plant J 43:568–586

Fettke J, Poeste S, Eckermann N et al (2005b) Analysis of cytosolic heteroglycans from leaves of transgenic potato (*Solanum tuberosum* L.) plants that under- or over-express the Pho2 phosphorylase isozyme. Plant Cell Physiol 46:1987–2004

Fettke J, Chia T, Eckermann N et al (2006) A transglucosidase necessary for starch degradation and maltose metabolism in leaves at night acts on cytosolic heteroglycans (SHG). Plant J 46:668–684

Fettke J, Nunes-Nesi A, Alpers J et al (2008) Alterations in cytosolic glucose-phosphate metabolism affect structural features and biochemical properties of starch-related heteroglycans. Plant Physiol 148:1614–1629

Fettke J, Hejazi M, Smirnova J et al (2009) Eukaryotic starch degradation: integration of plastidial and cytosolic pathways. J Exp Bot 60:2907–2922

Fettke J, Albrecht T, Hejazi M et al (2010) Glucose 1-phosphate is efficiently taken up by potato (*Solanum tuberosum*) tuber parenchyma cells and converted to reserve starch granules. New Phytol 185:663–675

Fettke J, Malinova I, Albrecht T et al (2011) Glucose 1-phosphate transport into protoplasts and chloroplasts from leaves of *Arabidopsis*. Plant Physiol 155:1723–1734

Fettke J, Fernie AR, Steup M (2012a) Transitory starch and its degradation in higher plants. In: Tetlow IJ (ed) Starch: origins, structure and metabolism, vol 5, Essential reviews in experimental biology. Society for Experimental Biology, London, pp 311–374

Fettke J, Leifels L, Brust H et al (2012b) Two carbon fluxes to reserve starch in potato (*Solanum tuberosum* L.) tuber cells are closely interconnected but differently modulated by temperature. J Exp Bot 63:3011–3029

Fontes CMGA, Gilbert HJ (2010) Cellulosomes: highly efficient nanomachines designated to deconstruct plant cell wall complex carbohydrates. Annu Rev Biochem 79:655–681

Fordham-Skelton AP, Chilley P, Lumbreras V et al (2002) A novel higher plant protein tyrosine phosphatase interacts with SNF1-related protein kinases via a KIS (kinase interaction sequence) domain. Plant J 29:705–715

Fu F-F, Xue H-W (2010) Coexpression analyses identifies rice starch regulator1, a rice AP2/EREBP family transcription factor, as a novel rice starch biosynthesis regulator. Plant Physiol 154:927–938

Fujita N, Nakamura Y (2012) Distinct and overlapping functions of starch synthase isoforms. In: Tetlow IA (ed) Starch: origin, structure and metabolism, vol 5, Essential reviews in experimental biology. Society for Experimental Biology, London, pp 115–140

Fujita N, Kubo A, Suh DS et al (2003) Antisense inhibition of isoamylase alters the structure of amylopectin and physicochemical properties of starch in rice endosperm. Plant Cell Physiol 44:607–618

Fujita N, Toyosawa Y, Yoshinori U et al (2009) Characterization of pullulanase (PUL)-deficient mutants of rice (*Oryza sativa* L.) and the function of PUL on starch biosynthesis in the developing rice endosperm. J Exp Bot 60:1009–1023

Fulton DC, Stettler M, Mettler T et al (2008) Beta-AMYLASE4, a noncatalytic protein required for starch breakdown, acts upstream of the active beta-amylases in *Arabidopsis* chloroplasts. Plant Cell 20:1040–1058

Garz A, Sandmann M, Rading M et al (2012) Cell-to-cell diversity in a synchronized Chlamydomonas culture as revealed by single cell analyses. Biophys J 103:1078–1086

Gayarre J, Duran-Trío L, Garcia OC et al (2014) The phosphatase activity of laforin is dispensable to rescue EPM2a$^{-/-}$ mice from Lafora disease. Brain 137:806–818

Gentry MS, Dowen RH III, Worby CA et al (2007) The phosphatase laforin crosses evolutionary boundaries and links carbohydrate metabolism to neutral disease. J Cell Biol 178:477–488

Gentry MS, Dixon JE, Worby CA (2009) Lafora disease: insights into neurodegeneration from plant metabolism. Trends Biochem Sci 34:628–639

Gentry MS, Pace RM (2009) Conservation of the glucan phosphatase laforin is linked to rates of molecular evolution and the glucan metabolism of the organism. BMC Evol Biol 9:138

Gentry MS, Romá-Mateo C, Sanz P (2013) Laforin, a protein with many faces: glucan phosphatase, adapter protein, et alii. FEBS J 280:525–537

Gilbert HJ (2010) The biochemistry and structural biology of plant cell wall deconstruction. Plant Physiol 153:444–455

Glaubitz U, Li X, Köhl K et al (2014) Differential physiological responses of different rice (*Oryza sativa*) cultivars to elevated night temperature during vegetative growth. Funct Plant Biol 41:437–448

Goldberg RN, Bell D, Tewari YB et al (1991) Thermodynamics of hydrolysis of oligosaccharides. Biophys Chem 40:69–76

Graf A, Smith AM (2011) Starch and the clock: the dark side of the plant productivity. Trends Plant Sci 16:169–175

Graf A, Schlereth A, Stitt M et al (2010) Circadian control of carbohydrate availability for growth in *Arabidopsis* plants at night. Proc Natl Acad Sci U S A 107:9458–9463

Guillén D, Sánchez S, Rodríguez-Sanoja R (2010) Carbohydrate-binding domains: multiplicity of biological roles. Appl Microbiol Biotechnol 85:1241–1249

Häusler RE, Heinrichs L, Schmitz J et al (2014) How sugars might coordinate chloroplast and nuclear gene expression during acclimation to high light intensities. Mol Plant 7:1121–1137

Heazlewood JL, Durek P, Hummel J et al (2009) PhosPhAt: a database of phosphorylation sites in Arabidopsis thaliana and a plant-specific phosphorylation site predictor. Nucleic Acid Res 36:D1015–D1021

Hehre EJ, Brewer CF, Henghof DS (1979) Scope and mechanism of carbohydrase action. Hydrolytic and nonhydrolytic actions of beta-amylase on alpha- and beta-maltosyl fluoride. J Biol Chem 254:5942–5950

Hehre EJ, Kitabata S, Brewer CF (1986) Catalytic flexibility of glycosidases. The hydration of maltal by β-amylase to form 2-deoxymaltose. J Biol Chem 261:2147–2153

Hejazi M, Fettke J, Haebel S et al (2008) Glucan, water dikinase phosphorylates crystalline maltodextrins and thereby initiates solubilisation. Plant J 55:323–334

Hejazi M, Fettke J, Paris O et al (2009) The two plastidial starch-related dikinases sequentially phosphorylate glucosyl residues at the surface of both the A- and the B-type allomorphs of crystalline maltodextrins but the mode of action differs. Plant Physiol 150:962–976

Hejazi M, Fettke J, Kötting O et al (2010) The laforin-like dual-specificity phosphatase SEX4 from Arabidopsis hydrolyses both C6- and C3-monophosphate esters introduced by starch-related dikinases and thereby affects phase transition of alpha-glucans. Plant Physiol 152:711–722

Hejazi M, Fettke J, Steup M (2012) Starch phosphorylation and dephosphorylation: the consecutive action of starch-related dikinases and phosphatases. In: Tetlow IA (ed) Starch: origins, structure and metabolism, vol 5, Essential reviews in experimental biology. Society for Experimental Biology, London, pp 279–309

Henry C, Bledsoe SW, Siekman A et al (2014) The trehalose pathway in maize: conservation and gene regulation in response to the diurnal cycle and extended darkness. J Exp Bot 65:5959–5973

Hong YF, Ho T-HD, Wu CF et al (2012) Convergent starvation signals and hormone crosstalk in regulating nutrient mobilization upon germination in cereals. Plant Cell 24:2857–2873
Huang X-F, Nazarin-Fironzabadi F, Vincken J-P et al (2014) Expression of an amylosucrase in potato results in larger starch granules with novel properties. Planta 240:409–421
Hussain H, Mant A, Seale R et al (2003) Three isoforms of isoamylase contribute different catalytic properties for the debranching of potato glucans. Plant Cell 15:133–149
Hwang SK, Nishi A, Satoh H et al (2010) Rice endosperm-specific plastidial alpha-phosphorylase is important for synthesis opf short-chain malto-oligosaccharides. Arch Biochem Biophys 495:82–92
Ingkasuwan P, Netrphan S, Prasitwattanaseree S et al (2012) Inferring transcriptional gene regulation network of starch metabolism in Arabidopsis thaliana leaves using graphical Gaussian model. MBC Syst Biol 6:100
Ishikawa K, Nakatani H, Katsuya Y et al (2007) Kinetic and structural analysis of enzyme sliding on a substrate: multiple attack in β-amylase. Biochemistry 46:792–798
Isshiki M, Matsuda Y, Takasaki A et al (2008) *Du3*, a mRNA cap-binding protein gene, regulates amylose content in Japonica rice seeds. Plant Biotechnol 25:483–487
Izumi M, Hidema J, Makino A et al (2013) Autophagy contributes to nighttime energy availability for growth in Arabidopsis. Plant Physiol 161:1682–1693
Jane JL, Kasemsuwaran T, Leas S et al (1994) Anthology of starch granule morphology by scanning electron microscopy. Starch-Starke 46:121–129
Kainuma K, French D (1970) Action of pancreatic alpha-amylase and sweet potato beta-amylase on 6^2 and 6^2 α-glucosylmaltooligosaccharides. FEBS Lett 6:182–186
Kammerer B, Fischer K, Hilpert B et al (1998) Molecular characterization of a carbon transporter in plastids from heterotrophic tissues: the glucose 6-phosphate/phosphate antiporter. Plant Cell 10:105–117
Kartal Ö, Mahlow S, Skupin A et al (2011) Carbohydrate-active enzymes exemplify entropic principles in metabolism. Mol Syst Biol 7:542
Keeling PL, Myers AM (2010) Biochemistry and genetics of starch synthesis. Annu Rev Food Sci 1:271–303
Kerk D, Conley TR, Rodriguez FA et al (2006) A chloroplast-localized dual-specificity protein phosphatase in Arabidopsis contains a phylogenetically dispersed and ancient carbohydrate-binding module, which binds the polysaccharide starch. Plant J 46:400–413
Kiessling LL, Young T, Gruber TD et al (2008) Multivalency in protein-carbohydrate recognition. In: Fraser-Reid B, Tatsuata K, Thiem J (eds) Glycoscience. Springer, Berlin/Heidelberg, pp 2483–2523
Kihara M, Kaneko T, Ito K et al (1999) Geographic variation of β-amylase thermostability among varieties of barley (*Hordeum vulgare*) and β-amylase deficiency. Plant Breed 118:453–455
Kim T-J, Kim M-J, Kim B-C et al (1999) Modes of action of acarbose hydrolysis and transglycosylation catalyzed by a thermo-stable maltogenic amylase, the gene for which was cloned from a Thermus strain. Appl Environ Microbiol 65:1644–1651
Kötting O, Santelia D, Edner C et al (2009) STARCH-EXCESS4 is a laforin-like phosphoglucan phosphatase required for starch degradation in *Arabidopsis thaliana*. Plant Cell 21:334–346
Kötting O, Kossmann J, Zeeman SC et al (2010) Regulation of starch metabolism: the age of enlightenment? Curr Opin Plant Biol 13:321–329
Kramhøft B, Bak-Jensen KS, Mori H et al (2005) Multiple attack, kinetic parameters, and product profiles in amylose hydrolysis by barley α-amylase 1 variants. Biochemistry 44:1824–1832
Kreis M, Williamson M, Buxton B et al (1987) Primary structure and differential expression of β-amylase in normal and mutant barley. Eur J Biochem 169:517–525
Kubo A, Rahman S, Utsumi Y et al (2005) Complementation of *sugary-1* phenotype in rice endosperm with the wheat *isoamylase1* gene supports a direct role for isoamylase1 in amylopectin biosynthesis. Plant Physiol 137:43–56
Kubo A, Colleoni C, Dinges J et al (2010) Functions of heteromeric and homomeric isoamylase-type starch-debranching enzymes in developing maize endosperm. Plant Physiol 153:956–969

Lastdrager J, Hanson J, Smeekens S (2014) Sugar signals and the control of plant growth and development. J Exp Bot 65:799–807

Leivar P, Quail PH (2011) PIFs: pivotal components in a cellular signaling hub. Trends Plant Sci 2011:19–28

Li J, Francisco P, Zhou W, Edner C et al (2009) Catalytically-inactive β-amylase BAM4 required for starch breakdown in Arabidopsis leaves is a starch-binding protein. Arch Biochem Biophys 489:92–98

Liu Y, Bassham DC (2012) Autophagy: pathways for self-eating in plant cells. Annu Rev Plant Biol 63:215–237

Lloyd JR, Kossmann J (2015) Transitory and storage starch metabolism: two sides do the same coin? Curr Opin Biotechnol 32:143–148

Lohmeyer-Vogel EM, Kerk D, Nimick M et al (2008) *Arabidopsis At5g39790* encodes a chloroplast-localized carbohydrate-binding coiled-coil domain-containing putative scaffold protein. BCM Plant Biol 8:120

López CA, de Vries AH, Marrink SJ (2012) Amylose folding under the influence of lipids. Carbohydr Res 364:1–7

Lu CA, Lin CC, Lee KW et al (2007) The SnRK1A protein kinase plays a key role in sugar signaling during germination and seedling growth of rice. Plant Cell 19:2484–2499

Luís AS, Venditto I, Temple MJ et al (2013) Understanding how noncatalytic carbohydrate binding modules can display specificity for xyloglucan. J Biol Chem 288:4799–4809

Lunn JE (2007) Gene families and evolution of trehalose metabolism in plants. Funct Plant Biol 34:550–563

Lunn JE, Delorge I, Figueroa CM et al (2014) Trehalose metabolism in plants. Plant J 79: 544–567

Ma J, Jiang Q-T, Wei L et al (2014) Conserved structure and varied expression reveal key roles of phosphoglucan phosphatase gene starch excess 4 in barley. Planta 240:1179–1190

Mahlow S, Hejazi M, Kuhnert F et al (2014) Phosphorylation of transitory starch by α-glucan, water dikinase during starch turnover affects the surface properties and morphology of starch granules. New Phytol 203:495–507

Malinova I, Steup M, Fettke J (2011) Starch related heteroglycans in roots from *Arabidopsis thaliana*. J Plant Physiol 168:1406–1414

Malinova I, Steup M, Fettke J (2013) Carbon transitions from either Calvin cycle or transitory starch to heteroglycans as revealed by ^{14}C-labeling experiments using protoplasts from Arabidopsis. Physiol Plant 149:25–44

Malinova I, Mahlow S, Alseekh S et al (2014) Double knock-out mutants of Arabidopsis thaliana grown under normal conditions reveal that the plastidial phosphorylase isozyme (PHS1) participates in transitory starch metabolism. Plant Physiol 164:607–621

Martins MCM, Hejazi M, Fettke J et al (2013) Feedback inhibition of starch degradation in Arabidopsis leaves mediated by trehalose 6-phosphate. Plant Physiol 163:1142–1163

Matsushima R, Maekawa M, Kurano M et al (2014) Amyloplast-localized SSG4 protein influences the size of starch grains in rice endosperm. Plant Physiol 164:623–636

Meekins DA, Guo H-F, Husodo S et al (2013) Structure of the *Arabidopsis* glucan phosphatase LIKE SEX FOUR2 reveals a unique mechanism for starch dephosphorylation. Plant Cell 25:2302–2314

Meekins DA, Raththagala M, Husodo S et al (2014) Phosphoglucan-bound structure of starch phosphatase starch excess4 reveals the mechanism for C6 specificity. Proc Natl Acad Sci U S A 111:7272–7277

Meléndez-Hevia E, Waddell TG, Shelton ED (1993) Optimization of molecular design in the evolution of metabolism: the glycogen molecule. Biochem J 295:477–483

Mikami B, Degano M, Hehre EJ et al (1994) Crystal structure of soybean β-amylase reacted with β-maltose and maltal: active site components and their apparent role in catalysis. Biochemistry 33:7779–7787

Minassian BA, Lee JR, Herbick JA et al (1998) Mutations in a gene encoding a novel protein tyrosine phosphatase cause progressive myoclonus epilepsy. Nat Genet 20:171–174

Mizushima N, Yoshimori T, Ohsumi Y (2011) The role of Atg proteins in autophagosome formation. Annu Rev Cell Dev Biol 27:107–132

Moghaddam MRB, Van den Ende W (2012) Sugar and plant immunity. J Exp Bot 63:3989–3998

Nielsen TH, Wischmann B, Enevoldsen K et al (1994) Starch phosphorylation in potato tubers proceeds concurrently with de novo biosynthesis of starch. Plant Physiol 105:111–117

Nielsen MM, Bozonnet S, Seo ES et al (2009) Two secondary carbohydrate binding sites on the surface of barley alpha-amylase I have distinct functions and display synergy in hydrolysis of starch granules. Biochemistry 48:7686–7697

Nielsen JW, Kramhøft B, Boyonnet S et al (2012) Degradation of the starch components amylopectin and amylose by barley α-amylase 1: role of surface binding site 2. Arch Biochem Biophys 528:1–6

Niittylä T, Messerli G, Trevisan M et al (2004) A previously unknown maltose transporter essential for starch degradation in leaves. Science 303:87–89

Niittylä T, Comparat-Moss S, Lue WL et al (2006) Similar protein phosphatases control starch metabolism in plants and glycogen metabolism in mammals. J Biol Chem 281:11815–11818

Nishimo H, Murakawa A, Mori T et al (2004) Kinetic studies of AMP-dependent phosphorolysis of amylopectin catalyzed by phosphorylase b on a 27 MHz microbalance quartz-crystal. J Am Chem Soc 126:14752–14757

Nishiyama Y, Mazeau K, Morin M et al (2010) Molecular and crystal structure of 7-fold V-amylose complexed with 2-propanol. Macromolecules 43:8628–8636

Nitschke F, Wang P, Schmieder P et al (2013) Hyperphosphorylation of glucosyl C6 carbons and altered structure of glycogen in the neurodegenerative epilepsy Lafora disease. Cell Metab 17:756–767

Nougué O, Corbi J, Ball SG et al (2014) Molecular evolution accompanying functional divergence of duplicated genes along the plant starch biosynthesis path. BMC Evol Biol 14(1):103. doi: 10.1186/1471-2148-103

Pal SK, Liput M, Piques M et al (2013) Diurnal changes of polysome loading track sucrose content in the rosette of wild-type Arabidopsis and the starchless *pgm* mutant. Plant Physiol 162:1246–1265

Palm DC, Rohwer JM, Hofmeyr J-HS (2013) Regulation of glycogen synthase from mammalian skeletal muscle – a unifying view of allosteric and covalent regulation. FEBS J 280:2–27

Paparelli E, Parlanti S, Gonzali S et al (2013) Nighttime sugar starvation orchestrates gibberellin biosynthesis and plant growth in Arabidopsis. Plant Cell 25:3760–3769

Park J-T, Shim J-H, Tran P et al (2011) Role of maltose enzymes in glycogen synthesis by *Escherichia coli*. J Bacteriol 193:2517–2526

Paul MJ, Primavesi F, Jhurreea D et al (2008) Trehalose metabolism and signalling. Annu Rev Plant Biol 59:417–441

Payne CM, Baban J, Horn SJ et al (2012) Hallmarks of processivity in glycoside hydrolases from crystallographic and computational studies of the *Serratia marcescens* chitinases. J Biol Chem 287:36322–36330

Payne CM, Resch MG, Chen L et al (2013) Glycosylated linkers in multimodular lignocellulose-degrading enzymes dynamically bind to cellulose. Proc Natl Acad Sci U S A 110:14646–14651

Pederson BA, Turnbull J, Epp JR et al (2013) Inhibiting glycogen synthesis prevents Lafora disease in a mouse model. Ann Neurol 74:297–300

Peng M, Gao M, Båga M et al (2000) Starch-branching enzymes preferentially associated with A-type starch granules in wheat endosperm. Plant Physiol 124:265–272

Peng C, Wang Y, Liu F et al (2014) *FLOURY ENDOSPERM6* encodes a CBM48 domain-containing protein involved in compound granule formation and starch synthesis in rice endosperm. Plant J 77:917–930

Pérez S, Bertoft E (2010) The molecular structure of starch components and their contribution to the architecture of starch granules: a comprehensive review. Starch-Starke 62:389–420

Pfister B, Lu K-J, Eicke S et al (2014) Genetic evidence that chain length and branch point distributions are linked determinants of starch granule formation in Arabidopsis. Plant Physiol 165:1467–1474

Pulido R, Hooft van Huijsduijnen R (2008) Protein tyrosine phosphatases: dual-specificity phosphatases in health and disease. FEBS J 275:848–866

Purdy SJ, Bussell JD, Nunn CP et al (2013) Leaves from the Arabidopsis maltose exporter1 mutant exhibits a metabolic profile with features of cold acclimation in the warm. PLoS ONE 8:e79412

Putaux J-L, Montesanti N, Véronèse G et al (2011) Morphology and structure of A-amylose single crystals. Polymer 52:2198–2205

Pyl E-T, Piques M, Ivakov A et al (2012) Metabolism and growth in *Arabidopsis* depend on the daytime temperature but are temperature-compensated against cool nights. Plant Cell 24:2443–2469

Qian M, Nahoum V, Bumiel J et al (2001) Enzyme-catalyzed condensation reaction in a mammalian α-amylase. High-resolution structural analysis of an enzyme-inhibitor complex. Biochemistry 40:7700–7709

Ragauskas AJ, Williams CK, Davison BH et al (2006) The path for biofuels and biomaterials. Science 311:484–489

Ragel P, Streb S, Feil R et al (2013) Loss of starch granule initiation has a deleterious effect on the growth of Arabidopsis plants due to an accumulation of ADP-glucose. Plant Physiol 163:75–85

Rao SNR, Maity R, Sharma J et al (2010) Sequestration of chaperones and proteasome into Lafora bodies and proteasomal dysfunction induced by Lafora disease-associated mutations. Hum Mol Genet 19:4726–4734

Raththagala M, Brewer MK, Parker MW et al (2015) Structural mechanism of laforin function in glycogen dephosphorylation and Lafora disease. Mol Cell 57:261–272

Regina A, Blazek J, Gilbert E et al (2012) Differential effects of genetically distinct mechanisms of elevating amylose on barley starch characteristics. Carbohydr Polym 89:979–991

Reiland S, Messerli G, Baerenfaller K et al (2009) Large-scale Arabidopsis phosphoproteome profiling reveals novel chloroplast-kinase substrates and phosphorylation networks. Plant Physiol 150:889–903

Reinhold H, Soyk S, Šimková K et al (2011) β-amylase-like proteins function as transcription factors in Arabidopsis, controlling shoot growth and development. Plant Cell 23:1391–1403

Rejek M, Stevenson CE, Southard AM et al (2011) Chemical genetics and cereal starch metabolism: structural basis of the non-covalent and covalent inhibition of barley β-amylase. Mol BioSyst 7:718–730

Ritte G, Lloyd JR, Eckermann N et al (2002) The starch-related R1 protein is an alpha-glucan, water dikinase. Proc Natl Acad Sci U S A 99:7166–7171

Ritte G, Scharf A, Eckermann N et al (2004) Phosphorylation of transitory starch is increased during degradation. Plant Physiol 135:2068–2077

Roach PJ, DePaoli AA, Hurley TD et al (2012) Glycogen and its metabolism: some new developments and old themes. Biochem J 441:763–787

Roberts EH (1973) Predicting the storage life of seeds. Seed Sci Technol 1:499–514

Roblin P, Potocki-Véronèse G, Guieysse D et al (2013) SAXS Conformational tracking of amylose synthesized by amylosucrase. Biomacromolecules 14:232–239

Ruan Y-L (2014) Sucrose metabolism: gateway to diverse carbon use and sugar signaling. Annu Rev Plant Biol 65:26.1–26.35

Ruzanski C, Smirnova J, Rejzek M et al (2013) A bacterial glucanotransferase can replace the complex maltose metabolism required for starch-to-sucrose conversion in leaves at night. J Biol Chem 288:28581–28598

Ryu J-H, Drain J, Kim JH et al (2009) Comparative structural analyses of purified glycogen particles from rat liver, human skeletal muscle and commercial preparations. Int J Biol Macromol 45:478–482

Salazar C, Höfer T (2009) Multiple protein phosphorylation – from molecular mechanisms to kinetic models. FEBS J 276:3177–3198

Sankhala RS, Koksai AC, Ho L et al (2015) Dimeric quarternary structure of human laforin. J Biol Chem 290:4552–4559

Santelia D, Kötting O, Seung D et al (2011) The phosphoglucan phosphatase like SEX Four2 dephosphorylates starch at the C3-position in *Arabidopsis*. Plant Cell 23:4096–4111

Satoh H, Shibahara K, Tokunaga T et al (2008) Mutation of the plastidial alpha-glucan phosphorylase gene in rice affects the synthesis and structure of starch in the endosperm. Plant Cell 20:1833–1849

Scheidig A, Fröhlich A, Schulze S et al (2002) Downregulation of a chloroplast-targeted β-amylase leads to a starch-excess phenotype in leaves. Plant J 30:581–591

Schmitz J, Heinrichs L, Scossa F et al (2014) The essential role of sugar metabolism in the acclimation response of *Arabidopsis thaliana* to high light intensities. J Exp Bot 65:1619–1636

Schönknecht G, Chen W-H, Ternes CM et al (2013) Gene transfer from bacteria and archaea facilitated evolution of an extremophilic eukaryote. Science 339:1207–1210

Shaik SS, Carciofi M, Martens HJ et al (2014) Starch bioengineering affects cereal grain germination and seedling establishment. J Exp Bot 65:2257–2270

Shang BZ, Chang R, Chu J-W (2013) Systems-level modelling with molecular resolution elucidates the rate-limiting mechanisms of cellulose decomposition by cellobiohydrolases. J Biol Chem 288:29081–29089

Shimonaga T, Konishi M, Oyama Y et al (2008) Variation in storage α-glucans of the Porphyridiales (Rhodophyta). Plant Cell Physiol 49:103–116

Silver DM, Silva LP, Issakidis-Bourguet E et al (2013) Insight into the redox regulation of the phosphoglucan phosphatase SEX4 involved in starch degradation. FEBS J 280:538–548

Silver DM, Kötting O, Moorhead GB (2014) Phosphoglucan phosphatase function sheds light on starch degradation. Trends Plant Sci 19:471–478

Sim L, Beeren SR, Findinier J et al (2014) Crystal structure of the Chlamydomonas starch debranching isoamylase ISA1 reveals insights into the mechanism of branch trimming and complex assembly. J Biol Chem 289:22991–23003

Skeffington AW, Graf A, Duxbury Z et al (2014) Glucan, water dikinase exerts little control over starch degradation in Arabidopsis leaves at night. Plant Physiol 165:866–879

Smirnova J (2013) Carbohydrate-active enzymes metabolising maltose: kinetic and structural features. Dissertation, University of Potsdam

Smith SM, Fulton DC, Chia T et al (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

Sokolov LN, Dominguez-Solis JR, Allary AL et al (2006) A redox-regulated chloroplast protein phosphatase binds to starch diurnally and functions in its accumulation. Proc Natl Acad Sci USA 103:9732–9737

Sonnewald U, Kossmann J (2013) Starches – from current models to genetic engineering. Plant Biotechnol J 11:223–232

Soyk S, Šimková K, Zürcher E et al (2014) The enzyme-like domain of Arabidopsis nuclear β-amylases is critical for DNA sequence recognition and transcriptional activity. Plant Cell 26:1746–1763

Sparks E, Wachsmann G, Benfey PN (2013) Spatiotemporal signaling in plant development. Nat Rev Genet 14:631–644

Sreenivaculu N, Usadel B, Winter A et al (2008) Barley grain maturation and germination: metabolic pathway and regulatory network commonalities and differences highlighted by new MapMan/PageMan profiling tools. Plant Physiol 146:310–327

Sreenivasulu N, Wobus U (2013) Seed-development programs: a systems biology-based comparison between dicots and monocots. Annu Rev Plant Biol 64:189–217

Steichen JM, Petty RV, Sharkey TD (2008) Domain characterization of a 4-alpha-glucanotransferase essential for maltose metabolism in photosynthetic leaves. J Biol Chem 283:20797–20804

Stettler M, Eicke S, Mettler T et al (2009) Blocking the metabolism of starch breakdown products in *Arabidopsis* leaves triggers chloroplast degradation. Mol Plant 2:1233–1246

Steup M, Schächtele C (1981) Mode of glucan degradation by purified phosphorylase forms from spinach leaves. Planta 153:351–361

Stitt M, Zeeman SC (2012) Starch turnover: pathways, regulation and role in growth. Curr Opin Plant Biol 15:282–292

Stoddard FL (1999) Survey of starch particle-size distribution in wheat and related species. Cereal Chem 67:59–63

Streb S, Delatte T, Umhang M et al (2008) Starch granule biosynthesis in Arabidopsis is abolished by removal of all debranching enzymes but restored by the subsequent removal of an endoamylase. Plant Cell 20:3448–3466

Streb S, Eicke S, Zeeman SC (2012) The simultaneous abolition of three starch hydrolases blocks transient starch breakdown in *Arabidopsis*. J Biol Chem 287:41745–41756

Sullivan MA, Aroney STN, Li S et al (2014) Changes in glycogen structure over feeding cycles sheds new light on blood-glucose control. Biomacromolecules 15:660–665

Sulpice R, Flis A, Ivakov AA, Apelt F et al (2014) *Arabidopsis* coordinates the diurnal regulation of carbon allocation and growth across a wide range of photoperiods. Mol Plant 7:137–155

Sun T-P (2011) The molecular mechanism and evolution of the GA-GID1-DELLA signalling module in plants. Curr Biol 21:R338–R345

Sun X, Jones WT, Rikkerink EHA (2012) GRAS proteins: the versatile roles of intrinsically disordered proteins in plant signalling. Biochem J 442:1–12

Sundberg M, Pfister B, Fulton D et al (2013) The heteromultimeric debranching enzyme involved in starch synthesis in Arabidopsis requires both isoamylase1 and isoamyoase2 subunits for complex stability and activity. PLos ONE 8:e75223

Szecowka M, Heise R, Tohge T et al (2013) Metabolic fluxes of an illuminated *Arabidopsis thaliana* rosette. Plant Cell 25:694–714

Tagliabracci VS, Heiss C, Karthlic C et al (2011) Phosphate incorporation during glycogen biosynthesis and Lafora disease. Cell Metab 13:274–282

Takeda Y, Hizukuri S (1981) Re-examination of the action of sweet-potato beta-amylase on phosphorylated $(1 \rightarrow 4)$-α-D-glucan. Carbohydr Res 89:174–178

Tanackovic V, Svenson JT, Jensen S et al (2014) The deposition and characterization of starch in Brachypodium distachyon. J Exp Bot 65:5179–5192

Tan-Wilson AL, Wilson KA (2012) Mobilization of seed protein reserves. Physiol Plant 145:140–153

Tsuji H, Aya K, Ueguchi-Tanaka M et al (2006) GAMYB controls different sets of genes and is differentially regulated by microRNA in aleurone cells and anthers. Plant J 47:427–444

Turgeon R, Wolf S (2009) Phloem transport: cellular pathways and molecular trafficking. Annu Rev Plant Biol 60:207–221

Turnbull J, Wang P, Girard J-M et al (2010) Glycogen hyperphosphorylation underlies lafora body formation. Ann Neurol 68:925–933

van Wijk KJ, Friso G, Walther D, Schulze WX (2014) Meta-analysis of *Arabidopsis thaliana* phospho-proteomics data reveals compartmentalization of phosphorylation motifs. Plant Cell 26:2367–2389

Vander Kooi CW, Taylor AO, Pace RM et al (2010) Structural basis for the glucan phosphatase activity of Starch Excess4. Proc Natl Acad Sci U S A 107:15379–15384

Vilaplana F, Hasjim J, Gilbert RG (2012) Amylose content in starches: towards optimal definition and validating experimental methods. Carbohydr Polym 88:103–111

Vriet C, Welham T, Brachmann A et al (2010) A suite of *Lotus japonicus* starch mutants reveals both conserved and novel features of starch metabolism. Plant Physiol 154:643–655

Walley JW, Shen Z, Sartor R et al (2013) Reconstruction of protein networks from an atlas of maize seed proteotypes. Proc Natl Acad Sci U S A 110:E4808–E4817

Wattebled F, Dong Y, Dumez S et al (2005) Mutants of Arabidopsis lacking a chloroplastic isoamylase accumulate phytoglycogen and an abnormal form of amylopectin. Plant Physiol 138:184–195

Wattebled F, Planchot V, Szydlowski N et al (2008) Further evidence for the mandatory nature of polysaccharide debranching for the aggregation of semicrystalline starch and for overlapping functions of debranching enzymes in Arabidopsis leaves. Plant Physiol 148:1309–1323

Webber BL, Abaloz BA, Woodrow IE (2007) Myrmecophilic food body production in the understorey tree, Ryparosa kurrangii (Archariaceae), a rare Australian rainforest taxon. New Phytol 173:250–263

Weidberg H, Shvets E, Elazar Z (2011) Biogenesis and cargo selectivity of autophagosomes. Annu Rev Biochem 80:125–156
Weise SE, Weber APM, Sharkey TD (2004) Maltose is the major form of carbon exported from the chloroplast at night. Planta 218:474–482
Weise SE, Kim KS, Stewart RP et al (2005) β-Maltose is the metabolically active anomer of maltose during transitory starch degradation. Plant Physiol 137:756–761
Weise SE, Aung K, Jarou ZJ et al (2012) Engineering starch accumulation by manipulation of phosphate metabolism of starch. Plant Biotechnol J 10:545–554
Weitbrecht K, Muller K, Leubner-Metzger G (2011) First off the mark: early seed germination. J Exp Bot 62:3289–3309
Wilson WA, Roach PJ, Montero M et al (2010) Regulation of glycogen metabolism in yeast and bacteria. FEMS Microbiol Rev 34:952–985
Worby CA, Gentry MS, Dixon JE (2006) Laforin, a dual specificity phosphatase that dephosphorylates complex carbohydrates. J Biol Chem 281:30412–30418
Yadav UP, Ivakov A, Feil R et al (2014) The sucrose-trehalose 6-phosphate (Tre6P) nexus: specificity and mechanisms of sucrose signalling by Tre6P. J Exp Bot 65:1051–1068
Yamaguchi S (2008) Gibberellin metabolism and its regulation. Annu Rev Plant Biol 59:225–251
Yin LL, Xue HW (2012) The *MADS29* transcription factor regulates the degradation of the nucellus and the nucellar projection during rice seed development. Plant Cell 24:1049–1065
Yu TS, Zeeman SC, Thorneycroft D et al (2005) α-Amylase is not required for breakdown of transitory starch in Arabidopsis leaves. J Biol Chem 280:9773–9779
Yun M-S, Kawagoe Y (2010) Septum formation in amyloplasts produces compound granules in the endosperm and is regulated by plastid division proteins. Plant Cell Physiol 51:1469–1479
Zeeman SC, Umemoto T, Lue WL et al (1998) A mutant of *Arabidopsis* lacking a chloroplastic isoamylase accumulates both starch and phytoglycogen. Plant Cell 10:1699–1712
Zeeman SC, Kossmann J, Smith AM (2010) Starch: its metabolism, evolution, and biotechnological modification in plants. Annu Rev Plant Biol 61:209–234
Zeng D, Yan M, Wang Y et al (2007) *Du1*, encoding a novel Prp1 protein, regulates starch biosynthesis through affecting the splicing of Wx^b pre-mRNAs in rice (*Oryza sativa* L.). Plant Mol Biol 65:501–509
Zhang Q, Wing R (2013) Genome studies and molecular genetics: understanding the functional genome based on the rice model. Curr Opin Plant Biol 16:129–132
Zhou S-R, Yin L-L, Xue H-W (2013) Functional genomics based understanding of rice endosperm development. Curr Opin Plant Biol 16:236–246
Zi J, Mafu S, Peters RJ (2014) To gibberellins and beyond! Surveying the evolution of (di)terpenoid metabolism. Annu Rev Plant Biol 65:10.1–10.28
Zirin J, Nieuwenhius J, Perrimon N (2013) Role of autophagy in glycogen breakdown and its relevance to chloroquine myopathy. PLoS Biol 11:e1001708
Zulawski M, Braginets R, Schulze WX (2013) PhosPhAt goes kinases – searchable protein kinase target information in the plant phosphorylation site database PhosPhAt. Nucleic Acids Res 41:D1176–D1184