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Introduction

The cereal endosperm is our largest single primary food source, and thus among the most economically important structures in biology. Development of the cereal seed is orchestrated by the coordinated activities of a large number of genes that encode metabolic and regulatory enzymes and other proteins. This results in a triploid endosperm, the embryo, pericarp, seedcoat and other tissues of the mature grain. The endosperm consists of two tissues, the interior starch-filled endosperm and the outer epidermal layer called the aleurone.

Starch is the predominant storage carbohydrate in plants and the second most abundant biopolymer on earth, after cellulose. Starch is a mixture of amylose and amylopectin, both glucose polymers. Amylose is a mostly linear polymer of 10^5-10^6 Da with 200-2000 α-1,4 bonded glucose moieties with rare α-1,6 branch points. Amylopectin, on the other hand, is highly α-1,6 branched, with a complex structure of 10^7-10^8 Da and up to 3 x 10^6 glucose subunits, making it the largest biological molecule in nature. Starch is used as a feedstock in a wide array of applications for both bulk and commodity products in food and non-food industry. The interest in starch as a renewable polymer and souce for monomers to supplement and replace segments of the petro-chemical industry, e.g. as fuel (bio-ethanol) or for the manufacure of bio-plastics, is progressively increasing, as are the prospects of tailor-production of starches in transgenic crops.

The coordinated modulation of gene expression in sink organs such as the barley endosperm is to a large extent choreographed by sugar signaling. Sugar signaling cascades are important components of regulatory networks in most organisms. Compared to the situation in bacteria, yeast and animals, participants of the sugar signaling pathways in plants are poorly understood. Sugar signaling can be dissected into three steps, sugar sensing, signal transduction, and target gene expression. The picture is clouded by the dual function of sugars as nutrients and signaling molecules, and by the interaction (in plants and animals) between sugar signaling and hormonal networks. In plants the complexity is further increased by the vital role of sugar production through photosynthesis. Hexoses, hexose phosphates, sucrose and trehalose might serve as elicitors of plant sugar signaling. Hexokinase, sucrose and glucose transporters, and various sugar receptors, have been proposed as components of the sugar sensing machinery. Furthermore, sugar signaling does not operate in splendid isolation but, rather, is integrated in cellular regulatory networks. Most notably, the molecular characterization of sugar signaling mutants has revealed tight and extensive interactions between sugar and hormonal signaling (see Jansson, 2004 for a recent review), particularly for abscisic acid (ABA) and ethylene.

Transcriptional regulation of starch synthesis

We have previosuly cloned and characterized a large number of genes that are central for starch synthesis in barley[1-5]. During the last couple of years we have isolated a novel family of transcription factors, the SUSIBAs (for Sugar Signaling in Barley) and demonstrated that two of the them, SUSIBA1 and SUSIBA2, are crucial for sugar signaling and endosperm-specific gene expression during starch synthesis[6-8]. This work was first published in Plant Cell[6]. Susequent DNA-microarray analyses with the Affymetrix Barley1 GeneChip revealed

that SUSIBA1 also controlls fructan synthesis in barley leaves (Rosenquist et al., manuscript in preparation). Orthologs to the *susiba1* and *susiba2* genes have been identified in rice, maize and wheat. We are also analyzing *Arabidopsis* mutants with knock-outs for putative *SUSIBA* orthologs. One review article describing sugar signaling in Arabidopsis has been published[9]. The work on maize has been carried out in collaboration with Pioneer Hi-Bred in USA.

We have previously suggested that the second intron of the starch branching enzyme gene *sbeIIb* confers endosperm-specific expression by serving as a silencer that prevents transcription in non-expressing organs such as leaves and embryo[1]. To learn more about the regulatory mechanisms of the barley *sbeIIb* intron, we examined the tissue-specific activity of the sorghum *sbe* promoter in transient assays of *gfp* reporter constructs. We have earlier shown that the sorghum *sbe* gene is expressed in both sink and source organs[10]. We found that, when linked to the barley *sbeIIb* second intron, the sorghum *sbeIIb* promoter could not drive *gfp* transcription in sorghum or barley embryonic cells. Similar results were obtained for the barley *sbeIIa* promoter. Database searches showed that sequences homologous to the barley *sbeIIb* intron exist also in introns and flanking regions of some other grass genes. These results confirm that the endosperm-specific expression of the *sbeIIb* gene in barley is, partly, controlled by the second intron[11].

Antisense ODN inhibition

Antisense oligodeoxynucleotide (ODN) inhibition is a method widely used in animal sciences and an important emerging therapeutic approach in clinical medicine, e.g. in gene therapy. Recently, we have demonstrated the applicability of antisense ODN inhibition also in plant biology and showed that it operates via RNase H activation[7]. Through the work in our group, antisense ODN inhibition has proven an efficient strategy for gene silencing in both vegetative and endoperm tissues in barley. This work was published in the 2005 October issue of Plant Journal where it was featured on the cover[7], and it is also published in one review article 2006[12].

Programmed cell death during barley endosperm development

Caspases are essential in animal programmed cell death both as initiator and executioner proteases. Plants do not have close caspase homologues but several instances of caspase-like proteolytic activity have been demonstrated in connection with programmed cell death in plants. We have found that programmed cell death (PCD) during early endosperm development involves a caspace-like activity (VEIDase) and that it can be *in vivo*-localized to autophagosomes in randomly distributed cells of the starchy endosperm[13]. Several manifestations of programmed cell death exist in developing barley caryopsis, indicating a connection between VEIDase activity and developmental programmed cell death in barley.

Characterization of the high-amylose barley mutant Amo1

We have launched a program with the objectives to identify barely lines with modified carbohydrate synthesis and assess their commercial value. Seed and leaf transcriptomes and proteomes are compared. DNA-microarray analyses are carried out using the Affymetrix Barley1 GeneChips. The synthesis of starch and the composition and structure of starch and the starch granules between Amo1 and its parental line Midas were compared[14]. Staining of

starch with 8-amino-1,3,6-pyrenetrisulfonic acid coupled with confocal laser scanning microscopy illustrated the high-amylose content of starch from Amo1 endosperm and visualized a prominent degree of cracking at the surface of the granules. The Amo1 amylopectin exhibited a considerably higher content of covalently bound phosphate as compared to Midas, whereas the branch size distribution of the amylopectin molecules were similar in both cultivars. Expression of the genes encoding different starch synthesis enzymes in Amo1 appears more or less unaffected at both the transcript and protein levels. However, zymogram analyses clearly showed that the starch-branching enzyme (SBE) activity differed between the two cultivars, particularly at the early stage of endosperm development. Based on our earlier establishement of the barley endosperm proteome[2], we performed a comparison of the starch granule proteomes of Amo1 and Midas. The results revealed no differences with obvious relevance to starch synthesis but we found that the protein content of the Amo1 granules was higher than for Midas[14].

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