REGULATION OF THE STARCH SYNTHESIS PATHWAY: TARGETS FOR BIOTECHNOLOGY

I. INTRODUCTION

Glycogen synthesis in mammalian cells is relatively well understood, including the specificity of glycogen synthase for UDPglucose as well as its regulation through hormonally induced posttranslational protein modification. Textbooks of biochemistry usually describe these metabolic schemes in detail. Conversely, the biosynthesis of polysaccharides in bacteria and plants is usually described only superficially. These organisms accumulate glycogen (bacteria) or starch (plants) by metabolic pathways that are different in a number of respects from those occurring in animals. Despite the different structures of the final products, in both bacteria and plants ADPglucose is the glucose donor for the elongation of the α-1,4-glucan chain. Moreover, in both systems, the main regulatory step of the metabolism takes place at the level of ADPglucose synthesis.

II. GENETIC ENGINEERING

The gene technology developed in the past few decades can be used to test scientific hypotheses and to alter plant metabolic pathways for commercial advantage. These two uses of technology go hand in hand: unless it is understood how a pathway works, it is very difficult to change that pathway in a particular direction.

Plant transformation is now an experimental tool that can be used on many species; a large number of genes, viral genomes, and plant-transposable elements have been transferred to the genomes of species such as potato, tobacco, oilseed rape, and so on.

Transforming a plant with a foreign DNA involves a number of steps. Exogenously added DNA has to be taken up by isolated plant cells, and the transformed cells must be able to regenerate a plant; the exogenous DNA can originate in bacteria, animals, or other plants. Breeding is no longer limited to existing varieties within the species but is limited only by imagination and good biochemistry.

The methodology required for successful modification of plant metabolism has been perfected for some plant species and for specific enzymes. This methodology includes transformation of plant cells or tissues; regeneration of a healthy plant from the transformed cells; isolation of the gene encoding the enzyme of interest; identification of promoter sequences for tissue-specific expression; and targetting of the protein into the cellular compartment desired.

A transgenic organism is defined as one whose genome has been modified by the addition of exogenous DNA. This exogenous DNA can be a manipulated sequence from the same species or DNA from another species (plant or otherwise) that has a desirable property. The operational gene in the exogenous DNA is called the *transgene.* The DNA can be introduced into a cell by a variety of techniques (e.g., injection, transformation, viral infection). Biotechnology refers to the area of research in which recombinant DNA techniques are used to design and produce genotypes profitable for agriculture or other commercial enterprises.

III. VECTORS

The vectors used routinely to produce transgenic plants are derived from the soil bacterium *Agrobacterium tumefaciens.* In its natural form, this bacterium causes the crown gall disease in which the infected plant produces tumors ("galls") usually at the base ("crown") of the plant.

Part of the process of infection and tumor formation requires the insertion of the T_i plasmid of the bacterium into the genome of the plant, a characteristic that makes the T_i plasmid an ideal vector for the introduction of foreign DNA into the plant. The DNA of interest is "spliced" into the T_i plasmid, and then the whole segment is inserted into a plant chromosome. To achieve this objective, some modifications must be made to the T_i plasmid—for example, its attenuation (deletion of tumor-inducing genes), the insertion of cloning sites so that the DNA of interest can be inserted easily into the vector, and the addition of selectable genes.

The gene of interest is spliced into the modified T-DNA by conjugation of *Agrobacterium* with *Escherichia coli* containing an "intermediate vector." Then, *Agrobacterium* cells containing the recombinant plasmid are selected after conjugation by growing the culture in the presence of a suitable antibiotic, and these are the cells used to infect cut segments of plant tissues. The infected plant tissue (e.g., leaf discs) is placed in a medium containing the other antibiotic (e.g., kanamycin) so that only plant cells that acquired antibiotic resistance from the T-DNA transfer survive. The transformed cells grow into clumps of cells that can be induced to form roots and shoots

when plant hormones and nutrients are present in suitable quantities in the growth medium. The plants obtained in this manner can then be screened to see whether the DNA of interest has been incorporated in their genomes. DNA that have been incorporated using this methodology include those that confer resistance to glyphosate (a herbicide) and a gene that delays ripening. Genes relevant to starch synthesis have been transformed into potato, tobacco, and tomato using *A. tumefaciens* T_i plasmid–derived vectors. *Agrobacterium* has also been used to transform seeds, and transgenic plants of *Arabidopsis thaliana* have been obtained by cocultivation of imbibed seeds with *Agrobacterium* (Feldman and Marks, 1987).

The majority of the attempts to transform plants in the 1970s failed or remained unsubstantiated. A major advance occurred when chimeric genes were constructed in which the coding regions of foreign genes were inserted between the signals controlling gene expression in plants—upstream promoters and downstream adenylation sites (Downey *et al.*, 1983). The criteria confirming the successful integration and expression of the chimeric gene in the plant cells included the phenotypic expression of the desired characteristic, Southern blots to demonstrate the presence of the DNA in transformed tissue, Northern blots to confirm the presence of the RNA transcript of the correct size, and activity of the enzyme. Later studies established the sexual transmission of the foreign DNA to progeny of the transgenic plants in segregation ratios typical of simply inherited genes.

IV. PROTOPLAST ISOLATION AND TRANSFORMATION

Not all plant species are amenable to transformation using the T_i-plasmid. In this case, protoplasts are prepared and then induced to take up exogenously applied DNA. Protoplasts can be isolated from a variety of plant tissues, although usually leaves are used. Protoplast isolation involves the enzymatic removal of the cell wall by incubating tissue slices in a medium including fungal cellulases, pectinases, and hemicellulases. The medium is prepared at a high osmotic potential to prevent the bursting of the protoplasts once the cell wall has been digested. The conditions required to prepare viable protoplasts depend on the plant species and the tissue, and must be determined empirically—that is, by trial and error. Once purified from cell debris and the enzymatic solution, the protoplasts are ready for transformation. A number of methods can be used in order to get the protoplasts to take up the exogenous DNA, including the use of polyvalent cations and electroporation, fusion of protoplasts with liposomes containing the foreign DNA, and microinjection.

If the DNA is maintained stably within the cell and if the protoplasts can be induced to regenerate a whole plant, transgenic plants can be obtained.

V. PLANT REGENERATION

A few days after isolation, the protoplasts in their culture medium begin to regenerate their cell walls and divide to form a microcallus. A *callus* is a mass of undifferentiated plant cells, and its formation is dependent on the presence in the culture medium of the plant hormones auxins and cytokinins. The callus may eventually differentiate to form shoots and/or roots, depending on the balance of plant hormones in the culture medium, as demonstrated by Skoog and Miller (1957) with tobacco callus. When the ratio of auxins to cytokinins in the culture medium was high, the calluses were induced to form roots. Conversely, a low ratio of auxins to citokinins induced the formation of shoots. Intermediate ratios promoted the growth as callus.

For some plant species it has not yet been possible to regenerate plants from protoplasts, and in these cases protocols have been designed to transform embryogenic explants rather than protoplasts. When using embryogenic explants, it may be necessary for the foreign DNA to travel through several layers before reaching the cells that will originate the germ line, and for this reason the DNA coating very small particles or tungsten or gold is delivered into the target cells using an explosive force. This approach has been used successfully on maize and soybean.

VI. TISSUE- AND ORGANELLE-SPECIFIC EXPRESSION

A promoter used for many applications in plant molecular biology is that from the cauliflower mosaic virus S35, which is very strongly expressed in plant cells, but this promoter does not provide the control over transcription required for successful expression. To express a foreign gene or to overexpress an endogenous gene it is essential to be able to control transcription. The best way to obtain this control is to use the plants own promoters, and the first step is to characterize these promoters.

Promoter sequences have been isolated and fused to reporter genes so that the expression pattern can be monitored easily. Many promoters that differ in their expression pattern with respect to tissues, environmental conditions, or developmental stages have been characterized following this approach.

Another problem in protein expression is how to obtain a subcellular targetting. The plant cells (like all eukaryotic cells) consist of several compartments (e.g., nucleus, chloroplasts, mitochondria). The proteins that are expressed in compartments other than the cytosol usually have signal sequences that direct them to their subcellular destination. To direct a protein to the desired compartment, a fusion is effected between the signal sequence and the mature protein. Many such signal sequences have been identified and are available to direct proteins to practically any compartment.

VII. ANTISENSE TECHNOLOGY

Genes encoding enzymes involved in starch biosynthesis or other relevant pathways (e.g., synthesis of sucrose) can be used for the overexpression of enzyme activity, as described in this chapter for ADPGlc PPase. Another approach is the use of antisense (complementary) DNA or RNA to decrease gene expression, a good way to assess the role of an enzyme and whether it limits the rate of the overall pathway.

A chimeric gene encoding antisense RNA for ADPGlc PPase, reducing the expression of the enzyme (Müller-Röber *et al.*, 1992) to between 2 and 5% of the wild type, reduced starch content of potato tubers by the same percentage; the number of tubers increased, but their weight decreased.

In wild type potatoes the amylose content varies from 18 to 23%, but potato plants with altered starch composition and content have been obtained by a number of different approaches. By using the antisense technology for genes encoding granule-bound starch synthase (GBSS) and branching enzyme, potato starches with different ratios of amylose and amylopectin were obtained (Visser *et al.*, 1991). Using antisense technology to decrease the expression of Waxy protein, it was seen that the amount of amylose deposited in the starch granule was related to the activity of the GBSS protein. The little amylose present in starch granules from such tubers was shown to be located at the hilum of the granule in a core of varying size that is surrounded by amylose-free starch (Visser *et al.*, 1991).

To test the hypothesis that phosphate supply from the can limit the rate of photosynthesis (Sivak and Walker, 1986), antisense experiments were performed by Schultz *et al.* (1993). A cDNA for the potato triose phosphate translocator was identified and a fragment of this cDNA in reverse orientation was expressed in trangenic potato plants under the control of the constitutive cauliflower mosaic virus 35S promoter (Rismeier *et al.*, 1993). This experiment confirmed that P_i supply can limit photosynthesis since a

reduction of just 30% of P_i transport activity resulted in a decrease of 50% in the maximum rate of photosynthesis. As predicted (Sivak and Walker, 1986), this limitation if P_i supply also resulted in an accumulation of transitory starch in the leaves.

VIII. OTHER USES OF GENE TECHNOLOGY

Foreign enzymes—that is, with no plant equivalent, have been introduced into plants; one of the uses of this approach was to address the nature of sucrose transport into the phloem. An invertase derived from the yeast enzyme was targetted to the cell wall of tobacco, potato, tomato, and *A. thaliana* (Sonnewald *et al.*, 1994). The introduction of invertase decreased yield, presumably through the inhibition of sucrose transport.

The inorganic pyrophosphatase of *E. coli* was expressed in the cytosol of transgenic tobacco and potato plants, using the constitutive promoter of 35S cauliflower mosaic virus and the poly-A site of the octopine synthase gene terminator. Pyrophosphatase activity increased twofold in the transgenic plants relative to the controls, and the concentrations of pyrophosphate and pyruvate (indicating flow of photosynthates towards glycolysis) decreased. Sucrose content increased more than tenfold in the source leaves of the transgenic tobacco plants.

It should be noted that it is not enough to increase the amount of enzyme protein in a plant tissue to ensure higher activity of the enzyme. This is because individual enzymes may be regulated by several metabolites and the concentration of these cannot be controlled easily. Also, plant metabolism is integrated in ways that may escape control by the plant technologist, and altering one pathway may somehow affect alternative pathways in unpredictable ways. For example, Zrenner *et al.* (1993) managed to reduce the amount of UDPGlc PPase in potato tuber to just 4% of the wild-type level without any visible effect on carbohydrate metabolism.

Genetic modification of many dicotyledoneous crops such as potato using gene transfer via *A. tumefaciens* is, at present, an efficient and reliable technique; the application of the antisense route to limit or neutralize the action of undesirable genes has also been applied successfully to potatoes. Conversely, for monocotyledoneous crops such as maize, wheat, and rice, transformation can be accomplished by the much less efficient particle gun technique. This technique has been improved, resulting in rapid progress in the development of maize and later wheat with improved agronomic properties and/or altered starch composition.

Modification of wheat is also hampered for commercial reasons: unlike maize, wheat seeds have no male sterility, allowing the farmer to obtain

new seeds from his wheat harvest. Introduction of male sterility into wheat by genetic engineering would make wheat breeding as attractive economically as maize breeding. In wheat the large variation in starch granule size is a negative factor for optimal use of wheat starch, a characteristic that is unlikely to be modified by conventional breeding and is awaiting a more original approach.

The information provided in this chapter is limited not only by matters of space and relevance, but also by an additional reason. A number of biotechnological companies and institutions are working in the development of new cultivars with altered starch compositions. Research done by, or on behalf of, commercial enterprises generally is not published in refered journals and is kept secret by the companies until applying for a patent. This policy of secrecy clearly slows down the dissemination of scientific information and deprives scientists of the very helpful peer review (affecting negatively the quality of the research). This tendency, unfortunately, is likely to become more dominant. Limited information is sometimes offered in scientific conferences, but hard data are often missing.

Most of our knowledge on storage starch and its biosynthetic enzymes comes from crop plants that have been genetically manipulated to increase starch content for thousands of years through plant breeding. It would not be surprising, then, if some of the peculiarities of the genetics of ADPGlc PPase (number of gene families; even variations in subcellular localization) were "artifacts" introduced by the selection towards high yield and high starch selection pressure by humans.

IX. TRANSFORMATION OF PLANTS WITH AN *Escherichia coli* ALLOSTERIC MUTANT *glg* C GENE INCREASES STARCH CONTENT

As discussed in the chapter, "The Biosynthetic Reactions of Starch Synthesis," there is a preponderance of evidence indicating that the rate-limiting and regulatory enzyme of starch synthesis in algae or bacterial glycogen synthesis is the ADPGlc PPase. With respect to higher plants, control analysis experiments have shown that ADPGlc PPase is important in the regulation of leaf starch synthesis (see the chapter, "Starch Accumulation in Photosynthesis Cells). Also, reduced ADPGlc PPase activity in mutants led to a reduction in the rate of starch synthesis in potato tubers (Müller-Röber *et al.*, 1992). Therefore, it was of interest to see if the starch content in a plant could be augmented by increased expression of activity of one of the enzymes involved in starch biosynthesis. Overexpression of a plant ADPGlc PPase activity, however, would require the expression of two distinct genes to reconstitute its ADPGlc PPase activity. Moreover, it

is possible that the plant would compensate for the overexpression by altering the ratio of the effector metabolites, 3PGA and P_i, so that starch synthesis would not increase. Thus, a different strategy was chosen: an *E. coli* ADPGlc PPase, *glg* C gene of allosteric mutant 618, referred to as *Glg* C16 (Leung *et al.*, 1986), which encodes for an enzyme independent of the presence of activator for activity was used for the transformation. Expression of the bacterial mutant gene would have two advantages. Only one gene has to be expressed for ADPGlc PPase activity, and the mutant enzyme would be less sensitive to inhibition by its allosteric inhibitor, 5′AMP, insensitive to the inhibitor of the plant enzyme, P_i and independent of the activator for good activity (Leung *et al.*, 1986) (Table I). A collaboration with the Monsanto group was initiated to transfect plant systems with *Glg* C16 to see if the starch content of plants could be increased (Stark *et al.*, 1992). Because starch synthesis occurs in the plastid, a nucleotide sequence encoding the transit peptide of the *A. thaliana* ribulose 1,5-bisphosphate carboxylase chloroplast transit peptide was fused to the translation initiation site of the *glg* C16 gene (Fig. 1). A promoter was also needed; the chimeric gene was cloned behind a cauliflower mosaic virus (CaMV)-enhanced 35S promoter, or a tuber-specific patatin promoter, or, in the case of tomato plants, the *Arabidopsis* plant promoter from the *rbc*S gene was used (Stark *et al.*, 1992; Fig. 1). A polyadenylation signal from the nopaline synthase gene (Nos) was fused on at the 3′ end of the chimeric gene. The chimeric gene-containing promoter was placed in a cloning vector with a 35S-neomycin phosphotransferase gene as a selectable marker (Stark *et al.*, 1992) and was used for the transformation of tobacco calli, tomato cotyledons, and potato plants.

TABLE I

ACTIVATION AND INHIBITION OF THE ADPGLC PPASE ACTIVITY PRESENT IN TOBACCO PROTOPLASTS TRANSFORMED WITH *glg* C16[a]

Source of protoplast extract additions to assay	ADPGlc formed (nmol)
Nontransformed cells + 10 m*M* inorganic phosphate	0.0
Transformed + 2.5 m*M* fructose 1,6-bis-P	20.2
Transformed + 2.5 m*M* fructose 1,6-bis-P + 10 m*M* inorganic phosphate	18.0
Transformed + 20 m*M* + 3-P-glycerate	18.4
Transformed + 10 m*M* inorganic phosphate	6.4

[a] Data from Stark *et al.* (1992).

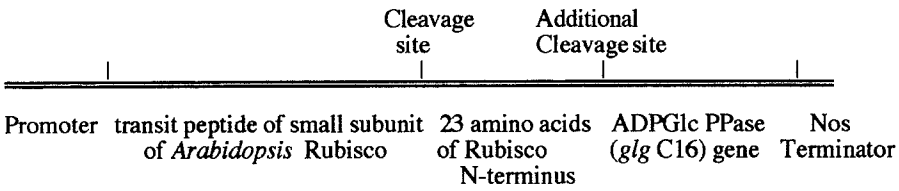

FIG. 1. Synthetic promoter–plastid transit peptide-*glg* C16 ADPGlc PPase gene. The chimeric gene is composed of the *Arabidopsis thaliana* chloroplast transit peptide portion of the ribulose bis-P carboxylase gene modified to have an extra cleavage site to eliminate the 23 amino acids of the N-terminal of the small subunit (Stark *et al.*, 1992) to prevent its possible interference with the catalytic or regulatory activity of the *glg* C16 gene product. The Nos terminator is the nopaline synthase 3′ poly A signal. The promoter can be either a constitutive promoter or a tissue-specific promoter.

In tobacco calli where the *glg*C gene product activity was detected, starch content was 1.7 to 8.7 times higher than in the controls lacking the *glg*C gene product (Stark *et al.*, 1992). The CaMV–chimeric gene was electroporated into tobacco protoplasts, and extracts of the transformed protoplasts gave rise to ADPGlc synthesis resistant to P_i inhibition and activated by fructose 1,6-bis-P (Table I). The synthesis of ADPGlc in the control protoplast extract was totally inhibited by P_i as expected since the tobacco and almost all plant ADPGlc PPases are most sensitive to inhibition by P_i. When the transgenic tobacco was examined by light microscopy and was compared with control calli, it showed a large increase in the number of starch granules (Stark *et al.*, 1992). Similarly, when tomato was transformed, with a construct transit peptide-*Glg* C16 gene, shoots excised from calli stained black with iodine reagent, whereas the controls were essentially negative.

Similar results have been obtained for Russet-Burbank potato tubers in which the chimeric gene, with its transit peptide under the control of a tuber-specific patatin promoter, increased starch in the tuber 25 to 60% over controls not containing the bacterial enzyme (Stark *et al.*, 1992; Table II). If the bacterial ADPGlc PPase *Glg* C16 gene was expressed in the tuber lacking the transit peptide gene portion, no increase in starch content was noted (Table II). Probably, ADPGlc PPase was expressed, but was not present in the amyloplast and, for this reason, was not able to supply ADPGlc to the starch synthases that are localized in the amyloplast.

A positive relationship between the expression levels of the ADPGlc PPase of *Glg* C16, as measured by immunoblotting of the potato extracts, and the increase in starch content was demonstrated, particularly in tubers at lower ranges of starch content. Lower levels of the expressed ADPGlc PPase resulted in increases of 21 to 63% in starch, intermediate levels of the expressed ADPGlc PPase gave increases of 33 to 118% in starch, and

TABLE II

STARCH CONTENT IN POTATO TUBERS TRANSFORMED WITH THE *glg* C16 AND *glg* C GENES[a]

	Plasmid used for transformation	Average starch content (% fresh weight)
A	Control; untransformed	12.3 ± 1.15
	Chloroplast transit peptide-*glg* C16	16.0 ± 2.00
	glg C16, no transit peptide	12.4 ± 0.24
B	Control; untransformed	13.2 ± 0.12
	Chloroplast transit peptide-*glg* C	13.1 ± 0.07

[a] Data from Stark *et al.* (1992).

the high expressed levels of the transit peptide-*Glg* C16 resulted in increases of 33 to 167%. It is of interest that when the wild-type *E. coli* ADPGlc PPase gene was expressed in the tuber, no increase in starch was noted (Table II). Thus, an important factor in increasing starch synthesis is to transform the tuber with an ADPGlc PPase with allosteric properties optimized to permit higher rates of ADPGlc synthesis under physiologic conditions.

X. ARE OTHER STARCH BIOSYNTHETIC ENZYMES RATE LIMITING?

Smith (1988) showed that in mutant *rr* pea leaves, in high light intensity, there was a 40% decrease in the rate of starch synthesis. A control coefficient analysis reported later (Smith *et al.*, 1990) showed that in low light intensity, there was essentially no effect on the rate of starch synthesis, whereas in high light intensity, the flux control coefficient value was 0.13, which is a small value (meaning very little control) and is only one-fifth the value seen for ADPGlc PPase (Neuhaus and Stitt, 1990). Thus an 86% reduction of branching enzyme activity had a small effect on regulation of starch synthesis.

It has been suggested that when plants are subjected to high temperature, starch synthase activity may be rate limiting. At temperatures higher than 30°C, both maize (Singletary *et al.*, 1994) and wheat endosperm (Hawker and Jenner, 1993; Keeling *et al.*, 1993, 1994; Jenner, 1994) had a reduction of starch deposition as compared with lower temperatures. In wheat, the starch biosynthetic enzyme affected was soluble starch synthase (SSS).

Using flux control coefficient analysis, Keeling *et al.* (1993) showed a control coefficient close to 1 between the rate of starch synthesis and the level of starch synthase activity in wheat endosperm extracts. It was also shown that in vitro, the endosperm starch synthase activity was sensitive to heat treatment in the range of 30 to 40°C if the treatment was for longer than 15 minutes. A similar study with maize endosperm showed a reduction of starch synthetic rate and a decrease in starch synthase activity in the heat-stressed maize endosperm (Singletary *et al.,* 1994). It was also noted, however, that in the heat-stressed maize, the endosperm ADPGlc PPase activity was also reduced to an even greater extent than the SSS (Singletary *et al.,* 1994).

Thus, in wheat and maize, under some environmental conditions, there might be a correlation between reduction of starch synthase activity and decreased starch synthesis. However, as the data obtained with maize suggest (Singletary *et al.,* 1994), other unknown factors, beside starch synthase activity, may be the primary reason for the reduction of starch synthesis in the heat-stressed plants. In the case of maize endosperm, another enzyme involved in starch synthesis. ADPGlc PPase, is also affected in the heat-stressed plant. It is also possible that other critical steps leading to starch biosynthesis are affected in both plants, such as carbon flow from source to sink tissues and invertase activity. Those processes were not studied in the heat-stressed plants. Thus, we believe that the published evidence does not warrant the designation of starch synthase as a major control point. Flux control coefficients for an enzyme within a process can only be determined if the activity of only that enzyme is affected. In the case of heat-stressed plants, it has not yet been shown that only the starch synthase activity is affected. A crucial test is whether the starch synthetic rate can be increased by overexpressing soluble starch synthase activity in the amyloplast. As shown in the preceding, starch accumulation can be increased by expressing a bacterial ADPGlc PPase allosteric mutant in plants (Stark *et al.,* 1992).

XI. OTHER PHYSIOLOGIC EFFECTS OF MANIPULATION OF STARCH SYNTHESIS

Starch phosphorylase and amylolytic enzymes are responsible for starch degradation during cold storage of potato tubers (see the chapter, "Starch Degradation"), and result in the formation of glucose-1-P and glucose from starch. Glucose-1-P may also be formed from the products of degradation of sucrose via invertase or sucrose synthase. Sugar accumulation, or "sweetening," decreases the quality of the tubers and makes them unsuitable for frying. Also, accumulation of sugars is eventually followed by the end of

dormancy, which is signaled by the onset of respiration and sprouting. Barry *et al.* (1994) found that by overexpressing ADPG PPase during cold storage they could delay cold sweetening and sprouting. They proposed that ADPG PPase would act as an active sink for the glucose-1-P and glucose (which can be converted into glucose-1-P) products of starch degradation and the ADPGlc formed is then converted back into starch. Overexpression of ADPG PPase during cold storage is achieved by a variation of the method used by Stark *et al.* (1992) and by using an *A. thaliana* cold-inducible promoter. The potato tubers obtained in this manner had better frying properties than the control after cold storage.

Giroux *et al.* (1996) described the effect of a single gene mutation in the *shrunken* 2 locus of maize (coding for the large subunit of the ADPGlc PPase), which involved the addition of 2 amino acids—tyrosine and serine. The mutation decreased the sensitivity of the enzyme to inhibition by phosphate and was introduced by using an in vivo, site-specific mutagenesis system that involved the use of the transposable element *ds* (dissociation). The mutated gene, named Rev6, increased seed weight by 11 to 18% without changing the proportion of the seed weight taken by starch. The authors proposed that increased ADPGlc PPase activity would affect the overall sink strength of the seeds, as it increased not just starch content but also other constituents of the seed.

XII. CONCLUSIONS

It is conceivable that methodology such as that used to increase starch quantity could be used to influence starch quality by manipulation of starch synthase and branching isoforms. These "new starches" may have greater usefulness in food and industrial processes. The production of modified "specialty" starches via molecular biology techniques is promising, and perhaps more beneficial and more economical than the chemical modification of starch for industrial purposes.

Because there has been an increased demand for starch for both specialized industrial and food uses since the mid-1980s (Katz, 1991), it appears that the study of basic questions on the structure–function relationships of the allosteric regulation of an enzyme involved in sugar nucleotide synthesis now may have a great impact on both agriculture and industry. It is of interest that this research on the routes and mechanism of regulation of bacterial glycogen and starch synthesis at the molecular level, which began in the mid-1960s, has led to opportunities for improving the quality of the uses for starch in industrial and food processes. This was never the original purpose of the studies, but is an example of how basic science, which

tries to answer basic questions, may lead to methods where nature can be manipulated for beneficial purposes.

In addition to altering quantity, starch quality could be changed via expression of the isoforms of starch synthase and branching enzymes in plants. These "designer" starches would be used in the food and other industries. One possible approach to modifying starch structure in a crop would be the replacement of the plant branching isozymes with other different properties. The foreign enzyme could be a chimeric construct.

Although construction of chimeric enzymes is done to elucidate the domains that determine the different properties of the isoforms (see the chapter, "Branching Enzymes"), a secondary benefit is that novel enzymes may be capable of branching starch differently than the wild-type enzymes, resulting in the production of starch with novel properties.

FURTHER READINGS

These sources provide additional in-depth coverage of this topic. For complete reference, please see the Reference section at the end of the book.

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