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Part I. Patterns of Gene Expression of ADPG-Pyrophosphorylase in Native and Transgenic Potato Plants.

The tuber ADPglucose pyrophosphorylase small subunit is encoded by only a single gene. Studies on the structure, organization and expression of the gene that encodes the ADPglucose pyrophosphorylase (sAGP) small subunit were completed and published (see Appendices 1 and 2). DNA sequence analysis revealed that the small subunit gene from potato is structurally similar to the rice endosperm *sAGP* in containing multiple introns at identical positions within the coding sequence. Unlike rice and other plants which contain multiple *sAGP* genes that are expressed in specific tissues (embryo vs. endosperm vs. leaves), potato contains only a single *sAGP* gene which is expressed in multiple tissues. This conclusion was supported by Southern and Northern blot data, primer extension analysis of leaf and tuber poly(A+)-RNAs and by directly comparing the sequences of isolated leaf and tuber cDNAs. This conclusion was verified by the observed spatial and tissue expression patterns in transgenic potato of a ß-glucuronidase reporter gene under the control of the *sAGP* promoter (Appendix 3).

Although the same *sAGP* gene is expressed in both leaves and tubers, the *sAGP* gene is controlled by different mechanisms in these tissues. Temporal analysis revealed that both subunits, large and small, exhibited coordinately increasing transcript and antigen levels during tuber development, suggesting that AGP expression is primarily under transcriptional control in this storage organ. In contrast, differential expression of the subunit transcripts was evident in leaf tissue with relative levels of the small subunit present at several-fold excess compared to large subunit levels. Immunoblot analysis, however, revealed much lower quantities of small subunit antigen than expected based on their mRNA levels, indicating the involvement of a post-transcriptional event(s) in coordinating subunit antigen levels. This post-transcriptional control of antigen abundance was also evident in leaf tissue subjected to a photoperiod regime and during sucrose induction of starch synthesis. Although transcript levels increased in most instances during periods of active starch synthesis, antigen levels remained virtually constant (large subunit) or decreased (small subunit) while *in vitro* enzyme activity levels decreased from 25-50%. These results emphasized the importance in the allosteric regulation of AGP in modulating starch biosynthesis. This differential control of *sAGP* expression in leaves and tubers is consistent with the type of starch metabolism in these tissues. In leaves, starch synthesis is regulated very tightly during the diurnal cycle presumably through the allosteric regulation of AGP enzyme activity, whereas processes such as transcription and translation have lesser roles. In contrast, starch metabolism in tubers is directed mainly towards synthesis resulting in transcription being the most important aspect. Overall, the described molecular studies revealed that regulation of AGP expression occurs at multiple levels and that the primary mode of control differs with tissue type.

Expression of the Potato Small Subunit Promoter-GUS Fusions (Appendix 3). To elucidate the mechanism controlling AGP expression during plant development, the expression of the potato tuber AGP small subunit (*sAGP*) gene was analyzed in transgenic potato plants using a promoter-ß-glucuronidase (ß-GUS) expression system. Consistent with results obtained by Northern blot analysis, *sAGP*-ß-GUS chimeric fusions were expressed at high levels in tubers and also in other starch-containing cells throughout the plant. Expression of *sAGP* in tubers reached levels as high as 120 nmol 4-MU/min/mg protein. Stolons exhibited *sAGP* expression

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levels of 33% compared to tubers while stems and roots displayed expression levels approximately 11-12 % compared to tubers. Low levels of *sAGP* expression were detected in leaves and flowers in which each exhibited only about 1 % the levels found in tubers.

Overall, the qualitative expression of the AGP-GUS chimeric gene correlates with the Northern profile of the endogenous *sAGP* gene with the notable exception of leaves. In leaves *sAGP* expression was much lower than anticipated based on the relative levels of *sAGP* mRNA observed in leaves and tubers. The low expression in leaves may be attributable to the absence of an essential cis-element and/or post-transcriptional regulation of the mRNA.

Although the same *sAGP* gene is expressed in different tissues, deletion analysis of the *sAGP* promoter revealed that different regions of the 5'-flanking sequence function in combination to confer the cellular and organ-specific patterns of *sAGP* expression. Stolon and tuber tissue revealed similar reductions in *sAGP* expression upon deletion of the 5'-promoter region as determined by fluorometric assay. On average, stolon and tuber tissue exhibited a 2- and 3-fold reduction of *sAGP* expression, respectively, upon removal of the first 2500 bp. Both tissues showed about 7-fold reduction upon deletion down to -670, relative to the transcriptional start site. Root tissue, on the other hand, revealed virtually no change in *sAGP* expression upon removal of the first 2500 bp. Deletion down to -670 resulted in only about a 4-fold decrease in expression. *sAGP* expression in tubers, stolons, and roots were barely detectable upon digestion down to -472 and completely eliminated when truncated to -160 bp of 5'-flanking sequence. Leaves showed a different pattern of expression in response to 5'-truncation of the -3500*sAGP*/GUS/NOS construct than the non-photosynthetic sink tissues. *sAGP* expression in leaves remained fairly constant, on average, upon removal of the first 2500 bp and exhibited about a 2-fold reduction when deleted down to -670. Low levels of *sAGP* expression was evident at -472 and elimination of expression occurred when digested down to -160. Stems, on the other hand showed a 4-fold decrease in *sAGP* expression upon deletion down to -1000 bp which is more similar to the reduction observed in the non-photosynthetic tissues than to leaves. Deletion down to -670 resulted in virtually no loss in expression while deletion down to -472 reduced expression slightly. As evident in all the other tissues, expression of the -160 bp construct was negligible. The non-coincident changes in gene activity displayed by these deletion constructs in the different tissues of the potato plant support the role of distinct cis-regulatory sequences in controlling the spatial expression of the *sAGP* gene.

The 3'-flanking sequences also contains cis-elements which enhance, suppress, and alter cell-specific expression of the *sAGP* gene (Appendix 3). This is best exemplified in stem and root tissue where the replacement of the 3' *nos* by 3' *sAGP* expands the spatial distribution of AGP expression to the parenchyma cells in addition to the starch sheath in stems and a reduction of reporter gene activity in roots. In addition, the cellular expression patterns of the *sAGP* gene only partially overlaps with the cellular expression pattern of the lAGP, and thus, allows for the formulation of a plausible mechanism for the post-transcriptional regulation of *sAGP* in leaves as discussed in more detail in the following section.

Differential Expression of the *sAGP* and *lAGP* Genes May Contribute to the Post-transcriptional Regulation of the *sAGP* in Leaves. Molecular studies have revealed the presence of three classes of large subunit *AGP* (*lAGP*) genes [32, 41]. Two of these classes are expressed, in addition to tubers, in leaves, albeit weakly, while expression patterns of the third class is restricted to tubers [32, 41]. In contrast to the multiple lAGP species, the *sAGP* is encoded by a single class of genes (Appendix 1). Transgenic analysis of one of the leaf lAGP genes has revealed specific expression exclusively in the midrib and guard cells of leaves [42], whereas expression of a *sAGP* gene is observed in the mesophyll and minor veins in addition to the midrib and guard cells. The more global and higher quantitative expression of the *sAGP* gene in leaves as compared to the *lAGP* provides a plausible explanation for the post-transcriptional control of the level of *sAGP* polypeptide during diurnal plant growth. As AGP

possesses a heterotetrameric enzyme structure consisting of two *lAGP* and two *sAGP*, excess unassembled *sAGP* is likely to be more susceptible to proteolysis, and hence, account for the apparent discrepancy between *sAGP* transcript and polypeptide levels (Appendix 3).

Part II. Studies on the Structure-Function Relationships of AGP

Mutagenesis of the Large Subunit cDNA of *AGP* (Appendix 4). In the previous funding period, we were able to express both the large and small subunit cDNAs in *Escherichia coli*. We demonstrated that functional enzyme was formed and that expression was able to complement a mutation in the bacterial *AGP* gene (*glg*C$^-$). This capacity to complement a mutation in *glg*C permitted a novel means to identify amino acid residues that are important in the allosteric and catalytic function of the AGP enzyme. To identify such residues, we initially subjected the large subunit cDNA to random mutagenesis using hydroxylamine and then co-expressed the DNA with wildtype small subunit cDNA in a *glg*C-deficient strain (Appendix 4). Over 346 mutants were obtained that accumulated normal levels of large subunits but produced little or no glycogen as viewed by the reduction in I_2 staining. These 346 mutants were then classified into 6 groups depending on their ability to accumulate glycogen, their levels of antigen and their levels of *in vitro* AGP enzyme activity. Groups I, II, and III exhibited no I_2 staining but contain normal, intermediate and low levels, respectively of enzyme activity. Classes IV-VI displayed the same corresponding levels of pyrophosphorylase activity but stained lightly with I_2.

Characterization of a Group I Allosteric Mutant. Of the 6 groups of mutants, the Group I type which contain only a single mutant #345 was of specific interest. Mutant 345 was unable to accumulate glycogen but possessed enzymatic and antigenic levels comparable to control cells when assayed in the presence of excess substrates and 3-PGA. Kinetic analysis of Mutant 345 indicated that it requires 45-fold greater amounts of the activator 3-PGA for maximum activity. Sequence analysis identified a single base substitution that resulted in the conversion of Pro_{52} to Leu (Fig. 1). This Pro residue is conserved among the plant, cyanobacterial, and bacterial AGPs. Of significance, Pro_{52} lies adjacent to Ala_{53} a conserved residue identified to be important in the allosteric regulation of the *E. coli* AGP enzyme [64]. In fact, the three amino acid sequence, PAV, is conserved in plants, cyanobacteria, and bacteria which supports a role of this hydrophobic sequence in enzyme function. We plan to conduct a site-directed mutagenesis of Val_{54} residue to determine whether this third residue is important for allosteric function of the enzyme.

Small Subunit Suppressors of Group I 345 Mutation. The isolation of single point mutation in one subunit type affords a unique opportunity to evaluate the interactions of these two different subunit types that are responsible for enzyme function. The identification of the $Pro_{52}\rightarrow$Leu (abbreviated Pro_{52}Leu) mutation in Mutant 345 indicates that the large subunit plays a critical role in allosteric function. To evaluate the role of the small subunit in this process we chemically mutagenized the small subunit cDNA and co-expressed it with Mutant 345 large subunit. By screening for the production of glycogen by I_2 staining, we were able to identify six suppressor mutants that produced varying quantities of glycogen. Several of these mutants have been characterized at the molecular level. Suppressor 5 contains a point mutation where Pro_{309}, located about 250 residues downstream from the conserved PAV motif, is converted into a Leu. In contrast, Suppressor 10 contains a $Pro_{46}\rightarrow$Leu conversion located adjacent to the PAV motif of the small subunit. This latter observation suggests that homologous regions of the large and small subunit interact with one another.

The Small Subunit is Capable of Forming a Homotetrameric Enzyme. In collaborative studies with J. Preiss (Michigan State University), the small subunit alone was able to form a homotetrameric enzyme when expressed in *E. coli* cells grown at room temperature (Appendix

5). Formation of the homotetramer small subunit enzyme was not evident in cells grown at 37°C (unpublished observations). The homotetramer enzyme required about 15- to 20-fold greater amounts of 3-PGA for activation as compared to the heterotetramer enzyme. Moreover, the homotetramer enzyme was more sensitive to Pi inhibition.

As the large subunit was unable to form an active enzyme by itself, these observations suggest that the small subunit may be the catalytic subunit with a very insensitive allosteric response towards activation by 3-PGA. In contrast, the large subunit plays a regulatory role and when combined with the small subunit increases the sensitivity of 3-PGA activation of the heterotetrameric enzyme. This view is supported by mutagenesis studies where expression of a mutated small subunit cDNA alone in *E. coli* grown at 37 C is able to complement the *glg*C mutation (unpublished data). The restoration of glycogen production suggests that the homotetrameric small subunit enzyme has a much lower $A_{0.5}$ for 3-PGA. We are currently purifying this enzyme to evaluate its kinetic parameters. The mutant phenotype is due to a single point mutation which converted Leu_{47}→Phe. Leu_{47} is only a single residue removed from the PAV motif in the small subunit and supports the role of this peptide region in allosteric regulation. It is interesting to note that a Phe residue is located at this position in the bacterial enzyme (Fig. 1).

Characterization of Group IV Mutants. The Group IV mutants are similar to the Group I type in possessing wildtype levels of AGP activity in the presence of excess activator 3-PGA. The group IV mutants, however, stain very lightly with I_2, indicating that they accumulate low levels of glycogen. Our present analysis of this group indicates that it contains mutants defective in allosteric properties, catalytic properties or both of these functions.

Allosteric Mutant - Mutant 27 possesses normal substrate binding for ATP, Glc 1-P and Mg^{2+}. In contrast, 6-fold greater levels of 3-PGA are required for 50% activation. This increase in $A_{0.5}$ was due to a point mutation resulting in an Asp_{415}Ser. Asp_{415} lies adjacent to the Lys residue that is reactive towards pyridoxal phosphate and, therefore, likely resides in the allosteric binding domain. The decrease in 3-PGA binding by the replacement of the adjacent Asp suggests that this residue also participates in 3-PGA binding. In addition to Mutant 27, two other group IV mutants appear to be defective in allosteric function and are presently being evaluated.

Catalytic Mutant - Unlike the response by Mutant 27, this Class IV mutant exhibited a normal hyperbolic activation by 3-PGA. Although Mutant 325 possesses a lower K_m for ATP, it is less sensitive to Glc 1-P and Mg^{2+}. Of these two parameters, the 3-fold decrease in affinity towards Glc 1-P is likely responsible for the significant decrease in glycogen production. The steady state levels of Glc 1-P in *E. coli* are estimated to be about 0.1 mM [67], comparable to the K_m of the wild type enzyme. Therefore, relatively minor changes in the K_m as reflected for the mutant 325 enzyme would have significant effects on ADPglucose formation and, in turn, glycogen production. The partial decrease in sensitivity toward Glc 1-P binding suggests that the mutation in 325 is only semi-dominant, having little effect on the catalytic properties of the wildtype small subunit in the heterotetrameric enzyme. This suggestion will be tested by identifying the mutation present in the large subunit and mutating the homologous residue in the small subunit.

Combined Allosteric/Catalytic Mutants - Three Group IV mutants are enzymes which display altered properties in both allosteric regulation and substrate binding. Mutants 143 and 336 have $A_{0.5}$ values for 3-PGA of 1.1 mM and 0.8 mM (10-fold and 8-fold higher, respectively, than wildtype), and also have reduced affinities for Glc 1-P with K_ms of 1.0 mM (11-fold higher than wildtype) and 0.65 mM (7.5-fold higher than wildtype), respectively. Mutant 332 also displays the same pattern with a $A_{0.5}$ of 1.2 mM for 3-PGA and a K_m of 2.9 for Glc 1-P. However, unlike the other Group IV mutants, 332 also shows the unusual feature of being

activated by Pi as shown below. In the presence of different concentrations of 3-PGA, the mutant displays a 2-fold activation up to 3 mM Pi. At higher Pi concentrations, inhibition begins to occur. Mutant 332 has been identified as minimally having an $Asp_{143} \rightarrow Asn$. If this is the only change in Mutant 332, the Asp_{136} could be an important focus of investigation since its change alters many properties of the enzyme at once.

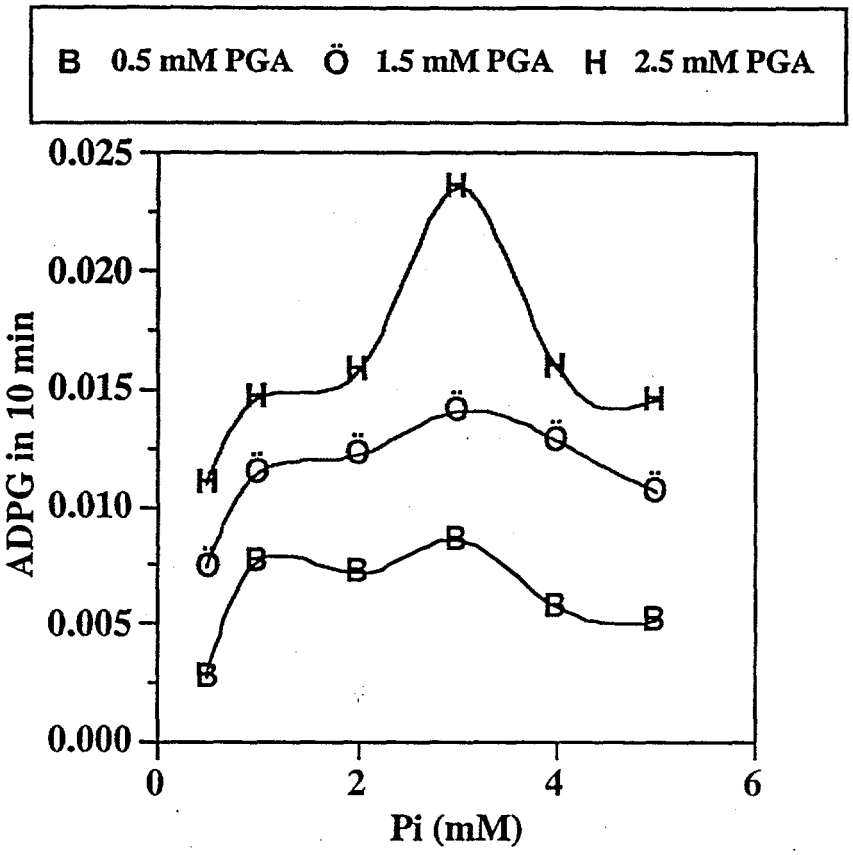

Fig. 2 Stimulation of Mutant 332 by Pi in the presence of varying levels of 3-PGA.

The Isolation and Characterization of Up-Regulated Mutants. The altered allosteric response exhibited by Mutant 345 is highly significant in that it demonstrates that dominant mutations in enzyme function can be obtained from a single point mutation in only one of the two structural genes for the plant AGP. Moreover, this observation suggests that it may be feasible to obtain mutations where the altered enzyme displays increase responsiveness to the activator 3-PGA or less sensitivity to the inhibitor Pi. Such mutants would be invaluable in efforts to genetically engineer plants for enhanced starch production.

UpReg-1 - During our studies, one mutant (UpReg-1) was identified which stained faster with I_2 and hence had increased glycogen accumulation. When the phenotypic response was examined at the enzyme level, the resulting heterotetrameric enzyme displayed an increased sensitivity to 3-PGA activation (from 160 μM to 13 μM) with no apparent change in the binding constants for the substrates Glc 1-P and ATP. UpReg-1 also displayed an increased resistance to Pi inhibition. In the presence of 0.25 mM 3-PGA, the enzyme showed a $I_{0.5}$ (concentration of Pi required for 50% inhibition) of 5 mM. This amount is 40- to 70-fold more than that required to inhibit the wildtype enzyme at the same concentration of 3-PGA. The increased sensitivity to 3-PGA is due to a single point mutation resulting in the conversion of acidic amino acid to a basic

residue (proprietary information). This allosteric mutant together with others afford us a unique opportunity to test the direct role of allosteric properties of ADPglucose pyrophosphorylase in controlling starch synthesis.

UpReg-2 Containing the *Shrunken 2* Revertant 6 (*Sh-2* Rev 6) Mutation in the Tuber cDNA. Professor C. Hannah (University of Florida) has identified a mutation in the large subunit gene (*Shrunken 2*) of the maize endosperm-specific ADPglucose pyrophosphorylase which confers higher starch accumulation in seeds. To determine what effect this mutation may have on the allosteric/catalytic properties of this enzyme, the *Sh-2* Rev6 mutation was incorporated in the tuber large subunit cDNA to produce UpReg-2 which was then co-expressed with wildtype small subunit cDNA. UpReg-2 appeared to accumulate higher amounts of glycogen than cells expressing the wildtype tuber cDNAs as viewed by I_2 staining. Preliminary kinetic analysis of the UpReg-2 enzyme indicates a lack of inhibition by Pi. If this initial assessment is accurate then we can test the role of Pi in modulating the *in situ* activity of the enzyme and its effect on starch production.

Characterization of N- and C-terminal Mutations. Preliminary studies by other laboratories on the maize and barley endosperm-specific AGP suggest that these enzymes are highly sensitive to proteolysis resulting in reduced sensitivity of the partially-cleaved enzyme towards activation by 3-PGA [29, 56]. As proteolysis appears to occur at the N- and/or C-termini [29, 56], we constructed large and small cDNA plasmids containing deletions at either end of the coding sequence. In one series of experiments we removed the last 19 residues that contained a lysine believed to be in the allosteric site from either the large and small subunit. No enzyme activity was evident when either truncated subunit cDNA was co-expressed with its corresponding wildtype counterpart. Subsequent analyses showed that although ample amounts of both subunits were present, assembly of these subunits into heterotetrameric structure did not occur. These results suggest that a small C-terminal peptide on both the large and small subunits is essential not only for allosteric function but also for enzyme assembly.

Deletions at the N-terminus were not as severe as that evident for the C-terminus but, nevertheless, significant effects were evident. Deletion of eight residues from the N-terminus of the small subunit had no significant effect on assembly with wildtype large subunit to form the holoenzyme. The catalytic parameters and sensitivity towards 3-PGA of the mutant enzyme also remain unchanged. The mutant enzyme, however, was very sensitive to heat treatment unlike the wildtype enzyme. Total loss of enzyme activity was observed after treating the enzyme at 58°C for 3 min whereas the wildtype enzyme activity was stable under these conditions (Appendix 5). The mutant enzyme was also more resistant towards Pi inhibition than the wildtype enzyme. These results indicate that the N-terminal end of the small subunit is required for proper polypeptide folding especially with regard to allosteric sensitivity to Pi inhibition and heat stability. A similar mutation at the N-terminus of the large subunit is presently under investigation.

Mutagenesis of the Small Subunit cDNA. Similar to the efforts made on the large subunit cDNA, the small subunit sequences were also subjected to mutagenesis by hydroxylamine. Several hundred mutants defective in glycogen synthesis were isolated and divided into 6 groups as before. Nine of the Group I and II mutants (which lack I_2 staining but contain significant levels of enzyme activity) have been characterized molecularly. Other than a mutation at the translation stop codon which results in a peptide addition at the C-terminus, one mutant showed a $Pro_{43} \rightarrow Ser$ (similar to Mutant 345), three of the mutants showed a $Asp_{121} \rightarrow Asn$, two mutants contained an $Asp_{252} \rightarrow Asn$, and two mutants displayed an $Ala_{106} \rightarrow Thr$. The redundancy of identical mutations suggests that saturation mutagenesis of the small subunit has been achieved with hydroxylamine.

The Asp_{121} Asn and Ala_{106} Thr mutants are interesting as these sites reside near Tyr_{114} which has been shown to lie at or near the ATP/ADPglucose binding site in the bacterial enzyme as viewed by photoaffinity labeling (64). Mutant Asp_{121} Asn has been characterized kinetically. Although the mutation lies near the putative ATP/ADPglucose binding site, the K_m for ATP was identical to the wildtype enzyme. The Asp_{121} Asn mutant, however, requires 8-fold higher amounts of 3-PGA for 50% activation and displays a 5.4 fold higher K_m for Glc 1-P than the wildtype enzyme.

Abbreviated List of Scientific Contributions during last 3 years of funding

1. Obtained direct evidence that there is only single *sAGP* gene which is expressed in leaves, tubers and other tissues in potato.

2. Showed that *sAGP* gene is differentially regulated in tubers and leaves. In tubers transcription is the most important level of control while in leaves allosteric control of AGP enzyme activity predominates.

3. Cis-elements of the *sAGP* gene reside at both the 5' and 3' sequences of the mRNA-intron sequences.

4. Demonstrated that expression of both the large and small subunit are required for functional expression in *E. coli* although under certain conditions the small subunit is capable of forming a homotetrameric enzyme requiring higher amounts of the activator 3-PGA for activity.

5. Identified mutations in the large and small subunits that produce enzymes defective in allosteric, catalytic or both of these activities.

6. Identified up-regulated mutations in the large subunit which when co-expressed with wildtype small subunit produce enzymes that requires less 3-PGA for activation.

List of Publications

1. Okita, T.W., Nakata, P., Ball, K., Smith-White, B. and Preiss, J. 1993 Enhancement of Plant Productivity by Manipulation of ADPglucose Pyrophosphorylase. *In* "Gene Conservation and Exploitation" (Gustafson, J.P., Appels, R. and Raven, P., ed.s), Plenum Press, New York, pp. 161-191.

2. Iglesias, A.A., Barry, G.F., Meyer, C., Bloksberg, L., Nakata, P.A., Greene, T., Laughlin, M.J., Okita, T.W., Kishore, G.M. and Preiss, J. 1993 Expression of the Potato Tuber ADP-glucose Pyrophosphorylase in *Escherchia coli*. J. Biol. Chem. **268**:1081-1086.

3. Preiss, J., Stark, D., Barry, G. F., Guan, H. P., Libal-Weksler, Y., Sivak, M. N., Okita, T. W. and Kishore, G. M. 1994 Prospects for the production of cereals with improved starch properties. In Proceedings of Improvement of Cereal Quality by Genetic Engineering, R. J. Henry, J.A. Ronalds, eds. Plenum Press, pp. 115-127.

4. Nakata, P. A., Anderson, J. M. and Okita, T. W. 1994 Structure and Expression of the Potato ADP-glucose Pyrophosphorylase Small Subunit Gene. J. Biol. Chem. **269**:30798-30807.

5. Nakata, P. N. and Okita, T. W. 1994 Studies to enhance starch biosynthesis by manipulation of ADPglucose pyrophosphorylase genes. *In* "Molecular and Cellular

Biology of the Potato, Second Edition" (Belknap, W. R., Vayda, M. E., and Park, W. D., eds), C.A.B. International, Wallingford, U.K.

6. Roberts, M. W., Preiss, J. and Okita, T. W. 1995 A capillary zone electrophoresis assay for the nucleoside transfer enzyme ADPglucose pyrophosphorylase. Anal. Biochem. **225**:121-126.

7. Nakata, P. A. and Okita, T. W. 1995 Differential Regulation of Potato ADP-Glucose Pyrophosphorylase in the Sink and Source Tissues of Potato. Plant Physiol. **108**:361-368

8. Ballicora, M. A., Laughlin, M. J., Fu, Y., Barry, G., Okita, T. W. and Preiss, J. 1995 ADP-glucose pyrophosphorylase from potato tuber. Significance of the N-terminal of the small subunit for heat stability and phosphate inhibition. Plant Physiol. in press.

9. Preiss, J., Ballicora, M. A., Laughlin, M. J., Fu, Y., Okita, T. W., Barry, G. F., Guan, H. and Sivak, M. N. 1995 Studies on the starch biosynthetic enzymes for manipulation of starch content and quality. in press.

10. Nakata, P.A. and Okita, T.W. 1995 Cis-elements important for the expression of the ADP-glucose pyrophosphorylase small subunit are located both upstream and downstream from its structural gene (submitted to MGG).

11. Greene, T.W., S.E. Chantler, M.L. Kahn, G.E. Barry, J. Preiss and T.W. Okita 1995 Mutagenesis of the potato tuber ADP-glucose pyrophosphorylase and characterization of an allosteric mutant defective in 3-phosphoglycerate activation. (Submitted to PNAS).