REGULATION OF ADP-GLUCOSE PYROPHOSPHORYLASE SUBUNIT EXPRESSION – A KEY ENZYME FOR STARCH BIOSYNTHESIS IN PLANTS

T.J. Gianfagna, X. Li, J. Xing, Y. Luo and H.W. Janes
Plant Science Department, Rutgers University, 59 Dudley Road, New Brunswick, NJ 08901-8520

INTRODUCTION
Starch is the predominant carbon sink of storage organs and leaves of many plants. Substantial evidence from the analysis of starch-deficient mutants, transgenic plants, and enzyme kinetics suggest that ADP-glucose pyrophosphorylase (AGPase) catalyzes a key step for starch biosynthesis in both photosynthetic and non-photosynthetic tissues. Fruit quality in tomato is largely a function of the soluble solids content of the fruit, and there is a direct relationship between starch levels early in fruit development and the soluble solids content at maturity. Moreover, the ability to synthesize starch shortly after fruit set, may be critical for successful organ development. Increased starch biosynthesis in tomato fruit may also lead to increased assimilate partitioning into fruit, and a decrease in non-edible biomass, reducing waste processing needs in the BLSS. Thus, AGPase appears to be an attractive biotechnological target for increasing starch synthesis in plants, both on earth and in space. The enzyme is a heterotetramer containing two $S$ and two $B$ subunits. We have identified three distinct genes for the $S$ subunit and one gene for the $B$ subunit (Chen et al., Plant Science 136:59-676, 1998). The purpose of this research is to determine the biochemical basis for regulation of AGPase activity in tomato, and to design novel ways of increasing starch content in plants by biotechnological manipulation of the gene(s) for this enzyme.

CURRENT STATUS OF RESEARCH
Methods
Total RNA was extracted from young tomato fruit, mRNA separated, and a cDNA expression library created by standard techniques. Identification of the multiple forms of the $S$ subunit transcripts was accomplished by PCR amplification using the following degenerate primers: T(GG)AGAGG (A)AAC(A)C(T)GC(T) AATGT and AAGTA(GA)AACC(ACG)GTA(AA)CAA(TG)ATACC, followed by 3’ and 5’ RACE (rapid amplification of cDNA ends) to create full length cDNAs. PCR products were cloned and sequenced.

$Agp S1$ was isolated from a lambda phage genomic library from tomato fruit using standard techniques. The 8.2 kb insert was cut with restriction enzymes, sub-cloned and sequenced. Truncated promoter regions were cloned into Agrobacterium tumefaciens LBA 4404 using pBI101, which contains the GUS gene. The results from three truncated promoters (0.8, 1.3 and 3.0 kb), all beginning upstream from the structural gene, are reported upon here. Leaf discs of tobacco were transformed with A. tumefaciens containing the three truncated promoter regions of $agp S1$ and plants were regenerated using antibiotic resistance for selection of transgenic plants. For tomato plants, cotyledons and hypocotyls were maintained on media supplemented with tobacco cell suspension cultures before and after transformation with A. tumefaciens.

For $agp S1$ promoter analysis, leaves, stem sections, roots and flowers were treated with GUS staining solution containing 5-bromo-4-chloro-3-indolyl β-D-glucuronide for 6 h at 37°C. Tissues were washed with water. Leaves were bleached with ethanol before staining. Sucrose induction experiments were carried out with normal tomato fruit and excised tobacco leaves from transgenic plants transformed with the three truncated promoters (0.8, 1.3 and 3.0 kb) of $agp S1$.

Results
1. Expression of subunits and effect of sucrose
Complete sequences for three $S$ subunit cDNAs ($agp S1$, $agp S2$, and $agp S3$; genebank accession numbers U81033, U81034, and U85497) and one for the $B$ subunit ($agp B$) were identified. Northern analysis revealed some major differences in the expression profiles of the isoforms between different tissues, and between tomato and potato. In the fruit, the expression of $agp B$ and $agp S1$ was very strong, $agp S2$ was expressed more weakly, while $agp S3$ was very low or absent. In the leaves, $agp B$ and $agp S3$ were the highly expressed isoforms, whereas expression of $agp S1$ was moderate and $agp S2$ very low. No obvious differences in transcript levels were observed for any isoform between mature and young leaves. In roots, neither $agp B$ or $agp S3$ were detected, although there was moderate expression of $agp S1$ and $S2$. 
When excised leaves were incubated in sucrose there was a significant induction of transcription for \textit{agp B}, \textit{agp S1}, and \textit{agp S2} within 8 h after incubation. Transcript levels remained elevated for at least 16 h. In contrast, \textit{agp S3} was not stimulated by sucrose. In fact, transcript levels declined by 16 h. When sucrose was injected into 10-d-old fruit, there was an increase in the level of \textit{agp S1} transcription within 3 h. The response to sucrose reached a maximum by 6 h and was maintained for 24 h after injection. When water alone was injected into fruit, activity declined relative to fruit that had not been injected.

2. Cloning of \textit{agp S1} and analysis of the promoter region

A lambda phage clone that hybridized with the AGPase S1 probe contained a tomato DNA insert with 8,200 base pairs (bp). Based upon comparison with the cDNA isolated from tomato fruit, the transcription initiation site is at bp 3178, the termination site at bp 7808 and there are 14 introns in the gene. The translation start codon (ATG) is at bp 3826 and the translation termination signal (TGA) at bp 7484. There is a TATAA box, a common eukaryotic regulatory sequence, at bp 3123, 55 bps upstream from the transcription initiation site.

Using transgenic tobacco with the \textit{agp S1} promoter and its truncated versions ligated to the GUS reporter gene, we will describe the histological location of \textit{agp S1} expression in all the organs of the plant, throughout their development. In the leaves e.g., \textit{agp S1} is located primarily in the guard cells and is conspicuously absent in the leaf mesophyll. All of the constructs (0.8, 1.3, and 3.0 kb) caused GUS expression. There were no differences between constructs; therefore, only the 0.8 kb region is required for guard cell expression. We determined the location of possible \textit{cis}-acting elements that confer sucrose sensitivity. Only guard cells from plants that contained the 3.0 kb region responded to sucrose treatment. There were no changes in expression when sucrose was fed to leaves containing the 0.8 or 1.3 kb promoter fragments, implying that the sucrose sensitive regulatory region is located in a 1.7 kb region upstream from the 1.3 kb fragment. There are four elements in the 1.7 kb segment of the 3.0 kb construct that are very similar to sucrose regulatory regions in other genes. It is likely, therefore, that one of the functions of this region of the \textit{agp S1} promoter is to confer responsiveness to sucrose.

\textit{Agp S1} is highly expressed in fruit tissue. In cv. Laura, fruit development from anthesis to red-ripe fruit takes approximately 60 d. Significant levels of \textit{agp S1} transcript appear about 10 d after anthesis. Transcript levels increase to a maximum about 15 d after anthesis and then decline significantly. Transcription of \textit{agp S1} is no longer detectable in fruit more than 35 d after anthesis. Both the \textit{B} and \textit{S2} genes are also activated during the same period of fruit development as \textit{agp S1}. There is, however, some additional transcriptional activity of the latter two genes in red fruit. This may be localized to the seed.

CONCLUSIONS

There are significant differences in \textit{S} gene expression between the different plant organs throughout their development. \textit{Agp S1} and \textit{S2}, but not \textit{S3}, are activated by sucrose. Transcriptional activity is consistent with the pattern of starch accumulation in tomato fruit, and suggests that AGPase is a key enzyme on the pathway in which sucrose is converted to starch.

FUTURE DIRECTIONS

Our transgenic tomato plants have begun to set fruit. We will extend our characterization of \textit{agp S1} expression in fruit using anti-sense constructs. In addition, and perhaps of greater significance, we can now use the \textit{agp S1} promoter to drive other genes that may increase carbon partitioning to the fruit, and perhaps produce plants with higher yield and nutritional quality for cultivation in space and on earth. The gene for isopentenyladenine transferase (\textit{ipt}) is a good candidate. When ligated to the \textit{agp S1} promoter, the \textit{ipt} gene should increase cytokinin production and cell division in the fruit only during the early stages of fruit development.

INDEX TERMS

Transgenic plants, tomato, starch, gene promoter