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ANNUAL PROGRESS REPORT

SOYBEAN RIBULOSE BISPHOSPHATE CARBOXYLASE SMALL SUBUNIT:
MECHANISMS AND DETERMINANTS OF RNA TURNOVER

AT

THE UNIVERSITY OF GEORGIA COMPLEX CARBOHYDRATE RESEARCH FOUNDATION
(UGRF)

A Department of Energy Unit of the
USDA/DOE/NSF Plant Science Centers Program

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Richard B. Meagher

1992 - 1993

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Project Summary for DOE's 1993 Annual Report

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Soybean Ribulose Bisphosphate Carboxylase Small Subunit: Mechanisms and Determinants of RNA Turnover in Higher Plants

R.B. Meagher, Department of Genetics

In order to investigate the mechanisms and determinants controlling RNA turnover in higher plants we have developed an the *in vivo* degradation system from petunia and soybean polysomes which faithfully degrades soybean ribulose-1,5-bisphosphate carboxylase small subunit (rbcS) mRNA. The most stable products represent a nested constellation of fragments, shortened from their 3' ends with intact 5' ends. Exogenously added rbcS RNA tagged with novel 5' sequence were degraded into a similar constellation of products but containing the extra 5' tag nucleotides. Detailed kinetics on the appearance of these exogenous products confirmed degradation proceeds in an overall 3' to 5' direction but suggested that there are multiple pathways of degradation. To further demonstrate a precursor product relationships, *in vitro* synthesized 3' truncated transcripts were shown to degrade into the expected smaller fragments. The system is now ready to examine the degradation of other mRNAs and various trans-acting determinants of turnover.

We have isolated three *Arabidopsis* genes encoding poly(A) binding proteins (PABP), *PAB1*, *PAB3*, and *PAB5*. Surprisingly, *PAB1* was expressed only in roots, while *PAB3* and *PAB5* were expressed only in flowers. A *PAB5* engineered for expression in yeast suppressed a lethal mutation in the yeast PABP gene. In yeast *PAB5* controlled poly(A) tail length and polysome loading of mRNAs. In the future we will examine the proteins activity *in vitro* in a yeast poly(A) nuclease system and our own plant RNA degradation system. The *PAB5* protein was over expressed in *E. coli* and is being purified. Interactive cloning and suppression cloning techniques will be used to identify the components interacting with the plant poly(A) binding proteins.

DOE Progress Report for 1992-1993

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**Soybean Ribulose Biphosphate Carboxylase Small Subunit:
Mechanisms and Determinants of RNA Turnover in Higher
Plants**

We have developed an *in vitro* degradation system from petunia and soybean polysomes in order to investigate the mechanisms and determinants controlling RNA turnover in higher plants. This system faithfully degrades soybean ribulose-1,5-bisphosphate carboxylase small subunit (rbcS) mRNA into the same products observed in total RNA preparations. In previous years we showed that the most stable products represent a nested constellation of fragments, which are shortened from their 3' ends, and have intact 5' ends. Exogenous rbcS RNA tagged with novel 5' sequence 15 or 56 bp long were synthesized *in vitro* as Sp6 and T7 runoff transcripts, respectively. When added to the system they were degraded faithfully into constellation of products which were 15 or 56 bp longer than the endogenous products, respectively. Detailed kinetics on the appearance of these exogenous products confirmed degradation proceeds in an overall 3' to 5' direction but suggested that there are multiple pathways through which the RNA may be degraded. To further demonstrate a precursor product relationships, *in vitro* synthesized transcripts truncated at their 3' ends were shown to degrade into the expected smaller fragments previously mapped in the 5' portion of the rbcS RNA. The *in vitro* system is now ready to examine the degradation of other mRNAs and various transacting determinants of turnover. Most of this work has been conducted by a senior graduate student, Matthew Tanzer, as the focus of his Ph.D. research. Mr. Tanzer has completed all the experiments needed to write this up for a publication which will be submitted to Plant Cell or Mol. Cell. Biol. in the next two months. A new graduate student Ravi Palanivelu has begun initial

work mapping the degradation products for *Arabidopsis* PUR5 (AIR synthetase) and ACT2 (actin) transcripts. Understanding the degradation of these RNAs will broaden our understanding of this process in plants.

Last year we proposed examining plant poly(A) binding proteins (PABP) in our *in vitro* system. This protein is found in animals and fungi and is presumed to be ubiquitous in all eukaryotic cells. Because of its high affinity for poly(A) it was presumed to protect the poly(A) tails of mRNAs from degradation, and in high concentrations will do just that *in vitro*. However, in the last few years a remarkable set of experiments in yeast has broadened our understanding of PABP function. The yeast gene and protein are required for yeast growth and when its expression is shut off cells cease to grow and die. Shortly after the expression of yeast PABP gene is stopped, poly(A) tails of the mRNA population get longer and the pool of monosomes gets larger. It turns out that PABP is required by the poly(A) specific nuclease. *In vitro* the nuclease requires PABP to degrade poly(A). The increase in the pool of monosomes can be explained in light of suppresser mutations isolated in conditional PABP mutants. These suppressers are factors which are involved in the 60S subunit addition to the ribosome initiation complex. Apparently the PABP is required for proper formation of the initiation complex. Thus PABP is now viewed as a central regulatory factor of post-transcriptional processes affecting the message.

We have isolated three *Arabidopsis* genes encoding PABPs, PAB1, PAB3, and PAB5, from what appears to be a very large family of genes. Surprisingly, PAB1 was expressed only in roots, while PAB3 and PAB5 were expressed only in flowers, suggesting that in plants PABPs may be involved in organ specific post-transcriptional processes. Sequence data on all three genes demonstrate that they have the expected four RNA recognition motifs (RRMs) covering the amino terminal two thirds of the proteins and unique carboxyterminal sequences. Furthermore, they represent a very diverse family of genes, far more diversity than is seen among all the previously isolated PABPs in animals and fungi.

The floral specific PAB5 cDNA was also cloned sequenced and the 5' and 3' ends of the RNA mapped. A *PAB5* promoter fusion to β -glucuronidase and a construct which will express *PAB5* antisense RNA in flowers have been transformed into *Arabidopsis*. These plants are maturing and primary transformants can be examined shortly. We anticipate that the *PAB5* antisense plants may be sterile in some way and an alternative strategy of for controlling the antisense expression with a Tet regulated promoter has been prepared if this is a problem.

PAB5 protein synthesized *in vitro* was shown to bind poly(A) with high specificity but not poly(G) or poly(C). These data, plus the presence of four RRM's suggest that PAB5 functions as a true poly(A) binding protein in plants. A PAB5 gene was engineered for expression in yeast and this construct suppresses a lethal mutation in the yeast PABP gene. In yeast the plant PAB5 gene controlled poly(A) tail length and the level of monosomes as does the yeast gene in wild type cells. The PAB5 protein was over expressed in *E. coli* and is being purified. In the future we will examine the proteins activity *in vitro* in a poly(A) nuclease system in collaboration with Alan Sachs at U.C. Berkeley and in our own plant *in vitro* degradation system. Interactive cloning and suppression cloning techniques will be used to identify other components interacting with the plant poly(A) binding proteins in plants. All this work was begun and carried out in the last year by a new postdoctoral fellow, Dima Belostotski, paid on the DOE grant.

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