Mechanisms and Determinants of RNA Turnover in Higher Plants

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We began an investigation of the post-transcriptional mechanisms controlling plant gene expression several years ago, by demonstrating that RNA levels for ribulose biphosphate carboxylase small subunit (*rbcS*) are partially controlled by the rate of RNA degradation (Shirley and Meagher, 1990; Thompson and Meagher, 1990). Plants supply all their carbon requirements by the fixation of carbon dioxide using the *rbc* enzyme. More recently it has become clear that a large number of plant genes are controlled at the post-transcriptional level and many at the level of RNA turnover. The determinants of RNA turnover were explored during the last four years with two diverse systems: the first dealt with the specific mechanisms by which *rbcS* mRNA was degraded in soybean, petunia and Arabidopsis; the second examined the roles of poly(A) binding proteins (PABP) in translation and RNA metabolism.

We have shown that *rbcS* mRNA is degraded into a discreet set of 5' proximal products (Thompson et al., 1992) and developed an in vitro system which would generate the same products from endogenous and exogenous *rbcS* mRNA (Tanzer and Meagher, 1994). Both the 5' and 3' proximal products are generated in random orders from full length RNA. More recently we mapped the precise 5' and 3' ends generated in vivo and in vitro by adapting a deoxynucleotide RNA sequencing technique, primer extension, RNase protection, and S1 mapping. Aligning the 5' and 3' processing sites demonstrated that these products are generated by endonucleolytic cleavage. Furthermore, the endonucleolytic cleavage precedes 3' poly(A) tail removal or 5' decapping, suggesting that this cleavage is the initial event in *rbcS* mRNA degradation (Tanzer and Meagher, 1995). These data clearly distinguished the processing of *rbcS* mRNA from the degradation of the majority of yeast mRNAs where deadenylation is followed by decapping and then by 5' to 3' exonucleolytic decay.

The poly(A) binding proteins (PABPs) are believed to regulate poly(A) tail length, mediate reinitiation of translation by interacting with initiation factors on the ribosomal subunits, and in link deadenylation with decapping and decay. This view is based on biochemical genetic studies in yeast. They are thought to be

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central elements in building post-transcriptional machinery effecting mRNA activity and stability. The *Arabidopsis* PABPs are encoded by a large diverse gene family whose members are differentially expressed (Belostotsky and Meagher, 1993). One of these genes, *PAB5*, is specifically expressed in developing pollen and ovules (Belostotsky and Meagher, submitted), while another *PAB2* is more constitutive. The *PAB2* and *PAB5* proteins are extremely diverse in sequence both from each other and from the yeast *PAB1* protein sharing only about 50% amino acid identity in any pairwise comparison. Thus, it was not clear if higher eukaryotic PABPs would share all the functions of the yeast *PAB1*. Both *PAB2* and *PAB5* bound poly(A) with specificity over other oligoribonucleotides. When expressed from a yeast promoter, both *PAB2* and *PAB5* rescued the viability of a yeast *PAB1* deletion (Belostotsky and Meagher, submitted). We are examining the specific properties of the plant PABPs using this yeast system and a combination of regulated yeast promoters and a temperature sensitive mutations in the plant PABPs which were constructed in yeast. So far we have demonstrated that in yeast, *Arabidopsis* *PAB5* restores nearly normal polysome structure and poly(A) tail processing (PABP dependent deadenylation), but did not restore the link between deadenylation and decay (Belostotsky and Meagher, submitted). This demonstrates that some of the major activities of PABPs previously identified only for yeast *PAB1* are conserved in PABPs from other eukaryotic kingdoms. Similar experiments are under way to determine if *PAB2* shares these properties. Since PABPs have been proposed to be at the center of the post-transcriptional apparatus we were interested in identifying other proteins to which they bound. Using the yeast dihybrid cloning system we have identified candidate proteins which may interact with *PAB2*. They passed the genetic tests for a "correct" dihybrid interaction and we are now in the process of exploring the biochemical validity of these interactions with co-immune precipitations reactions. Examining the role of PABPs in post-transcriptional regulation has become the major focus of our future DOE funded research.

Publications resulting from this research

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