Partial, 9 pgs

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An AU-Rich Element in the 3′ Untranslated Region of the Spinach Chloroplast *petD* Gene Participates in Sequence-Specific RNA-Protein Complex Formation

QIUYUN CHEN,† CYNTHIA C. ADAMS,‡ LYNN USACK, JIANJUN YANG,
RITA-ANN MONDE, AND DAVID B. STERN*

Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, New York 14853

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In chloroplasts, the 3′ untranslated regions of most mRNAs contain a stem-loop-forming inverted repeat (IR) sequence that is required for mRNA stability and correct 3′-end formation. The IR regions of several mRNAs are also known to bind chloroplast proteins, as judged from in vitro gel mobility shift and UV cross-linking assays, and these RNA-protein interactions may be involved in the regulation of chloroplast mRNA processing and/or stability. Here we describe in detail the RNA and protein components that are involved in 3′ IR-containing RNA (3′ IR-RNA)–protein complex formation for the spinach chloroplast *petD* gene, which encodes subunit IV of the cytochrome b_6/f complex. We show that the complex contains 55-, 41-, and 29-kDa RNA-binding proteins (ribonucleoproteins [RNPs]). These proteins together protect a 90-nucleotide segment of RNA from RNase T_1 digestion; this RNA contains the IR and downstream flanking sequences. Competition experiments using 3′ IR-RNAs from the *psbA* or *rbcL* gene demonstrate that the RNPs have a strong specificity for the *petD* sequence. Site-directed mutagenesis was carried out to define the RNA sequence elements required for complex formation. These studies identified an 8-nucleotide AU-rich sequence downstream of the IR; mutations within this sequence had moderate to severe effects on RNA-protein complex formation. Although other similar sequences are present in the *petD* 3′ untranslated region, only a single copy, which we have termed box II, appears to be essential for in vitro protein binding. In addition, the IR itself is necessary for optimal complex formation. These two sequence elements together with an RNP complex may direct correct 3′-end processing and/or influence the stability of *petD* mRNA in chloroplasts.

The roles of mRNA 3′ untranslated regions (UTRs) in regulating gene expression have become prominent (1, 18, 30, 44). In chloroplasts, the 3′ UTRs of most mRNAs are flanked by stem-loop structure-forming inverted repeat (IR) sequences that participate in 3′-end formation and are also thought to impede the progress of processive exoribonucleases (32, 36, 39, 40). Both the 3′ IR and the 5′ UTR have been shown by in vitro assays to associate with proteins which may regulate RNA processing, RNA stability, and/or translation initiation (10, 28, 32, 38, 46).

The mechanism of chloroplast mRNA 3′-end formation has been studied by using both in vitro assays and chloroplast transformation. In vitro transcription experiments were used to show that although the chloroplast RNA polymerase is capable of recognizing rho-independent prokaryotic transcription termination signals (8, 36), most chloroplast 3′ IRs have little or no transcription termination activity (36). Data obtained by comparing in vivo transcription rates upstream and downstream of the *Chlamydomonas atpB* 3′ IR are consistent with the in vitro observations (39). This finding suggests that most chloroplast mRNAs are synthesized as precursors, which are subsequently processed to yield the uniform 3′ termini found in vivo.

Both RNases and RNA-binding proteins (ribonucleoproteins [RNPs]) are likely to be required for chloroplast mRNA 3′-end maturation and for mRNA stability. Although RNases can process mRNA in the absence of additional factors, IRs present only transient barriers to 3′-5′ exoribonucleases such as *Escherichia coli* RNase II, as shown by in vitro studies using either bacterial or chloroplast 3′ IR-containing RNAs (3′ IR-RNAs) (24, 37). Therefore the IR alone cannot account for the measured in vivo lifetimes of mRNAs. This implies that additional protein factors are involved in processing and/or stabilization of the IR. A number of RNPs have been isolated from chloroplasts, and cDNA sequences have revealed consensus RNA-binding domains in most cases (reviewed in reference 12). However, the in vivo functions of most of these proteins are unknown. The exceptions are a 28-kDa spinach protein which appears to participate in 3′-end processing (32), a 54-kDa mustard protein that copurifies with an endoribonuclease activity that may be involved in RNA processing (27), and one or more 47-kDa *Chlamydomonas* proteins that may regulate translation initiation of *psbA* and *psbC* mRNAs (10, 46).

The correct action of chloroplast RNPs is likely to depend on the presence of *cis*-acting RNA sequences. Although little information is currently available for chloroplasts, sequence-specific motifs in mRNA which mediate protein binding are well known, for example, in the cases of cell cycle-regulated histone mRNAs (13, 23), the iron-responsive element in ferritin and transferrin receptor mRNAs (20), and a series of AU-rich elements in lymphokine and proto-oncogene mRNAs that negatively affect mRNA stability (5, 34). Interestingly, these elements have similar effects on plant mRNAs (29). Furthermore, AU-rich elements are found in the 3′ UTRs of chloroplast mRNAs, where preliminary work by us and others suggests that they may act as binding sites for RNPs (7, 26).

In this report, we describe the protein factors that associate

* Corresponding author. Phone: (607) 254-1306. Fax: (607) 254-1242. Electronic mail address: ds28@cornell.edu.

† Present address: Department of Biology, University of Utah, Salt Lake City, UT 84112.

‡ Present address: Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213.