Plant Molecular Biology **20**: 1185–1188, 1992.

Update section

Short communication

The *psbL* gene from bell pepper (*Capsicum annuum*): plastid RNA editing also occurs in non-photosynthetic chromoplasts

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Received 12 May 1992; accepted in revised form 3 August 1992

Key words: Capsicum annuum, bell pepper, chromoplast, chloroplast

Abstract

We have determined the nucleotide sequence of the plastid *psbL* gene from bell pepper. This gene has an ACG as a first codon. Isolation of RNA from pepper leaves and ripe fruits and subsequent sequencing of the *psbL* cDNA revealed that this ACG codon is post-transcriptionally edited into an AUG initiation codon in both leaves and fruits. These data indicate that the RNA editing machinery which exists in chloroplasts is still functional in chromoplasts from ripe fruits.

During plant development the plastid compartment is undergoing important structural and biochemical changes. During fruit ripening in pepper or tomato, for example, chloroplasts differentiate into non-photosynthetic chromoplasts. This transition is characterized by the degradation of chlorophylls, the accumulation of large amounts of carotenoids and a structural reorganization (for a review, see [2]).

We have chosen bell pepper (*Capsicum annuum*) as a model system to study chromoplast differentiation. We have previously shown that changes in both nuclear and plastid gene expression are involved in this developmental process [2, 9, 10, 12]. We could demonstrate that plastid genes are transcribed in chromoplasts and that the transcriptional activity of chromoplasts is not significantly different from that of chloroplasts from mature green fruits [2, 9]. Furthermore, the mRNAs for all the plastid genes tested are detected in chromoplasts [2, 9]. However, in contrast to chloroplasts, chromoplasts do not have significant translational activity in bell pepper as shown by two different methods, i.e. either by incubating isolated organelles in the presence of radiolabelled methionine or by *in vivo* protein labelling and subsequent organelle isolation [2, 9]. Thus, these data indicated the prevalence of a

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X65570.

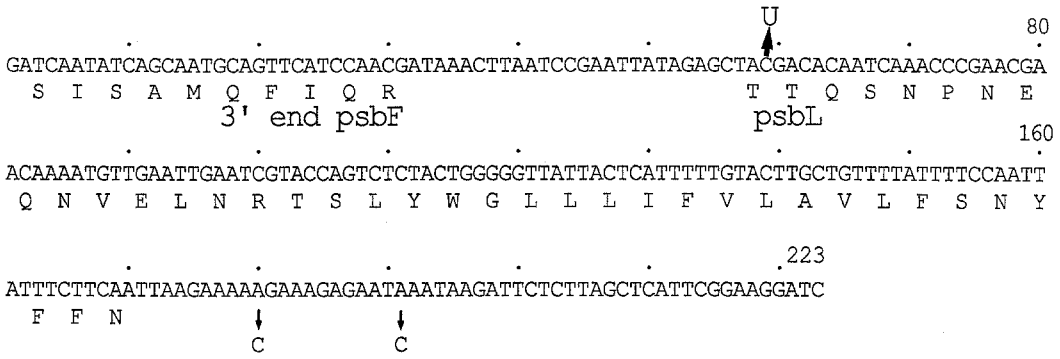

Fig. 1. Nucleotide sequence of the bell pepper *psbL* gene, its flanking regions and the 3′ end of the *psbF* gene. The C which is post-transcriptionally edited into U is indicated by a thick arrow. Differences with the corresponding sequence from tobacco are indicated by thin arrows.

post-transcriptional/translational control of plastid gene expression in this system.

RNA editing is a post-transcriptional process first observed in the case of trypanosome mitochondrial mRNAs [1] and then in the case of a number of mRNAs (for a review, see [3]), including various plant mitochondrial mRNAs [4–6]. More recently, Hoch *et al.* [7] and Kudla *et al.* [8] have reported RNA editing in the case of the maize *rpl2* and tobacco *psbL* plastid mRNAs (in both cases, an ACG codon is converted into an AUG initiation codon).

In order to examine the possible involvement of RNA editing in the expression of plastid genes during chromoplast differentiation, we have cloned a 223 bp *Sau*3A DNA fragment from the bell pepper plastid genome, containing the 3′ end of the *psbF* gene and the entire *psbL* gene (Fig. 1). When compared with the corresponding region of the tobacco plastid genome, only two base substitutions are observed in the bell pepper sequence, both of which are located in the 3′-flanking region of *psbL* (Fig. 1). Bell pepper *psbL* has an ACG as a first codon as is the case of tobacco. Using a synthetic oligonucleotide complementary to the RNA (spanning position 76 to 105 in Fig. 1) and which was radiolabelled in the presence of [γ-^{32}P]ATP and polynucleotide kinase, we have performed an RNA gel blot analysis of the *psbL* transcript during the differentiation of chloroplasts into chromoplasts (Fig. 2). These experiments revealed that the *psbL* transcript level does not significantly change during this developmental process, as already observed for other plastid transcripts (e.g. the *rbcL* transcript [9]). Using the same oligonucleotide

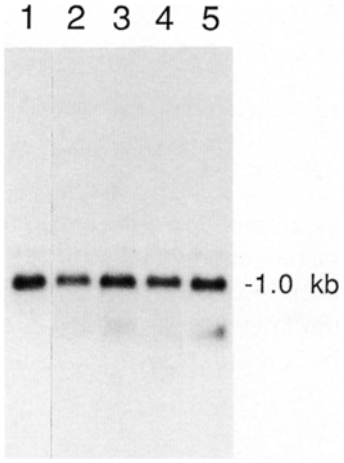

Fig. 2. RNA gel blot analysis of the *psbL* transcript during the chloroplast to chromoplast transition in bell pepper fruits. Total RNAs were isolated from a fruit at the green mature stage (lane 1), from an almost entirely red fruit (lane 5) and from fruits at three intermediate stages of ripening (lanes 2–4). Identical amounts of total RNA (7 μg) were separated by formaldehyde/agarose (1.2% w/v) gel electrophoresis, transferred to a Hybond-N (Amersham) membrane and subsequently hybridized to a radiolabelled oligonucleotide specific for the *psbL* transcript (see in the text). A faint hybridization signal corresponding to a larger transcript is also visible in each lane of the original autoradiograph. The additional band seen in the lower part of the autoradiograph (smaller than 1 kb) is due to a previous hybridization experiment using the same blot but a different probe.

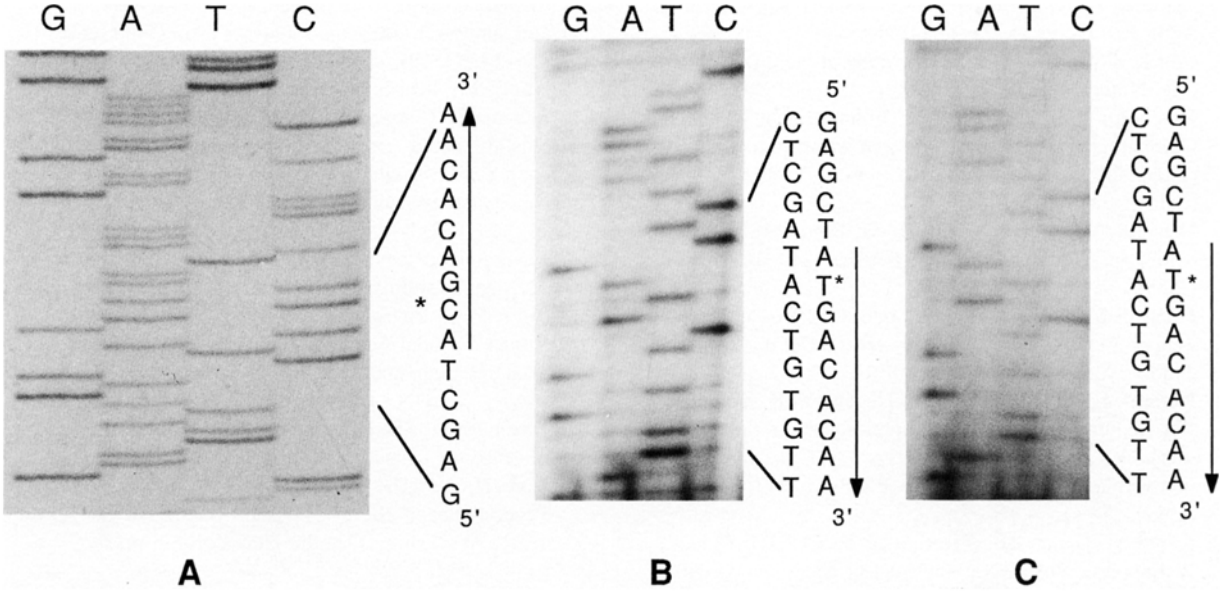

Fig. 3. Comparison of sequencing gel autoradiographs showing the 5′ end of the bell pepper *psbL* gene (A) and the corresponding cDNA from leaves (B) and ripe fruits (C). The editing position is indicated by an asterisk. The beginning of the coding region is shown by an arrow.

and poly(A)$^+$RNA isolated from bell pepper leaves and from ripe fruits, we have sequenced the cDNA corresponding to the 5′ end of the *psbL* gene. As expected, the leaf cDNA sequence was edited to convert the ACG codon into an AUG initiation codon (Fig. 3). This conversion even occurs in non-photosynthetic chromoplasts from ripe fruits (Fig. 3) in which the *psbL* gene product (a photosystem II 3.2 kDa polypeptide) is unlikely to be synthesized (as discussed above [8, 9]). Furthermore, this polypeptide has no function in chromoplasts since the photosynthetic membrane system is disorganized and progressively disappears [8, 11].

These data suggest that the editing machinery is still functional in non-photosynthetic plastids and exclude the involvement of editing as a post-transcriptional control mechanism during the differentiation of chloroplasts into chromoplasts, at least as far as *psbL* is concerned. Since we did not determine if other transcripts are edited in chromoplasts, we cannot exclude the involvement of RNA editing in the regulation of expression of other genes. However, our data indicate that RNA editing is not a general mechanism of regulation. As proposed for plant mitochondria [5], it seems more likely that RNA editing acts only as a correction mechanism in plastids.

Acknowledgements

We are grateful to A. Klein for technical assistance, to Dr S. Römer for providing us with pepper fruit RNAs and to Dr J.M. Grienenberger for critical reading of the manuscript.

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