Arabidopsis thaliana small subunit leader and transit peptide enhance the expression of Bacillus thuringiensis proteins in transgenic plants

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Abstract

The expression of the modified gene for a truncated form of the cryIA(c) gene, encoding the insecticidal portion of the lepidopteran-active CryIA(c) protein from Bacillus thuringiensis var. kurstaki (B.t.k.) HD73, under control of the Arabidopsis thaliana ribulose-1,5-bisphosphate carboxylase (Rubisco) atslA promoter with and without its associated transit peptide was analyzed in transgenic tobacco plants. Examination of leaf tissue revealed that the atslA promoter with its transit peptide sequence fused to the truncated CryIA(c) protein provided a 10-fold to 20-fold increase in cryIA(c) mRNA and protein levels compared to gene constructs in which the cauliflower mosaic virus 35S promoter with a duplication of the enhancer region (CaMV-En35S) was used to express the same cryIA(c) gene. Transient expression assays in tobacco protoplasts and the whole plant results support the conclusion that the transit peptide plus untranslated sequences upstream of that region are both required for the increase in expression of the CryIA(c) protein. Furthermore, the CaMV-En35S promoter can be used with the Arabidopsis atslA untranslated leader and transit peptide to increase expression of this protein. While subcellular fractionation revealed that the truncated CryIA(c) protein fused to the atslA transit peptide is located in the chloroplast, the increase in gene expression is independent of targeting of the CryIA(c) protein to the chloroplast. The results reported here provide new insight into the role of 5′ untranslated leader sequences and translational fusions to increase heterologous gene expression, and they demonstrate the utility of this approach in the development of insect-resistant crops.

Introduction

Rubisco is the bifunctional enzyme found in the chloroplasts of plants that catalyzes the initial carbon dioxide fixation step in the Calvin cycle and functions as an oxygenase in photospiration. In higher plants it consists of eight each of two subunits, a large subunit (LSU) encoded by the chloroplast genome and the small subunit (SSU) polypeptide encoded in the nuclear genome [6]. The SSU polypeptides are formed as precursors containing an amino-terminal extension termed a transit peptide that is involved in the transport of the SSU polypeptide into the chloroplast during which the transit peptide is removed [6]. Rubisco is the most abundant pro-
tein found in plant leaves, representing up to 50% of the soluble protein [17]. Thus, the SSU promoters and their transit peptides are attractive candidates for expression of genes at high levels in green tissue and for targeting of different proteins into the chloroplast. The SSU polypeptides are encoded by a multigene family each with a different pattern of expression. In Arabidopsis thaliana, the SSU family consists of four members [15]. Earlier work on transgenic expression of two marker genes under the control of the promoter from one of the Arabidopsis SSU genes, ats1A, showed that sequences encoding the transit peptide increase expression levels compared to the promoter fragment alone [3]; however, specific sequences or regions required for this increase in expression were not identified.

Insect-resistant tomato, tobacco and cotton plants have been developed through the expression of insecticidal proteins from Bacillus thuringiensis (B.t.) [7, 26, 21]. A major challenge in using this approach to develop crops with commercial levels of insect control has been to increase the level of gene expression. One successful approach to increasing expression has been through the use of modified coding sequences which dramatically increase B. thuringiensis gene expression in plants [21, 22]. For control of certain insect pests, however, it may still be necessary to increase B. thuringiensis expression overall or in specific plant tissues. In the work reported here, expression in transgenic tobacco plants of the modified gene for a truncated form of the crylA(c) gene, which encodes an insecticidally active fragment of the CrylA(c) protein of B. thuringiensis var. kurstaki (B.t.k.) HD73, under the control of the ats1A promoter of Arabidopsis and the CaMV-En35S promoter with and without the ats1A transit peptide is compared. We were interested in determining if CrylA(c) protein levels could be increased by this method and if the increase in expression was directly due to targeting of protein into the chloroplast or the sequences encoding the transit peptide. Examination of crylA(c) gene expression in leaves at both the mRNA and protein levels reveals that the ats1A promoter directs approximately 10-fold to 20-fold higher levels of expression than the CaMV-En35S promoter when ats1A untranslated leader and transit peptide coding sequences are included and that the differences seen at the protein level are also reflected at the mRNA level. Chloroplast isolation experiments show that when the CrylA(c) protein is fused to the SSU transit peptide, the B. thuringiensis protein is translocated to the chloroplast. For the crylA(c) gene, whole plant expression and a tobacco protoplast transient expression assay indicate that both the 5’untranslated leader and the transit peptide of ats1A are essential for the increase in expression. Targeting of the CrylA(c) protein to the chloroplast does not play an important role in gene expression. However, transient expression analysis of the Escherichia coli β-glucuronidase gene (GUS) fused to segments of the ats1A untranslated leader and transit peptide demonstrates that the ability of these sequences to increase gene expression are dependent on the coding sequence to which they are attached. The results reported here provide new insight into the role of 5’ untranslated leader sequences and translational fusions to increase heterologous gene expression, and they demonstrate the utility of this approach in expressing a gene of agricultural importance.

Material and methods

Vector construction

The crylA(c) gene utilized in the vectors described in this report is the fully modified truncated crylA(c) gene described by Perlak et al. [22], and referred to in that reference as FM crylA(c). The nucleotide sequence of this gene can be found in Perlak et al. [21]. This gene is a synthetic truncated version of the B.t.k. HD73 crylA(c) gene containing multiple nucleotide substitutions which increase CrylA(c) protein production in transgenic plants as demonstrated by Perlak et al. [21, 22]. In this report we refer to this modified truncated gene as crylA(c) and its protein product as CrylA(c) protein.