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Stable expression of a biodegradable protein-based polymer in tobacco chloroplasts

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Abstract Bioelastic protein-based polymers (PBP) have several medical (prevention of post-surgical adhesions) and non-medical (biodegradable plastic) applications. This study compares expression levels of PBP genes (synthetic) integrated into the nuclear genome or the large single-copy (LSC) or inverted repeat (IR) region of the chloroplast genome in transgenic tobacco plants. Polymer transcripts accumulated up to 100-fold higher in the IR plants than in those of nuclear transgenic plants. Integration of foreign genes into all of the chloroplast genomes (homoplasmy) and higher levels of polymer transcripts were observed only in the IR and not in LSC transgenic plants. Expression of the polymer protein was further confirmed by Western blot analysis.

Key words Chloroplast transformation · Biopolymer · Protein-based polymer · Molecular farming

Introduction

The chloroplast genome is an attractive target for expression of foreign genes due to its ability to express extraordinarily high levels of foreign proteins and efficient containment of foreign genes through maternal inheritance (Daniell et al. 1998). Stable complementation of chloroplast deletion mutants was first observed in Chlamydomonas reinhardtii, a unicellular alga (Boynton et al. 1988). The first expression of foreign genes was observed in isolated cucumber plastids (Daniell and McFadden 1987) and, via particle bombardment, in plastids of cultured tobacco cells (Daniell et al. 1990) and regenerable plant tissues (wheat embryos, Daniell et al. 1991). Stable expression of antibiotic resistance genes using autonomously replicating chloroplast expression vectors (Daniell et al. 1990) or via integration into the plastid genome was later observed in tobacco (Svab and Maliga 1993). More recently, commercially useful chloroplast transgenic plants expressing very high levels of herbicide resistance (Daniell et al. 1998) or insect resistance (McBride et al. 1995; Kota et al. 1999) have been engineered. Here we report exploitation of the chloroplast transformation approach for molecular farming by the genetic engineering of a value added trait that is not advantageous or required by transgenic plants.

The synthetic biopolymer gene (EG121) that codes for a bioelastic protein-based polymer (PBP) was designed after repeated amino acid sequences of GVGVP, which is observed in all sequenced mammalian elastin proteins (Yeh et al. 1987). Elastin is one of the strongest known natural fibers and is present in ligaments and arterial walls. PBPs offer a wide range of materials similar to those of the petroleum-based polymers, such as hydrogels, elastomers, and plastics. Inserting sheets of the polymer GVGPV has been shown to prevent post-surgical adhesions and scars (Urry et al. 1993). Other medical applications of bioelastic PBPs include tissue reconstruction (synthetic ligaments and arteries, bones, wound coverings, artificial pericardia, catheters, and programmed drug delivery (Urry 1995; Urry et al. 1993, 1996). Non-medical applications include transducers, molecular machines, superabsorants, and biodegradable plastics.

The elastic PBP, (GVGVP)_{121} has been expressed to such high levels in E. coli that polymer inclusion bodies occupied up to 90% of the cell volume (Guda et al.
Materials and methods

Plasmid vectors and E. coli strains

The protein-based polymer gene (GVGVP)121mer (designated as EG121) was synthesized and cloned into pET11d plasmid for bacterial expression (McPherson et al. 1996; Daniell et al. 1997). The IR chloroplast integration vector (pSBL-ctV2) was constructed earlier in our laboratory, while the LSC vector pZS197 (Svab and Maliga 1993) was obtained from Dr. P. Maliga’s laboratory (Walksman Institute, Rutgers University, N.J.).

Gene expression in E. coli

Plasmid vectors pSBL-CG-EG121 or pZS-CG-EG121 were transformed into E. coli strain XL-1 Blue and grown in Terrific Broth (Tartof and Hobbs 1987) in the presence of Ampicillin (100 μg/ml) at 37°C for 24 h. SDS-PAGE was carried out according to Laemmli (1970) using a 12% resolving gel and a 5% stacking gel and run for 5 h at a constant current of 30 mAmps. Crude protein extracts from E. coli cells were prepared and electrophoresed as described by Guda et al. (1995). After electrophoresis, polypeptides were visualized by negative staining with CuCl₂ (Lee et al. 1987). Gels were destained in 0.25 M sodium EDTA and 0.25 M Tris-Cl, pH 9.0 with three changes of buffer at 10 min intervals. Immunoblotting and staining was carried out as described by Zhang et al. (1996) using the monoclonal antiserum raised against the polymer AVGVP which cross-reacts well with polymer GVGVP (kindly provided by Dr. Urry, Bioelectronics Research Ltd, Birmingham, AL) and the “Immuno-Blot Assay Kit” (Bio-Rad, respectively).

Results and discussion

Chloroplast integration and expression vectors

The universal chloroplast vector, pSBL-CG-EG121, and the tobacco vector, pZS-CG-EG121, target integration of the foreign genes into the IR or the LSC region of the tobacco chloroplast genome (Daniell et al. 1998; Svab and Maliga 1993). The flanking chloroplast DNA sequence of the universal vector is highly conserved among higher plants and, therefore, could be used to transform chloroplast genomes of higher plants. On the other hand, the tobacco vector is useful for integrating foreign genes specifically into the tobacco chloroplast genome because this gene order is not conserved among other plant chloroplast genomes (Daniell et al. 1998). The pSBL vector integrates the aadA and EG121 genes into the 16S-23S-spacer region, while the pZS vector integrates into the intergenic region between the rbcL and accD genes of the chloroplast genome. Expression cassettes of both chloroplast integration vectors possess the chimeric aadA gene and the EG121 gene driven by a constitutive Prm promoter and regulated by a 3’ untranslated region of the plastid psbA gene. The chimeric aadA gene encoding aminoglycoside 3’-adenyltransferase, confers spectinomycin resistance in chloroplasts (Goldschmidt-Clermont 1991), thereby enabling selection of the transformants on spectinomycin dihydrochloride. To facilitate translation of the dicistronic mRNA, independent Shine-Dalgarno (SD) sequences were provided to the aadA gene.