PHOTOSYSTEM I FUNCTION AND ASSEMBLY IN TOBACCO CHLOROPLAST MUTANTS

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Introduction

Photosystem I is a thylakoid membrane complex that functions as a plastocyanin-ferredoxin oxidoreductase (1). Light energy is captured by antenna pigments and rapidly transferred to the reaction center chlorophylls, P_{700} and electron transfer is initiated. Electrons pass through the intermediary electron acceptors A_0, A_1, F_X, F_A and F_B to ferredoxin. Photosystem I consists of at least thirteen individual polypeptides named PsaA-PsaN (depending on the organism). All electron transfer intermediates between plastocyanin and ferredoxin are coordinated by three chloroplast encoded proteins, PsaA, PsaB and PsaC. The chloroplast genome also encodes PsaI, PsaJ, Ycf3 and Ycf4 that are either associated with photosystem I or required for its stable accumulation. The function of these open reading frames in photosystem I biogenesis is not well established. Deletion of PsaI and PsaJ in cyanobacteria (2,3) and *Chlamydomonas* (4) has no significant impact on photosystem I phenotype. Biochemical analysis suggests PsaI and PsaJ may be required for structural organization of PsaL and PsaF, respectively (2, 3). Ycf3 and Ycf4 have not yet been identified as structural components of photosystem I, but are required for its normal accumulation (5, 6). As part of a wider study of the function of unidentified chloroplast open reading frames in tobacco we are generating deletion and site-directed mutations of the aforementioned genes that should improve our understanding of the role of the chloroplast encoded proteins in photosystem I biogenesis.

Methods

Approximately 2 kbp DNA fragments containing the chloroplast *psaI*, *psaJ*, *ycf3* or *ycf4* genes (figure 1) were PCR amplified from total tobacco DNA. Each DNA fragment was subcloned into pUC19. The chimeric *atpA-aadA* cassette was then blunt ended and inserted into the cloned DNA fragments, at convenient restriction sites interrupting or deleting each of the chloroplast genes (figure 1).

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Each plasmid was precipitated onto tungsten particles and bombarded into leaf discs. The leaf discs were then used to regenerate plants following published protocols (7).

Results

The chimeric *aadA* construct was used to delete exon 3 and interrupt exon 2 of *ycf3* (figure 1). *AadA* was also cloned into an *Nsi*I site within an intron between exon 2 and exon 3 of *ycf3*. Each plasmid was then introduced into tobacco chloroplasts by particle bombardment of leaf discs, and spectinomycin resistant plants regenerated in approximately 8-10 weeks.

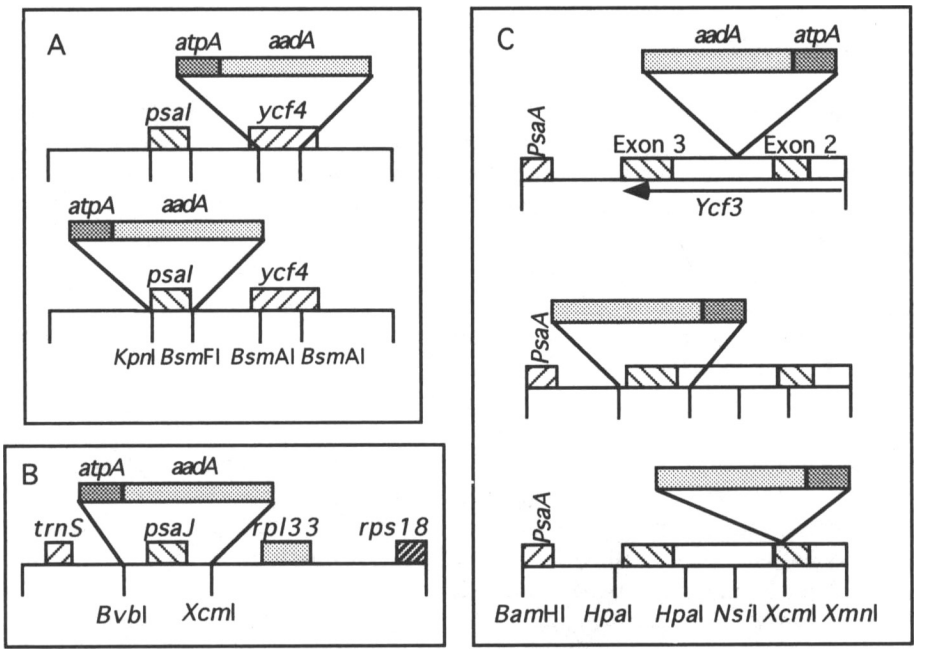

Figure 1. Restriction enzyme maps of cloned chloroplast DNA fragments used for tobacco plastid transformation. The *aadA* cassette was used to delete of *psaI* (A), *ycf4* (A), *psaJ* (B) and *ycf 4* (C). The *ycf3* gene is interrupted by two introns in tobacco chloroplast DNA. As a control, *aadA* was placed within intron 2 separating exon 2 and 3 (C, upper). The *aadA* cassette was used to delete exon 3 (C, middle) and interrupt exon 2 (C, Lower).