Nicotiana chloroplast genome

7. Expression in E. coli and B. subtilis of tobacco and Chlamydomonas chloroplast DNA sequences coding for the large subunit of RuBP carboxylase

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Summary. RuBPCase, the enzyme responsible for carboxylation and oxidation of RuBP in a wide variety of photosynthetic organisms, is the major protein found in the chloroplast. Here we present the first evidence for direct expression in E. coli and B. subtilis of tobacco and Chlamydomonas ct-DNA sequences coding for the LS of RuBPCase as demonstrated by a simple in situ immunoassay.

Key words: Nicotiana – Chlamydomonas – Chloroplast DNA – RuBPCase – E. coli – B. subtilis

Ribulose-1, 5-bisphosphate carboxylase (RuBPCase) (Ec 4.1.1.39), the enzyme responsible for both CO2 fixation and evolution in a wide variety of photosynthetic organisms, is the major protein found in the chloroplast (Kung 1977). In higher plants it occurs as an oligomer of eight large subunits (LS) (MW 53,000) and eight small subunits (SS) (MW 12,000–15,000) (Jensen and Bahr 1977). The LS, containing the catalytic sites, are coded by the chloroplast genome (Chan and Wildman 1972) and are synthesized in the chloroplast (Gray and Kekwick 1973). The SS, whose function remains uncertain, are coded by the nuclear genome and synthesized in the cytoplasm as a precursor of higher molecular weight (MW 20,000) that is cleaved and transported into the chloroplast (Chua and Schmidt 1978; Highfield and Schmidt 1978). This enzyme provides an attractive model for studying nucleus-chloroplast cooperation and gene regulation in plants. Genes for the LS of RuBPCase from maize (Coen et al. 1977), spinach (Bottomley and Whitfield 1978; Erion et al. 1981) and C. reinhardii (Malnoè et al. 1979) have been expressed in vitro in coupled transcription-translation systems, and maize and wheat LS genes have been expressed in vivo in E. coli (Gatenby et al. 1981). Here we present the first evidence for direct expression in E. coli and B. subtilis of chloroplast DNA (ct-DNA) sequences from tobacco and Chlamydomonas coding for the LS of RuBPCase using a simple in situ immunoassay.

A clone library of Nicotiana otophora ct-DNA has been constructed and mapped with respect to BamHI and SmaI sites (Zhu et al. 1982). Unique restriction fragments generated by cleavage of the ct-DNA with SmaI, SalI, HindIII or EcoRI contain the intact LS gene suitable for expression studies, whereas BamHI and PstI cut within the gene. This was determined by using the spinach LS gene as a probe previously inserted into plasmid pBR322 (pJEA4) by Erion et al. (1981). Figure 1A shows the identification of the tobacco LS gene by the hybridization of 32P-labeled pJEA4 to N. otophora ct-DNA digested with SmaI, SalI, HindIII or EcoRI. The fragments Sal6 (14 kb) and Hind2 (11 kb) containing the intact LS gene were isolated from total N. otophora ct-DNA and cloned in E. coli using pBR325 and pBR322 as the respective vectors (Fig. 1B). Cloned fragments Sal6 (in plasmid PRCZ2) and Hind2 (in plasmid PRCZ1) hybridized with 32P-labeled spinach LS gene probe, confirming that both clones contain the LS gene, although these fragments are considerably larger than the LS gene itself (1.3–1.6 kb).

The LS gene from Chlamydomonas reinhardii was used for cloning in Bacillus. This LS gene was previously cloned in plasmid pBR322 (pLM401) by L. Metz (personal communication, Fig. 2A). Plasmid PRCZ3 (Fig. 2B) was constructed by substituting the 0.7 kb EcoRI – HindIII region of the B. subtilis expression plasmid pPL608 (Williams et al. 1981) with the EcoRI – HindIII fragment (1.15 kb and 0.75 kb) spanning the anterior region of the Chlamydomonas LS gene. This substitution is clearly illustrated with the
dotted lines in Fig. 2 between A and B. Two contiguous HindIII fragments (0.75 kb and 1.25 kb) of pLM401 (Fig. 2A), which contain the major posterior region of the LS gene, were inserted into the HindIII site of plasmid pPL608, generating pRCZ4. This insertion is also marked with the dotted lines in Fig. 2 between A and C. Both recombinant plasmids were transformed into B. subtilis strain SS53 with selection for the neomycin-resistance trait specified by pPL608.

For detection of the LS gene product a simple in situ immunoassay was employed (Anderson et al. 1980). The clones CSR603 (PRCZ1) and CSR603 (PRCZ2) containing the N. otophora LS gene were inoculated and incubated on a N-Z bottom agarose plate overnight at 37°C. CSR603 cells containing pBR322 and pBR325 were used as controls. After incubation, the cells in the plates were lysed with chloroform vapor and lysozyme. A channel was made in the center of the plate into which was added antiserum against the LS of RuBPCase. Incubation was continued for another day at room temperature. A sharp immunoprecipitation line was formed between CSR603 (PRCZ1) and the central channel demonstrating that this clone has produced the LS polypeptide (Fig. 3A). B. subtilis cells carrying PRCZ3 and PRCZ4 and control cells containing the vector pPL608 were similarly cultured on N-Z bottom agarose plate, lysed with chloroform vapor and lysozyme, and overlaid with molten agarose mixed with the antiserum against LS. Incubation continued for one week at 4°C. Colonies harboring both recombinant plasmids showed an immunoprecipitation ring, whereas the control cells exhibited no immunoreaction (data not shown). This result was confirmed by immunoprecipitation of extracts of these clones with antiserum against LS in an Ouchterlony double diffusion test (Fig. 3B). Figure 3B shows expression in B. subtilis of anterior portion of the LS gene from Chlamydomonas and in E. coli of the LS...