

Sequence of the cyanobacterial tRNA^W gene in *Synechocystis* PCC 6803: requirement of enzymatic 3' CCA attachment to the acceptor stem

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The cyanobacterial group, which is endowed with two photosystems that are tandemly linked and oxygenic as in plants, is considered important for understanding the endosymbiotic evolutionary origin of chloroplasts. While many of its photosystem and N₂-fixation genes have been characterized, much less is known about the components of its translational apparatus. Since many of the components of the translational system in chloroplasts are presently known (1) a similar understanding of the components of the cyanobacterial system would be very useful.

Here we describe the cloning and characterization of a tRNA gene from the cyanobacterium *Synechocystis* PCC6803 showing that the 3' CCA end of the functional molecule is not encoded in the genome. The 3' CCA ends of the tRNAs in all investigated chloroplasts (2) and also in the mitochondria of the lower land plant liverwort (3) are not genome-encoded and require obligatory post-transcriptional attachment.

A 4.1 kb *AccI*-*EcoRI* fragment (pJS EA 145; Figure 1) from a 15 kb long λEMBL3-LCSII genomic clone of *Synechocystis* PCC 6803 previously described by Sibold and Subramanian (4) was cloned out and both DNA strands were completely sequenced. The data revealed the presence of six ORFs (four of which were identified), and a gene for tryptophanyl-tRNA (*trnW*[CCA]) as inferred by its homology to the *trnW* genes in data bases. The *trnW* in *Synechocystis* is flanked by *rpl19* at the 5' end and *nusG* at the 3' end (Figure 1 and ms. in preparation). An *E.coli* consensus tRNA promoter-like sequence was not detected in the 5' bacterial tRNA gene. In *E.coli*, the *trnW* gene (named *trpT*) occurs in a different context, in the *rrnC* operon, at 85 min on the chromosomal map (5).

The nucleotide sequence of the cyanobacterial *trnW* and its alignment to the homologous genes of various organisms/organelles is given in Figure 2. The closest

resemblance (80–90% identity) was found to chloroplast tRNA^Ws and the lowest resemblance was to cytosolic homologues. A cloverleaf model of the *Synechocystis* and *Chlamydomonas reinhardtii* chloroplast tRNA^W sequences show that the few substitutions between the two are found between positions 16 and 17 the D- and anticodon loops, TΨC stem/loop, and the aminoacyl stem. An extra U is found in the D-loop of all land plant *trnW*s (Figure 2), indicating its probable insertion during evolution at the emergence of land plants.

Southern blot analysis of *Synechocystis* PCC 6803 DNA cleaved with various restriction enzymes was performed, using a radiolabeled 45-mer synthetic oligonucleotide consisting of the 5' sequence (1–45) of this tRNA. The results revealed a single copy of the *trnW* gene in the *Synechocystis* genome (data not shown). Thus the gene described here is the functional gene in this organism.

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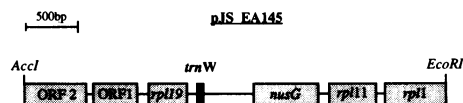


Figure 1. Schematic representation of the 4.1 kb pJS EA145 clone

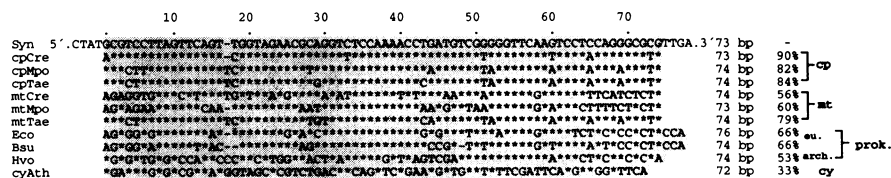


Figure 2. Alignment of *Synechocystis* tRNA^W to the homologous sequences from other genomes. cp, chloroplastic; mt, mitochondrial; cy, cytosolic. Cre, *Chlamydomonas reinhardtii* (green alga); Mpo, *Marchantia polymorpha* (lower land plant); Zma, *Zea mays* (maize); Tae, *Triticum aestivum* (wheat); Eco, *Escherichia coli*; Bsu, *Bacillus subtilis*; Hvo, *Halobacterium volcanii* (archaeobacterium); Ath, *Arabidopsis thaliana*.

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