Mapping of genes on the chloroplast DNA of *Spirodela oligorhiza*

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Abstract. With the use of spinach chloroplast RNAs as probes, we have mapped the rRNA genes and a number of protein genes on the chloroplast DNA (cpDNA) of the duckweed *Spirodela oligorhiza*. For a more precise mapping of these genes we had to extend the previously determined [14] restriction endonuclease map of the duckweed cpDNA with the cleavage sites for the restriction endonucleases *Sma*I and *Bgl*I. The physical map indicates that duckweed cpDNA contains two inverted repeat regions (18 Md) separated by two single copy regions with a size of 19 Md and 67 Md, respectively.

By hybridization with spinach chloroplast rRNAs it could be shown that each of the two repeat units contains one set of rRNA genes in the order: 16S rRNA gene – spacer – 23S rRNA gene – 5S rRNA gene.

A spinach chloroplast mRNA preparation (14S RNA), which is predominantly translated into a 32 Kilodalton (Kd) protein [9], hybridized strongly to a DNA fragment in the large single copy region, immediately outside one of the inverted repeats. With another mRNA preparation (18S), which mainly directs the *in vitro* synthesis of a 55 Kd protein [9], hybridization was observed with two DNA regions, located between 211° and 233° and between 137° and 170°, respectively. Finally, with a spinach chloroplast genomic probe for the large subunit of ribulose 1,5-bisphosphate carboxylase [17], hybridization was found with a DNA fragment located between 137° and 158° on the map.

Introduction

The duckweed *Spirodela oligorhiza* is a widely used organism in plant molecular biology for the study of light-induced changes in gene activity [15]. In order to study the regulated gene expression of the chloroplast genome we have first determined the physical organization of the cpDNA of this plant. As previously shown, *Spirodela oligorhiza* cpDNA has a buoyant density of 1.698 g.cm⁻³, a G + C content of 37% and an exceptionally large contour length of 54.1 μm, corresponding to a molecular weight of 120 Md [13]. Physical maps of this cpDNA, constructed with the aid of the restriction endonucleases *Xho*I, *Sac*I and *Pst*I [14], showed that the structural organization of the duckweed cpDNA is very similar to those found for other cpDNAs from higher plants. It contains two invertedly repeated DNA segments, which are separated by a small single copy region and a large single copy region.
In this paper we present the location of the chloroplast rRNA genes and the genes for the large subunit of ribulose 1,5-bisphosphate carboxylase, for a 55 Kd protein and for a 32 Kd protein on the physical map of *S. oligorhiza*. For fine mapping purposes, the *Xho I, Pst I* and *Sac I* map determined previously [14], was extended with the cleavage sites of the restriction endonucleases *Sma I* and *Bgl I*.

**Materials and methods**

a. *Isolation of chloroplast DNA*

*Spirodela oligorhiza* plantlets were grown and harvested, and the cpDNA was isolated as described previously [13].

b. *Restriction endonucleases/Digestion of chloroplast DNA*

Digestion with the endonucleases *Sac I, Xho I, Pst I, Bgl I, Bam HI, Eco RI* (New England, Biolabs, Beverly) and *Sma I* (Boehringer, Mannheim) were carried out as recommended by the suppliers.

c. *Agarose gel electrophoresis/physical mapping*

A physical map, showing the order of the fragments obtained by digestion with the endonucleases *Sma I* and *Bgl I*, was constructed following the procedures described by Herrmann et al. [10] and in our previous paper [14].

d. *Nomenclature*

In the order of declining sizes, the fragments generated by primary digestion of cpDNA with *Sac I* are called SA, SB etc., those obtained by *Sma I* digestion are called SmA, SmB etc. and those generated by *Pst I, Xho I* and *Bgl I* are called PA, PB etc., XA, XB etc. and BA, BB etc., respectively. The subfragments obtained by double digestion with *Pst I* plus *Sac I* are called PS 1, PS 2 etc. For those obtained by other combinations of enzymes an analogous nomenclature is used.

e. *Isolation, purification and iodination of chloroplast RNA*

Isolation, purification and iodination of heterologous spinach chloroplast RNAs, *viz.* 5S rRNA, 16S rRNA, 23S rRNA and two mRNA fractions — the one enriched in mRNA coding for a 32 Kd protein and the other enriched in mRNA encoding a 55 Kd protein —, was performed exactly as described by Bohnert *et al*. (6) and Driesel *et al*. [9].

f. *RNA/DNA hybridization*

Purified, $^{125}$I-labelled chloroplast RNA was hybridized to filter immobilized restriction fragments or subfragments of cpDNA, separated by agarose gel electrophoresis, using the Southern procedure [12] as described by Bohnert *et al*. [6].