Cyanelle DNA from *Cyanophora paradoxa* exists in two forms due to intramolecular recombination

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The DNA of cyanelles which are described as endosymbiotic cyanobacteria from *Cyanophora paradoxa* (strain LB555UTEX) is equivalent to 127 kilobasepairs. It is characterized by two inverted repeat segments, 10 kilobasepairs in size, which are separated from each other by long single-copy DNA segments of unequal size. This morphology of the chromosome is also found in the chloroplast DNA of most higher plants and some green algae. The cyanelle DNA exists in two forms of circular molecules which differ only in the orientation of the two single-copy DNA segments relative to each other. This is likely due to intramolecular recombination within the inverted repeat segments.

Cyanelle DNA *Chloroplast DNA* Cyanophora paradoxa *Inverted repeat* *Intramolecular recombination*

1. INTRODUCTION

The cyanelle DNA from *Cyanophora paradoxa* is distinguished by a 10 kilobasepair (kbp) repeat unit [1] in inverted orientation [2] separated by two single-copy DNA regions. The size of the molecule is 127 kbp [3]. By these characteristics the DNA from cyanelles, which have been described as endosymbiotic cyanobacteria in a flagellate host [4], resembles chloroplast DNAs (review [5]). It had been argued [6,7] that such a DNA organization might lead to intramolecular recombinational events within the two copies of the repeat unit. This would result in two populations of molecules which differ in the orientation, yet not the position, of the two single-copy DNA regions relative to each other. The two forms of the molecule may be distinguished by the use of restriction endonucleases which do not cleave within the repeat unit, which cleave however asymmetrically adjacent to the repeat unit. Such a situation has now been found in the cyanelle DNA from *Cyanophora paradoxa*.

2. MATERIALS AND METHODS

The growth of cells (*Cyanophora paradoxa*, strain LB555UTEX) and the isolation of DNA from purified cyanelles were as in [2,3]. Restriction endonucleases from Boehringer (Mannheim) or PL (St Goar) were used as recommended. DNA fragments were separated on 0.5–1.8% agarose gels (Seakem, MCI, Rockland). DNA fragment transfer to nitrocellulose (BA 85, Schleicher and Schüll, Dassel), nick-translation of 16 S and 23 S rRNA gene probes and hybridization conditions and autoradiography were as in [1–3]. In some cases, isolated 16 S and 23 S rRNA either from *Escherichia coli* MRE600, or 16 S plus 23 S rRNA from cyanelles was used in the hybridizations after labelling with T4 polynucleotide kinase (New England BioLabs, Schwalbach) according to [8]. The 16 S and 23 S rRNA gene probes were from spinach chloroplast DNA fragments cloned into pBR322 at the *Hin*dIII or *Bam*HI site. The spinach 16 S rRNA gene cloned into the *Bam*HI site of pBR322 was a gift of Dr W. Bottomley (Canberra ACT). In

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some experiments also the *E. coli* rRNA genes recovered from the transducing phage λ rif d^{18} [9], which was given by Dr H. Delius (Heidelberg), were used as probes.

3. RESULTS AND DISCUSSION

The cyanelle DNA from *Cyanophora paradoxa* (strain LB555UTEX) has a circle size of 127 ± 1 kbp [3]. It contains a repeat unit ~10 kbp in size [1] and we have now found that the two copies of the unit are present in inverted orientation [2]. In the course of mapping the cyanelle genome of this strain we had encountered problems when using specifically the restriction endonucleases *Sal*I and *Bam*HI, neither of which cleaves [1,2] the inverted repeat unit. Both enzymes produce only a limited number of relatively large DNA fragments (table 1). Among the 8 subfragments visible, 4 are present in sub-stoichiometric amounts (fig. 1,2). The M_r-values of all of these add up to ~180 kbp. This is in contradiction to experiments using several other restriction endonucleases which cleave the repeat unit at least once and which lead to the reported genome size of 127 kbp [2,3].

The sub-stoichiometric DNA fragments (*Sal*I or *Bam*HI) all hybridized with probes for rRNA genes from spinach chloroplasts (fig. 1) or from *E. coli* (fig. 2) or with isolated cyanelle rRNA. The cyanelle rRNA genes are located as a set (16 S, 23 S, 5 S) once in each repeat unit [1,2]. According to their size, all bands of stoichiometry <1 could contain the whole repeat unit (table 1). With the help of further mapping studies (detailed in [2]) these 4 DNA bands could be grouped into two pairs for either *Bam*HI or *Sal*I (table 1). The two members of each pair appear to be produced by the mirror-like switch of an asymmetric cleavage site around the center of the small single-copy DNA region.

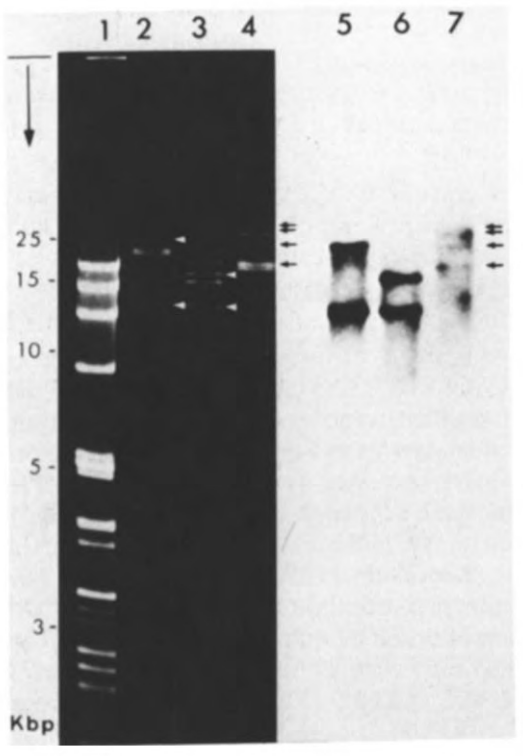

Fig. 1. Hybridization of a spinach cloroplast 23 S rRNA gene probe to DNA fragments from cyanelle DNA: (1) spinach chloroplast DNA/*Bam*HI; (2,5) cyanelle DNA/*Xho*I; (3,6) cyanelle DNA/*Xho*I × *Sal*I; (4,7) cyanelle DNA/*Sal*I. The average M_r of the cyanelle DNA was ~50 kbp; (⇒) DNA fragments (*Xho*I and *Xho*I × *Sal*I) containing one inverted repeat each (1); (➞) bands after *Sal*I digestion to which hybridization occurred.

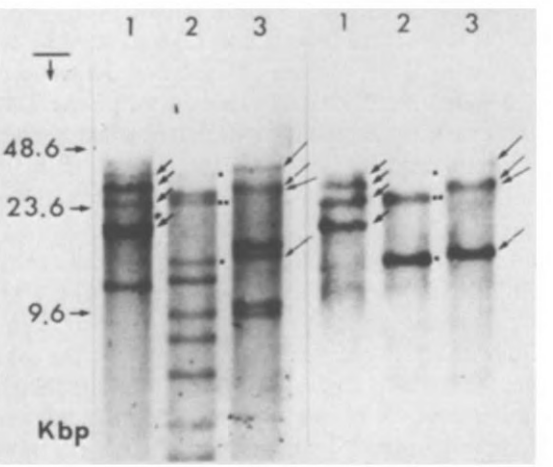

Fig. 2. Hybridization of *E. coli* 23 S rRNA (right) to restriction fragments from cyanelle DNA separated on a 0.5% agarose gel (left): (1) *Sal*I; (2) *Sal*I × *Bam*HI; (3) *Bam*HI. The sub-stoichiometric fragments which contain the rDNA region are indicated with arrows (single digests) or points (double digests), respectively.

This situation can be explained by the map of this region (fig. 3, table 1). A *Sal*I and a *Bam*HI cleavage site each have been mapped there [2]. These sites apparently appear on either side of a symmetric axis therein which divides the small single-copy DNA region (17.9 kbp, as measured from the 5'-ends of the 16 S rRNA genes on each end). The distances from this axis are 7.6 kbp for the *Bam*HI cleavage site and 2.5 kbp for the *Sal*I cleavage site, resulting in a difference of ~15 kbp (*Bam*HI) and 5 kbp (*Sal*I) between the members of a pair of DNA fragments (table 1). The sub-stoichiometric DNA bands were present in all of >20 different DNA preparations tested. A similar situation was found in a second *Cyanophora* strain whose cyanelle DNA shows a different restriction pattern (unpublished).

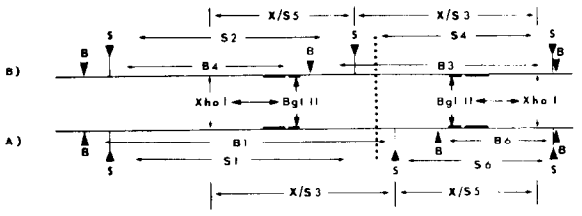

Fig. 3. Partial map of the cyanelle DNA including the small single-copy and inverted repeat regions. The two orientations (A) and (B) are numbered as in table 1. The minimal size of the repeat unit is given by the *Bgl*II and *Xho*I cleavage sites (see [1]); (···) symmetry axis of the small single-copy DNA region; (▬) 16 S rRNA gene (containing a *Bgl*II site) and the adjacent 23 S rRNA gene. X/S3 and X/S5 are DNA subfragments after *Xho*I/*Sal*I double digestion, 16 kbp and 11.8 kbp in size.

When the DNA was cleaved both with *Sal*I and *Xho*I (which cleaves within the repeat unit) no sub-stoichiometric bands appeared and the rRNAs or gene probes hybridized only to two DNA fragments (fig. 1). Due to the symmetry conferred by the *Xho*I site in the repeat unit the *Xho*I/*Sal*I fragments 3 and 5 change their relative positions but not their size (fig. 3). However, in the *Sal*I/*Bam*HI double digest again 4 sub-stoichiometric bands were found which all showed positive hybridization (fig. 2).

A well-known example for the described phenomenon is the 2 μm DNA from yeast, which contains two separated inverted repeat segments of 599 bp each, dividing the 6318 bp molecule into about equal halves [10]. This molecule is found as a mixed population of equal amounts of two distinct forms which differ in the orientation of the unique regions relative to each other. Recombination occurs at a specific site within the repeat unit

Table 1

Size and stoichiometry of cyanelle DNA fragments after digestion with restriction endonucleases *Sal*I and *Bam*HI

	*Sal*I (kbp)	Stoichio-metry	Conformations			*Bam*HI (kbp)	Stiochio-metry	Conformations	
			A	B				A	B
S1	33.3	<1	33.3		B1	41.1	<1	41.1	
S2	28.6	<1		28.6	B2	37.9	1	37.9	37.9
S3	28.0	1	28.0	28.0	B3	29.5	<1		29.5
S4	23.2	<1		23.2	B4	25.3	<1		25.3
S5	18.6	1	18.6	18.6	B5	15.2	1	15.2	15.2
S6	18.5	<1	18.5		B6	13.8	<1	13.8	
S7	18.2	1	18.2	18.2	B7	9.8	1	9.8	9.8
S8	11.2	1	11.2	11.2	B8	9.4	1	9.4	9.4
	179.7		127.8	127.8		182.0		127.2	127.1

DNA fragments to which 16 S and 23 S rRNA gene probes hybridized are underlined

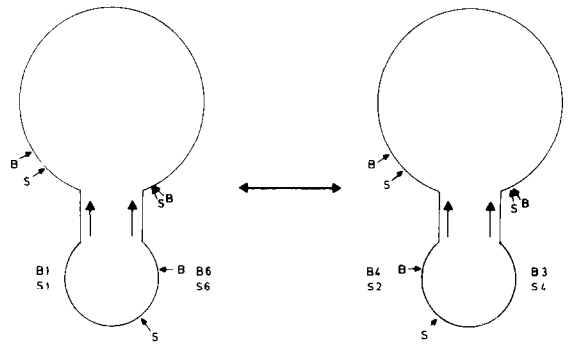

Fig. 4. Two forms of cyanelle DNA with opposite orientation of the small single-copy DNA segment. The positions of cleavage sites for *Bam*HI and *Sal*I in the large single-copy DNA region are not drawn to scale.

[11]. It is dependent upon a gene product encoded by 2 μm DNA. The existence of a mixed population of cyanelle DNA molecules (fig. 4) which contain a much larger repeat unit of ~10 kbp is probably indicative of a similar mechanism. In many chloroplast DNAs which contain an even larger repeat unit (22–28 kbp in size) no restriction endonuclease has yet been found which would allow a similar observation.

In search of a possible function for a recombinational flip–flop mechanism it has been argued that it might help preventing errors in replication which would lead to changes of the repeat unit [12]. An influence extending beyond just the proof reading of repeat segments is however suggested by the observation that chloroplast DNAs from some members of the legume family which have lost one of their repeat units are considerably more subject to intramolecular rearrangements than those containing an inverted repeat [13]. Whether the intramolecular recombination system found in *Cyanophora* cyanelle DNA, which most probably represents an ancient preserved chloroplast or a side line of plastid evolution, causes the observed evolutionary stability of chloroplast DNAs will have to be studied.

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