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Genetics

The chloroplast genome exists in multimeric forms

(spinach/chloroplast DNA/*in vivo* DNA forms/DNA oligomers/leaf maturation)

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ABSTRACT **Chloroplast DNA conformation was analyzed by pulse-field gel electrophoresis. We found that spinach leaf chloroplast DNA molecules exist in at least four distinct forms with the apparent molecular weights of monomer, dimer, trimer, and tetramer. Two-dimensional gel analysis of DNA after UV nicking and in the presence of ethidium bromide indicates that they are not isomers that differ in superhelical density. DNA gyrase decatenation analysis demonstrates that the majority of the DNA molecules are oligomers rather than catenanes. The relative amounts of monomer, dimer, trimer, and tetramer forms, quantitated by molecular hybridization, are 1, 1/3, 1/9, and 1/27, respectively, and do not change during leaf maturation. The possible mechanisms of chloroplast DNA oligomer formation are discussed.**

Chloroplast DNA exists as a negatively supercoiled double-stranded circle varying in size between 120 and 200 kilobases (kb) among almost all chloroplast-containing organisms (1). DNA recombination in chloroplasts has been documented in *Chlamydomonas* (2, 3), and evidence strongly suggests that recombination also occurs in higher plant chloroplasts (4–6). Each chloroplast contains many copies of its genome (7), thus potentially allowing intermolecular recombination of individual DNA molecules to occur. Inter- and intramolecular recombination events could result in a dynamic equilibrium between polymerization and depolymerization of chloroplast multimeric DNAs. However, the impact of recombination on conformations of the chloroplast genome has not been well explored, although the presence of a small number of DNA dimers was observed by electron microscopy in cesium chloride-purified chloroplast DNA samples more than a decade ago (8, 9). In their studies, Kolodner and Tewari (8) found that 3.6% and 1.9% of circular DNA molecules existed as dimers and catenated dimers, respectively. They later showed that the majority (80%) of the spinach chloroplast dimer DNA molecules were in a head-to-head configuration and 20% of them were in a head-to-tail configuration (9). By taking advantage of the pulse-field gel electrophoresis technique (10, 11), we have analyzed the conformations of chloroplast genomes. We show here that the chloroplast genome exists in monomer, dimer, trimer, and tetramer forms. The relative amounts of these different chloroplast DNA forms in spinach are 1, 1/3, 1/9, and 1/27, respectively, and do not change during leaf maturation. These results suggest that a dynamic recombination process in chloroplasts establishes a unique equilibrium of oligomeric DNA forms.

MATERIALS AND METHODS

Preparation of Chloroplast DNA Samples and Pulse-Field Gel Electrophoresis. Intact chloroplasts from young and mature spinach leaves were isolated according to published procedures (12). One hundred microliters of the chloroplast sample (≈5 mg of chlorophyll per ml) was warmed to room temperature and gently mixed with 300 μl of 0.9% low-melting agarose (FMC InCert agarose) warmed to 42°C, containing 0.33 M sorbitol, 25 mM citrate hydrochloride (pH 7.0), 90 mM 2-mercaptoethanol, and 125 mM EDTA. The mixture was poured into a 10-mm × 4-mm × 2-mm mold made with 1% agarose in a Petri dish. After the low-melting agarose solidified, 15 ml of a solution containing 450 mM EDTA, 1% sodium *N*-laurylsarcosinate, 10 mM Tris·HCl (pH 8.0), and 15 mg of proteinase K were added, and the mixture was incubated at 50°C overnight with shaking. The sample was washed three times with 500 mM EDTA (pH 9.0) at 50°C with shaking (4–5 hr each). The Petri dish was covered with aluminum foil during all the procedures to avoid light damage to the DNA molecules. The low-melting agarose with the embedded chloroplast DNA was excised and analyzed in an alternating contour-clamped homogeneous electric field gel electrophoresis apparatus (11). The gel (1% agarose in 0.5× TBE) was subjected to electrophoresis in 0.5× TBE (45 mM Tris borate/45 mM boric acid/1 mM EDTA, pH 8.0) at a constant 9°C temperature, with 45-sec pulse time at 220 volts for 16 hr unless otherwise indicated. The gel was stained with ethidium bromide (EtdBr) and photographed.

Blotting and Hybridization of Pulse-Field Gel Separated Chloroplast DNA Samples. After EtdBr staining and photography, the DNA in the gel was partially depurinated and blotted to a Nytran membrane (Schleicher & Schuell) overnight. The membrane was baked at 80°C for 2 hr in a vacuum according to Maniatis *et al.* (13). Prehybridization, hybridization, and filter washing conditions were according to published procedures (14). The 1.2-kb *Bgl* II–*Xba* I spinach DNA fragment, which contains most of the *psbA* gene (15), was random primer labeled to a high specific activity (≈10^9 cpm per μg of DNA) and used as a hybridization probe.

Preparation of the Control Catenane Dimer Plasmid. Five μg of plasmid pBR51A^2-ΔRI DNA was incubated with 5 μg of Tn*3* resolvase in 500 μl of solution containing 150 mM NaCl, 10 mM $MgCl_2$, and 20 mM Tris·HCl (pH 8.0) at 37°C for 2 hr to convert most of the DNA to catenane dimers. The reaction was terminated by heating the reaction mixture to 65°C for 15 min, followed by phenol extraction and ethanol precipitation. The DNA mixture was resuspended in distilled H_2O and subjected to restriction enzyme digestion or embedded in the low-melting agarose as described for the chloroplast sample. The plasmid pBR51A^2-ΔRI and Tn*3* resolvase were kindly provided by Jan Dungan and N. R. Cozzarelli (University of California, Berkeley) and were purified according to published procedures (16).

DNA Gyrase Decatenation Assay. The agarose with embedded plasmid or chloroplast DNAs was further washed with 50 mM EDTA (pH 9.0) three times at 37°C for 3 hr each. The samples were then washed with decatenation buffer (25 mM Tris·HCl, pH 7.6/2 mM $MgCl_2$/1 mM dithiothreitol/5 mM

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Abbreviation: EtdBr, ethidium bromide.

spermidine/bovine serum albumin at 50 μg/ml/60 mM KCl) three times at 37°C for 2 hr each. The samples were then added to tubes containing 100 μl of the decatenation buffer containing 0.5 mM ATP and held at room temperature for 20 min. Variable amounts (1- to 16-fold) of *Escherichia coli* DNA gyrase were added and incubated at 30°C for 1 hr. The *E. coli* DNA gyrase was kindly provided by Jan Dungan and N. R. Cozzarelli and purified according to published procedures (17, 18).

RESULTS

Spinach Chloroplast DNA Exists as Different Distinct Forms. Fig. 1 shows patterns of DNAs from intact chloroplasts of freshly harvested young and mature spinach leaves after lysis, deproteinization, pulse-field gel electrophoresis, and EtdBr staining. In both chloroplast samples, identical multiple DNA bands are evident. By using the λ DNA oligomer ladder as a size marker, the DNA in band 1 is approximately 160 kb in size, which coincides with the estimated spinach chloroplast genome size of 150 kb. Bands 2, 3, and 4 are approximately 2, 3, and 4 times the size of band 1. Under the experimental conditions used for this gel, DNA molecules with sizes larger than 700 kb cannot be resolved (band 5). We demonstrated that the distinct DNA bands are chloroplast DNA in two ways. First, these bands hybridized to the spinach chloroplast fragment encoding the *psbA* gene (the gene encoding the 32-kDa D1 protein of the photosystem II reaction center), which has no detectable sequence similarity in either nuclear or mitochondrial genomes (Fig. 2). The fluorescent background material below each DNA band most likely represents partially degraded chloroplast DNA, since it hybridizes to the *psbA* probe. The amount of degradation varies among different preparations (compare Figs. 1 and 2) but does not significantly affect the ratio of the different bands of intact DNA molecules. Second, digestion of the DNAs eluted from individual bands (Fig. 2, bands 1–4) with *Bam*HI resulted in DNA fragments that are identical to those seen when cesium chloride gradient-purified chloroplast DNA is digested with *Bam*HI (Fig. 3). However, the above results do not differentiate whether the observed higher molecular weight chloroplast DNA forms are linear or circular molecules. If the DNA bands were linear molecules, we would expect that each molecule had been broken only once. The results in Fig. 3 would exclude the possibility that

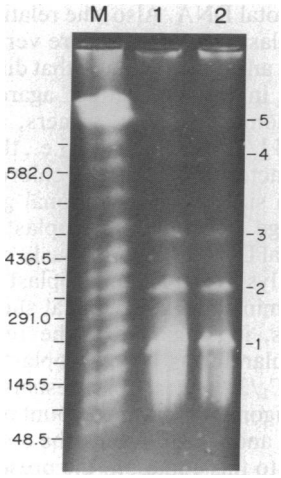

FIG. 1. Spinach chloroplast DNA from young (lane 1) and mature (lane 2) leaves exists as multimeric forms. Lane M contains a ladder of ligated λ DNA (11), for which the sizes are marked in kilobases. The distinct chloroplast DNA bands are numbered 1–5. Bands 1–4 have approximate molecular weights of a monomer, dimer, trimer, and tetramer chloroplast DNA molecule.

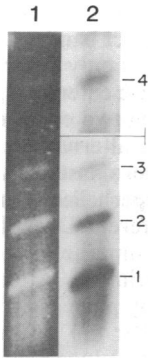

FIG. 2. The four spinach chloroplast DNA forms hybridize to a chloroplast DNA gene-specific probe. The chloroplast DNA forms from mature spinach leaves were separated by pulse-field gel electrophoresis and the gel was stained with EtdBr and photographed (lane 1). The DNA was blotted and hybridized to a spinach chloroplast *psbA* gene-specific probe (lane 2). The exposure times were 1 hr for visualization of bands 1–3 and 4 hr for visualization of band 4.

linearization occurred at a specific site, since we do not detect any restriction fragment length polymorphism between the purified control chloroplast DNA and the different DNA bands excised from the agarose gel. If the chloroplast DNA was randomly linearized during sample preparation and the bands in the pulse-field gel represent linear molecules, then these results would be consistent with the interpretation that chloroplast DNA exists as oligomeric forms. However, we cannot exclude the possibility that the chloroplast DNA forms are circular molecules. In this case, different structural

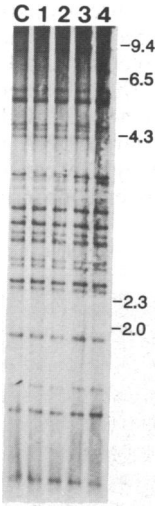

FIG. 3. The *Bam*HI restriction fragment patterns of purified spinach chloroplast DNA (lane C) and DNAs with sizes of monomer (lane 1), dimer (lane 2), trimer (lane 3), and tetramer (lane 4) as shown in Figs. 1 and 2. The locations of the DNA sizes markers (in kb) are marked. The chloroplast DNA was separated in a pulse-field gel as described in *Materials and Methods* except that the 1% regular agarose gel was substituted by a 1.1% low-melting agarose gel (FMC InCert agarose). After electrophoresis, the agarose slices corresponding to the four different forms of chloroplast DNA were excised, washed with *Bam*HI digestion buffer (supplied by BRL) three times at 37°C for 30 min, and completely digested with 20 units of *Bam*HI for 3 hr at 37°C. The reaction mixtures (with agarose slices) were heated to 65°C for 15 min and extracted once with phenol, once with phenol/chloroform, 1:1 (vol/vol), and once with chloroform. The DNA was precipitated with ethanol and resuspended in water. The *Bam*HI-digested DNA fragments were end-labeled with Klenow and [α-^{32}P]dCTP. The end-labeled DNA fragments (≈200,000 cpm per lane) were separated on a 0.8% agarose gel. After electrophoresis, the gel was dried onto Whatman DE-81 filter paper at 60°C for 1 hr and exposed to x-ray film for 4 hr.

forms could potentially cause the circular DNA molecules to migrate as different distinct bands. Circular DNA molecules could exist as isomers that only differ in superhelical density, as a mixture of oligomeric catenanes, or as a mixture of true oligomers. The following two experiments were designed to distinguish among these alternatives.

Different Spinach Chloroplast DNA Forms Are Not Isomers That Only Differ in Superhelical Density. We excluded the possibility that the different DNA forms potentially result from differences in the superhelicity of the chloroplast DNA by the following experiment. After the chloroplast DNA forms were separated by pulse-field gel electrophoresis, the gel was stained in EtdBr (10 μg/ml) for 30 min and UV-irradiated for 2 min to introduce nicks and therefore relax the DNA molecules. To monitor the efficiency of the UV treatment, we used supercoiled pUC plasmid DNAs as a control, which mostly converted to nicked forms under the same conditions (data not shown). Following UV irradiation, the agarose slice containing the DNA (with the EtdBr present) was excised, inserted into another agarose gel slot, and subjected to pulse-field electrophoresis in the second dimension. As shown in Fig. 4, the relative mobility of the different chloroplast DNA forms is the same as for the original DNA sample even in the presence of EtdBr and after UV nicking. We interpret this result as evidence that the different chloroplast DNA bands are not simply due to superhelicity differences of the monomeric form DNA molecule.

Different Spinach Chloroplast DNA Forms Are True Oligomers Rather Than Oligomeric Catenanes. The other two alternatives could lead to the multiple DNA bands with apparent sizes of dimers, trimers, and tetramers—e.g., the chloroplast DNA exists as a mixture of true oligomers or the chloroplast DNA is a mixture of oligomeric catenanes. After examining purified supercoiled spinach chloroplast DNA by electron microscopy, Kolodner and Tewari (8, 9) found 3.6% circular dimers and 1.9% catenated dimers. Trimer and tetramer molecules were not detected, most likely due to the ease of breaking such large DNA molecules during their isolation. *E. coli* DNA gyrase can resolve oligomeric catenanes to monomers under appropriate conditions (17, 18).

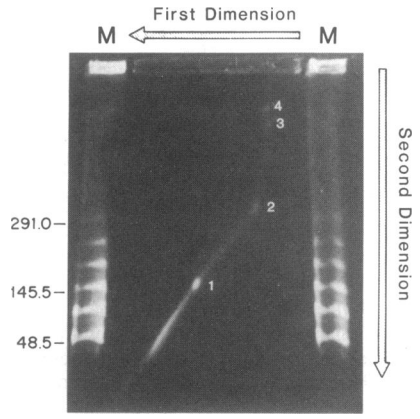

FIG. 4. The presence of EtdBr and the introduction of DNA nicks do not affect the migration of different spinach chloroplast DNA forms. The chloroplast DNA sample was first electrophoresed for 12 hr under the same conditions described in Fig. 1 except that the pulse time was 30 sec at 120 V. The gel was stained in EtdBr (10 μg/ml) for 30 min and illuminated with UV light (302 nm) for 2 min. The lane containing chloroplast DNAs was excised and inserted into the well of a 1% agarose gel. The second gel was electrophoresed at a pulse of 30 sec at 220 V for 18 hr, stained in EtdBr (20 μg/ml) for 30 min, and photographed. The DNA spots labeled 1, 2, and 3 migrate with the apparent molecular weight of monomer, dimer, and trimer molecules. Under the electrophoresis condition used, DNAs of sizes equal to or larger than a tetramer molecule migrate together (spot 4). The locations of DNA size markers (in kb) are marked.

We used DNA gyrase to estimate the contribution of true oligomers and oligomeric catenanes to the multiple chloroplast DNA bands. As a control, Fig. 5*A* shows that *E. coli* DNA gyrase can completely resolve catenane dimers to monomers when the catenated pBR51A^2-ΔRI plasmid DNA (see legend of Fig. 5) is embedded in the low-melting agarose. As is evident in lane 1 of Fig. 5*A*, a significant amount of the pBR51A^2-ΔRI catenane dimers are nicked. The production of nicked monomers after *E. coli* DNA gyrase incubation indicates that the nicked catenane dimers can also be resolved, though with slightly lower efficiency (18). After extensive washing of the agarose-embedded chloroplast DNA samples, they were incubated with *E. coli* DNA gyrase under identical conditions as the control DNA sample and analyzed by pulse-field gel electrophoresis (Fig. 5*B*). There are no apparent changes in the relative amounts of the different chloroplast DNA bands after treatment with *E. coli* DNA gyrase. Thus, although catenated dimers were reported (8, 9), they were not detectable under our experimental conditions. It is possible that pulse-field gel electrophoresis cannot resolve catenated chloroplast DNA molecules from monomer molecules or that the amount of catenated dimer molecules is below the detectable limits. Taken together, we conclude from these results that oligomeric catenanes, if any, represent only a very small portion of the lower mobility chloroplast DNA bands. The majority of the larger sized chloroplast DNAs therefore are true dimers, trimers, and tetramers.

Relative Amounts of Different Spinach Chloroplast DNA Oligomers Are Unique and Do Not Change During Leaf Maturation. The relative amounts of chloroplast DNA monomers, dimers, trimers, and tetramers were quantitated by molecular hybridization (Table 1). Since the majority of the chloroplast DNA bands represent the true monomers, dimers, trimers, and tetramers, the quantitation of their relative amounts may be considered a close estimation of the amount of true oligomer forms. We quantitated the different DNA bands by the amount of *psbA* probe hybridized in Southern hybridization experiments similar to those shown in Fig. 2, assuming that the amount of DNA on the membrane is proportional to the amount of probe hybridized. We noted that some DNA is always retained in the embedding agarose after pulse-field electrophoresis, regardless of the source of DNA. For the chloroplast DNA samples, the percentage of DNA retained in the embedding agarose varied in different preparations and different gels, but in most cases it was less than 20% of the total DNA. Also, the relative amounts of the different chloroplast DNA forms were very similar in different preparations, and thus it is likely that different forms were equally trapped in the embedding agarose. The relative amounts of monomers, dimers, trimers, and tetramers are $\approx$1, 1/3, 1/9, and 1/27, respectively; i.e., the amount of DNA decreases by a factor of 3 when the chloroplast DNA oligomer increases in size by one additional genome copy. The higher percentage of dimer chloroplast DNA molecules ($\approx$22% of the total DNA) than previously reported (8) is most likely due to the lysis of intact chloroplasts in the embedding agarose, which minimizes mechanical shearing of the DNA molecules. Thus, it appears that the relative amounts of different molecular forms of chloroplast DNA follow the equation $C_n = C_1/3^{n-1}$, were n represents the genome copy number in the oligomer, C_n is the amount of oligomer with the genome copy n, and C_1 represents the amount of the monomer. According to this equation, the presence of pentamers, hexamers, and even higher order oligomers would be expected; however, their low abundance will make detection practically difficult. Also, the pulse-field gel electrophoresis conditions used in our experiments cannot resolve DNAs of sizes larger than 700 kb, so any oligomer larger than a tetramer molecule will migrate as one band (see Fig. 1).

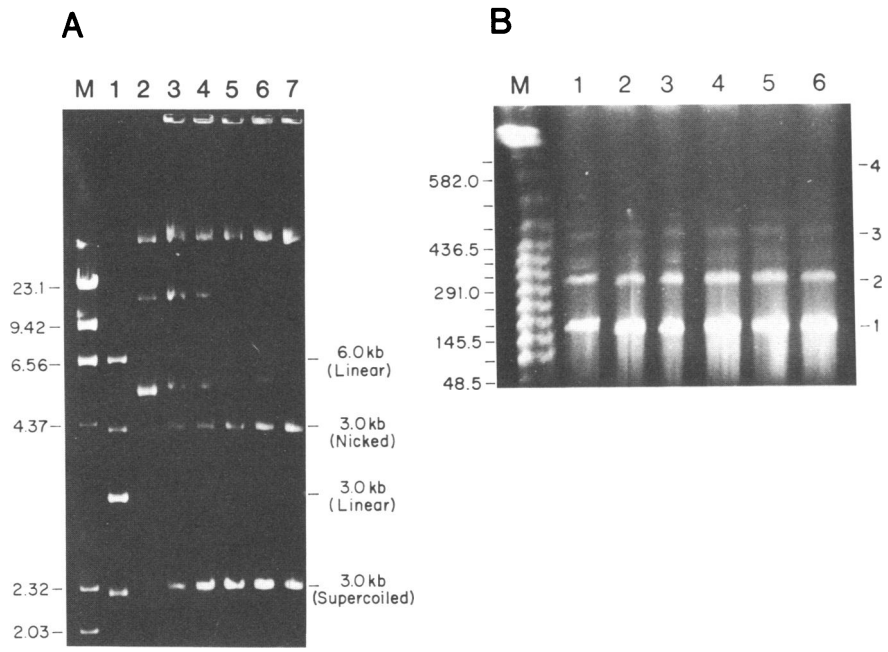

FIG. 5. Decatenation analysis of a plasmid catenane dimer (*A*) and spinach chloroplast DNA (*B*) by *E. coli* DNA gyrase. (*A*) Lane 2, a mixture of plasmid pBR51A^2-ΔRI and the catenane dimer that is produced by Tn*3* resolvase-mediated recombination between the two recombination sequences in pBR51A^2-ΔRI. The plasmid pBR51A^2-ΔRI is a dimer of plasmid pBR51A (16), which has a Tn*3* resolvase recognition sequence containing fragment inserted between the *Eco*RI and *Pvu* II site of pBR322, except that the single *Eco*RI site in one copy was destroyed. Lane 1, an *Eco*RI digestion of the DNA shown in lane 2. *Eco*RI digestion of plasmid pBR51A^2-ΔRI generates 6.0-kb linear DNA molecules. *Eco*RI digestion of the catenane dimer generates the 3.0-kb linear DNA molecules and 3.0-kb nicked or supercoiled circular DNA molecules depending on whether nicked or non-nicked forms existed in the *Eco*RI site-depleted DNA circle of the catenane dimer molecule. The same DNA mixture of pBR51A^2-ΔRI and catenane dimers as shown in lane 2 was embedded in low-melting agarose and treated the same way as chloroplast DNA. The agarose with the embedded plasmid DNAs was incubated with *E. coli* DNA gyrase under decatenation conditions for 1 hr with 1-, 2-, 4-, 8-, and 16-fold the amount of enzyme required to completely resolve the catenane dimers of the same DNA sample in solution (lanes 3–7, respectively). The DNAs were separated on a 1.2% regular agarose gel. Lane M, linear DNA size markers (in kb). (*B*) Low-melting agarose-embedded chloroplast DNA (lane 1) was treated with *E. coli* DNA gyrase under decatenation conditions for 1 hr with increasing DNA gyrase concentrations as in the pBR51A^2-ΔRI plasmid DNA control in *A* (lanes 2–6, respectively). The chloroplast DNA was then separated by pulse-field gel electrophoresis. The positions of chloroplast DNA monomers, dimers, trimers, and tetramers are marked (labeled 1–4). Lane M, ligated oligomer λ DNA ladders as size markers (in kb).

DISCUSSION

Our experimental results provide strong evidence for the existence of spinach leaf chloroplast DNA as a mixture of true oligomers. However, they cannot differentiate whether the oligomers resolved by pulse-field gel electrophoresis are circular or randomly linearized molecules. Based on previous work, it is generally accepted that the *in vivo* form of chloroplast DNA is that of a circular and negatively supercoiled molecule (1, 4, 19). However, it is possible that most or all chloroplast DNA molecules are randomly linearized by an unknown mechanism during the isolation of intact chloroplasts and the following sample preparation. Such linearization could occur at DNA membrane attachment sites. If the membrane attachment occurs at more than one site in some chloroplast DNA molecules, this would also explain the hybridizable DNA background, which differs in size from the expected sizes of monomer, dimer, trimer, and tetramer molecules. Despite such potential random linearization, our results still allow us to conclude that the chloroplast DNA exists as a mixture of circular oligomeric forms, whether or not the chloroplast DNA in the pulse-field gel is present as circular or linear molecules.

Table 1. Relative amounts of the mutimeric forms of spinach chloroplast DNA

DNA form	Relative mass amount	Approximate mass amount	Approximate molar concentration
Monomer	1.0	1	1
Dimer	0.35 ± 0.02	1/3	1/6
Trimer	0.112 ± 0.002	1/9	1/27
Tetramer	0.035 ± 0.002	1/27	1/108

The relative mass amounts represent the mean ± SD of five measurements except for the tetramer, for which the mean was calculated from two measurements. The amount of the monomer form of DNA was arbitrarily set to 1.0. The chloroplast DNA electrophoresis, DNA transfer, and hybridization conditions were as described. After exposure to x-ray film and localization of the hybridization signals, the bands corresponding to monomer, dimer, trimer, and tetramer chloroplast DNA molecules were excised from the membrane, and the amount of hybridized probe was quantitated by scintillation spectroscopy.

The mechanism of formation of multimeric chloroplast DNA forms is unknown. At least two possibilities could be considered. First, it has been reported that chloroplast DNA replicates by both the Cairns and the rolling-circle mechanism (19, 20). The true oligomeric DNA forms could be generated by circularizing the rolling-circle intermediate tails with two, three, or four times the length of the genome. However, the rolling-circle replication mechanism was proposed to be a supplemental replication process to the Cairns mechanism, and no tail of the rolling-circle intermediates was found to be longer than 1.5 times the size of the genome (19). Thus, it is unlikely that the high percentage of oligomeric chloroplast DNA molecules are generated by a rolling-circle replication mechanism. The second and more likely mechanism of oligomeric chloroplast DNA formation is through inter- and intramolecular recombination. Several lines of evidence, including the reversibility of the single-copy regions between the inverted repeats (4), production of a

recombinant chloroplast genome after fusion of two different tobacco somatic cell lines (5) or during the formation of the sexual zygote in *Chlamydomonas* (2), and rescue of a *Chlamydomonas* chloroplast mutant by site-directed recombination after introduction of a wild-type chloroplast DNA fragment (4), indicate that chloroplasts maintain an active recombination system. Our results also suggest that the recombination process is dynamic and, in the case of spinach chloroplasts, has reached a unique equilibrium during leaf maturation. If this is the case, the equilibrium constants (e.g., the ratio of the rate constant for intramolecular recombination and that for intermolecular recombination of any recombination process) can be calculated.

The recombination processes that lead to the DNA form conversion can be generalized as $F_n + F_m \rightleftharpoons F_{n+m}$, where F_n, F_m, and F_{n+m} represent the oligomer forms with n, m, and $n + m$ genome units and their relative amounts are C_n, C_m, and C_{n+m}, respectively. From the observation that the relative amounts of different DNA forms do not change during leaf maturation, we tentatively conclude that the recombination process is dynamic and has reached a unique equilibrium. Thus, the equilibrium constant can be written as $K = C'_{n+m}/[C'_n \times C'_m]$, where C'_n, C'_m, and C'_{n+m} represent the relative molar concentrations of oligomeric forms with n, m, and $n + m$ genome units. C'_n, C'_m, and C'_{n+m} can be calculated according to the formulas $C'_n = C_n/n$, $C'_m = C_m/m$, and $C'_{n+m} = C_{n+m}/(n + m)$. The relative molar concentrations of different oligomeric forms are shown in Table 1. If we further assume that a recombination process is only responsible for the formation of all oligomeric chloroplast DNAs, the data from Table 1 can be used to calculate the K values (Table 2). The equilibrium constant K can also be expressed as $K = k_1/k_2$. Here k_1 represents the rate constant of the intermolecular recombination process leading to chloroplast DNA polymerization, and k_2 represents the rate constant of intramolecular recombination events resulting in depolymerization. For example, the K value for the conversion of monomer and dimer ($F_1 + F_1 \rightleftharpoons F_2$) is 1/6. In other words, the rate constant for intramolecular recombination of a dimer to two monomers is 6 times that for intermolecular recombination of the two resolved monomers into a dimer.

In comparison to circular DNA molecules in *E. coli* and yeast, the high percentage of chloroplast DNA molecules existing as oligomeric forms is unusual. Although in *E. coli* (21) and yeast (22) circular plasmid DNAs normally yield certain amounts of dimers, such dimers are only a minor component of total plasmid DNA, and larger oligomeric forms have rarely been observed, probably due to their extremely low abundance. A different mechanism would be expected in chloroplast to maintain the observed ratios of different oligomeric chloroplast DNA forms. A preliminary survey of chloroplast DNAs from the green algae *Mesotaenium caldariorum* and three other higher plants (pea, maize, and tomato) indicates that it is common for chloroplast DNAs to exist as multimeric forms (data not shown). Since the pea chloroplast DNA does not contain inverted repeats (1), the detection of multimeric forms of pea chloroplast DNA argues against the requirement of an inverted repeat for the formation of multimeric DNA forms. The presence of a high percentage of chloroplast DNAs as oligomeric forms will have an impact on virtually all processes in which chloroplast DNA participates, including replication, transcriptional competence, and DNA segregation during chloroplast division. In the case of the yeast 2-μm plasmid dimeric molecules, only the replication origin of one copy has been reported to be used during replication (22). It will be interesting to determine how the chloroplast DNA origins of replication in oligomers are utilized during replication. Also, it will be interesting to know if the existence of multimeric forms of chloroplast DNA molecules is of functional importance to the plastid and the plant or if it is simply a consequence of the coexistence of recombination activity and genome polyploidy in the chloroplast.

Table 2. Summary of predicted equilibrium constants of some possible recombination processes of different chloroplast DNA oligomers

Recombination process	Predicted equilibrium constant
$F_1 + F_1 \rightleftharpoons F_2$	1/6
$F_1 + F_2 \rightleftharpoons F_3$	2/9
$F_1 + F_3 \rightleftharpoons F_4$	1/4
$F_2 + F_2 \rightleftharpoons F_4$	1/3
$F_1 + F_1 + F_1 \rightleftharpoons F_3$	1/27
$F_1 + F_1 + F_2 \rightleftharpoons F_4$	1/18
$F_1 + F_1 + F_1 + F_1 \rightleftharpoons F_4$	1/108

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