DNA Strand-Transfer Activity in Pea (*Pisum sativum* L.) Chloroplasts

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The occurrence of DNA recombination in plastids of higher plants is well documented. However, little is known at the enzymic level. To begin dissecting the biochemical mechanism(s) involved we focused on a key step: strand transfer between homologous stromal extracts from pea (*Pisum sativum* L.) chloroplasts. Formation of joint molecules requires Mg++, ATP, and homologous substrates. This activity is inhibited by excess single-stranded DNA (ssDNA), suggesting a necessary stoichiometric relation between enzyme and ssDNA. In a novel assay with Triton X-100-permeabilized chloroplasts, we also detected strand invasion of the endogenous chloroplast DNA by 32P-labeled ssDNA complementary to the 165 rRNA gene. Joint molecules, analyzed by electron microscopy, contained the expected displacement loops.

Genetic recombination is an essential cellular function common to all organisms (Bernstein et al., 1985; Hotta et al., 1985; Roca and Cox, 1990; Eggleston and Kowalczykowski, 1991). It has been argued that its primary biological role is the repair of damaged DNA rather than the production of variation by the exchange of genetic information (Bernstein et al., 1985; Roca and Cox, 1990). In higher plants, DNA recombination occurs in the three genetic systems of the cell, namely, nuclear, mitochondrial, and chloroplast genomes (Hotta et al., 1985; Medgyesy et al., 1985; Levings and Brown, 1989).

Chloroplast DNA recombination has been demonstrated in the unicellular green alga *Chlamydomonas* (Boynton et al., 1991). Several lines of evidence suggest that recombination and related processes, such as gene conversion, also occur in the chloroplast genome (plastome) of higher plants. The existence of multimeric forms of the plastome has been interpreted as resulting from intermolecular recombination (Kolodner and Tewari, 1979; Deng et al., 1989). Several

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Abbreviations: D-loop, displacement loop; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA.
three to four nucleotide residues. During synapsis, the nu-
cleoprotein filament binds and eventually pairs with homol-
ogous dsDNA to form joint molecules. The first contacts are
between nonhomologous regions, resulting in the formation
of a large network of molecules that contributes to acceleration
of pairing. This is followed by homologous alignment
and the formation of joint molecules, with limited unwinding
of the dsDNA. In the final step, homologously aligned mol-
ecules undergo a unidirectional, RecA-mediated extension of
the heteroduplex region that results in strand exchange.

The key role played by RecA homologs in general recom-
bination in every eubacterium studied in some detail (Roca
and Cox, 1990; Eggelston and Kowalczykowski, 1991), and
the endosymbiotic origin of chloroplasts (Gray, 1989), made
a search for a RecA-like activity a reasonable first step in
understanding the biochemical mechanism(s) involved in
plastid DNA recombination. In addition, we have recently
cloned an Arabidopsis thaliana cDNA encoding a protein
highly homologous to E. coli RecA and containing a predicted
chloroplast transit peptide at its amino terminus (Cerutti et
al., 1992). By using assays devised to study the strand ex-
change catalyzed by E. coli RecA, we have found a strand
transfer activity in pea (Pisum sativum L.) chloroplasts. As
with the E. coli protein, formation of joint molecules requires
homologous DNA substrates, Mg2+, and ATP. These results
suggest, for the first time, the involvement of a RecA-like activity in chloroplast DNA recombination.

MATERIALS AND METHODS

Materials

Restriction enzymes, T4 polynucleotide kinase, and T4
DNA ligase were from New England Biolabs. Calf intestinal
alkaline phosphatase and BSA were from Boehringer-Mann-
heim. Proteinase K, creatine kinase, creatine phosphate, and
ATP were from Sigma. Escherichia coli RecA protein was
from Pharmacia. Exonuclease III, mung bean nuclease (Gray, 1989), and ATP. These results
suggest, for the first time, the involvement of a RecA-like activity in chloroplast DNA recombination.

Preparation of DNA Substrates for Strand-Transfer Assays

Linear [32P]dsDNA and Circular M13 ssDNA

This method was modified from that of McCarthy et al.
(1988). A 1.87-kb HaeII fragment from pUC8 was blunt
ended with mung bean nuclease and cloned into M13mp19.
ssDNA was purified by standard procedures (Sambrook et
al., 1989). 32P-labeled homologous dsDNA was prepared by
digesting supercoiled pUC8 with HaeII, followed by treat-
ment with mung bean nuclease. After separation on 1%
agarose gels, the 1.87-kb segment was isolated as described
by Tautz and Renz (1983) and labeled at the 5' end with T4
polynucleotide kinase and [γ-32P]ATP (Sambrook et al.,
1989).

Linear 32P-ssDNA and Closed Circular Duplex DNA

This method was modified from that of Konforti and Davis
(1987). Supercoiled pUC8 was isolated by CsCl/ethidium
bromide isopyknic centrifugation (Sambrook et al., 1989).
Homologous linear ssDNA was prepared by digesting pUC8
with PstI and EcoRI. Then, one strand was digested with
exonuclease III (Henikoff, 1984) and the remaining strand
purified by agarose gel electrophoresis (Tautz and Renz,
1983). The ssDNA was labeled with T4 polynucleotide kinase
and [γ-32P]ATP (Sambrook et al., 1989).

Linear 32P-ssDNA Homologous to the Plastid 16S rRNA

Gene

The pea chloroplast 16S rDNA was cloned into the
pBluescriptII KS+ vector (Cerutti and Jagendorf, 1991). A
0.45-kb EcoRI/DraI fragment was excised by standard pro-
cedures and labeled with T4 polynucleotide kinase and [γ-
32P]ATP (Sambrook et al., 1989). Labeled DNA was dena-
tured with urea and moderate heat and separated on 5%
nondenaturing polyacrylamide gels (James and Bradshaw,
1984). The two bands of ssDNA were cut out of the gel and
DNA purified by a crush-and-soak procedure (Dybczynski
and Plucieniczak, 1988).

Linear ssDNA with Ends Homologous to Plastid DNA (for
EM Analysis)

The pBluescriptII KS+ vector containing a 2.0-kb SacI/
HindII chloroplast fragment, comprising the 16S rRNA gene
(Cerutti and Jagendorf, 1991), was linearized with EcoRI. This
leaves 0.5 and 1.5 kb of sequences homologous to chloroplast
DNA (Fig. 6) flanking vector sequences. The DNA was de-
natured (James and Bradshaw, 1984) and separated on 1.3%
agarose gels, and the ssDNA bands were purified as described
by Tautz and Renz (1983).

Strand-Transfer Assays

Stromal Extract

Chloroplasts were isolated from pea leaves as described by
Nivison et al. (1986). They were resuspended in incubation
buffer (25 mm Hepes-KOH, pH 7.5; 330 mm sorbitol; 20 mm
KCl; 1 mm DTT; 0.05% BSA) at approximately 1.5 to 2.0 mg
of Chl mL-1 and broken by adding Triton X-100 to a final
centration of 0.1%. Membranes were pelleted by centrifu-
gation in a microfuge for 5 min at 4°C. Eighty-microliter
aliquots of the supernatant were used for the reactions. When
needed, ATP and MgCl2 were added to 10 mm final cen-
tration. The standard reactions contained 100 ng of ssDNA
and 750 ng of dsDNA and were adjusted to a final volume
of 100 μL with incubation buffer. The dsDNA was added
last, followed, after 5 min, by SDS to a 0.5% final cen-
tration. Samples were incubated in the dark for 40 min at 25°C.
As reported before (McKown and Tewari, 1984), the stromal

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extract has significant nuclease activity, and the addition of SDS was found to improve the signal-to-noise ratio. Presumably, SDS inactivates the nuclease(s) much faster than the recombinase(s). However, the effective concentration of SDS is unknown because it depends on the amount of interacting proteins, membranes, etc. present in the extract. This caused some variability in the experiments and, occasionally, complete loss of the strand-transfer activity.

Reactions were stopped by adding SDS, sodium sarcosinate, and EDTA to 1.5%, 1.5%, and 50 mM final concentration, respectively. All subsequent manipulations were done at 4°C to minimize branch migration. DNA was purified by standard phenol/chloroform extractions (Sambrook et al., 1989) and separated by agarose gel electrophoresis. Gels were dried as described by Silhavy et al. (1984) and exposed to Kodak XAR-5 film.

Permeabilized Chloroplasts

Chloroplasts were isolated and broken with Triton X-100 as described above. Eighty-microliter aliquots of the permeabilized chloroplasts were used for the reactions. The substrates were the endogenous chloroplast DNA (presumably dsDNA) and 100 ng of 32P-ssDNA homologous to the plastid 16S rRNA gene. Incubation conditions and subsequent DNA isolation were as described for stromal extract. Purified DNA was digested with PstI at 20°C for 1 to 2 h, using a 30-fold excess of enzymic activity. Under these conditions the digestion was found to proceed to completion while minimizing loss of D-loop structures by branch migration. Agarose gel electrophoresis and autoradiographic detection were as described above.

Control Assays with E. coli RecA

When using as substrates linear 32P-dsDNA from pUC8 and circular M13 ssDNA, strand-transfer reactions were carried out as described by McCarthy et al. (1988) for 5 min, to allow formation of stable joint molecules. In the assays with linear 32P-ssDNA and closed circular dsDNA from pUC8, we used the conditions described by Konforti and Davis (1987) with 10 min of incubation without adding E. coli ssDNA-binding protein. All subsequent manipulations were as described above.

EM Analysis

The strand-transfer reactions were performed using permeabilized chloroplasts and, as substrates, the endogenous chloroplast DNA and unlabeled ssDNA with chloroplast rDNA sequences flanking vector sequences (see above). After purification, the DNA was digested with BamHI and separated on 0.8% agarose gels. A broad band containing the joint molecules, previously identified by using labeled ssDNA, was cut out of the gel, and DNA was isolated as described by Tautz and Renz (1983). Purified DNA was spread for EM using the formamide/Cyt c technique (Inman and Schnös, 1970). Grids were rotary shadowed with Pt/Pd (4:1) and examined in a Phillips 300 electron microscope. Micrographs were projected onto a digitizing tablet to determine the contour length of the DNA molecules (Zidas Image Analysis; Carl Zeiss).

RESULTS

Strand-Transfer Activity in Chloroplast Extracts

E. coli RecA promotes the exchange of DNA strands between a variety of cDNA substrates. Gel electrophoresis has been used extensively to identify the products of this reaction by their mobility (Konforti and Davis, 1987; Griffith and Harris, 1988; McCarthy et al., 1988). However, in crude cellular extracts, other enzymic activities can lead to artifactual products as discussed in detail by Griffith and Harris (1988). Therefore, we used several assays to identify a true strand-transfer activity in chloroplasts of pea.

In one assay, modified from that of McCarthy et al. (1988), a recombinase catalyzes strand transfer between a 32P-labeled linear dsDNA fragment and a complementary segment cloned into the ssDNA E. coli phage M13mp19. A scheme for such a reaction, consistent with the mechanism of strand transfer by RecA (Roca and Cox, 1990; Kowalczykowski, 1991; Radding, 1991), is shown in Figure 1A. The DNA

\[ [32P] \text{Haell} \]

\[ \text{pUC 8 fragment} \]

**Figure 1.** DNA substrates used to assay for a chloroplast strand-transfer activity. A, Formation of joint molecules by strand transfer between the 1.87-kb Haell fragment from pUC8 (linear dsDNA) labeled with 32P and the unlabeled M13mp19 vector into which this fragment has been cloned (circular ssDNA). In the linear dsDNA, thin and thick lines indicate complementary strands. In the circular ssDNA, the thick line indicates the sequence corresponding to the Haell fragment (complementary to the strand indicated by a thin line in the linear dsDNA). The M13mp19 vector sequence is indicated by a thin line in the circular molecule. B, Formation of joint molecules by strand transfer between 32P-labeled linear ssDNA and unlabeled supercoiled dsDNA. The thick and thin lines indicate complementary strands.
The formation of joint molecules requires a heat-sensitive factor, Mg$^{2+}$, and ATP, and it is inhibited by excess of ssDNA. A strand-transfer assay was carried out as described in “Materials and Methods” using the substrates depicted in Figure 1A. Lane 1, DNA substrates incubated with boiled stromal extract; lane 2, DNA substrates incubated with native stromal extract in the absence of Mg$^{2+}$; lane 3, DNA substrates incubated with native stromal extract in the absence of ATP; lane 4, complete reaction; lane 5, DNA from a complete reaction was purified and heated to 65°C for 5 min before loading the gel; lane 6, control reaction with purified E. coli RecA.

B. Inhibition of the chloroplast strand-transfer activity by excess homologous ssDNA. Lanes 1 to 4, Reactions carried out in the presence of 100, 200, 800, and 1600 ng of ssDNA substrate, respectively.

The formation of joint molecules requires a heat-sensitive factor(s), because boiling of the stromal extract abolished detection of any product (Fig. 2A). Incubation of the dsDNA with nonhomologous ssDNA also failed to generate a product (data not shown). However, a product with the same mobility that generated by purified E. coli RecA was clearly detected (Fig. 2A). On the other hand, this activity was too low to generate a visible signal in conventional ethidium bromide-stained gels (data not shown).

The formation of joint molecules requires a heat-sensitive factor(s), because boiling of the stromal extract abolished detection of any product (Fig. 2A). Incubation of the dsDNA with nonhomologous ssDNA also failed to generate a product (data not shown). The strand-transfer activity was dependent on Mg$^{2+}$ and ATP (Fig. 2A) and, as predicted by the strand-transfer scheme shown in Figure 1A, the reaction products were partially unstable to heating at 65°C for 5 min (Fig. 2A). However, they were stable to treatment with 1.5% SDS, 1.5% sodium sarcosinate, and phenol/chloroform, routinely used for DNA purification before gel electrophoresis.

In this assay, the conversion of the labeled dsDNA substrate to a partially or completely single-stranded molecule by a nuclease or helicase, followed by annealing to the complementary ssDNA substrate, would generate an artificial product. As a control, we incubated labeled linear dsDNA and circular ssDNA separately with the stromal extract, under the conditions used for the strand-transfer reaction. These DNA substrates were incubated with a stromal extract from pea chloroplasts, under the conditions described in “Materials and Methods.” As previously reported (McKown and Tewari, 1984), this stromal extract contains a significant level of nuclease and/or phosphatase activity (data not shown). However, a product with the same mobility as that generated by purified E. coli RecA was clearly detected (Fig. 2A).

A further indication that the activity forming most of the joint DNA molecules is not a nuclease or helicase is given by the fact that an excess of homologous ssDNA substrate inhibited the reaction (Fig. 2B). This rules out the mechanism in which a nuclease or helicase generates ssDNA, which can anneal with the added ssDNA. We are left with a very high probability of a chloroplast enzyme which, like E. coli RecA, accomplishes strand transfer by binding to ssDNA with an optimal stoichiometry to form a nucleoprotein filament (Roca and Cox, 1990; Kowalczykowski, 1991; Radding, 1991; West, 1992).

A second strand-transfer assay involved $^{32}$P-labeled linear ssDNA and homologous closed circular dsDNA (Shibata et al., 1979; Konforti and Davis, 1987), as depicted in Figure 1B. These substrates are more representative of recombinogenic DNA existing in a cell (Siddiqi and Fox, 1973; Konforti and Davis, 1987; Sun et al., 1991). In addition, this assay serves as a control against a role of DNA ligases in product formation, because these enzymes are unable to link free ssDNA molecules (Lehman, 1974).

Incubation of the substrates with the stromal extract resulted in the formation of a product comigrating with that chloroform extractions, combined, and incubated in a standard reaction lacking the protein extract. The amount of annealed product detected was less than 10% of that generated by the recombinase activity in the stromal extract (data not shown).

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generated by *E. coli* RecA (Fig. 3). As before, this activity was abolished by boiling the extract. The formation of joint molecules also exhibits an absolute dependence on MgATP (Fig. 3). The joint molecules migrate slightly slower than a relaxed circle of dsDNA. This argues against the involvement of a protein covalently binding to the ends of the ssDNA and forming a circle (Griffith and Harris, 1988) or circularization of the ssDNA by RNA ligase (Tessier et al., 1986), because these products would migrate faster on an agarose gel.

**Strand-Transfer Activity in Permeabilized Chloroplasts**

The assays with naked exogenous DNA substrates indicated the existence of a strand-transfer activity in chloroplasts of pea. However, in vivo chloroplast DNA is organized in nucleoids, interacting with several proteins (Nemoto et al., 1988; Kuroiwa, 1991). Thus, we were interested in finding whether this activity can operate on exogenous naked DNA and the endogenous chloroplast DNA, the predicted substrates in experiments of chloroplast genome transformation by recombination (Boynton et al., 1991; Staub and Maliga, 1992).

We used Triton X-100-permeabilized chloroplasts in a reaction equivalent to that depicted in Figure 1B. The $^{32}$P-ssDNA was a 450-nucleotide segment internal to the plastid 16S rRNA gene (Cerutti and Jagendorf, 1991), and the complementary substrate was the 120-kb polyploid chloroplast genome (Palmer and Thompson, 1981; Palmer, 1985). After incubation, the DNA was purified and digested with PstI, under conditions that minimize branch migration. The fragments were separated by agarose gel electrophoresis, and the radioactive signal was detected by autoradiography.

The 16S rRNA gene is located in a 12.5-kb PstI fragment in the chloroplast genome of pea (Palmer and Thompson, 1981; Meeker et al., 1988). Consistent with the interaction of the exogenous $^{32}$P-ssDNA with the chloroplast 16S rDNA, a band of approximately 12.3 kb was detected in the complete reaction (Fig. 4). The formation of this product was dependent on MgATP and the presence of $^{32}$P-ssDNA homologous to the chloroplast DNA (Fig. 4). As before, the reaction was inhibited by the addition of excess unlabeled nonhomologous ssDNA (Fig. 4).

**Characterization of the Joint Molecules by EM**

To obtain more direct evidence for the formation of joint molecules, we analyzed by EM the products of the assay with permeabilized chloroplasts. In this case, we used as ssDNA substrate the pBlueScript vector flanked by sequences homologous to chloroplast rDNA, as described in "Materials and Methods." Thus, the strand-transfer reaction is expected to stop at the region of nonhomology (vector sequences), generating a D-loop with a tail of ssDNA (Shibata et al., 1979; Konforti and Davis, 1987; Roca and Cox, 1990; Kowalczykowski, 1991).

In pea chloroplast DNA, there are two replication origins that have been mapped as D-loops by EM analysis. They are located in the spacer region between the 16S and 23S rRNA genes and downstream from the 23S rRNA gene (Meeker et al., 1988). To avoid artifactual results due to the interaction of DNA and proteins in "Materials and Meth-
ods." The linear 8-kb fragments isolated with the joint molecules were used as internal standards for size determination.

We found several molecules with the expected D-loop structures and ssDNA tails (Fig. 5). Under the spreading conditions used, ssDNA is thinner and kinkier than dsDNA. Thus, one side of the D-loops seems single stranded, whereas the other side appears to be double stranded, with a protruding tail of ssDNA (Fig. 5). In all cases, the D-loops were found in the corresponding region of homology, and the joint molecules were of the expected size (Fig. 6). However, the ssDNA tails were shorter than expected, and in a few cases they were missing. Presumably, this is due to breakage during the DNA manipulations (McEntee et al., 1979) and/or digestion by nucleases in the chloroplast extract (McKown and Tewari, 1984).

Other structures, presumably formed by annealing of the added ssDNA to complementary ssDNA gaps in the chloroplast DNA, were also detected (data not shown). Because the plants were irradiated with UV light before chloroplast isolation, noncoding lesions (such as pyrimidine dimers) could have caused DNA synthesis to become discontinuous, generating ssDNA gaps when replication reinitiates downstream from the lesions (McLennan, 1988; Sassanfar and Roberts, 1990). We also observed long, free ssDNA fragments (Fig. 5 and data not shown), presumably resulting from breakage and protein-independent branch migration. Such a phenomenon has been observed before for the strand-transfer products of RecA (Kahn et al., 1981).

As a control we incubated permeabilized chloroplasts, under standard conditions, in the absence of exogenous ssDNA. Chloroplast DNA was purified and complementary ssDNA added before the digestion with BamHI. After separation by agarose gel electrophoresis, DNA was isolated from the 8.0- to 9.1-kb region and analyzed by EM. However, we were unable to find any molecule with a D-loop structure or ssDNA hybridizing to a ssDNA gap. We could not detect free ssDNA fragments either (data not shown).

**DISCUSSION**

There is convincing evidence that DNA recombination takes place in chloroplasts of higher plants (Medgyesy et al., 1985; Thanh and Medgyesy, 1989; Fejes et al., 1990; Staub and Maliga, 1992). However, our understanding of the biochemical mechanism(s) involved is still extremely limited. Current models of general recombination require initiation of the reaction by strand exchange between ssDNA from one molecule and a homologous region of dsDNA from the other (Holliday, 1964; Meselson and Radding, 1975; Szostak et al., 1983). This strand exchange is uniquely catalyzed by recombinases, like E. coli RecA (Roca and Cox, 1990; Dykstra et al., 1991; Eggleston and Kowalczykowski, 1991; Johnson and Kolodner, 1991; Sanders et al., 1991; West, 1992).

Several assays have been devised to analyze the strand-exchange activity of RecA (Shibata et al., 1979; McEntee et al., 1980; Konforti and Davis, 1987; Griffith and Harris, 1988; McCarthy et al., 1988). During this reaction, invading ssDNA from one molecule pairs with its complementary recipient in dsDNA, thereby displacing the noncomplementary strand of dsDNA and forming a joint molecule.

**Figure 5.** Electron micrographs of joint molecules formed in the assay with permeabilized chloroplasts. Note the presence of D-loops with the expected ssDNA tails (open arrows). Long free pieces of ssDNA were also found (closed arrow) (Bar, 0.5 μm).

**Figure 6.** In the molecules analyzed by EM, the D-loops were found in the expected region of homology with the chloroplast DNA. The 8-kb BamHI fragment containing the 16S rRNA gene in pea chloroplast DNA is shown at the top. The thick line indicates the 2-kb SacI/HincII chloroplast DNA fragment cloned into the pBluescript vector. The positions of the D-loops and contour lengths of observed individual molecules are indicated below. The size of the standard 8-kb fragment is expressed as the mean ± so (n = 30). Restriction enzymes: B, BamHI; E, EcoRI; H, HincII; S, SacI.
Using several assays based on this general mechanism, we have found a strand-transfer activity in pea chloroplasts. The formation of joint molecules by chloroplast extracts requires DNA homology, Mg\(^{2+}\), and ATP. One can envision several alternative pathways whereby two homologous DNA substrates become joined by heteroduplex regions or artificial products with similar migration are formed (Griffith and Harris, 1988; McCarthy et al., 1988). However, as already discussed, results of several control experiments argue against a role of nucleases, ligases, or a DNA-binding protein in product formation. Perhaps the most convincing evidence for strand transfer is the direct visualization by EM of molecules with the expected D-loop structures. We cannot exclude the possibility that these molecules are formed by a helicase, which unwinds the dsDNA, allowing annealing of one strand to the complementary ssDNA. However, recent experiments have shown that DNA helicases either have no effect or inhibit the homologous pairing step in bacteriophage T4 UvsX-mediated and E. coli RecA-mediated strand-exchange reactions (Kodadek, 1991). Moreover, the formation of joint molecules in chloroplasts is inhibited by excess ssDNA, which is not consistent with a mechanism involving a helicase.

Proteins able to carry out DNA strand transfer have been isolated from a number of eukaryotes (Hotta et al., 1985; McCarthy et al., 1988; Roca and Cox, 1990; Dykstra et al., 1991; Eggleston and Kowalczykowski, 1991; Johnson and Kolodner, 1991; Sanders et al., 1991; West, 1992). Two genes involved in DNA repair and recombination in Saccharomyces cerevisiae have recently been isolated and shown to encode proteins structurally similar to eubacterial RecA (Bishop et al., 1992; Shinozaka et al., 1992; West, 1992). However, the actual mechanism of action of these proteins is still unknown. Several other eukaryotic proteins promote DNA strand exchange without the need for a nucleoside triphosphate cofactor and can function at nearly catalytic concentrations (McCarthy et al., 1988; Dykstra et al., 1991; Eggleston and Kowalczykowski, 1991; Johnson and Kolodner, 1991; Sanders et al., 1991; West, 1992). Moreover, these strand-exchange proteins also contain an intrinsic DNA exonuclease (Dykstra et al., 1991; Johnson and Kolodner, 1991; Sanders et al., 1991; West, 1992). None of these proteins has been shown yet to form a nucleoprotein filament, and they apparently act by a mechanism different from that of E. coli RecA (Eggleston and Kowalczykowski, 1991; Radding, 1991; West, 1992).

In contrast, the activity detected in chloroplasts of pea appears to be similar to that of E. coli RecA. The requirement for ATP and the inhibition by excess ssDNA, suggesting a required stoichiometric relation between the enzyme and ssDNA, are consistent with the mechanism of strand transfer that has been demonstrated for RecA. In further support, we have found in chloroplasts of pea a 39-kD protein, immunologically related to E. coli RecA, that is induced by DNA-damaging agents (H. Cerutti et al., 1993). A similar protein, with an apparent molecular mass of 40.5 kD, was also detected in A. thaliana chloroplasts (Cerutti et al., 1992). Moreover, a gene encoding a RecA-like protein, including a predicted chloroplast transit peptide, was cloned from an A. thaliana cDNA library (Cerutti et al., 1992). The combination of these four lines of evidence strongly suggests that the chloroplast strand-transfer activity is due to a RecA protein homolog.

A role of ssDNA in initiating DNA strand exchange has been proposed in several models of recombination (Holliday, 1964; Meselson and Radding, 1975; Szostak et al., 1983). In S. cerevisiae an early event in meiosis is the formation at or near meiotic hot spots for recombination of double-strand breaks having 3' single-stranded tails. Results of biochemical and genetic analyses strongly suggest that molecules with ssDNA ends are early intermediates in yeast meiotic recombination (Padmore et al., 1991; Sun et al., 1991; Bishop et al., 1992; Shinozaka et al., 1992). Moreover, in studies of E. coli Hfr conjugation, Siddiqi and Fox (1973) found that donor DNA integrates as a single strand, preferentially into the newly synthesized recipient strands. Because Triton X-100 apparently does not affect the structure of plastid nucleoids (Nemoto et al., 1988; Kuroiwa, 1991), our results with permeabilized chloroplasts suggest that ssDNA can also act as an efficient substrate for homologous invasion of the chloroplast DNA. This reaction probably mimics the initial events in the integration of homologous donor DNA during chloroplast transformation (Boynton et al., 1991; Staub and Maliga, 1992).

The RecA-like activity shown here may function in chloroplast DNA recombination and the integration of homologous donor DNA during chloroplast transformation. Further research on the biochemical mechanism(s) involved in chloroplast recombination is needed and may provide insights into a wide range of problems such as chloroplast DNA evolution, chloroplast DNA repair, sequence conservation of plastid DNA, and uniparental inheritance of the chloroplast genome. From a practical point of view, this information might be useful for improving the current technology of chloroplast genome transformation.

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