Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 3094–3098, April 1996
Genetics

RecA protein stimulates homologous recombination in plants

(intrachromosomal recombination/mitomycin C resistance/plant transformation/nuclear targeting)

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ABSTRACT **A number of RecA-like proteins have been found in eukaryotic organisms. We demonstrate that the prokaryotic recombination protein RecA itself is capable of interacting with genomic homologous DNA in somatic plant cells. Resistance to the DNA crosslinking agent mitomycin C requires homologous recombination as well as excision repair activity. Tobacco protoplasts expressing a nucleus-targeted RecA protein were at least three times as efficient as wild-type cells in repairing mitomycin C-induced damage. Moreover, homologous recombination at a defined locus carrying an endogenous nuclear marker gene was stimulated at least 10-fold in transgenic plant cells expressing nucleus-targeted RecA. The increase in resistance to mitomycin C and the stimulation of intrachromosomal recombination demonstrate that *Escherichia coli* RecA protein is functional in genomic homologous recombination in plants, especially when targeted to the plant nucleus.**

The process of homologous recombination requires search for homology, recognition of sequence similarity, and strand exchange between two DNA molecules. In *Escherichia coli* these different steps are mediated by a single protein, the RecA protein (for review see ref. 1), which plays a central role in the recombination pathway of this bacterium. However, additional proteins are needed to initiate recombination and to resolve the intermediates created by RecA. Recombination is initiated by the generation of single-stranded DNA (ssDNA) and DNA ends in *E. coli* and presumably in all organisms. In *E. coli*, the combined action of the products of the *recB, recC*, and *recD* genes initiates a major recombination pathway (for review see ref. 2). ssDNA is recognized by RecA protein, and homologous double-stranded DNA is actively searched for. Exchange of complementary strands leads to the formation of recombination intermediates (Holliday structures). The intermediates can be resolved by different pathways; the major one involves the action of the RuvA, RuvB, and RuvC proteins. All of the recombination proteins have to work in concert to complete recombination successfully.

A number of proteins with similarity to RecA have been found in eukaryotic cells such as budding yeast, fission yeast, humans, mice, chicken, and plants (ref. 3; references in ref. 4). The best-characterized ones are the Dmc1 and Rad51 proteins from *Saccharomyces cerevisiae*. Both proteins show considerable sequence homology to RecA; in addition, Rad51 forms DNA/protein filaments, strikingly similar in tertiary structure to those formed with RecA (5). In addition to RecA-like proteins, a different class of strand-exchange proteins was found in eukaryotic cells (for review see ref. 4). The distinguishing feature of most of these proteins is that they do not require ATP to stimulate strand exchange. Rather than a search for homology and promotion of strand invasion, recombination involves exposure of ssDNA by exonuclease and subsequent reformation of double-stranded DNA from complementary ssDNAs. The mechanism is therefore inherently different from that of RecA-mediated recombination. Therefore, in eukaryotic cells, several RecA-like proteins seem to cooperate and another class of strand-exchange proteins might be needed at the same time. In light of the complexity of eukaryotic recombination the question arises whether true RecA-type recombination is possible and can be mediated by a single protein in a higher eukaryote such as a plant cell. To address this question, the key protein of the *E. coli* recombination pathway, RecA, was expressed in transgenic plants, and its effect on homologous recombination was analyzed in two different ways.

Mitomycin C is known to intercalate *in vivo* into DNA, leading to crosslinking of complementary strands (6). Crosslinking leads to inhibition of DNA synthesis in bacteria without concomitant effects on RNA or protein synthesis (7). The data indicate that mitomycin C efficiently blocks DNA replication. The resultant daughter-strand blocks can be repaired by homologous recombination (sister-chromatid exchange) and excision repair. We found that high mitomycin C concentrations kill plant cells efficiently, presumably because the capacity of this repair/recombination system is exhausted. Plant cells expressing RecA, however, exhibited a considerably higher resistance to this drug. This suggests that RecA can function in plant cells, interacting with or supplementing the endogenous plant recombination machinery. Furthermore, RecA was shown to stimulate intrachromosomal recombination in plants directly. To be maximally effective, the *E. coli* RecA had to be targeted to the plant nucleus. This was achieved by expressing a RecA construct coding for a fusion protein of a nuclear targeting sequence from the large T antigen of simian virus 40 (SV40) and the RecA protein (nt-RecA) in transgenic tobacco cells.

MATERIALS AND METHODS

Plasmid Constructions. Modified *recA* genes were derived from plasmid pDR1453 (8). The plasmid was digested with the restriction enzyme *Sac* II, the ends were made blunt with DNA polymerase I large fragment, and the amino-terminus-encoding part of the *recA* gene was subcloned as a *Sac* II/*Eco*RI fragment in plasmid pUC18, which had been cut with *Eco*RI and *Sma* I, yielding plasmid pRecA-1. The same plasmid was digested with *Hin*fI, the ends were rendered blunt, and the carboxyl-terminus-encoding part of the *recA* gene was subcloned in pUC19 (*Eco*RI/*Sma* I) as a *Hin*fI/*Eco*RI fragment, yielding plasmid pRecA-2. The amino-terminus-encoding part was further modified. A *Bst*XI/*Eco*RI and a *Taq* I/*Bst*XI fragment obtained from pRecA-1 encoding the amino-terminal part of *recA* without its initiation codon and two complementary oligonucleotides (5′-GGG GAC TCC TCC TAA GAA GAA GCG TAA GGT TAT GGC GAT-3′ and 5′-CGA TCG CCA TAA CCT TAC GCT TCT TCT TAG

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Abbreviations: ssDNA, single-stranded DNA; SV40, simian virus 40; CaMV, cauliflower mosaic virus; Sul^r, sulfonamide resistance.

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GAG GAG TCC CC-3′) encoding the missing codons as well as the SV40 nuclear localization sequence were inserted into plasmid pUC18 that was had been digested with *Eco*RI and *Sma* I, yielding plasmid pRecA-3. The DNA sequence of relevant junctions confirmed the expected structures of the constructs.

For expression of nt-RecA in plants, the leader sequence and the codons encoding the first four amino acids of the ribulose-bisphosphate carboxylase/oxygenase (Rubisco) small subunit (SSU) gene were fused to the *recA* gene. Plasmid pSP64/TPNPTII (9) contains the amino-terminus-encoding part of the SSU gene. This plasmid was digested with *Eco*RV and *Sal* I and a *Sma* I/*Eco*RI fragment derived from pRecA-3 carrying the amino-terminus-encoding portion of the *recA* gene and a *Eco*RI/*Sal* I fragment from pRecA-2 containing the carboxyl-terminus-encoding portion inserted into it to yield pRecA-4. The complete *nt-recA* gene was excised from pRecA-4 by digestion with *Hin*dIII and *Sal* I. The *Hin*dIII ends were made blunt and the fragment was inserted into plasmid pDH51 (10), which had been modified to contain an additional *Hin*dIII site upstream of the unique *Eco*RI site. This step fused the cauliflower mosaic virus (CaMV) 35S promoter and the polyadenylylation signal, respectively, to the *nt-recA* gene. For expression of RecA in plants, the *Sma* I/*Eco*RI fragment from pRecA-3 carrying the amino-terminus-encoding portion of the *recA* gene and the *Eco*RI/*Sal* I fragment from pRecA-2 containing the carboxyl-terminus-encoding portion were inserted into the modified pDH51 plasmid via corresponding sites.

The binary vector carrying the selectable marker gene conferring sulfonamide resistance (Sul^r) was constructed as follows: Plasmid pJIT119 (11) was digested with *Hin*dIII, the ends were filled in with DNA polymerase I large fragment, and the *Hin*dIII/*Sal* I fragment carrying the Sul^r gene was inserted into plasmid pDH51, which had been digested with *Sma* I and *Sal* I. To obtain pS001, the Sul^r gene fused to the CaMV 35S promoter and polyadenylylation signal was exchanged for the methotrexate-resistance gene in pM001 (12) by using the *Nco* I and *Sst* I sites. The *recA* and *nt-recA* genes were excised by digestion with *Hin*dIII and inserted into the unique *Hin*dIII site of plasmid pS001, leading to plasmids pS/recA and pS/nt-recA, respectively.

Plant Transformation and Tissue Culture Procedures. Plasmids pS/recA and pS/nt-recA were transferred to *Agrobacterium tumefaciens* strain GV3101/pMP90RK (13) by electroporation, and the resulting strains (G64 and G63) were used to inoculate leaf disks made from sterile tobacco SR1 plants according to published procedures (13). Transformed shoots were selected on sulfadiazine (100 mg/liter). Plants were regenerated and tested for rooting on sulfadiazine (100 mg/liter). Transgenic plants were grown to maturity in the greenhouse and seeds were harvested. The inheritance of the transgenes was tested by germination of seeds in the presence of sulfadiazine on the same medium. SR1hph2 plants (14) were grown from seedlings which were selected on hygromycin (15 mg/liter) under sterile conditions. G63 and G64 plants were crossed to SR1hph2 plants in the greenhouse. Siblings harboring the *recA* and *hph2* transgenes were selected by growth of seedlings in the presence of both sulfadiazine (100 mg/liter) and hygromycin (15 mg/liter) under sterile conditions. Plants were grown to maturity without further selection.

Mitomicin C Resistance Assays. Protoplasts were prepared from leaves of axenically grown G64/2 and G63/19 plants as described by Negrutiu *et al.* (15), with some modifications. Cut leaves (3 g) were digested in 50 ml of K3 (15)/0.4 M sucrose containing naphthaleneacetic acid at 1 mg/liter, kinetin at 0.2 mg/liter, 0.6% cellulase Onozuka R10 (Serva), and 0.3% Macerozyme R10 (Serva) in 145-mm Petri dishes at 22°C, in the dark, for 16 hr. Protoplasts were purified by filtration through steel sieves (250-μm and 100-μm mesh width) and washed once in W5 medium (15). Protoplasts were suspended in 1 ml of MaMg buffer (0.5 M mannitol/15 mM $MgCl_2$/0.1% Mes, pH 5.7), counted under a light microscope, and diluted to a final concentration of 10^6 cells per ml with K3/0.4 M sucrose. A 1-ml aliquot of protoplast solution was diluted with 9 ml of K3/0.4 M sucrose medium and mitomycin C was added from a stock solution, to the final concentrations indicated in Fig. 2. After incubation for 2 days in the dark at 22°C, the protoplasts were cultivated, using the bead technique of Shillito *et al.* (16) with some modifications. Protoplasts were embedded by dilution with an equal volume of medium containing 0.8% low-melting-point agarose (FMC), as a gelling agent, and grown on a solid support carrier system in 20 ml of liquid medium. Cultures were grown for approximately 4 weeks with weekly changes of medium. Survival was scored when the microcalli reached diameters of 2–4 mm. Plants were regenerated from representative samples. These plants showed no obvious growth abnormalities.

Intrachromosomal Recombination. Protoplasts were prepared from G64/2 × SR1hph2 and G62/2 × SR1hph2 plants as described above. To determine the number of intrachromosomal recombination events, cultures were grown for 6–8 weeks in the presence of kanamycin at 100 μg/ml after embedding. To determine the regeneration frequency, protoplasts were grown under identical conditions without kanamycin. From representative samples of microcalli, plants were regenerated to verify resistance to kanamycin.

RESULTS

Generation of Plants Expressing RecA and a Nucleus-Targeted Variant. Nuclear proteins concentrate in the nucleus irrespective of their size. "Nuclear localization signals," short stretches of amino acids, are believed to be responsible for targeting of nuclear proteins (for reviews see refs. 17 and 18). The size of the *E. coli* RecA monomer is close to the exclusion limit of eukaryotic nuclear pores. RecA protein is unlikely to contain nuclear localization signals and therefore might be excluded from the nucleus and hence from its target. To cover this eventuality we expressed RecA protein in plants not only in its authentic form but also fused to a nuclear localization signal.

First the *recA* gene was modified to remove most bacterial sequences from its up- and downstream untranslated regions (for details see *Materials and Methods*). In a second step, the coding sequence was attached 5′ to the nuclear localization sequence of the SV40 large T-antigen (19), yielding a fusion protein (nt-RecA). To optimize nt-RecA translation, a leader sequence derived from the Rubisco SSU gene (20) carrying a translation initiation codon was fused 5′ to the nt-RecA coding sequence. Both *recA* and *nt-recA* sequences were placed under transcriptional control of the CaMV 35S promoter and supplied 3′ with a eukaryotic polyadenylylation signal (Fig. 1). Finally, the genes were inserted into a binary vector suitable for *Agrobacterium*-mediated plant transformation, using the Sul^r gene to select transformed plants (Fig. 1). In plasmid pS/recA the orientation of the *recA* transgene relative to the Sul^r gene was such that the two genes are transcribed in opposite directions. In plasmid pS/nt-recA the *nt-recA* and the Sul^r genes are transcribed in the same direction.

Agrobacteria harboring binary vectors carrying the *recA* or the *nt-recA* transgene were used to infect tobacco leaf disks. Transgenic plants were generated and selected for sulfonamide resistance. Plants transgenic for *recA* were designated G64, whereas the plants transgenic for *nt-recA* were designated G63. Individual transgenic plants were numbered consecutively. Sulfonamide-resistant transformants were selected which contained intact single-copy inserts and transmitted the Sul^r marker to their progeny as a single Mendelian trait. Two plants were chosen that expressed RecA (G64/2) or nt-RecA (G63/19) to similar high levels. Immunofluorescence studies of protoplasts and root squashes indicated that nt-RecA protein efficiently accumulated in the nucleus, whereas no

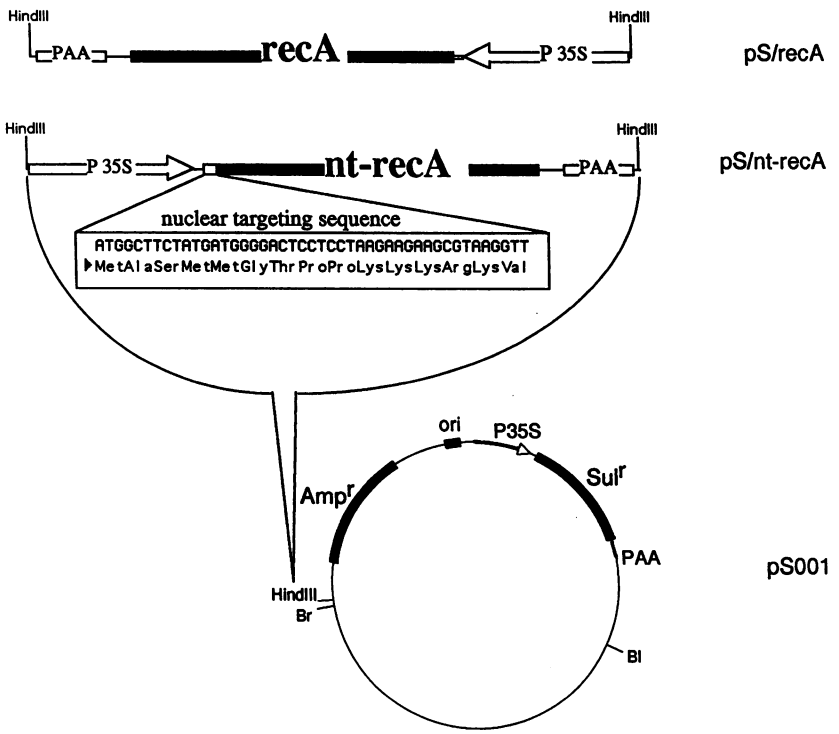

FIG. 1. Schematic representation of transgenic *recA* genes. Coding sequences are indicated by solid bars. In *recA* transgenes, open boxes indicate sequences which were added to the RecA coding region. Small boxes indicate sequences not coding for proteins. The nucleotide sequence which was added to make pS/nt-recA and the corresponding amino acid sequence are indicated. Promoters are shown as open arrows, polyadenylylation signals, as open boxes. HindIII, recognition sequence for *Hin*dIII endonuclease; P35S, CaMV 35S RNA promoter; PAA, polyadenylylation signal from CaMV; ori, *E. coli* origin of replication; Sul^r, sulfonamide-resistance gene, Amp^r, ampicillin-resistance gene; Br, *Agrobacterium* T-DNA right border sequence; Bl, T-DNA left border sequence.

particular enrichment in the nucleus was detected for RecA (data not shown).

Expression of RecA and nt-RecA Leads to Increased Resistance to Mitomycin C. To analyze the effect of mitomycin C on plant growth, a quantitative and reproducible assay was used. The system described by Lebel *et al.* (21), which allows one to follow the fate of single plant cells, was developed further to obtain greater sensitivity to mitomycin C and a monotonic survival-dose-response curve.

In the control, protoplasts were prepared from axenic tobacco SR1 wild-type plants, and parallel preparations were treated with various concentrations of mitomycin C. Subsequently the protoplasts were cultivated in a bead-type culture in the presence of mitomycin C. Untreated protoplasts actively divided and formed microcalli within a period of 4–8 weeks. In a typical experiment, 10–20% of the untreated protoplasts plated grew to microcalli. Increasing concentrations of mitomycin C progressively inhibited the formation of microcalli (Fig. 2). No growth (less than 10^{-5} of control values) was observed at concentrations of 40 μg/ml and above. The survival curve showed a low-dose shoulder which could be explained by the activity of repair mechanisms leading to resistance to mitomycin C. The survival curve became semilogarithmic at high doses of mitomycin C. This damage could apparently no longer be repaired by the endogenous repair mechanisms.

Protoplasts of a plant homozygous for the *recA* transgene (G64/2) were significantly more resistant to the toxic effect of mitomycin C than were control cells. At mitomycin C concentrations of 40 μg/ml more than 0.1% of the cells survived and grew to microcalli (Fig. 2). However, no *recA* transgenic cells (less than 10^{-5} of control values) grew at mitomycin C concentrations of 50 μg/ml and above. In contrast, more than 0.1% *nt-recA* transgenic protoplasts (homozygous G63/19) were able to grow and regenerate at mitomycin C concentrations of up to 60 μg/ml, the highest concentration tested (Fig. 2).

Intrachromosomal Recombination of a Chromosomal Marker Is Stimulated by RecA and nt-RecA. To study the process of intrachromosomal recombination in plants, Peterhans *et al.* (14) have developed a transgenic system. A pair of deletion derivatives of the selectable marker gene neomycin phosphotransferase II (*nptII*) were stably integrated into the tobacco genome. The deletions removed portions of either the 5′ or the 3′ end of the gene, rendering it nonfunctional. The segments in line SR1hph2 (14) were oriented as direct repeats

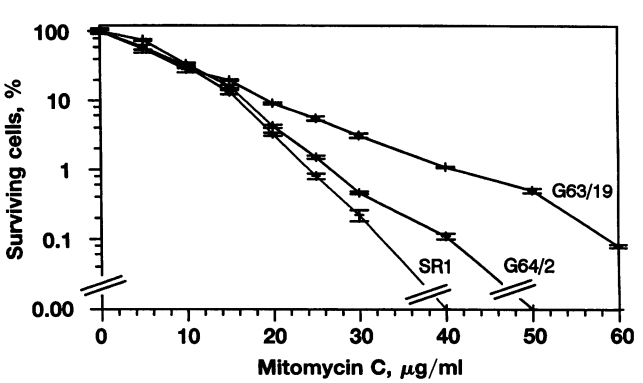

FIG. 2. RecA and nt-RecA transgenic protoplasts are more resistant to the toxic effect of mitomycin C. Protoplasts obtained from SR1, G64/2, and G63/19 plants were regenerated to microcalli in the presence or absence of mitomycin C. Survival frequencies are defined by the number of microcalli regenerated in the presence of mitomycin C divided by the number of microcalli regenerated in its absence. Mean values obtained from three different data sets are shown. The error bars represent standard deviations calculated for each data point. Data points were reproduced within ±25% for mitomycin C at 0, 10, 25, and 50 μg/ml in three additional, independent experiments.

with a 352-bp homologous overlap, interrupted by a functional hygromycin phosphotransferase gene. In this line, the basic module was present in three tightly linked copies in the genome (14). Intrachromosomal recombination events which lead to restoration of a functional gene can easily be detected by selection of kanamycin-resistant cells in tissue culture.

To study the influence of RecA and nt-RecA expression on intrachromosomal recombination, homozygous plants of line SR1hph2 containing the defective *nptII* genes were crossed to the homozygous lines G64/2 and G63/19. Progeny plants carrying the *recA* or *nt-recA* genes as well as the defective genes were selected by germinating seeds on hygromycin and sulfonamide. Plants resistant to both antibiotics were grown under sterile culture conditions without further selection, and leaf mesophyll protoplasts were prepared. Protoplasts were plated and cultured until microcalli appeared. The number of protoplasts forming microcalli in the absence of selection (regeneration frequency) was determined for each batch of protoplasts and found to be about 20–30% of all protoplast preparations. The number of protoplasts regenerating in the presence of kanamycin was determined. The frequency of intrachromosomal recombination was calculated from the number of microcalli which grew on kanamycin versus the total number of calli appearing on nonselective medium. Twenty kanamycin-resistant microcalli were selected at random for regeneration into plants. All regenerated plants formed roots on kanamycin-containing medium, confirming that calli which grew in the presence of kanamycin were indeed resistant to the antibiotic.

The frequency of intrachromosomal recombination was found to be 1.04×10^{-5} in the control line SR1hph2. In contrast, the frequencies in G64/2 × SR1hph2 and G63/19 × SR1hph2 were found to be 5.37×10^{-5} and 10.3×10^{-5}, respectively (Fig. 3). Since the control line SR1hph2 was homozygous, but all individuals resulting from crosses are heterozygous for the *hph2* locus and since Peterhans *et al.* (14) have shown that the frequency of intrachromosomal recombination is directly proportional to the number of target loci, the stimulation of homologous recombination by RecA and nt-RecA, respectively, might actually be twice as much as found experimentally. These data show that the RecA protein, especially if targeted to the nucleus, is able to interact with the plant chromosome and the host recombination machinery and to markedly increase the level of intrachromosomal somatic recombination.

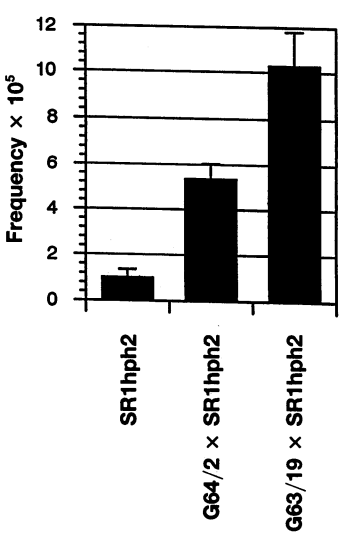

FIG. 3. RecA and nt-RecA expression leads to a stimulation of intrachromosomal recombination. At least 5×10^6 protoplasts, prepared from each of the three lines, were regenerated in the presence of kanamycin in three independent experiments. The frequency of intrachromosomal recombination was calculated from the number of calli growing in the presence of kanamycin, divided by the regeneration frequency obtained in each experiment. Mean values are shown and error bars represent standard deviations.

DISCUSSION

The *E. coli* RecA protein was expressed in tobacco plants both in its authentic form and as a nucleus-targeted variant, and its effect on homologous recombination reactions in somatic plant cells was analyzed. The effects of mitomycin C on wild-type and (nt-)RecA-expressing plant cells was tested first. For this purpose, a reproducible and quantitative assay for mitomycin C resistance, based on protoplast survival, was developed. When this assay was used, wild-type protoplast survival under mitomycin C treatment followed a dose-response curve similar to those frequently seen with bacteria and yeast: a shoulder at low doses and a semilogarithmic decrease at higher doses (Fig. 2).

At higher mitomycin C concentrations it is expected that the DNA sustains damage at many sites, saturating the repair system. In RecA- and, more clearly, in nt-RecA-expressing plant cells, the low-dose shoulder was shifted to higher mitomycin C concentrations. Mitomycin C induces formation of interstrand crosslinks (7). These might give rise to potentially lethal but recombinogenic DNA structures, which might result from excision repair as is the case for psoralen crosslinks (22) or blockage of semiconservative DNA replication (23). If the mechanism responsible for repair of mitomycin C damage at lower concentrations is based on homologous recombination, this would lead to lethality at higher mitomycin C concentrations when the number of such structures exceeds the capacity of repair by homologous recombination. Evaluation of the dose-response curves (Fig. 2) as described by Friedberg (24) suggests that nt-RecA expression provides the cells with the capacity to repair damage caused by mitomycin C at concentrations up to 50 μg/ml, whereas the endogenous repair mechanism in wild-type tobacco protoplasts can repair damage caused by mitomycin C at concentrations only up to 15 μg/ml.

Intrachromosomal recombination, between incomplete 5′ and 3′ segments of *nptII* with a 352-bp region of homology and separated by a hygromycin resistance gene in line SR1hph2 (14), was stimulated 10-fold by nt-RecA. Since Peterhans *et al.* (14) have shown that all kanamycin-resistant plants resulting from this target line had generated an intact functional gene, these results suggest that RecA is mediating homologous recombination in the plant chromosome. The plants used in this assay were heterozygous for the *hph2* locus. Therefore, restoration of functional and therefore intact *nptII* genes must have occurred by intramolecular homologous recombination. We have not determined which of the three modules present in SR1hph2 had undergone recombination; it is likely, however, that various combinations of recombination events within and between individual modules have occurred, as observed by Peterhans *et al.* (14).

Stimulation of resistance to mitomycin C and promotion of intrachromosomal recombination thus both suggest that bacterial RecA protein is able to interact with or supplement the plant recombination machinery in somatic cells. This suggests in turn that inefficient homology searching and/or strand exchange is one factor limiting homologous recombination in somatic plant cells. It may be important to note that although both assays used to analyze recombination, resistance to mitomycin C and intrachromosomal recombination, are based on regenerating protoplasts, they are very likely to reflect events in whole plants. In both cases, protoplasts were isolated from leaves of intact plants and challenged immediately with the selective agents.

RecA needs ssDNA as a substrate *in vitro*. In *E. coli*, the RecBCD enzyme is one activity generating ssDNA *in vivo*. In

mitomycin C-damaged plant cells, ssDNA may arise from interruption of semiconservative DNA replication (23) and/or excision repair of crosslinks (22). In the case of intrachromosomal recombination, ssDNA regions may be created in the loose chromatin structure by the transcription mechanism or during replication. Stimulation of homologous recombination was observed with both RecA and nt-RecA, although only the latter efficiently accumulated in the nucleus (data not shown). Therefore, authentic RecA must be able to reach the nucleus, either by passing through the nuclear pores or by reaching the DNA in the nucleus at stages during the cell cycle when the nuclear membrane is broken down. However, nt-RecA was consistently more effective than RecA. Since RecA does not accumulate in the nucleus (data not shown), the concentration of active protein at target sites within the nucleus will be considerably lower than with nt-RecA, thus explaining these differences. The degree of stimulation of mitomycin C resistance and intrachromosomal recombination with RecA and nt-RecA was different. In mitomycin C resistance, nt-RecA had a dramatic effect while the stimulation by RecA was moderate. In contrast, nuclear targeting of nt-RecA as compared with RecA led to only a further doubling in stimulation of intrachromosomal recombination. These differences might directly relate to the time at which RecA reaches the targets in the nucleus and hence its subcellular localization at different stages in the cell cycle.

Proteins with sequence homology to RecA play a role in homologous recombination in yeast. One of them, RAD51, by itself was shown to possess RecA-like activities *in vitro* (25). However, interaction with other proteins may be necessary *in vivo*—e.g., with DMC1 (26) and RAD52 (27). Although higher eukaryotic cells, including plant cells, have genes homologous to *Dmc1*, *Rad51*, or *recA* (ref. 3; see references in ref. 4), their exact role in genomic homologous recombination is unknown. The functional homology of RAD51 to RecA suggests that RecA-like homologous recombination operates in yeast, and possibly in higher eukaryotes. However, the recombination machinery of eukaryotes appears to be far more complex than in *E. coli*, since several RecA homologues seem to interact with one another and pairing proteins exist which were shown to act by nucleolytic degradation of one strand and pairing of the generated single strands (for review see ref. 4).

The fact that prokaryotic RecA protein by itself is able to stimulate genomic homologous recombination in plant cells suggests that a single protein is sufficient to perform search for homology and strand exchange in a higher eukaryotic cell. Therefore it is possible that the redundancy observed in eukaryotic RecA homologues is not of functional origin and that RAD51 by itself, or any other homologue with strand-exchange activity, may play a role in recombination similar to the one played by RecA in *E. coli*. The other proteins may serve auxiliary functions or may be the result of evolutionary divergence. However, it cannot be excluded at present that RecA in plants mediates an independent pathway leading to recombination intermediates which are recognized by the endogenous plant recombination machinery.

We thank Petra Becker, Melanie Cole, Stefanie Reusch, Gisela Simons, and Edelgard Wendeler for excellent technical help and Wolfgang Schmalenbach for synthesizing the oligonucleotides. We are indebted to Prof. Dr. John Hays for critically reading the manuscript and many helpful suggestions. The European Union financed part of this work (Contract BIOT CT-900207).

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