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A plant cDNA that partially complements *Escherichia coli recA* mutations predicts a polypeptide not strongly homologous to RecA proteins

(*Arabidopsis thaliana*/plant DNA repair/*recA* gene/UV resistance/recombination)

QISHEN PANG, JOHN B. HAYS*, AND INDIRA RAJAGOPAL

Department of Agricultural Chemistry, Oregon State University, Corvallis, OR 97331-6502

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ABSTRACT **A plant (*Arabidopsis thaliana*) cDNA previously selected for its ability to partially complement the UV sensitivity of *Escherichia coli* RecA⁻ UvrC⁻ Phr⁻ mutants and designated *DRT100* (*D*NA-damage *r*epair/*t*oleration) was subcloned into a high-copy-number plasmid and expressed via a bacterial promoter. It increased resistance of RecA⁻ UvrB⁻ Phr⁻ bacteria to mitomycin C and methyl methanesulfonate as well as to UV light. This lack of specificity, and its ability to increase resistance in both UvrB⁻ and UvrC⁻ mutants, suggested that Drt100 activity might be complementing RecA⁻ phenotypes. *DRT100* partially complemented three RecA⁻ phenotypes thought to reflect deficiencies in homologous recombination—namely, inability to plate *λred⁻gam⁻* phages and P1 phages and to recombinationally integrate donor DNA during conjugal crosses—but did not complement inability to induce *E. coli* SOS functions. The 395-amino acid *DRT100* open reading frame encodes an apparent N-terminal chloroplast transit peptide and a putative 322-residue mature protein with a conserved nucleotide binding motif, but otherwise little global homology with bacterial RecA proteins. There are several tandemly repeated leucine-rich motifs. DNA from two closely related plants, but not from maize, hybridized strongly to a *DRT100* cDNA probe.**

Plants and other organisms will be subjected to biologically significant increases in UV-B light (290–320 nm) during the next few decades, as a result of stratospheric ozone depletion. Plants employ high levels of UV-B-absorbing flavonoids to shield vital structures, but UV light nevertheless damages their genomes (1). Organisms survive UV-light damage to DNA by removing photoproducts via photoreactivation and excision repair or by tolerating their presence. In *Escherichia coli*, photoreactivation is mediated by photolyase, the product of the *phr* gene, and UV excision repair requires all three members of the protein system encoded by the *uvrA*, *uvrB*, and *uvrC* genes. Recombinational and mutagenic toleration both require the *recA* gene product, as well as other proteins. Photoreactivation and excision repair occur in *Arabidopsis thaliana* (1) and other plants, but there has been little evidence for toleration processes. Recently we isolated four *Arabidopsis* cDNAs that increased survival of UV-irradiated *E. coli* RecA⁻ UvrC⁻ Phr⁻ mutants (Q.P. and J.B.H., unpublished work). Here we show that one, *DRT100* (*D*NA-damage *r*epair/*t*oleration),† partially complements seven RecA⁻ phenotypes related to defective resistance to DNA-damaging agents and inability to mediate homologous recombination.

MATERIALS AND METHODS

Bacterial Strains. Genotypes are indicated in italics (wild-type with terminal + superscript; mutant otherwise)—e.g., *recA*⁺, *recA*, *recA*::*cat*, Δ(*recA–srl*), *recA56* (2). Phenotypes (not italicized) begin with capital letters and are superscripted + or − for wild-type or mutant—e.g., RecA⁺, RecA⁻. Strain FD2566 is Δ(*recA–srl*)::Tn*10* Δ(*uvrB–chlA*) Δ(*kdp–phr*)*214* (3). The three deletions eliminate all UV resistance, by making the cells RecA⁻ [deficient in all homologous recombination and lacking recombinational toleration of DNA damage and the ability to mount DNA-damage-induced induction of repair and toleration (SOS) functions (4)], Uvr⁻ (lacking excision repair), and Phr⁻ (lacking photoreactivation). Strain FD2565 is the same as FD2566, but *recA*⁺. Strain DJ1 is *recA56* and therefore RecA⁻ Uvr⁺ Phr⁺ (5). The above three strains are derivatives of the RecA⁺ Uvr⁺ Phr⁺ strain C600 (6). Strain QP2895 is *uvrC34* Δ(*kdp–phr*)*214* (i.e., Uvr⁻ Phr⁻); it was derived from strain TS11 [itself a *uvrC34* derivative of strain N99 (7)] by phage P1-mediated transduction using strain FD2565 as a Δ(*kdp–phr*)*244* donor. Strain QP2897 is the same as QP2895, but *recA*::*cat*, and thus also RecA⁻; it was constructed by P1 transduction of QP2895 using strain DPB302 as a *recA*::*cat* donor (8). Strain QP2898, the same as QP2897 but F′(*proA*⁺ *proB*⁺ *lacI*^{q} *lacZΔM15* Tn*10*), remains RecA⁻ Uvr⁻ Phr⁻; the F′ *lacI*^{q} episome, which overproduces the *lac* repressor, was transferred from strain XL-1 Blue (9).

Plasmids. Plasmid pQP1000 was previously selected, from a library of plasmids excised from a phage λYES-R *Arabidopsis* cDNA library (10) (obtained from R. Davis, Stanford University), by virtue of its ability to increase the resistance of strain QP2898 to UV light (Q.P. and J.B.H., unpublished work). We obtained a 1.4-kilobase product by standard polymerase chain reaction (PCR) using pQP1000 DNA templates with primers corresponding to the *GAL1* promoter and *lac* promoter regions of λYES, which flank the cDNA insert. A 1.4-kilobase *Eco*RI restriction fragment of the PCR product was inserted into the *Eco*RI site of plasmid pUC19 (12). In this pUC19-*DRT100* plasmid, designated pQP1001, the first full codon of the *DRT100* reading frame (see Fig. 2) is in frame with the pUC19 *lacZ* ATG start that is 29 codons upstream and under control of the *lac* promoter (P_{lac}). In the pUC19-(INV)*DRT100* plasmid, designated pQP1002, *DRT100* is inverted with respect to P_{lac}. Plasmid pQP1003 is the same as pQP1001, and plasmid pQP1004 the same as pQP1002, except that the *DRT100 Eco* RI fragments were excised directly from pQP1000 DNA.

Deletion plasmids were constructed by cleavage of pQP1003 with restriction endonucleases (sites indicated in Fig. 2), electrophoretic purification of appropriate fragments, and insertion into sites in the pUC19 polylinker region, putting all coding sequences in frame with *lacZ* translation signals and oriented for transcription initiated at P_{lac}.

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Abbreviations: Ap, ampicillin; Tc, tetracycline; IPTG, isopropyl β-D-thiogalactopyranoside; LRM, leucine-rich motif; cfu, colony-forming unit(s); pfu, plaque-forming unit(s).

*To whom reprint requests should be addressed.

†The sequence reported in this paper has been deposited in the GenBank data base (accession no. X66482).

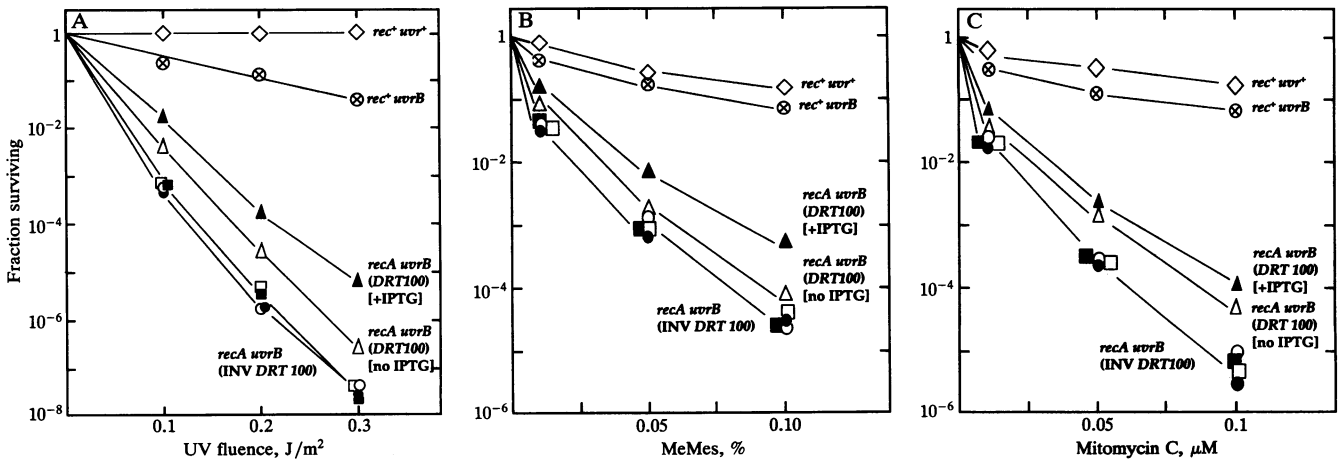

FIG. 1. Complementation of *E. coli* DNA-damage-sensitivity mutations by *Arabidopsis DRT100* cDNA. *E. coli* strains C600 [*rec*$^+$ *uvr*$^+$ (◇)] and FD2565 [*rec*$^+$ *uvrB* (⊗)] containing plasmid pUC19, and strain FD2566 (*recA uvrB*) containing plasmid pQP1001 [*DRT100* (△, ▲)], pQP1002 [INV(*DRT100*) (□, ■), or pUC19 (○, ●), were grown in the presence (filled symbols) or absence (open symbols) of IPTG, treated with DNA-damaging agents, and plated to assess survival. Surviving fraction equals cfu in treated resuspensions divided by cfu in untreated suspensions. Each determination corresponds to at least 50 surviving cfu. Data are averages for two independent experiments; ranges were less than twice the size of the symbols in all cases. Nearly identical results were obtained with plasmid pQP1003 (Fig. 2, legend) in place of pQP1001. (*A*) Irradiated with 254-nm UV light at 0.025 J/m^2 per sec to indicated fluences. (*B*) Treated with methyl methanesulfonate (MeMes) (Fluka) at indicated concentrations (% by volume) for 30 min at 37°C and washed five times in 10 mM Mg_2SO_4. (*C*) Treated with mitomycin C (Sigma) at indicated concentrations for 30 min at 37°C and washed five times.

Bacterial Media and Antibiotics. Tryptone/yeast extract (TBY) broth, tryptone/maltose (TBMM) broth, LB plates, trypticase/magnesium (TCMB) plates, tryptone/yeast extract/Ca^{2+} (R) plates, and tryptone (TA) soft agar have been described (7, 13, 14). Concentrations of ampicillin (Ap) and tetracycline (Tc) in plates were 75 μg/ml and 13 μg/ml, respectively.

Bacterial Resistance to DNA-Damaging Agents. Cells were grown overnight in TBY/Ap broth and inoculated into fresh broth. After 3 hr of growth at 37°C, isopropyl β-D-thiogalactopyranoside (IPTG, 2 mM) was added (where indicated), and growth was continued for 2 hr (to late logarithmic phase). The bacteria were then harvested and suspended in 10 mM $MgSO_4$, to 5 × 10^8 colony-forming units (cfu) per ml. Cell suspensions were treated with UV light, mitomycin C, or methyl methanesulfonate, spread on triplicate LB/Ap plates, and incubated overnight at 37°C.

Bacteriophage Plating Efficiencies. λ phage stocks were prepared by plate-stock growth (7). Phage concentrations were determined by spotting 10-μl aliquots of various dilutions onto soft agar (TA) lawns of bacteria (previously grown in TBMM broth plus 2 mM IPTG) after the lawns had been layered onto TCMB plates and incubating overnight at 38°C. Stocks of P1*vir* bacteriophages (clear-plaque formers) were prepared by plate-stock growth (13). Phage concentrations were determined by spotting aliquots on lawns of bacteria (previously grown in TBY broth plus 5 mM $CaCl_2$ and 2 mM IPTG) in TA soft-agar layers on R plates and incubating the plates overnight at 38°C.

Measurement of Conjugal Recombination Frequencies. Procedures were those of Miller (15), with minor modifications. The Hfr donor strain EG333 (5) transfers DNA counterclockwise, beginning at 12 min, and is *pyrA*::Tn*10*Δ(*pro–lac*)XIII; thus, it transfers linked Tc-resistance and Lac$^-$ markers. The donor for F′ transfer was XL-1 Blue, in which the F′ episome confers Tc resistance (9). Recipients contained plasmids conferring Ap resistance, but donors were Ap-sensitive. Total concentrations of recipients in mating mixtures were scored on LB/Ap plates, and recombinants (Hfr × F′ cross) or transconjugants (F′ transfer) were scored on LB/Ap/Tc plates. On lactose-fermentation indicator plates with Tc and Ap, Tc-resistant Lac$^+$ cells, presumably products of Tn*10* transposition from Hfr DNA, were about 0.1% as frequent as Tc-resistant Lac$^-$ recombinants.

Determination and Analysis of DNA Sequences. The *Eco*RI fragment from pQP1003 was used to produce subfragments

Table 1. Apparent efficiencies of complementation of RecA$^-$ phenotypes

DNA-damage treatment	DNA-damage resistance factor* [efficiency (%) of RecA$^-$ phenotype complementation]	
	RecA$^+$	RecA$^-$(Drt100)
UV to cells, 0.1 J/m^2	516	32 [6.2]
UV to cells, 0.2 J/m^2	3.2 × 10^4	56 [0.17]
UV to cells, 0.3 J/m^2	1.1 × 10^6	160 [0.014]
Mito C to cells, 0.01 μM	14	2.3 [17]
Mito C to cells, 0.05 μM	338	10 [2.9]
Mito C to cells, 0.10 μM	1.1 × 10^4	11 [0.10]
MeMes to cells, 0.01%	11	3.6 [33]
MeMes to cells, 0.05%	233	8.8 [3.8]
MeMes to cells, 0.10%	1640	16 [1.0]
UV to λ phages,† 6 J/m^2	9.7	3.5 [36]
UV to λ phages,† 8 J/m^2	16	5.2 [32]

Values are from analyses of data from Fig. 1 *A* (lines 1–3), *B* (lines 4–6), and *C* (lines 7–9) and relative survival fractions for plating UV-irradiated λ*red*$^-$*gam*$^+$ phages. Mito, mitomycin; MeMes, methyl methanesulfonate.

*Relative DNA-damage resistance factor equals bacterial (lines 1–9) or phage (lines 10 and 11) survival fraction for RecA$^+$ Uvr$^-$Phr$^-$ [FD2565(pUC19)] or RecA$^-$ Uvr$^-$ Phr$^-$(Drt100) [QP2898(pQP100)] bacteria divided by survival for RecA$^-$ Uvr$^-$ Phr$^-$ bacteria [QP2898(pUC19), QP2898(pQP1002), and QP2898 (no plasmid); data are average values among strains used (not all in every case; see legend to Fig. 1)]. Survival fractions for RecA$^-$ Uvr$^-$ Phr$^-$ were 7.6 × 10^{-4} (line 1), 4.3 × 10^{-6} (2), 5.1 × 10^{-8} (3), 3.9 × 10^{-2} (4), 4.7 × 10^{-4} (5), 8.4 × 10^{-6} (6), 5.9 × 10^{-2} (7), 9.4 × 10^{-4} (8), 7.7 × 10^{-2} (9), 1.1 × 10^{-4} (10), and 1.9 × 10^{-5} (11). Apparent RecA$^-$ complementation efficiency (values in brackets) equals survival factor divided by survival factor for RecA$^+$ bacteria, × 100%.

†Efficiencies of plating of λ*plac5 lacZ118 red3 cI857* phages, irradiated at 254 nm to indicated fluences at a concentration of 5 × 10^7 pfu/ml in Tris/magnesium buffer (14), were determined using TCMB plates and the following bacteria (all grown in TBMM broth plus IPTG): RecA$^-$, strain QP2898 with no plasmid or pUC19 or pQP1002; RecA$^+$, strain FD2565 with pUC19; RecA$^-$(Drt100), strain QP2898(pQP1001). Similar results were obtained when plates also contained IPTG. Range between the two independent determinations was less than ±12% of averages, except ±25% for line 11, RecA$^-$(Drt100).

for automated DNA sequence determination (Applied Biosystems) in both directions, in two runs. Disagreements were resolved by manual dideoxy sequencing.

RESULTS

Partial Complementation of Sensitivity to DNA-Damaging Agents. *Arabidopsis DRT100* cDNA, when inserted in correct orientation directly downstream of the *lac* promoter in a high-copy-number plasmid and fully derepressed by IPTG, increased the resistance to UV light of *E. coli* hypersensitive mutants by as much as 160-fold (Fig. 1*A*). *DRT100* increased UV resistance even in the absence of IPTG, presumably because multiple copies of the *lac* operator titrated out endogenous *lac* repressor (17). Drt100 activity complemented the UV sensitivity of RecA$^-$ UvrC$^-$ Phr$^-$ mutants equally well (data not shown). Relative survival factors are compared in Table 1 (lines 1–3). UV-sensitive bacteria typically show reduced plating efficiencies for UV-irradiated phage (14). Plating efficiencies on RecA$^-$ Uvr$^-$ Phr$^-$ bacteria were increased by Drt100 activity, nearly to RecA$^+$ Uvr$^-$ Phr$^-$ levels (Table 1, lines 10 and 11).

Fully derepressed Drt100 activity also increased resistance of RecA$^-$ UvrB$^-$ bacteria to a DNA-alkylating agent, methyl methanesulfonate, and a DNA-crosslinking chemical, mitomycin C (Fig. 1 *B* and *C*). Relative survival factors are compared in Table 1 (lines 4–9).

Partial Complementation of Some Other RecA$^-$ Phenotypes. The fact that Drt100 increased resistance to methyl methanesulfonate, whose products are recognized poorly by the UvrABC system (18), suggested that it might be complementing RecA$^-$ rather than Uvr$^-$ phenotypes. We tested the effect of Drt100 on another RecA$^-$ phenotype, namely, inability to support growth of λred^-gam^- phages. These phages propagate themselves only if homologous recombination between λ monomer circles produces sufficient levels of dimer circles, which are substrates for encapsidation (19); λred^-gam^- phages therefore provide a very sensitive test for RecA recombination activity. Drt100 increased by 18-fold the efficiency of plating of λred^-gam^- phages on RecA$^-$ Uvr$^-$ Phr$^-$ bacteria (Table 2, column 2) and increased plating on RecA$^-$ Uvr$^+$ Phr$^+$ bacteria (strain DJ1) by 13-fold (data not shown). RecA (and RecBCD) functions are also needed for normal replication of wild-type P1 phages (20), although here the role of homologous recombination is less well understood. Drt100 increased the plating efficiency of P1 phages on *recA::cat uvrC34* Δ*phr* bacteria 65-fold (Table 2, column 3).

We further determined the effect of Drt100 activity, in RecA$^-$ cells, on formation of stable transconjugants during Hfr × F$^-$ conjugal crosses, a well-studied recombination process. Drt100 increased the recombinant frequency in RecA$^-$ recipients nearly 50-fold (Table 2, column 4), corresponding to an apparent complementation efficiency of nearly 1%.

Drt100 failed to complement a third RecA$^-$ phenotype, namely, inability to stimulate autoproteolysis of the LexA and λ repressors and thus mediate induction of *E. coli* SOS functions by DNA damage (4) (data not shown). Drt100 did not increase the efficiency of spontaneous or UV-stimulated induction of λc^+ prophages in Δ*recA* bacteria under conditions where UV-treated RecA$^+$ bacteria released an average of 2 pfu per cell and RecA$^-$ bacteria (*recA56*) released 3×10^{-6} pfu per cell. It did not increase the frequency of rifampicin resistance in RecA$^-$ bacteria irradiated to 5 J/m^2 (21). Drt100 did not increase expression of the known SOS gene *sfiA* above background levels in RecA$^-$ bacteria containing both λ*cI*(ind$^-$) *red3 xis1* and λ*cI*(ind$^-$) *sfiA::lacZ* prophages (22) under conditions (UV light, mitomycin C) where *sfiA::lacZ* expression was induced up to 25-fold in RecA$^+$ strains.

DNA Sequence Analysis. The *DRT100* reading frame is open from the beginning of the cDNA, for 1187 base pairs (Fig. 2). The N-terminal portion of the corresponding 395-amino acid polypeptide is rich in serine and threonine and small hydrophobic residues and has a net positive charge; it thus closely resembles chloroplast transit peptides (23). The Gly-Arg-Val-Thr sequence encoded by nucleotides 208–219 is identical to the proposed consensus site for processing of chlorophyll a/b-binding proteins (23). Therefore the cDNA isolated here most likely encodes the entire mature protein. The putative protein contains 322 residues, slightly less than bacterial RecA proteins (26). Nucleotides 553–588 of *DRT100* encode

Table 2. Partial correction of Rec$^-$ phenotypes by *DRT100* cDNA

RecA phenotype of Uvr$^-$ Phr$^-$ bacteria (plasmids)*	Relative λred^-gam^- plating efficiency,† no. × 10^8		Relative phage P1 plating efficiency,‡ no. × 10^4		Recombinants per 10^6 recipients, (Hfr × F$^-$ cross)§	
RecA$^+$ (none)	10^8	[100%]	10^4	[100%]	2690	[100%]
RecA$^-$ (none)	0.8 ± 0.06	[0%]	0.4 ± 0.3	[0%]	0.3	[0%]
RecA$^-$(Drt100)	14 ± 4	[0.000013%]	26 ± 8	[0.0026%]	16	[0.6%]
RecA$^-$(INV Drt100)	0.9 ± 0.2	[0%]	0.3 ± 0.1	[0%]	ND	

Values in brackets are RecA$^-$ complementation efficiencies.

*Bacterial strains: RecA$^+$ Uvr$^-$ Phr$^-$, FD2566(pUC19) (phage plating) or QP2895(pUC19) (conjugation); RecA$^-$ Uvr$^-$ Phr$^-$, QP2898(pUC19) (phage plating) or QP2897(pUC19) (conjugation); RecA$^-$ Uvr$^-$ Phr$^-$(Drt100), QP2898(pQP1001 or pQP1003) (phage plating) or QP2897(pQP1003) (conjugation); RecA$^-$ Uvr$^-$ Phr$^-$(INV Drt100), QP2898(pQP1002 or pQP1004).

†Stocks of *λplac5 lacZ118 red3 cI857* (= red^-gam^+) and *λbio11 imm^{21} c*(ts) (= red^-gam^-) phage were prepared, and plaque-forming-units (pfu) were measured using the indicated bacterial strains. Relative plating efficiency equals pfu on RecA$^-$ bacteria containing indicated plasmids, divided by pfu on RecA$^+$ control bacteria. For λred^-gam^- phage, data correspond to means and SDs for five experiments (each with triplicate plates): two with pQP1001 or pQP1002 on TCMB plates, one with pQP1001 or pQP1002 on TCMB plates with 1 mM IPTG, one with pQP1003 or pQP1004 on TCMB plates, and one with pQP1003 or pQP1004 on TCMB plates with IPTG. There were no systematic differences. Control phages (λred^-gam^+) plated with the same efficiencies on RecA$^+$, RecA$^-$, and RecA$^-$(Drt100) bacteria.

‡The concentration of pfu in a stock of P1*vir* phage was measured using the indicated bacteria. Strains and definition of relative plating efficiency (RecA$^-$/RecA$^+$) were as described above for phage λ plating. Data correspond to a single experiment (triplicate plates).

§Conjugal matings between indicated recipients and F′ donor (strain XL-1 Blue) or Hfr donor (strain EG333) were at a ratio of five donors to one recipient, using a mating period of 30 min [very similar results were obtained using a donor/recipient ratio of 0.2 and a 120-min mating period (60-fold Drt100 effect)]. Mating efficiencies, as determined by transfer of F′ episomes from strain XL-1 Blue, were 0.32 to 0.35 for RecA$^+$, RecA$^-$, and RecA$^-$(Drt100) recipients. ND, not determined.

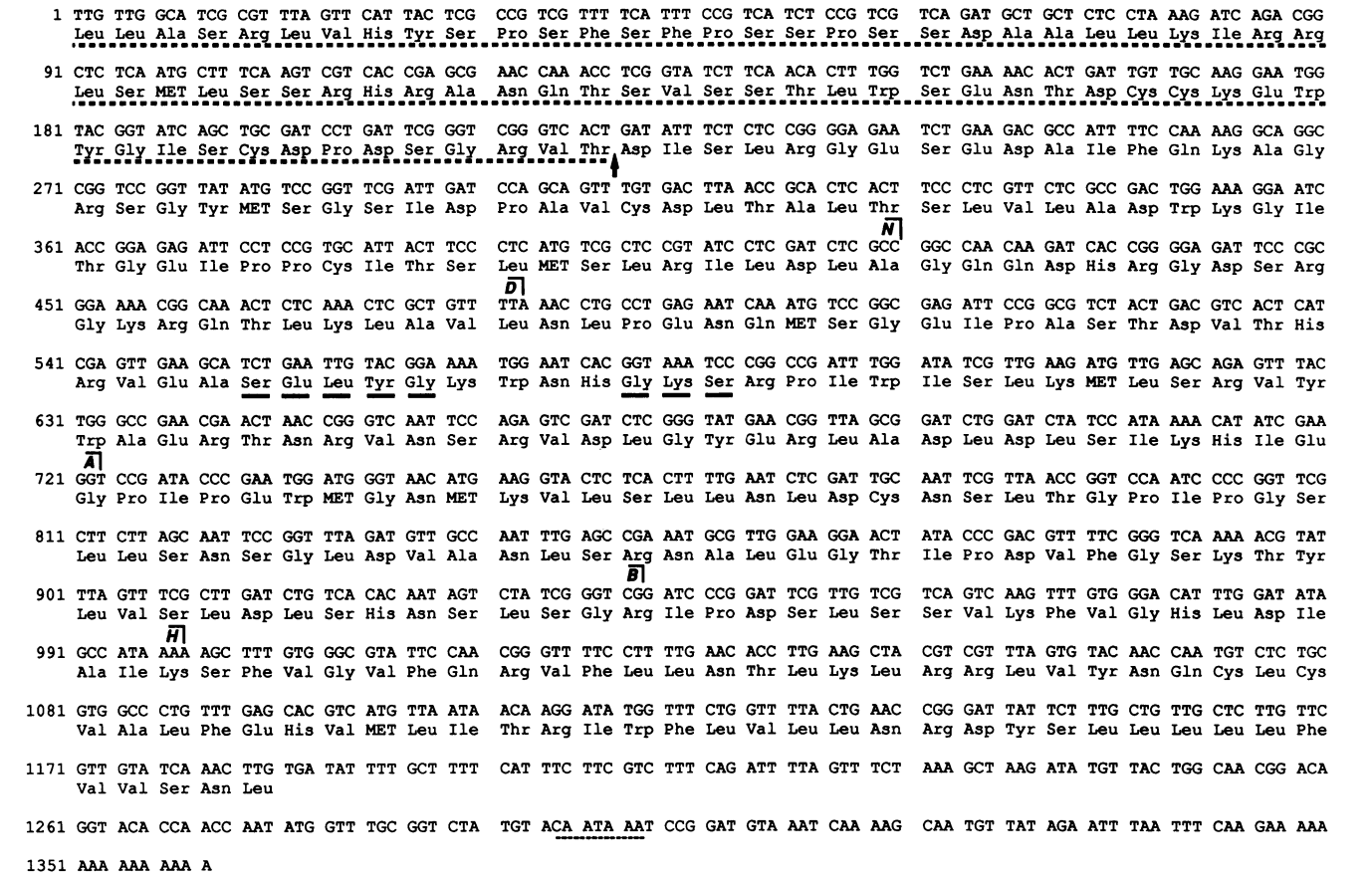

FIG. 2. Sequences of *Arabidopsis DRT100* cDNA and protein encoded by longest open reading frame. Indicated are the putative chloroplast transit peptide sequence (heavy dotted underline) (23); putative site for protease cleavage during processing in chloroplasts (arrow) (23); amino acids in putative nucleotide binding site (residues underlined) (24); putative polyadenylylation signal (light dotted underline) (25); and endpoints of *DRT100* coding sequences retained in deletion plasmids pQP1003N, 1003D, 1003A, 1003B, and 1003H (*N, D, A, B,* and *H*). These plasmids increased the resistance of RecA$^-$ Uvr$^-$ Phr$^-$ bacteria (strain FD2566) to 0.3-J/m^2 UV light by factors of 1.3, 1.5, 1.2, 73, and 102, respectively; the factor for pQP1003 was 83. (The cDNA isolated begins with GG at nucleotide −2.)

eight amino acids identical or highly conserved with respect to putative nucleotide-binding motifs (Walker boxes) in RecA-like yeast DMC1 and RAD51 proteins, and in a bacterial RecA consensus sequence (24, 27, 28) (Fig. 3*A*). However, Drt100 does not show significant global homology with any of these.

The Drt100 amino acid sequence reveals an extended region thought to be involved in specific protein–protein interactions, a repetition of LRMs. LRM repeats contain 22–25 amino acids, including several conserved leucine positions. The superfamily of proteins with LRM domains includes two yeast repair proteins, RAD1 and RAD7 (29). In Drt100 we note four 24-residue repeats, with 4 or 5 conserved leucines and 11 or 12 other positions of conservation, encoded by nucleotides 685–972 (Fig. 3*B*). These C-terminal repeats are preceded, immediately in register, by five less-perfect 21- to 24-residue leucine-rich repeats. Two deletion plasmids, pQP1003H and pQP1003B, complemented the UV sensitivity of RecA$^-$ UvrB$^-$ Phr$^-$ bacteria (strain FD2566) as well as pQP1003. These plasmids respectively lack 62 or 82 C-terminal amino acids of Drt100 (Fig. 2) and therefore encode all or all but one LRM (Fig. 3*B*). Plasmid pQP1003A, in which none of the four C-terminal LRM sequences is intact, and plasmids pQP1003D and pQP1003N, encoding smaller Drt100 peptides (Fig. 2), were inactive. In *E. coli* RecA protein, at least 35 C-terminal residues are dispensable (30).

The first (internal) ATG in *DRT100*, at nucleotide 97, is preceded by purine-rich sequences that might function as rudimentary ribosome-binding sites (31) in bacteria. We do not know whether the *recA*-complementing activity expressed by plasmids pQP1001 and pQP1003 corresponds to a putative 363-amino acid translation product initiated at the internal ATG, or to the expected 426-residue LacZ–Drt100 fusion polypeptide, or to both. Plasmid pQP1000, which did not encode a LacZ–Drt100 fusion, conferred only slightly less UV resistance (data not shown).

Genomic DNA from two other (dicotyledonous) Brassicaceae, Chinese cabbage and broccoli, but not from maize, a monocot, hybridized to a *DRT100* probe at high stringency (data not shown).

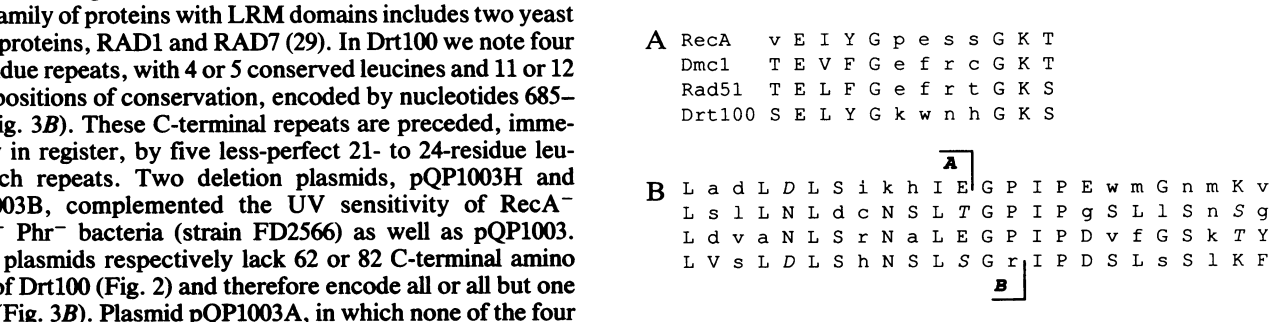

FIG. 3. Protein motifs in Drt100. (*A*) Comparison of predicted Drt100 protein sequence with those of a bacterial RecA consensus (27) and two yeast RecA-like proteins (27, 28), in the putative nucleotide-binding region (24). (*B*) Folding of predicted Drt100 sequence on itself to reveal repeated 24-residue leucine-rich motifs (LRMs) (29). Uppercase letters, positions of identity or high conservation; lowercase, positions of nonconservation; A and B, C-terminal endpoints of deletion proteins corresponding to plasmids pQP1003A and pQP1003B (see legend to Fig. 2), respectively.

DISCUSSION

We have tested a plant cDNA for ability to complement seven different phenotypes of *E. coli* RecA$^-$ Uvr$^-$ Phr$^-$ mutants, which lack the principal cellular responses to DNA-damaging UV light and chemicals: photoreactivation, nucleotide excision repair, and recombinational and mutagenic toleration. *Arabidopsis DRT100* cDNA partially complemented four repair/toleration deficiencies of these mutants—sensitivity to UV light, mitomycin C and methyl methanesulfonate and inability to propagate UV-irradiated phages and three homologous-recombination deficiencies—inability to propagate λred^-gam^- and P1 phages and failure to form stable transconjugants in Hfr × F$^-$ crosses. What might be the biochemical nature of the Drt100 activity? It increased the UV resistance of mutants lacking UvrB, a function involved in recognition of DNA damage sites in *E. coli* (18), and of mutants lacking UvrC, the damaged-DNA-incising activity (18), and increased resistance to methyl methanesulfonate, whose DNA products are not good UvrABC substrates (18). Therefore, Drt100 appeared not to function by replacing a UvrABC component. Its ability to increase resistance to several DNA-damaging agents and to act in the dark suggests that *DRT100* encodes neither a single-polypeptide UV endonuclease (32), nor a photolyase, nor an enzyme catalyzing biosynthesis of a UV-absorbing compound. Drt100 might be a novel excision repair enzyme, but it seems more likely that it is a RecA functional analog. Below we compare *DRT100* effects with those of bacterial *recA*$^+$ genes.

Apparent efficiencies for complementation by Drt100 of RecA$^-$ DNA-damage sensitivity relative to RecA$^+$ are compared in Table 1 (values in brackets). Efficiencies were high for UV-irradiated phages (lines 10 and 11) and for low levels of bacterial DNA damage (lines 1, 4, and 7). They decreased markedly with increasing bacterial DNA damage (lines 2, 3, 5, 6, 8, and 9), as if Drt100 activity were being saturated. Complementation efficiencies were moderate for homologous recombination during Hfr × F$^-$ crosses and were low, but significant, for propagation of homologous-recombination-dependent λ and P1 phages (Table 2). Translation efficiency might be reduced by the presence of codons poorly used by *E. coli*. Drt100 may be unstable in *E. coli*. Even if significant levels of stable Drt100 protein were produced, the presence of N-terminal β-galactosidase residues and the chloroplast transit peptide may have inhibited activity.

The ratios discussed above may underestimate Drt100 complementation efficiencies for DNA-damage toleration, because RecA$^+$ DNA-damage-resistance activities include not only recombinational processes but also induction by DNA damage of SOS functions, such as error-prone and recombinational toleration of DNA damage. In fact, at equivalent UV doses, RecA$^-$ (Drt100) cells and RecA$^+$ LexA$^-$ (Ind$^-$) mutants, which cannot induce SOS responses, show similar UV resistance (Fig. 1*A* and ref. 33).

Complementation of RecA$^-$ deficiencies in growth of λred^-gam^- and P1 phages may appear inefficient because of highly nonlinear relationships between burst size and ability to make a visible plaque (19, 34). The DNA substrates with which RecA or Drt100 interacts during conjugal recombination, phage DNA recombination, and recombinational toleration of DNA damage may differ with respect to effects of the chloroplast transit peptide. Further characterization of Drt100 awaits identification of the processed form in chloroplasts and production of a facsimile thereof in *E. coli*.

To our knowledge, this is the first report of complementation of bacterial RecA$^-$ phenotypes by the product of a eukaryotic gene. In addition to *DRT100*, an *Arabidopsis* cDNA encoding a chloroplast-targeted protein with significant homology to bacterial RecA proteins has recently been isolated (ref. 11, the preceding paper in this issue). We suggest that Drt100 increases tolerance of unrepaired DNA lesions in bacteria by recombinational mechanisms, such as daughter-strand gap filling or lesion bypass via copy choice (16). Our studies of Drt activity here and the identification of a chloroplast-targeted RecA homolog by Jagendorf and coworkers (11) together suggest that plants may also employ recombinational toleration mechanisms. The function in plants of neither protein has been determined. The extensive domain of LRM repeats (29) in Drt100 suggests that it may interact with other plant Drt proteins, or self-associate. The divergence between the primary sequences of the yeast and *Arabidopsis* RecA homologs on the one hand, and that of Drt100 on the other, poses an intriguing evolutionary puzzle and provides a tool for structure–function analyses of these versatile proteins.

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