

Modulation of DNA Repair and Recombination by the Bacteriophage λ Orf Function in *Escherichia coli* K-12

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The *orf* gene of bacteriophage λ , fused to a promoter, was placed in the *galK* locus of *Escherichia coli* K-12. Orf was found to suppress the recombination deficiency and sensitivity to UV radiation of mutants, in a $\Delta(\textit{recC ptr recB recD})::P_{\textit{lac}} \textit{gam bet exo pae cl} \Delta \textit{recG}$ background, lacking *recF*, *recO*, *recR*, *ruvAB*, and *ruvC* functions. It also suppressed defects of these mutants in establishing replication of a pSC101-related plasmid. Compared to *orf*, the *recA803* allele had only small effects on *recF*, *recO*, and *recR* mutant phenotypes and no effect on a *ruvAB* mutant. In a fully wild-type background with respect to known recombination and repair functions, *orf* partially suppressed the UV sensitivity of *ruvAB* and *ruvC* mutants.

Conjugative recombination in a *recBC sbcB sbcC* mutant of *Escherichia coli* depends upon the *recF*, *recO*, and *recR* genes, as well as other *rec* genes and the *ruv* genes, and is said to proceed via the RecF pathway (for a review, see reference 14). In the same cell, however, homologous recombination between bacteriophage λ chromosomes does not depend on *recF*, *recO*, or *recR*, even when the phage lacks its own recombination system, Red, and is thus dependent upon the host's recombination system (33). Sawitzke and Stahl (33) showed that λ 's RecORF independence is due to its *orf* gene (previously called *ninB*). Further studies showed that *orf* encodes a protein and that plasmid-borne *orf* can act in *trans* to promote phage recombination, but not conjugative recombination, in the absence of RecORF (34).

Recombination between phage chromosomes via the Red pathway requires neither RecORF nor Orf, but Orf nonetheless is a participant (39). The result of its action is to focus crossovers near the initiating double-strand break in both the RecF (35) and Red (39) pathways.

An additional observation suggested that Orf can promote recombination events other than crossovers between phage chromosomes. In a cell in which the *recC-ptr-recB-recD* gene cluster is replaced by the λ Red system genes (*gam*, *bet*, and *exo*) and from which the *recG* gene is deleted, recombination between the bacterial chromosome and a 3.6-kbp linear double-stranded DNA (dsDNA) molecule is dependent upon *recF*, *recO*, and *recR*. However, in such a cell, ectopic expression from the bacterial chromosome of a segment of the λ chromosome, including the *orf* gene, partially decreases this dependence (29).

For this study, the *orf* gene was installed by itself in the *galK* locus. The expression of chromosomally encoded *orf* was found to have surprisingly pleiotropic effects on recombination, replication, and repair in *E. coli*. Orf suppressed the mutant phenotypes of not only *recF*, *recO*, and *recR*, but *ruvAB* and *ruvC*

as well. In contrast, the *recA803* allele, which partially suppresses the phenotypes of *recF*, *recO*, and *recR* mutations affecting RecF pathway recombination and repair (44, 45), had no comparable effect on the Red pathway.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used for this study are described in Table 1. Some of the constructions involved the replacement of chromosomal genes by means of λ Red-mediated recombination with linear DNA species introduced into the cell via electroporation.

The λ *orf* gene was inserted into a plasmid vector and then crossed into the *galK* locus, essentially as previously described for the *rap* and *gfp* genes (31). The primers used for *orf* amplification had the sequences 5'-GGAGAGGGAACAT ATGAAAAAATAACC-3' and 5'-ATATGCTGAGCTCCTTCAACCGGAG AA-3'. The *orf*-containing plasmid intermediate was designated pTP913.

A variant of the $\Delta(\textit{recC ptr recB recD})::P_{\textit{lac}} \textit{gam bet exo pae cl} \Delta \textit{recG}$ substitution lacking *red* functions was constructed. Plasmid pTP822 contains *recC*-flanking sequences, a *red*-expressing cassette, PaeR7 restriction modification genes, the λ *cl* gene, and *recD*-flanking sequences (30). Parts of the *red* sequences were deleted by digestion of the plasmid with the HpaI restriction endonuclease and ligation in the presence of nonphosphorylated NotI linkers (AGCGGCCGCT) to make plasmid pTP964. The deleted sequences included codons for the C-terminal 63 amino acid residues of the Beta protein and the N-terminal 72 residues of the exonuclease.

Plasmid pTP980, bearing a deletion-substitution of *recC-ptr-recB-recD*, was made by replacing DNA sequences between the BglII and SacI sites in pTP822 with the *tetR* and *tetA* genes of transposon Tn10. The *tet* genes were amplified by PCR with primers 5'-CTGCTGAGATCTCTCGACATCTTGGTTACCGT-3' and 5'-GCAGCATCTAGACGCGGAATAACATCATTGG-3'. The plasmid and PCR product were digested with BglII and SacI and then ligated together.

Deletion-substitutions of the *recA* and *ruvC* genes were made by electroporation of Red-expressing bacteria with PCR-generated linear DNA species consisting of the *tetR* and *tetA* genes of transposon Tn10 flanked by 40 bases matching sequences in and near the ends of the coding sequences of the target genes. The primers used for PCRs were as follows: for *recA*, 5'-ATTACCCGG CATGACAGGAGTAAAAATGGCTATCGACGAA CTCGACATCTTGGTT ACCGT-3' and 5'-GACCCCTGTGTATCAAACAAGACGATTAATAATCT TCGTT CGCGGAATAACATCATTGG-3'; and for *ruvC*, 5'-CTAAACAGC AAAACGGAGACGCGTGATGGCTATTATCTCCTCGACATCTTGGTT ACCGT-3' and 5'-GAACTGACCGAGGCGGTATACTTAACGACGTCGC CCTCTCGCGGAATAACATCATTGG-3'.

The *recG* and *sulA* genes were deleted by means of a variation of the ampicillin enrichment technique described by Murphy et al. (25). The targeted genes were first replaced with a *gfp-cat* cassette, which makes the resulting strain fluorescent and chloramphenicol resistant. The *gfp-cat* cassette was subsequently replaced by means of Red-mediated recombination with a linear DNA species consisting only of sequences flanking the target gene (*recG602* allele) (25). Recombinants

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TABLE 1. Bacterial strains used for this study

Strain	Relevant genotype	Source, reference, or construction
AB1157 background		
AB1157	<i>F⁻ thr-1 ara-14 leuB6 Δ(gpt-proA)62 lacY1 tsx-33 supE44 galK2 λ⁻ Δrac hisG4 rfbD1 mgl-51 rpsL31 kdgK51 cyl-5 mtl-1 argE3 thi-1 Δqsr'</i>	2
JC15329	<i>Δ(srl-recA)306::Tn10</i>	A. J. Clark
KM32	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-cat</i>	30
MV1222	<i>recA803</i>	M. Volkert
SS185	<i>recJ284::Tn10</i>	15
TP538	<i>ΔrecG6200::tet</i>	29
TP540	<i>ΔruvAB6203::tet</i>	29
TP577	<i>ΔrecF6206::tet</i>	29
TP609	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6209::tet lexA71::Tn5</i>	29
TP641	<i>ΔrecO6218::tet</i>	29
TP643	<i>ΔrecQ6216::tet</i>	29
TP645	<i>ΔrecR6213::tet</i>	29
TP656	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6211</i>	31
TP657	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6211 lexA71::Tn5</i>	TP609 × linear psulAΔ (25)
TP664	<i>ΔrecN6205::tet</i>	31
TP672	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6211 galK::P_{mac}-gfp-kan915</i>	31
TP681	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6211 galK::P_{mac}-gfp-kan915 Δ(srl-recA)306::Tn10</i>	31
TP682	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6211 galK::P_{mac}-gfp-kan915 ΔrecF6206::tet</i>	31
TP684	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6211 galK::P_{mac}-gfp-kan915 ΔrecQ6216::tet</i>	31
TP685	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6211 galK::P_{mac}-gfp-kan915 ΔrecR6213::tet</i>	31
TP702	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6211 galK::P_{mac}-gfp-kan915 ΔruvAB6203::tet</i>	31
TP749	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6211 galK::P_{mac}-orf-kan913</i>	TP656 × linear pTP913 (31)
TP753	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6211 galK::P_{mac}-orf-kan913 Δ(srl-recA)306::Tn10</i>	TP749 × P1 (JC15329)
TP754	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6211 galK::P_{mac}-orf-kan913 ΔrecF6206::tet</i>	TP749 × P1 (TP577)
TP758	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6211 galK::P_{mac}-orf-kan913 ΔrecQ6216::tet</i>	TP749 × P1 (TP643)
TP759	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6211 galK::P_{mac}-orf-kan913 ΔrecR6213::tet</i>	TP749 × P1 (TP645)
TP760	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6211 galK::P_{mac}-orf-kan913 ΔruvAB6203::tet</i>	TP749 × P1 (TP540)
TP761	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6211 galK::P_{mac}-gfp-kan915 ΔrecO6218::tet</i>	TP672 × P1 (TP641)
TP762	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-pae-cl822 ΔrecG6202 ΔsulA6211 galK::P_{mac}-orf-kan913 ΔrecO6218::tet</i>	TP749 × P1 (TP641)
TP787	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-cat ΔrecG6202 ΔsulA6211 galK::P_{mac}-gfp-kan915</i>	TP672 × P1 (KM32)
TP788	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-bet-exo-cat ΔrecG6202 ΔsulA6211 galK::P_{mac}-orf-kan913</i>	TP749 × P1 (KM32)
TP790	<i>galK::P_{mac}-gfp-kan915</i>	AB1157 × P1 (TP672)
TP791	<i>galK::P_{mac}-orf-kan913</i>	AB1157 × P1 (TP749)
TP793	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-tet-pae-cl964 ΔrecG6202 ΔsulA6211 galK::P_{mac}-gfp-kan915</i>	TP787 × linear pTP964 ^a
TP794	<i>Δ(recC-ptr-recB-recD)::P_{tac}-gam-tet-pae-cl964 ΔrecG6202 ΔsulA6211 galK::P_{mac}-orf-kan913</i>	TP788 × linear pTP964 ^a
TP799	<i>galK::P_{mac}-gfp-kan915 ΔruvAB6203::tet</i>	TP790 × P1 (TP540)
TP800	<i>galK::P_{mac}-orf-kan913 ΔruvAB6203::tet</i>	TP791 × P1 (TP540)
TP801	<i>galK::P_{mac}-gfp-kan915 ΔruvC6208::tet</i>	TP790 × P1 (TP797)
TP802	<i>galK::P_{mac}-orf-kan913 ΔruvC6208::tet</i>	TP791 × P1 (TP797)
TP807	<i>galK::P_{mac}-gfp-kan915 ΔrecA6207::tet</i>	TP790 × P1 (TP796)
TP808	<i>galK::P_{mac}-gfp-kan915 ΔrecF6206::tet</i>	TP790 × P1 (TP577)
TP809	<i>galK::P_{mac}-gfp-kan915 ΔrecG6200::tet</i>	TP790 × P1 (TP538)
TP810	<i>galK::P_{mac}-gfp-kan915 recJ284::Tn10</i>	TP790 × P1 (SS185)
TP811	<i>galK::P_{mac}-gfp-kan915 ΔrecN6205::tet</i>	TP790 × P1 (TP664)
TP812	<i>galK::P_{mac}-gfp-kan915 ΔrecO6218::tet</i>	TP790 × P1 (TP641)
TP813	<i>galK::P_{mac}-gfp-kan915 ΔrecQ6216::tet</i>	TP790 × P1 (TP643)
TP814	<i>galK::P_{mac}-gfp-kan915 ΔrecR6213::tet</i>	TP790 × P1 (TP645)
TP815	<i>galK::P_{mac}-gfp-kan915 Δ(recC-ptr-recB-recD)::tet980</i>	TP790 × P1 (TP838)
TP817	<i>galK::P_{mac}-orf-kan913 ΔrecA6207::tet</i>	TP791 × P1 (TP796)
TP818	<i>galK::P_{mac}-orf-kan913 ΔrecF6206::tet</i>	TP791 × P1 (TP577)
TP819	<i>galK::P_{mac}-orf-kan913 ΔrecG6200::tet</i>	TP791 × P1 (TP538)
TP820	<i>galK::P_{mac}-orf-kan913 recJ284::Tn10</i>	TP791 × P1 (SS185)
TP821	<i>galK::P_{mac}-orf-kan913 ΔrecN6205::tet</i>	TP791 × P1 (TP664)
TP822	<i>galK::P_{mac}-orf-kan913 ΔrecO6218::tet</i>	TP791 × P1 (TP641)
TP823	<i>galK::P_{mac}-orf-kan913 ΔrecQ6216::tet</i>	TP791 × P1 (TP643)
TP824	<i>galK::P_{mac}-orf-kan913 ΔrecR6213::tet</i>	TP791 × P1 (TP645)
TP825	<i>galK::P_{mac}-orf-kan913 Δ(recC-ptr-recB-recD)::tet980</i>	TP791 × P1 (TP838)
TP838	<i>Δ(recC-ptr-recB-recD)::tet980</i>	KM32 × linear pTP980 ^a

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TABLE 1—Continued

Strain	Relevant genotype	Source, reference, or construction
TP851	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA6211\ lexA71::Tn5\ recA\ \Delta recA6207::tet$	TP657 \times P1 (TP796)
MDS12 background		
MDS12	Derivative of wild-type <i>E. coli</i> strain MG1655 bearing 12 large deletions of nonessential genes amounting to an 8.1% reduced genome	9
TP748	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}cat$	MDS12 \times P1 (KM32)
TP750	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822$	TP748 \times linear pTP822
TP765	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG::gfp\text{-}cat$	TP750 \times linear precGgc4 ^a
TP769	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202$	TP765 \times linear precGΔ (25)
TP770	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA::gfp\text{-}cat$	TP769 \times linear psulAgc4 ^a
TP772	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA6211$	TP770 \times linear psulAΔ (25)
TP773	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA6211\ \Delta(srl\text{-}recA)306::TnI0$	TP772 \times P1 (JC15329)
TP774	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA6211\ recA803$	TP773 \times P1 (MV1222)
TP775	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA6211\ \Delta recF6206::tet$	TP772 \times P1 (TP577)
TP776	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA6211\ \Delta recO6218::tet$	TP772 \times P1 (TP641)
TP777	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA6211\ \Delta recR6213::tet$	TP772 \times P1 (TP645)
TP778	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA6211\ \Delta ruvAB6203::tet$	TP772 \times P1 (TP540)
TP779	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA6211\ recA803\ \Delta recF6206::tet$	TP774 \times P1 (TP577)
TP780	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA6211\ recA803\ \Delta recO6218::tet$	TP774 \times P1 (TP641)
TP781	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA6211\ recA803\ \Delta recR6213::tet$	TP774 \times P1 (TP645)
TP782	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA6211\ recA803\ \Delta ruvAB6203::tet$	TP774 \times P1 (TP540)
TP783	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA6211\ galK::P_{mac}\text{-}gfp\text{-}kan915$	TP772 \times linear pTP915 (31)
TP784	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA6211\ galK::P_{mac}\text{-}orf\text{-}kan913$	TP772 \times linear pTP913 (31)
TP796	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recA6207::tet$ (substitution of <i>tet</i> for <i>cdn6-349</i>)	TP750 \times linear DNA ^a
TP797	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta ruvC6208::tet$ (substitution of <i>tet</i> for <i>cdn6-168</i>)	TP750 \times linear DNA ^a
TP804	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA6211\ galK::P_{mac}\text{-}gfp\text{-}kan915$	TP783 \times P1 (TP797)
TP806	$\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ \Delta recG6202\ \Delta sulA6211\ galK::P_{mac}\text{-}orf\text{-}kan913$	TP784 \times P1 (TP797)
	$\Delta ruvC6208::tet$	

^a Plasmids and PCR-generated linear DNA species used for these constructions are described in Materials and Methods.

lacking *gfp-cat* were enriched in the resulting mixed culture as previously described, except that their growth was arrested with 10 μ g of chloramphenicol/ml before the addition of ampicillin to kill the growing cells. Only one cycle of enrichment was required, as the recombinants were readily detected among large numbers of nonrecombinant colonies by their lack of fluorescence under long-wavelength UV radiation. The *gfp-cat* cassette contains the *gfp* gene from pGreenTIR (21) fused to the strong, *lacI*-controllable promoter $P_{A1/04}$ (11, 38). Its putative DNA sequence and details of its use will be provided upon request.

The reduced-genome *E. coli* strain MDS12 (9) was used to stabilize the $\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ ruvC$ genotype, which has a tendency to acquire suppressor mutations in the AB1157 background (31). The suppressor mutations presumably activate *nusA* (18, 19). The cryptic prophage encoding *nusA* is deleted from MDS12, and a $\Delta(recC\text{-}ptr\text{-}recB\text{-}recD)::P_{lac}\text{-}gam\text{-}bet\text{-}exo\text{-}pae\text{-}cI822\ ruvC$ derivative of MDS12 did not appear to acquire suppressors.

The *recA803* allele was introduced via P1 transduction into strains bearing $\Delta(srl\text{-}recA)306::TnI0$, with selection for growth on sorbitol-containing minimal medium. The presence of the *recA803* substitution V37M (17) was verified in all strains employed for this study. A segment of the chromosome containing the *recA* gene was PCR amplified, and the dsDNA product was sequenced by automated fluorescent dideoxynucleotide chain termination methods at the University of Massachusetts Medical School Nucleic Acids Facility (data not shown).

Phages. The *lac::cat819* substitution borne by $\lambda\text{-}lac::cat819\ nin5$ (30) replaces bp 23,135 to 33,498 of the λ chromosome, including *attP*, *int*, *xis*, *exo*, *bet*, and *gam*; the *nin5* deletion removes a number of other genes, including *orf* and *rap*. The phage thus lacks all of its known recombination functions. Infection of a host bearing the *PaeR7* restriction modification system by unmodified $\lambda\text{-}lac::cat819\ nin5$ results in intracellular cutting of the phage chromosome at the boundaries of the *lac::cat819* substitution, releasing it as a linear dsDNA species. λ GB2, a hybrid between λ and the spectinomycin and streptomycin resistance-conferring plasmid pGB2 (5) was constructed by crossing wild-type λ with plasmid pTP933 and by isolating recombinant phages as described previously (28). In the hybrid, pGB2 sequences replace bp 23,135 to 33,498 of the λ chromosome. Plasmid pTP933 was constructed by ligating *XhoI*-digested pTP819 (30) with *SalI*-digested pGB2. The resulting *XhoI*-*SalI* joints are not cuttable by *XhoI* or its isoschizomer *PaeR7*; therefore, λ GB2 is not cut by *PaeR7* in the infected cell. Phages were propagated and titrated on strain KM32.

Crosses. Bacterial strains were tested for recombination proficiency in groups of four. Single colonies were used to inoculate Luria-Bertani (LB) medium (1%

tryptone, 0.5% yeast extract, and 0.5% NaCl, with 1 mM added NaOH). Cultures were incubated motionless for 18 to 24 h at 37°C. Cells were harvested by centrifugation for 10 min at 4,300 \times g at 4°C, resuspended with 0.05 to 0.1 volume of TM (10 mM Tris-HCl [pH 7.4], 10 mM MgSO₄), and kept on ice. The absorbance values at 600 nm of the resuspended cells were determined, and all were adjusted to the same value, that of the least dense culture or 4.0, whichever was lower. The actual adjusted values of A_{600} were in the range of 2.5 to 4.0. Dilutions of the adjusted resuspended cells were counted in a Petroff-Hauser chamber for total cell counts and were plated on LB agar (LB medium with 1.5% agar) for the determination of total viable titers. Cells (100 μ l) were added to the phage mixture (10 μ l) and incubated at 37°C for 15 min. The phage mixture consisted of $\lambda\text{-}lac::cat819\ nin5$ and λ GB2, at approximately 10⁹/ml and 10⁷/ml, respectively, in TM plus 0.01% gelatin, resulting in nominal multiplicities of infection of approximately 0.1 and 0.001, respectively. Both phages have wild-type λ early regulatory systems and thus are prevented from expressing their genes, or replicating, by the *cI* repressor present in the infected cells. Mixtures were subsequently diluted 100-fold into TM with CHCl₃ for the determination of unadsorbed phage titers as well as into prewarmed LB medium for 1 h of further incubation, with aeration, at 37°C, after which they were plated on LB agar containing 10 μ g of chloramphenicol/ml and on LB agar containing 20 μ g of spectinomycin/ml. Measurements of viability (viable titer divided by total cell count), numbers of chloramphenicol-resistant colonies per infected viable cell, and numbers of spectinomycin-resistant colonies per infected viable cell were recorded. Each infection was done in triplicate. Measured efficiencies of phage adsorption ranged from 76% to over 99%.

Measurement of UV killing. Bacterial strains were tested for sensitivity to UV radiation in groups of four. Dilutions of cells prepared and titrated as described above were spotted, in 20- μ l portions, onto LB agar plates which had been previously incubated for 1 to 2 h uncovered at 37°C to promote quick drying of the spots. Three identical plates, each spotted with dilutions of four bacterial strains, were simultaneously irradiated with a set dose of short-wave UV in a Stratalinker apparatus (Stratagene). The parallel array of cylindrical UV lamps in this apparatus was expected to produce a uniform intensity of irradiation; in practice, a good agreement of colony counts among simultaneously irradiated plates was obtained (see Tables 2 and 6). The irradiated plates were removed from the apparatus under dim fluorescent light, wrapped in aluminum foil, and incubated at 37°C.

TABLE 2. Phenotypes of Orf⁺ and Orf⁻ strains

Strain	Relevant genotype ^a	Orf	% Recombination ^b	Viability ^c	% Plasmid replication ^d	UV survival ^e
AB1157 background						
TP672	Wild type	—	1.1009 ± 0.0492	1.00 ± 0.11	63.6 ± 2.3	2.8 × 10 ⁻¹ ± 3.3 × 10 ⁻²
TP681	<i>recA</i>	—	0.0015 ± 0.0004	0.42 ± 0.04	35.8 ± 3.1	2.3 × 10 ⁻⁶ ± 1.2 × 10 ⁻⁶
TP682	<i>recF</i>	—	<0.0005	0.15 ± 0.02	9.5 ± 2.1	1.2 × 10 ⁻⁵ ± 2.4 × 10 ⁻⁶
TP761	<i>recO</i>	—	0.0006 ± 0.0006	0.35 ± 0.06	9.3 ± 1.4	1.6 × 10 ⁻⁴ ± 1.0 × 10 ⁻⁴
TP685	<i>recR</i>	—	0.0003 ± 0.0003	0.21 ± 0.05	7.0 ± 1.1	4.5 × 10 ⁻⁵ ± 1.3 × 10 ⁻⁵
TP684	<i>recQ</i>	—	0.1657 ± 0.0030	0.95 ± 0.14	64.6 ± 2.9	9.1 × 10 ⁻² ± 8.3 × 10 ⁻³
TP702	<i>ruvAB</i>	—	0.0055 ± 0.0016	0.29 ± 0.03	0.9 ± 0.2	1.4 × 10 ⁻⁵ ± 6.2 × 10 ⁻⁶
TP793	<i>red</i>	—	0.0001 ± 0.0000	0.60 ± 0.08	43.1 ± 1.8	5.1 × 10 ⁻² ± 5.0 × 10 ⁻³
TP749	<i>Wild</i>	+	0.9057 ± 0.0387	0.70 ± 0.06	60.4 ± 4.0	2.5 × 10 ⁻¹ ± 2.5 × 10 ⁻²
TP753	<i>recA</i>	+	0.0065 ± 0.0005	0.46 ± 0.03	54.3 ± 3.7	4.4 × 10 ⁻⁵ ± 2.7 × 10 ⁻⁵
TP754	<i>recF</i>	+	0.3722 ± 0.0163	0.75 ± 0.04	47.9 ± 1.9	1.3 × 10 ⁻² ± 1.8 × 10 ⁻³
TP762	<i>recO</i>	+	0.3152 ± 0.0077	0.54 ± 0.14	41.0 ± 5.3	2.9 × 10 ⁻² ± 5.2 × 10 ⁻³
TP759	<i>recR</i>	+	0.4063 ± 0.0123	0.55 ± 0.02	61.0 ± 6.0	9.5 × 10 ⁻³ ± 1.4 × 10 ⁻³
TP758	<i>recQ</i>	+	0.1097 ± 0.0061	0.51 ± 0.05	56.2 ± 3.4	1.5 × 10 ⁻² ± 2.1 × 10 ⁻³
TP760	<i>ruvAB</i>	+	0.4778 ± 0.0346	0.51 ± 0.01	42.7 ± 6.2	2.2 × 10 ⁻² ± 2.0 × 10 ⁻³
TP794	<i>red</i>	+	<0.0005	0.22 ± 0.04	53.4 ± 3.4	1.6 × 10 ⁻² ± 7.8 × 10 ⁻³
MDS12 background						
TP783	Wild type	—	0.7377 ± 0.0362	1.00 ± 0.23	90.3 ± 20.2	5.3 × 10 ⁻¹ ± 4.8 × 10 ⁻²
TP804	<i>ruvC</i>	—	0.0263 ± 0.0044	0.19 ± 0.03	20.3 ± 3.5	3.2 × 10 ⁻⁵ ± 6.7 × 10 ⁻⁶
TP784	<i>Wild</i>	+	0.3843 ± 0.0096	1.00 ± 0.49	27.5 ± 1.8	5.1 × 10 ⁻¹ ± 3.9 × 10 ⁻²
TP806	<i>ruvC</i>	+	0.5933 ± 0.0434	0.47 ± 0.05	50.7 ± 19.6	4.9 × 10 ⁻² ± 3.2 × 10 ⁻³

^a All strains are additionally Δ(*recC ptr recB recD*::*P_{tac} gam bet exo pae c1822 ΔrecG6202 ΔsulA6211*).
^b Data are percentages of infected viable cells converted to chloramphenicol-resistant recombinants after infection with λ *lac*::*cat819 nin5*. Means and standard errors of measurement are given for three or more determinations in all cases.
^c Viability measurements were normalized to those of TP672 or TP783.
^d Data are percentages of infected viable cells converted to spectinomycin resistance after infection with λGB2.
^e Fraction of cells surviving a UV dose of 30 J/m².

RESULTS

Orf is deleterious to *E. coli*, as previously noted by Sawitzke and Stahl (34). Expression of the *orf* gene from a multicopy plasmid (a pBR322 derivative) directed by the moderate, *lac* repressor-controllable promoter *P_{mac}* was found to be lethal to wild-type *E. coli*, even in a strain bearing *F' lacI^Q*. A nonlethal level of expression was obtained by supplying an additional *lac* repressor in *trans* from a compatible plasmid. In the chromosome, the *galK*::*P_{mac} orf* insertion conferred a slow-growth, small-colony phenotype on both wild-type and Δ(*recC ptr recB recD*::*P_{tac} gam bet exo pae cI* strains. This growth retardation phenotype was exacerbated by the addition to the medium of IPTG (isopropyl-β-D-thiogalactopyranoside), which induces an elevated level of expression from *P_{mac}* in the strains used for this study, all of which bear the wild-type *lacI* gene. Otherwise identical constructs containing *gfp* or *rap* in place of *orf* (31) did not exhibit this phenotype (data not shown).

The ability of the Orf protein to substitute for various *E. coli* recombination and repair functions was tested by the construction of isogenic *galK*::*orf* and *galK*::*gfp* strains and comparisons of the effects of null mutations in *rec* or *ruv* genes in the two backgrounds.

The first set of experiments was done with strains in which RecBCD had been replaced by Red and from which *recG* was deleted. The reason for employing a background lacking RecG was that it exhibits elevated levels of Red-mediated recombination (29). Efficient recombination between the chromosomes of these strains and double-stranded linear DNA molecules depends upon the *red*, *recA*, *recF*, *recO*, *recR*, *recQ*, *ruvAB*, and *ruvC* genes (24, 29, 30). The recombining linear

dsDNA molecule was released by intracellular PaeR7 restriction endonuclease digestion of the chromosome of infecting λ *lac*::*cat nin5*, which was prevented from replicating or expressing lytic genes by the host-encoded cI repressor (30). The efficiencies of formation of chromosomal *lacZ*::*cat* recombinants by the wild-type and mutant strains are shown in Table 2. Orf effects (the ratios of recombinants of the *galK*::*orf* strain to recombinants of the corresponding *galK*::*gfp* strain) are shown in Table 3. Orf strongly stimulated recombination in *recF*, *recO*, and *recR* mutant cells, to nearly the level seen for the wild type. In addition, and unexpectedly, Orf significantly stimulated recombination in *ruvAB* and *ruvC* mutants. Orf had little or no effect on recombination in the wild type or in *recA*, *recQ*, or *red* mutants.

TABLE 3. Orf effects^a

Genotype	Recombination	Viability	Plasmid replication	UV survival
Wild type	0.8	0.7	1.0	0.9
<i>recA</i>	4.4	1.1	1.5	19
<i>recF</i>	>700	4.9	5.0	1,100
<i>recO</i>	520	1.5	4.4	180
<i>recR</i>	1,500	2.6	8.7	210
<i>recQ</i>	0.7	0.5	0.9	0.2
<i>ruvAB</i>	87	1.7	48	1,500
<i>ruvC</i>	23	2.4	2.5	1,600
<i>red</i>	ND ^b	0.4	1.2	3.1

^a Ratios of values were determined for corresponding pairs of Orf⁺ and Orf⁻ strains (see Table 2 for further details).
^b ND, not detectable.

TABLE 4. Phenotypes of strains with *recA* wild type and *recA803* alleles^a

Strain	Genotype ^b	<i>recA</i> allele	% Recombination	% Plasmid replication	UV survival
TP772	<i>rec</i> ⁺	Wild type	1.2012 ± 0.0669	88.7 ± 2.1	4.8 × 10 ⁻¹ ± 4.6 × 10 ⁻²
TP775	<i>recF</i>	Wild type	0.0082 ± 0.0015	28.1 ± 1.7	6.8 × 10 ⁻⁵ ± 1.1 × 10 ⁻⁵
TP776	<i>recO</i>	Wild type	0.0051 ± 0.0010	34.7 ± 2.6	2.3 × 10 ⁻⁴ ± 8.3 × 10 ⁻⁵
TP777	<i>recR</i>	Wild type	0.0022 ± 0.0002	26.9 ± 3.0	8.5 × 10 ⁻⁵ ± 1.6 × 10 ⁻⁵
TP778	<i>ruvAB</i>	Wild type	0.0147 ± 0.0018	5.6 ± 0.1	7.6 × 10 ⁻⁶ ± 2.3 × 10 ⁻⁶
TP774	<i>rec</i> ⁺	803	1.4836 ± 0.1102	90.8 ± 2.0	5.3 × 10 ⁻¹ ± 3.1 × 10 ⁻²
TP779	<i>recF</i>	803	0.0253 ± 0.0026	48.2 ± 1.4	4.2 × 10 ⁻³ ± 5.2 × 10 ⁻⁴
TP780	<i>recO</i>	803	0.0056 ± 0.0005	27.3 ± 2.8	8.1 × 10 ⁻³ ± 1.2 × 10 ⁻³
TP781	<i>recR</i>	803	0.0051 ± 0.0005	23.5 ± 5.3	1.6 × 10 ⁻³ ± 2.9 × 10 ⁻⁴
TP782	<i>ruvAB</i>	803	0.0088 ± 0.0005	11.7 ± 0.9	1.5 × 10 ⁻⁵ ± 8.1 × 10 ⁻⁶

^a Phenotypes are described further in Table 2.
^b All strains are additionally Δ(*recC ptr recB recD*)::*P*_{tac} *gam bet exo pae cI822 ΔrecG6202 ΔsulA6211*, in the MDS12 background.

The viability of a Δ(*recC ptr recB recD*)::*P*_{tac} *gam bet exo pae cI822* strain is significantly reduced by the elimination of *recF*, *recO*, or *recR*. This viability defect is partially suppressed by deletion of the *sulA* gene (29). The remaining viability defects of these strains are more prominent in logarithmically growing cells than in stationary-phase cells (data not shown). To minimize the role of viability effects in experiments on recombination, we deleted *sulA* from all Red-substituted strains used for this study; in addition, we used saturated or near-saturated cultures grown without active aeration for infections. Even so, some mutants exhibited reduced viability—as much as seven-fold in the case of *recF* (Table 2). As indicated in Table 3, Orf partially compensated for the viability defects of the *recF*, *recR*, and *ruvC* mutants; less activity was seen in the cases of *recO* and *ruvAB* mutants, but this may not be significant. Orf made no contribution to the viability of *recA*, *recQ*, and *red* mutants.

Some of the mutant strains have a reduced ability to become spectinomycin resistant after infection with λGB2 (Table 2). This phage, a hybrid between λ and the pSC101-based plasmid vector pGB2, converts an immune (λ repressor-expressing) wild-type host to spectinomycin resistance with nearly 100% efficiency. The *ruvAB* strain exhibited an especially marked defect in conversion; smaller defects were seen in the *recF*, *recO*, *recR*, and *ruvC* mutants. The mutants exhibited no defect in plaque formation when they were infected with heteroimmune λ strains (data not shown). This observation indicates that phage-borne genes are efficiently taken up and expressed in the mutant strains and therefore that the mutants probably are defective in replication from the pSC101 origin. Consistent with this interpretation, we observed that many of the *ruvAB* cells, but not wild-type cells, infected with λGB2 formed microcolonies on spectinomycin-containing plates (data not shown). Orf restored the efficiencies of conversion of all of the defective mutants to nearly the wild-type level (Table 3).

The “wild-type” strain used for these experiments lacks the *recG* gene, and as a consequence, is UV sensitive relative to the true wild type. As shown in Table 2, the deletion of additional *rec* or *ruv* genes increased the UV sensitivity to various degrees, as was previously reported (29). Orf greatly decreased the UV sensitivity of *recF*, *recO*, *recR*, *ruvAB*, and *ruvC* mutants, slightly decreased the UV sensitivity of the *recA* mutant, and had little or no effect on the UV sensitivity of the wild type or the *red* mutant (Table 3).

The *recA803* allele was tested in the same way as Orf, by the

construction of isogenic strains bearing the wild-type and 803 alleles of *recA* and comparisons of the effects of knocking out *rec* or *ruv* genes in the two backgrounds. The data in Tables 4 and 5 show that the *recA803* allele had little or no effect on the ability of *recF*, *recO*, *recR*, and *ruvAB* mutants to recombine. The *recA803* allele modestly decreased the UV sensitivity of the *recF*, *recO*, and *recR* mutants but had almost no effect on that of the *ruvAB* mutant. The *recA803* allele additionally had little effect on the ability of the *ruvAB* mutant to convert to spectinomycin resistance after infection with λGB2. The effect of *recA803* on this phenotype in the other mutants was not meaningfully measurable; the experiments were done in the MDS12 background, in which the phenotype is less pronounced than in the AB1157 background.

The inability of RecA803 to bypass the need for RecORF raised the question of whether RecA is directly involved in Red-mediated recombination. It might, alternatively, be required only for the production of an SOS function which participates directly. To test this idea, the recombination proficiencies of *lexA*::Tn5 and *lexA*::Tn5 *recA*Δ::*tet* strains with λ *lac*::*cat819 nin5* were compared. These strains (TP657 and TP851) produced 0.24% ± 0.036% and 0.004% ± 0.0004% recombinants, respectively, per infected viable cell (compare with the recombination figures shown in Table 2). The RecA dependence of recombination in the constitutively SOS-induced *lexA*::Tn5 strain, while not as strong as that of the wild type (60-fold versus 700-fold), indicates that RecA has a role other than helping the induction of SOS functions.

The large effects of Orf on the UV sensitivity of *recF*, *recO*, *recR*, *ruvAB*, and *ruvC* mutants in the Δ(*recC ptr recB recD*)::*P*_{tac} *gam bet exo pae cI822 ΔrecG* genetic background raised the question of whether Orf would have similar effects in a genetic background more closely related to the wild type with regard to

TABLE 5. *recA803* effects^a

Genotype	Recombination	Plasmid replication	UV survival
Wild type	1.2	1.0	1.1
<i>recF</i>	3.1	1.7	61
<i>recO</i>	1.1	0.8	35
<i>recR</i>	2.3	0.9	19
<i>ruvAB</i>	0.6	2.1	2.0

^a Ratios of values were determined for corresponding pairs of *recA* wild-type and *recA803* strains (see Table 4).

TABLE 6. Orf effects on UV survival in a non-Red-substituted background^a

Genotype ^b	Strain no.		UV survival		Orf effect
	Orf ⁻	Orf ⁺	Orf ⁻	Orf ⁺	
Wild	790	791	$6.4 \times 10^{-1} \pm 4.0 \times 10^{-2}$	$7.6 \times 10^{-1} \pm 3.4 \times 10^{-2}$	1.2
<i>recA</i>	807	817	$6.6 \times 10^{-5} \pm 1.2 \times 10^{-5}$	$1.3 \times 10^{-4} \pm 1.1 \times 10^{-5}$	1.9
<i>recBCD</i>	815	825	$5.6 \times 10^{-2} \pm 2.5 \times 10^{-2}$	$1.5 \times 10^{-2} \pm 5.0 \times 10^{-3}$	0.3
<i>recF</i>	808	818	$4.1 \times 10^{-2} \pm 4.6 \times 10^{-4}$	$1.2 \times 10^{-1} \pm 1.2 \times 10^{-3}$	3.0
<i>recG</i>	809	819	$6.8 \times 10^{-2} \pm 6.5 \times 10^{-4}$	$2.0 \times 10^{-1} \pm 8.8 \times 10^{-3}$	2.9
<i>recJ</i>	810	820	$2.5 \times 10^{-1} \pm 1.7 \times 10^{-2}$	$1.4 \times 10^{-1} \pm 5.1 \times 10^{-3}$	0.5
<i>recN</i>	811	821	$2.6 \times 10^{-1} \pm 1.7 \times 10^{-2}$	$9.2 \times 10^{-2} \pm 5.9 \times 10^{-3}$	0.3
<i>recO</i>	812	822	$4.4 \times 10^{-2} \pm 1.0 \times 10^{-3}$	$2.0 \times 10^{-1} \pm 3.8 \times 10^{-3}$	4.5
<i>recQ</i>	813	823	$4.1 \times 10^{-1} \pm 2.1 \times 10^{-2}$	$2.6 \times 10^{-1} \pm 1.5 \times 10^{-2}$	0.6
<i>recR</i>	814	824	$6.0 \times 10^{-2} \pm 7.4 \times 10^{-3}$	$1.8 \times 10^{-1} \pm 1.2 \times 10^{-2}$	3.0
<i>ruvAB</i>	799	800	$4.4 \times 10^{-2} \pm 4.7 \times 10^{-3}$	$3.6 \times 10^{-1} \pm 2.1 \times 10^{-2}$	8.1
<i>ruvC</i>	801	802	$2.2 \times 10^{-3} \pm 1.4 \times 10^{-4}$	$2.8 \times 10^{-2} \pm 2.1 \times 10^{-3}$	12.6

^a UV doses were 30 J/m² for *recA* and *ruvAB* strains and 75 J/m² for all others. See Table 2 for descriptions of phenotype values.
^b All strains are *galK::gfp* or *galK::orf*, but otherwise they are unaltered AB1157.

known recombination and repair functions. To test this, isogenic *galK::orf* and *galK::gfp* variants of strain AB1157 were constructed, followed by corresponding pairs of mutants lacking *rec* or *ruv* genes. The data in Table 6 show that Orf significantly reduced the UV sensitivity of the *ruvAB* and *ruvC* mutants; this Orf effect is further illustrated by the survival curves in Fig. 1. Orf additionally modestly increased the survival of *recF*, *recO*, *recR*, *recG*, and *recA* strains after UV irradiation (Table 6).

DISCUSSION

The Orf protein can partially substitute for RecORF in Red-mediated recombination between the bacterial chromosome and short linear dsDNA molecules, in promoting the replication of a pSC101-derived plasmid, and in recovery of a cell from UV-induced damage. These findings are superficially inconsistent with those of a previous study, which suggested a more limited capability of Orf to substitute for RecORF (34). However, in the previous study, *orf* was expressed from a plasmid. As noted by the investigators, in a *recBC sbcB sbcC* background, RecORF and Orf both might be expected to influence the tendency of a plasmid to produce potentially lethal linear multimers (10). Moreover, there is indirect evidence that plasmid linear multimers can inhibit recombination, perhaps by competing for a limited supply of recombination proteins (24). The ability of Orf to substitute for RecORF has suggested all along that Orf may perform the same mechanistic function as RecORF (33). The term “RecORF” (also appearing in the literature as “RecFOR”) recognizes that the three polypeptide products of the *recF*, *recO*, and *recR* genes are thought to function as a physically interacting complex in the same step(s) in recombination and gap repair (7, 22, 45). Genetic (44) and biochemical (4, 22, 41, 42) evidence indicates that the key RecORF-promoted step is the loading of RecA protein onto single-stranded DNA (ssDNA), displacing the *E. coli* single-stranded DNA-binding protein (SSB). That RecORF’s main role in recombination is, in effect, to assist RecA was indicated by the isolation of *srf* (suppressor of *recF*) alleles of *recA* (44). The protein encoded by one such allele, RecA803, has been shown to compete more effectively than wild-type RecA with SSB for binding to ssDNA (16). Both

a *recA803 recBC sbcB sbcC* strain and a $\Delta(recC ptr recB recD)::P_{tac} gam bet exo pae cI \Delta recG galK::orf$ strain are capable of recombining efficiently in the absence of RecORF. This observation led to the prediction that RecA803 might do for the $\Delta(recC ptr recB recD)::P_{tac} gam bet exo pae cI \Delta recG$ cell what Orf does: promote efficient RecORF-independent recombination. The inability of RecA803 to promote RecORF-independent recombination via the Red pathway (Table 5) raised the question of why RecA803 needs RecORF to load it onto ssDNA, given that RecA803 can displace SSB by itself. One interesting possible answer is that for Red recombination, RecA needs to displace the Beta protein, not SSB, from ssDNA.

The idea that a dsDNA end acted on by Red is converted to a Beta protein-coated 3'-ended ssDNA is consistent with the enzymology of λ exonuclease (Exo) (12), the interaction between Exo and Beta (32), and the DNA-binding properties of

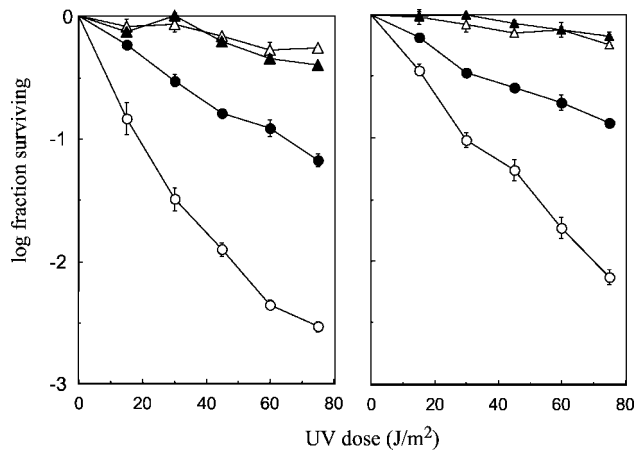


FIG. 1. Partial suppression by Orf of the UV sensitivity phenotype of *ruv* mutants. The strains are wild type for known recombination and repair functions, except as noted. Symbols for left panel: open triangles, wild type (TP790); filled triangles, *galK::orf* (TP791); open circles, *ruvAB* (TP799); filled circles, *ruvAB galK::orf* (TP800). Symbols for right panel: open triangles, wild type (TP790); filled triangles, *galK::orf* (TP791); open circles, *ruvC* (TP801); filled circles, *ruvC galK::orf* (TP802). Error bars (some of which are smaller than the symbols) indicate standard errors for three measurements of titers.

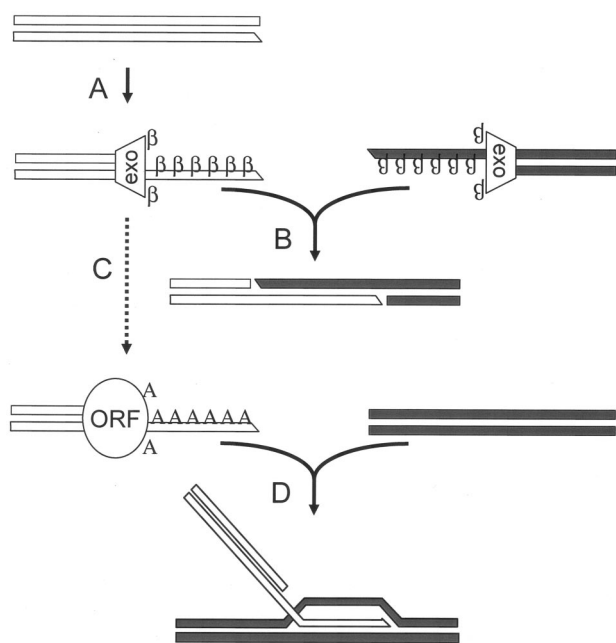


FIG. 2. Roles of recombination proteins in the early steps of the Red pathway. DNA intermediates are drawn according to the Stahl model (37). (A) A free dsDNA end is acted on by the Exo- β complex, leaving a 3'-overhanging ssDNA species coated with β . (B) If a complementary ssDNA is available, β pairs the two, generating a spliced recombinant. (C) If a complementary ssDNA is not available, eventually RecORF (or possibly λ Orf) facilitates the polymerization of RecA on the ssDNA, displacing β . (D) The RecA-coated ssDNA can invade an unbroken homologous duplex. Recombinant formation in this case depends upon further processing by RuvABC; this requirement is partially bypassed in the presence of the λ Orf protein.

Beta (8, 23). The idea that RecA has to displace Beta to act on this DNA end has implications for our understanding of the Red pathway.

According to the model for Red recombination developed by Stahl and coworkers (37), the Red pathway is best understood as having two branches, with one proceeding via strand annealing and the other proceeding via strand invasion. The strand invasion mechanism requires RecA, while strand annealing is RecA independent. RecA-promoted strand invasion is necessary when only one of the two recombining partners has a free end and the other does not, as is the case in the recombination event monitored in this study involving the *lac::cat* dsDNA segment and the (uncut) bacterial chromosome.

The inference, outlined above, that RecA must displace Beta to form recombinants via the strand invasion pathway suggests that the progression of a double-strand-break repair-recombination event down one or the other branch of the Red pathway is kinetically regulated by the type of partner available to the linear DNA species (Fig. 2). The sequence of events in Red recombination, according to this view, would be as follows. (i) An end produced by a double-strand cut to a chromosome is acted on by the Exo-Beta complex. Exo digests the 5'-ended strand, and at the same time, deposits the Beta protein on the exposed 3'-ended single strand in a way which is analogous to the deposition of RecA protein on ssDNA by RecBCD after the interaction of the latter with a χ site (1, 43).

(ii) If a complementary ssDNA is present in the cell, Beta promotes annealing, forming a recombinant. (iii) If a complementary ssDNA is not present and a dsDNA with shared sequences is present, recombination will still take place, but only after RecORF (or possibly Orf) removes Beta from the 3'-ended ssDNA and replaces it with RecA.

The idea of sequential and mutually exclusive actions by Beta and RecA suggests that strand annealing is the primary Red pathway while strand invasion is a salvage pathway, only taking place when strand annealing is blocked. In the salvage pathway, perhaps the only role of Red is to convert a dsDNA end into a 3' overhang, and a large number of additional cellular recombination proteins may be needed to make a recombinant.

The sequential Beta-RecA hypothesis raises the following question: why is Beta required at all in the strand invasion pathway? Beta is specifically required for Red activity in both strand annealing and strand invasion events (26). Possibly, Beta modulates the exonuclease activity of Exo, which otherwise would destroy recombination intermediates.

The surprising finding of this study was that Orf complements null mutations in *ruvAB* and *ruvC*. In the study that originally characterized Orf functions, this activity could not have been detected, as the recombination event under investigation was Ruv independent (33). The Ruv-complementing activity was observable in a cell which was mutated only in *ruvAB* or *ruvC*, not in genes for other known repair-recombination functions (Fig. 1), implying that it is not a peculiarity of the highly engineered $\Delta(recC\ ptr\ recB\ recD)::P_{tac}\ gam\ bet\ exo\ pae\ cI\ \Delta recG$ genetic background in which most of the experiments were done.

The RuvA, RuvB, and RuvC proteins have been shown to act in concert to resolve branched DNA molecules which model recombination intermediates (46). RuvB is a helicase which drives branch migration and which is targeted to Holliday junctions by the RuvA protein (40). RuvC is an endonuclease (resolvase) which specifically cleaves Holliday junctions at symmetrically related strands (3). The phage λ -encoded Rap protein, which is also a junction-targeted endonuclease (36), can, like Orf, partially substitute for RuvC, but not for RuvAB or other *E. coli* recombination proteins (31).

The pleiotropy of Orf action suggests that Orf does not directly replace the proteins whose functions it renders nearly unnecessary. It is readily conceivable that Orf might have the same functional activity as RecORF. If a single amino acid substitution in RecA, turning it into RecA803, is almost all that is needed to dispense with RecORF, there is no obvious reason why the same effect could not be achieved by even the small Orf protein, which has a monomer molecular mass of 16.6 kDa. It is harder to imagine that Orf could have the same enzymatic activities as RuvABC and is nearly inconceivable that it could mimic both RecORF and RuvABC.

One way in which Orf might indirectly suppress the phenotypes of strains lacking RecORF or RuvABC components is by inducing the expression of a set of cryptic genes which have RecORF- and RuvABC-like activities. Indeed, *ruv* mutations are known to be suppressed by mutational activation of the cryptic *rusA* gene (19). The ability of Orf to suppress a *ruvC* mutation is *rusA* independent, as it occurs in a genetic background from which *rusA* and all the cryptic prophage genes in

its vicinity have been deleted (Table 4) (9), but the possibility that Orf activates some other set of genes cannot be ruled out.

A second way in which Orf might work is by modifying some other protein or multiprotein complex, making it RecBCD-like, i.e., capable of carrying out moderately efficient recombination in the absence of RecORF or RuvABC (see references 14 and 27 for reviews). This hypothetical mechanism of Orf action is constrained by three observations, as follows. (i) In the $\Delta(\text{recC ptr recB recD})::P_{\text{tac}} \text{gam bet exo pae cI } \Delta\text{recG galK::orf}$ background, recombination and repair are independent of RecORF and RuvABC but are still highly dependent upon Red, despite the presence of Orf (Table 2). It follows from this observation that there is no Orf-modified protein complex in the cell, other than Exo-Beta, which can operate on dsDNA ends to promote efficient exchanges, and also that RecG cannot be the Orf target. (ii) Suppressing effects of Orf are also seen in cells in which RecBCD is present (Table 6). Therefore, having both RecBCD and Orf gives cells capabilities beyond those which result from having RecBCD without Orf. (iii) Orf does not suppress a *recQ* mutant (Table 3), leaving open the possibility that RecQ might be the Orf target. This possibility is made unlikely, however, by the observation that the slow growth phenotype conferred by Orf is not suppressed by a *recQ* null mutation, as would be expected if Orf acted only by modifying RecQ; the *recQ* mutation itself does not noticeably affect the growth rate (data not shown).

A third way in which Orf might work is by modulating the activity of SSB. This SSB modulation hypothesis provides a ready explanation for how Orf could help RecA displace SSB from ssDNA. Orf-modulated SSB may also help RecA to compete with Beta for ssDNA binding. The expression level-dependent growth slowing or stopping activity of Orf can also be readily understood as an effect of its interfering to various degrees with SSB's function in DNA replication (see reference 20 for a review). It is not obvious how SSB modulation could account for Orf's RuvABC-bypassing activity. The essential role of SSB in replication makes a precise evaluation of its role in recombination difficult. However, there is evidence indicating that SSB has an essential role in recombination as well as in replication (6). The current understanding of recombination mechanisms permits speculation that the resolution of recombination intermediates can occur by a number of different pathways (13, 47), but at least in a $\Delta(\text{recC ptr recB recD})::P_{\text{tac}} \text{gam bet exo pae cI } \Delta\text{recG}$ strain, only RuvABC is fast enough to resolve them in such a way as to form recombinants efficiently. If RuvABC is not present, perhaps other fast processes resolve the intermediates without forming recombinants. If SSB has a critical role in any of these other processes, then modulation of its activity by Orf might slow them down or accelerate still other recombinant-forming processes.

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