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Recombination-Promoting Activity of the Bacteriophage λ Rap Protein in *Escherichia coli* K-12

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The *rap* gene of bacteriophage λ was placed in the chromosome of an *Escherichia coli* K-12 strain in which the *recBCD* gene cluster had previously been replaced by the λ *red* genes and in which the *recG* gene had been deleted. Recombination between linear double-stranded DNA molecules and the chromosome was tested in variants of the *recGΔ red*$^+$ *rap*$^+$ strain bearing mutations in genes known to affect recombination in other cellular pathways. The linear DNA was a 4-kb fragment containing the *cat* gene, with flanking *lac* sequences, released from an infecting phage chromosome by restriction enzyme cleavage in the cell. Replacement of wild-type *lacZ* with *lacZ*::*cat* was monitored by measuring the production of Lac-deficient chloramphenicol-resistant bacterial progeny. The results of these experiments indicated that the λ *rap* gene could functionally substitute for the *E. coli ruvC* gene in Red-mediated recombination.

The *rap* gene of phage λ has a number of properties suggesting that it functions in homologous recombination. It is required for efficient formation or stability of RecBCD-generated cointegrates between λ and plasmids bearing homologous sequences (3). This observation is responsible for its name: recombination adept with plasmids. The *rap* gene also influences both the nature and clustering of recombination events in λ crosses (17). On the basis of the *rap* gene's map location and apparent involvement in recombination, it was proposed that *rap* is a functional analog (though it is not a homolog) of the *rusA* genes of phage 82 and a cryptic *Escherichia coli* prophage (6). The RusA protein is a Holliday junction resolvase, which can substitute for the RuvC protein of *E. coli* in promoting recombination (7, 15). Consistent with this proposal, it was found that the *rap* gene encodes a nuclease which cleaves at the branch points of Holliday junctions; in addition, it cleaves three-stranded junctions (D-loops) (16).

An *E. coli* strain in which the RecBCD recombinase is replaced with the Red system of phage λ is hyper-rec. In such a strain recombination involving short stretches of sequence identity (30 to 1,000 bp) occurs at an elevated level, relative to wild-type *E. coli*, and is strongly dependent upon both Red function and double-strand breaks (2, 9, 11, 18, 19). This hyper-rec state of a bacterium expressing some of the phage recombination genes (*gam*, *bet*, and *exo*) partially models the conditions which prevail in a phage-infected cell. It has been found that expression in a *red*$^+$ cell of genes from the *nin* region of the λ chromosome, including *rap*, does not further stimulate recombination but makes recombination partially independent of *recF*, *recO*, *recR*, and *ruvC* (12). In this study we show that the *rap* gene accounts for some of this activity, complementing the recombination defect of a *ruvC* mutant.

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Strains. Bacterial strains constructed for this study are described in Table 1. λ *lac*::*cat819 nin5* has been described previously (13).

The *rap* gene was PCR amplified from λ *cI857 S7* DNA with primers having the sequences 5′-CACGAGGAAGCATATG ATGGCTA-3′ and 5′-GTTTCAACGAGCTCTTATGGAAT GGTT-3′. Following digestion with *Nde*I and *Sac*I restriction endonucleases (sites are underlined in the primer sequences), it was ligated between the *Nde*I and *Sac*I sites of an expression vector bearing a promoter/ribosome binding site cassette with the sequence GGGCCCGCACCCCAGGC**TTTACA**TTGTG AGCGGATAACAA**TATAAT**GAAGCTTAATAAGGAGGA AAAACATATG. The promoter (−35 and −10 hexamers are in bold type) is designated P_{mac}. Its design is based on the studies of Lanzer and Bujard (4). It promotes a moderate level of transcription and is controllable by the *lac* repressor (unpublished observations). The P_{mac}-*rap* assembly was sequenced and found to contain wild-type *rap* (data not shown). It was cloned between the *Apa*I and *Sac*I sites of a derivative of pTP838 (10) in which the *Nde*I site had been removed by digestion with *Nde*I, filling in with *E. coli* DNA polymerase I large fragment, and religation. The resulting plasmid, pTP914, contains the following elements in order: an *Aat*II site; *galK* amino terminal-encoding sequences; P_{mac}-*rap*; a kanamycin resistance determinant derived from Tn*903*; *galK* carboxy terminal-encoding sequences; a *Bam*HI site; and the pBR322 replication origin and beta lactamase gene. pTP914 was digested with *Bam*HI and was partially digested with *Aat*II (the *rap* gene contains an *Aat*II site). The digested DNA was introduced into *E. coli* strain TP656 via electroporation. Kanamycin-resistant transformants were screened for ampicillin sensitivity and were tested by colony PCR for the presence of an insertion of the expected size in *galK*. An otherwise identical strain, bearing the *gfp* (green fluorescent protein) gene of pGreenTIR (8) in place of *rap*, was constructed as a *rap* mutant control.

Starting with an *E. coli* strain in which the *recBCD* gene cluster was replaced with P_{tac}-*gam-bet-exo pae cI*, variants lacking Red functions were constructed by transformation with

TABLE 1. Bacterial strains used in this study[a]

Strain	Genotype	Source, reference, or description
AB1157	*F⁻ thr-1 ara-14 leuB6* Δ(*gpt-proA*)*62 lacY1 tsx-33 supE44 galK2* λ⁻ *rac hisG4 rfbD1 mgl-51 prsL31 kdgK51 cyl-5 mtl-1 argE3 thi-1 qsr'*	1
CS85	*eda-51*::Tn*10 ruvC53*	14
JC15329	Δ(*srl-recA*)*306*::*Tn10*	A. J. Clark
N2057	*ruvA60*::*Tn10*	14
SS185	*recJ284*::*Tn10*	5
TP507	*AB1157* Δ(*recC-ptr-recB-recD*)::P_{tac}-*gam-bet-exo-pae-cI822*	13
TP540	Δ*ruvAB6203*::*tet*	12
TP554	Δ*recG6202*	12
TP577	Δ*recG6202* Δ*recF6206*:*tet*	12
TP607	Δ*recG6202* Δ*sulA6209*Δ::*tet*	12
TP643	Δ*recG6202 galK*::P_{tac}-*nin-kan878* Δ*recQ6216*::*tet*	12
TP644	Δ*recG6202 galK*::P_{tac}-*nin-kan878* Δ*recO6218*::*tet*	12
TP645	Δ*recG6202 galK*::P_{tac}-*nin-kan878* Δ*recR6212*::*tet*	12
TP656	*AB1157* Δ*recBCD*::P_{tac}-*gam-bet-exo-pae-cI822* Δ*recG6202 sulA6211*(Δ*cdn9-153*)	TP607 × linear DNA fragment[b]
TP664	*recN6205*(substitution of *tet857* for *cdn4-519*)	TP507 × linear DNA fragment[b]
TP671	*galK*::P_{mac}-*rap-kan914*	TP656 × linear DNA fragment[c]
TP672	*galK*::P_{mac}-*gfp-kan915*	TP656 × linear DNA fragment[c]
TP675	*galK*::P_{mac}-*rap-kan914* Δ(*srl-recA*)*306*::*Tn10*	TP671 × P1(JC15329)
TP676	*galK*::P_{mac}-*rap-kan914* Δ*recF6206*::*tet*	TP671 × P1(TP577)
TP677	*galK*::P_{mac}-*rap-kan914* Δ*recO6218*::*tet*	TP671 × P1(TP644)
TP678	*galK*::P_{mac}-*rap-kan914* Δ*recQ6216*::*tet*	TP671 × P1(TP643)
TP679	*galK*::P_{mac}-*rap-kan914* Δ*recR6212*::*tet*	TP671 × P1(TP645)
TP680	*galK*::P_{mac}-*rap-kan914 ruvC53 eda*::*Tn10*	TP671 × P1(CS85)
TP681	*galK*::P_{mac}-*gfp-kan915* Δ(*srl-recA*)*306*::*Tn10*	TP672 × P1(JC15329)
TP682	*galK*::P_{mac}-*gfp-kan915* Δ*recF6206*::*tet*	TP672 × P1(TP577)
TP683	*galK*::P_{mac}-*gfp-kan915* Δ*recO6218*::*tet*	TP672 × P1(TP644)
TP684	*galK*::P_{mac}-*gfp-kan915* Δ*recQ6216*::*tet*	TP672 × P1(TP643)
TP685	*galK*::P_{mac}-*gfp-kan915* Δ*recR6212*::*tet*	TP672 × P1(TP645)
TP686	*galK*::P_{mac}-*gfp-kan915 ruvC53 eda*::*Tn10*	TP672 × P1(CS85)
TP695	*galK*::P_{mac}-*rap-kan914 recJ284*::*Tn10*	TP672 × P1(SS185)
TP696	*galK*::P_{mac}-*rap-kan914* Δ*recN6205*::*tet*	TP671 × P1(TP664)
TP697	*galK*::P_{mac}-*rap-kan914* Δ*ruvAB6203*::*tet*	TP671 × P1(TP540)
TP698	*galK*::P_{mac}-*rap-kan914 ruvA60*::*Tn10*	TP671 × P1(N2057)
TP700	*galK*::P_{mac}-*gfp-kan915 recJ284*::*Tn10*	TP672 × P1(SS185)
TP701	*galK*::P_{mac}-*gfp-kan915* Δ*recN6205*::*tet*	TP672 × P1(TP664)
TP702	*galK*::P_{mac}-*gfp-kan915* Δ*ruvAB6203*::*tet*	TP672 × P1(TP540)
TP703	*galK*::P_{mac}-*gfp-kan915 ruvA60*::*Tn10*	TP672 × P1(N2057)
TP740	Δ(*recC-ptr-recB-recD*)::*tet-pae-cI963*	TP656 × linear DNA fragment[c]
TP741	Δ(*recC-ptr-recB-recD*)::P_{tac}-*gam-tet-pae-cI967*	TP656 × linear DNA fragment[c]

[a] Strains TP671 through TP741 were all constructed in the TP656 background; they bear deletions of the coding sequences of *recG* and *sulA*. They are presumed to bear all the other genetic markers of AB1157 as well, but these were not tested. Strains TP671 through TP703 bear the substitution (*recC-ptr-recB-recD*)Δ::P_{tac}-*gam-bet-exo-pae-cI822*; strains TP740 and TP741 bear variants lacking Red functions.

[b] The *sulA6211* and *recN6205* alleles were constructed by the use of methods previously described (10).

[c] Details of the construction are given in the text.

linear plasmids. One plasmid, pTP963, was constructed by replacing the Bgl2 site-bounded P_{tac}-*gam-bet-exo* module of plasmid pTP822 (13) with a segment of DNA containing the tetracycline resistance determinant of Tn*10*, previously cloned in plasmid pTP802 (10). The other plasmid, pTP967, was constructed in two steps. First, DNA between the *Hpa*I sites in *bet* and *exo* was replaced by a linker for the restriction enzyme *Not*I. Then a segment of DNA containing the tetracycline resistance determinant of Tn*10*, previously cloned in plasmid pTP857 (10), was inserted into the *Not*I site.

Crosses. Bacterial strains were grown overnight with aeration in Luria-Bertani (LB; 1% tryptone, 0.5% yeast extract, 0.5% NaCl, 1 mM NaOH) medium supplemented with 25 μg of kanamycin/ml and, in the case of tetracycline-resistant strains, 25 μg of tetracycline/ml. Overnight cultures were diluted 100-fold into LB supplemented with 10 mM $MgSO_4$ plus 0.2% maltose, grown with aeration at 37°C, chilled on ice, and adjusted, if necessary, to a density of approximately 2×10^8/ml. Phage was added at a multiplicity of 10, along with isopropyl-β-D-thiogalactopyranoside (IPTG) to a concentration of 1 mM. Tubes containing mixtures of phage and bacteria were incubated on ice for 10 min, shifted to a 37°C water bath for 5 min, and then aerated by rolling at 37°C for 55 min. Cultures were plated on LB agar for determination of viable titer and

TABLE 2. Effects of *rap* on λ Red-mediated recombination in *E. coli* strains

Strain no.		Relevant genotype[a]	% Recombination[b]		Rap effect[c]
rap deficient	*rap*$^+$		*rap* deficient	*rap*$^+$	
672	671	Wild type	1.05 ± 0.11	1.89 ± 0.30	1.8
681	675	*recA*	0.01 ± 0.003	0.02 ± 0.01	2.6
682	676	*recF*	0.20 ± 0.02	0.85 ± 0.08	4.3
700	695	*recJ*	1.35 ± 0.40	1.19 ± 0.69	0.9
701	696	*recN*	0.81 ± 0.20	0.80 ± 0.15	1.0
683	677	*recO*	0.02 ± 0.01	0.01 ± 0.00	0.7
684	678	*recQ*	0.06 ± 0.01	0.12 ± 0.02	2.1
685	679	*recR*	0.01 ± 0.005	0.02 ± 0.01	2.6
703	698	*ruvA*	0.03 ± 0.02	0.04 ± 0.01	1.2
702	697	*ruvAB*	0.03 ± 0.01	0.06 ± 0.02	2.2
686	680	*ruvC*	0.03 ± 0.02	0.80 ± 0.27	23.1

[a] All strains bear the *recBCDΔ*::P_{tac}*-gam-bet-exo-pae-cI822* substitution as well as in-frame deletions of all but the first and last few codons of *recG* and *sulA*. Those listed under the column headed "*rap* deficient" bear the *galK*::P_{mac}*-gfp-kan915* insertion; those listed under the column headed "*rap*$^+$" bear the *galK*::P_{mac}*-rap-kan914* insertion.

[b] Recombination frequencies were measured as described in the text. Averages and standard errors of the ratios of recombinants (white colonies on chloramphenicol–X-Gal–IPTG plates) to total viable titers were determined from 2 to 13 measurements.

[c] Ratio of recombination frequency in the *rap*$^+$ and *rap*-deficient background.

were plated on LB agar supplemented with 10 μg of chloramphenicol/ml plus 1 mM IPTG plus 80 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal)/ml for determination of *lac*::*cat819* recombinants.

The recombination event monitored in these crosses involves replacement of a small segment of the *lacZ* gene with the chloramphenicol resistance-conferring *cat* gene. The *cat* gene is brought into the cell by infection with λ *lac*::*cat819 nin5*. The phage injects its chromosome, which circularizes but is not transcribed or replicated due to the presence of *cI* repressor in the cell. The PaeR7 restriction endonuclease, also present in the cell, cuts the phage chromosome in two places, releasing a double-stranded DNA fragment consisting of the *cat* gene flanked on either side by 1.5-kb segments of *lac* sequence.

To verify that recombination between the cell chromosome and λ *lac*::*cat819 nin5* is dependent upon Red function in the Red-for-RecBCD-substituted *E. coli* strain TP656, variants lacking Red functions were constructed (Table 1). In one strain the entire P_{tac}*-gam-bet-exo* module was eliminated; in another, the last 64 codons of *bet* and the first 46 codons of *exo* were eliminated. Formation of *lac*::*cat819* recombinants was reduced approximately 700- and 400-fold by these two modifications, respectively (averages of two measurements; data not shown).

Rap effects on Red-mediated recombination. Starting with an *E. coli* strain bearing a (*recC-ptr-recB-recD)*Δ::P_{tac}*-gam-bet-exo-pae-cI* substitution as well as deletions of *recG* and *sulA*, two isogenic sets of *rap*$^+$ and mutant *rap* strains with mutations in various other recombination genes were constructed. The reason for employing a background lacking *recG* is that deletion of *recG* elevates the frequency of Red-mediated recombination; deletion of *sulA* was found to enhance the viability of *recBCDΔ*::*red* strains lacking *recF*, *recO*, or *recR* function (12). The results of crosses in these strains are summarized in Table 2. As seen previously (12), recombination in the non-*rap*-containing background depends on *recA*, *recF*, *recO*, *recQ*, *recR*, *ruvAB*, and *ruvC*. The data in Table 2 indicate that mutation of *recJ* in the *red*$^+$ *recGΔ* background had little effect on recombination; in contrast, it significantly reduced recombination in the previous study. We attribute the difference in outcomes to the absence of *sulA* in the strains constructed for this study, which improves the viability of the *recG recJ* double mutant. In addition, the data in Table 2 show that deletion of *recN* has little effect on Red-mediated recombination.

Expression of *rap* alters the genetic dependency of Red-mediated recombination, as shown in Table 2. Recombination in the *rap*$^+$ cell is much less dependent upon *ruvC*, as predicted. In addition, recombination is somewhat less dependent upon *recF*. Slight differences are also seen in *recA*, *recQ*, *recR*, and *ruvAB* mutants, but these are of doubtful significance, representing small changes in low recombination frequencies.

Other Rap phenotypes. The restoration of recombination proficiency to a *recGΔ ruvC* mutant by *rap* suggested that *rap* might restore some degree of resistance to DNA-damaging agents as well, but this appears not to be the case. Testing UV sensitivity, as described previously (12), we found that there was no reliably measurable difference between *rap*$^+$ and mutant *rap* versions of the *recGΔ ruvC* strain (data not shown).

Introduction of the *ruvC53* allele into *E. coli* (*recC-ptr-recB-recD)*Δ::P_{tac}*-gam-bet-exo-pae-cI recGΔ sulAΔ* produces a cell which grows quite slowly relative to its *ruvC*$^+$ parent. When *rap* is expressed in the cell, the growth defect is less pronounced but is still detectable. However, cultures of both *rap*$^+$ and non-*rap*-containing variants are frequently taken over by fast-growing, recombination-proficient, and relatively UV-resistant mutants. We assume these mutants are pseudorevertants, with mutations activating the *rusA* gene as described by Mandal et al. (7), but we have not tested them further. To ensure that the results shown in Table 2 were not influenced by the presence of such revertants, *ruvC* mutant strains were reconstructed by P1 transduction for each experiment. Only crosses which showed no evidence of fast-growing revertants after plating were used in determining recombination frequencies.

None of the phenotypes of the *rap*$^+$ strains is affected by the presence or absence of the *lac* inducer IPTG, although the control *gfp*$^+$ strain fluoresces more brightly when grown in the presence of IPTG (data not shown). We infer that the basal

level of expression from P_{mac} is sufficient to suppress the recombination and cell growth phenotypes of *recBCD*Δ:: *red-pae-cI recG*Δ *sulA*Δ *ruvC53*, but neither the basal nor induced level suppresses the UV sensitivity phenotype. The inability of the wild-type, chromosomal *lacI* gene to exert tight negative control over *rap* expression may be due to the presence in the cell of four different promoters capable of binding (and thus partially titrating) the *lac* repressor: wild-type P_{lac}, P_{tac}-*gam-bet-exo*, P_{lacUV5}-*cI*, and P_{mac}-*rap*. However, this hypothesis has not been tested.

The properties of rap^+ cells suggest that Rap can substitute for RuvC in some cellular pathways but not in others. On the other hand, the Rap protein's partial complementation of a *recF* mutant (Table 2) and its broader range of nucleolytic activities (16) suggests that, conversely, Rap may carry out an additional function(s), most likely in phage replication, repair, or recombination, that RuvC does not.

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